The Management and Outcomes of Neuroblastoma in South Africa

Jaques van Heerden

Thesis presented for the degree of
Doctor of Philosophy
in the Department of Paediatrics and Child Health
Stellenbosch University

March 2021

Supervisor: Professor Mariana Kruger

Stellenbosch University https://scholar.sun.ac.za

DECLARATION

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in the Department of Paediatrics and Child Health, Stellenbosch University. By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated), and in the case of multi-authored published papers, constitutes work for which the candidate was the lead author. The contribution of the candidate to multi-authored papers is outlined in the introduction to each included paper as appropriate. I have not previously in its entirety, or in part, submitted the thesis for obtaining any qualification at this or any other university.

Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the funders.

PLAGIARISM DECLARATION

This thesis has been submitted to the Turnitin module and I confirm that my supervisors have seen my report and any concerns revealed by such have been resolved with my supervisors.

Jaques van Heerden
March 2021

Copyright ©2021 Stellenbosch University

All rights reserved. No part of this publication may be reproduced or transmitted in any form or by any means electronic or mechanical including photocopy, recording, or any information storage or retrieval system, without written permission of the author.

Stellenbosch University https://scholar.sun.ac.za

DEDICATION

Halfway Down an A. A. Milne poem

Halfway down the stairs
is a stair
where i sit.
there isn't any
other stair
quite like
it.
i'm not at the bottom,
i'm not at the top;
so this is the stair
where
I always
stop.

Halfway up the stairs
Isn't up
And it isn't down.
It isn't in the nursery,
It isn't in town.
And all sorts of funny thoughts
Run round my head.
It isn't really
Anywhere!
It's somewhere else
Instead!

And this is to that child who was unreminded – that halfway down was also halfway up instead.

Stellenbosch University https://scholar.sun.ac.za

THE NATURE AND SCOPE OF CONTRIBUTIONS

Jaques van Heerden conceptualized most the studies, unless noted differently in each manuscript, and developed the protocols, participated in recruitment of the patients for the prospective studies, and performed data analysis, assisted by the statisticians as indicated per article or manuscript. The student drafted and finalized all manuscripts for publication in chapters 2-10.

Mariana Kruger conceptualized some studies as indicated per manuscript, and assisted in concept development of all studies, critically reviewed and edited all manuscripts.

Anita Brink assisted in the conceptualization of the tumour markers and mIBG study, and assisted in concept development, critically reviewed and edited the manuscript of chapter 6.

Larry Hadley assisted in concept development, critically reviewed and edited the manuscript of chapter 8.

Judy Schoeman provided data from the South African Children's Tumour Registry (SACTR).

Tonya Esterhuizen (Chapter 5,6,8,10), *Kirstien Wouters* (Chapter 7) and *Benn Sartorius* (Chapter 4) were the statisticians that performed the data analysis and critically reviewed and edited all data related reporting contained in the manuscripts.

All co-authors critically reviewed manuscripts and agreed that they could be included in the thesis my PhD.

Stellenbosch University https://scholar.sun.ac.za

SUMMARY (English)

Neuroblastoma (NB) is the second most diagnosed childhood solid tumour in high-income countries (HIC), but the incidence has not accurately been described in low- and middle-income countries (LMICs). The diagnostic difficulty, with limited treatment modalities, contributes to poor outcomes in LMICs. This PhD dissertation investigates the management of NB in South Africa with the aim to develop the first prospective national neuroblastoma treatment protocol/clinical trial to improve overall survival (OS).

Using the South African Cancer Study Group's Tumour Registry data, between 2000 and 2016 the incidence of NB in South Africa was found to be between 1.74 to 2.6 cases/million children, which was lower than the 10.5 cases per million children reported in HICs. South Africa had a higher number of patients with high-risk (HR) tumours (75.6%), mainly due to advanced disease (70%). The 2-year OS was excellent for very low risk (VLR) (94.1%) and low risk (LR) disease (81.6%), while acceptable for intermediate risk (IR) disease (66.7%) but poor for HR disease (27.6%) ($p<0.001$, 95% CI). Limitations in risk stratification included the low number of tumours tested for MYCN (38.4%), with more than half being MYCN-amplified (54%), and no other NB related genetic characteristics.

Several treatment protocols were used in the different paediatric oncology units in South Africa during the study period (2000-2014) and the OPEC/OJEC (carboplatin, cisplatin, etoposide, cyclophosphamide and vincristine) induction chemotherapy regimen proved to be the least toxic with better metastatic remission rates for HR-NB. Ferritin had predictive value for complete metastatic remission rate, while LDH had predictive value for two-year OS and were found to be suitable tumour markers to use as surrogates for sophisticated genetic testing and mIBG-scans in the context of limited resources. Age at diagnosis, specifically the 18-month cut-point value, remained a significant prognostic factor, similar to HICs.

Due to limited access to autologous stem cell transplants, the role of surgery and radiotherapy in the management of HR disease were investigated and found to significantly improve five-year OS with surgery and marginally with radiotherapy ($p<0.001$, 95% CI). Furthermore, the disparities in neuroblastoma health care provision in the different provinces in South Africa was found to exist and should be addressed to ensure equitable health care provision for all children as per the South African Constitution.

The implementation of the newly developed national NB single arm clinical trial in South Africa, adjusted to available national resources, was a complex process with major navigational bureaucratic challenges. Yet the process might serve as a guideline for similar processes in LMICs. The recruitment of patients into the national NB clinical trial proved to be difficult due to both the COVID-19 pandemic and reluctance to recruit advanced stage patients into clinical trials. However, with careful investigation and in collaborative spirit, rare diseases such as neuroblastoma in South Africa could be managed in national management protocols, aimed at improving overall survival and cure.

Stellenbosch University https://scholar.sun.ac.za

OPSOMMING (Afrikaans)

Neuroblastoom (NB) is die tweede mees gediagnoseerde soliede tumor by kinders in hoëinkomstelande (HIL'e). Tog word die voorkoms daarvan in lae- en middelinkomstelande (LMIL'e) nie akkuraat beskryf nie. Die diagnostiese uitdaging, sowel as beperkte behandelingsmetodes, lei dus tot swak uitkomste in LMIL'e. Hierdie PhD dissertasie ondersoek die behandeling van NB in Siud Afrika ten einde die algehele oorlewingsyfer (AO) te verbeter.

Deur van data tussen 2000 en 2016 van die Suid Afrikaanse Kinderkanker Studie Groep se Tumor Register gebruik te maak, was die voorkomssyfer van NB in Suid-Afrika as 1,74 tot 2,6 gevalle per miljoen kinders bereken, wat veel laer was as die aangemelde 10,4 gevalle per miljoen kinders in HIL'e. Suid-Afrika het 'n hoër getal pasiënte met hoërisiko- (HR-)tumore (75,6%), hoofsaaklik vanweë gevorderde siekte (70%). Die AO oor twee jaar het was uitstekend vir uiters laerisiko- (ULR-) (94,1%) en laerisiko- (LR-)siekte (81,6%), aanvaarbaar vir matigerisiko- (MR-) (66,7%), maar sleg vir HR-siekte (27,6%) ($p<0,001$, 95% CI). Beperkings in risikostratifikasie het ingesluit die klein aantal tumore wat vir MYCN getoets is (38,4%), met meer as die helfde met MYCN-amplifikasie (54%), en die onvermoë om NB-verwante genetiese kenmerke te bepaal.

Verskeie behandelingsprotokolle was gebruik deur die verskillende kinderkanker eenheide in Suid Afrika gedurende hierdie periode (200-2014) en dit was getoon dat die OPEC/OJEC-regimen (karboplatien, sisplatien, etoposied, siklofosfamied en vinkristien) die grootste voordeel vir HR-NB inhou wat toksisiteit en metastatiese remissiesyfers betref. Ferritien het waarde om die metastatiese algehele-remissiesyfer te voorspel, terwyl LDH by die voorspelling van die AO oor twee jaar waardevol was en kan albei tumormerkers gebruik word as surogate van gesofistikeerde genetiese toetse en mIBG-skandering in omstandighede met beperkte hulpbronne. Soos wat in HIL'e bevind is, bly ouderdom ten tyde van diagnose, maar spesifiek die 18 maande afsnypunt, 'n beduidende prognostiese faktor.

Met beperkte toegang tot outoloë stamseloorplantings, was die rol van sowel chirurgie as radioterapie ondersoek en dit was bewys dat chirurgie die AO oor vyf jaar noemenswaardig verbeter terwyl tumorbestraling 'n effens beter AO oor vyf jaar getoon het ($p<0.001$, 95% CI). Verder het ons aangetoon dat daar noemenswaardige hulpbron verskille tussen provinsies bestaan wat aangespreek moet word om regverdige gesondheidsorg aan alle kinders te besorg volgens die Soud Afrikaanse Grondwet.

Met die implementering van 'n nasionale NB-protokol enkel arm kliniese studie in Suid-Afrika, met die doel om die benutting van nasionale hulpbronne te optimaliseer, was 'n kompleks onderneming waartydens vele burokratiese uitdagings genavigeer moes word, maar kan as 'n riglyn vir ander LMIL'e dien. Die proses om patiente vir die nasionale NB kliniese studie in te win was was uitdagend as gevolg van beide COVID-19 pandemie en die onwilligheid om patiente met gevorderde siekte by die studie in te sluit. Egter, met respekvolle ondersoek en 'n gees van samewerking, kan seldsame siektes soos neuroblastoom in Suid Afrika volgens 'n nasionale behandelingsprotokol behandel word met die doel om die algemene oorlewing te verbeter en patiente te genees.

Stellenbosch University https://scholar.sun.ac.za

SAMENVATTING (Dutch)

Neuroblastoom (NB) is de tweede, meest gediagnosticeerde solide tumor bij kinderen in 'high-income countries' (HIC), maar de incidentie is niet nauwkeurig beschreven in 'low- and middle-income countries' (LMIC's). De diagnostische problemen met de beperkte behandelingsmodaliteiten dragen bij aan een slechte prognose in LMIC's. Dit proefschrift onderzoekt de behandelingsresultaten van NB in Zuid-Afrika met als doel de prognose te verbeteren.

De data van het Tumor Register van de Zuid Afrikaanse Kinkerkanker Studie tussen 2000 en 2006 laten een incidentie van NB in Zuid-Afrika zien van 1,74-2,6 gevallen per miljoen kinderen, hetgeen lager is dan de 10,4 gevallen per miljoen kinderen die gerapporteerd worden in HIC. Zuid-Afrika heeft een groter aantal patiënten met hoog risico (HR) tumoren (75,6%), voornamelijk vanwege de gevorderde ziekte bij presentatie (70%). De 2-jaars 'overal survival' (OS) waren uitstekend bij zeer laag risico (VLR) (94,1%) en laag risico (LR) (81,6%), aanvaardbaar bij gemiddeld risico (IR) (66,7%), maar slecht bij HR-ziekte (27,6%) ($p < 0,001$, 95% CI). Beperkingen in risicostratificatie waren onder meer, het lage aantal tumoren dat op MYCN werd getest (38,4%), met meer dan het helft (54%) MYCN geamplificeerd, en het onvermogen om NB-gerelateerde genetische kenmerken te bepalen.

Tijdens de studie periode (2000-2014) werden meerdere behandelingsprotocollen door verschillende pediatrische oncologie-eenheden gebruikt en bleek het OPEC/OJEC-regime (carboplatine, cisplatine, etoposide, cyclofosfamide en vincristine) de meeste voordelen te bieden met betrekking tot de toxiciteit en tumorremissie bij HR-NB. Ferritine blijkt belangrijk te zijn bij het voorspellen van het percentage van complete remissie, terwijl LDH van waarde is bij het voorspellen van tweejarige OS. Beiden zijn geschikte tumormarkers om te gebruiken als surrogaten voor geavanceerde genetische tests en mIBG-scans in de context van beperkte middelen. Leeftijd bij diagnose, met name de 18-maanden 'cut-off', is een belangrijke prognostische factor zoals in HIC's.

Gezien de beperkte toegang tot autologe stamceltransplantaties, werden de rollen van zowel chirurgie als radiotherapie bij HR ziekte bestudeerd, en blijkt de vijfjarige OS significant te verbeteren met chirurgie en marginaal met radiotherapie ($p<0.001$, 95% CI). Bovendien blijken er verschillen in de gezondheidszorg voor neuroblastoom in de verschillende provincies in Zuid-Afrika te bestaan en deze zouden moeten worden aangepakt om te zorgen voor een rechtvaardige gezondheidszorg voor alle kinderen volgens de Zuid-Afrikaanse grondwet.

De implementatie van de nieuw ontwikkelde nationale NB klinisch studie in Zuid-Afrika, aangepast aan de beschikbare nationale middelen, was een complex proces met grote bureaucratische uitdagingen die moesten worden overwonnen. Toch zou het proces als richtlijn kunnen dienen voor soortgelijke processen in LMIC's. De rekrutering van patiënten voor de nationale NB klinische studie bleek moeilijk te zijn vanwege zowel de COVID-19 pandemie als de onwil om patiënten in een gevorderd stadium voor klinische studies te werven. Met zorgvuldig onderzoek en in een samenwerkingsgeest zouden zeldzame ziekten zoals neuroblastoom in Zuid-Afrika echter kunnen worden behandeld met behulp van nationale managementprotocollen, gericht op het verminderen van de morbiditeit en mortaliteit.

Stellenbosch University https://scholar.sun.ac.za

RĒSUMĒ (French)

Le neuroblastome (NB) est la deuxième tumeur solide de l'enfant la plus diagnostiquée dans les pays à revenu élevé (High-Income Countries en anglais, HIC), mais son incidence n'a pas été décrite avec précision dans les pays à faible et moyen revenu (Low and Middle-Income Countries en anglais, LMIC). La difficulté diagnostique, avec des modalités de traitement limitées, contribue à de mauvais résultats dans les pays à faible et moyen revenu. Cette thèse de doctorat a pour objectif de structurer la prise en charge du NB en Afrique du Sud dans le but éventuel de développer le premier protocole national/ essai clinique afin d'améliorer la survie globale (Overall Survival en anglais, OS).

Les données provenant du registre du South African Cancer Study Group entre 2000 et 2016, rapportent une incidence du NB en Afrique du Sud de 1,74 à 2,6 cas/million d'enfants, ce qui est inférieur aux 10,4 cas/million d'enfants déclarés dans les HIC. L'Afrique du Sud a un nombre plus élevé de patients atteints de tumeurs de haut risque (HR) (75,6%), principalement en raison d'une maladie avancée (70%) et d'une amplification MYCN de 54% des tumeurs. La survie globale à 2 ans est excellente pour les maladies de très faible risque (VLR) (94,1%) et de faible risque (LR) (81,6%), tandis qu'elle est acceptable pour la maladie de risque intermédiaire (IR) (66,7%) mais pauvre pour la maladie HR (27,6%) ($p < 0,001$, 95% CI). Les limites de la stratification des risques comprennent le faible nombre de tumeurs testées pour MYCN (38,4%), avec plus de la moitié étant MYCN-amplifié (54%) et sans autres caractéristiques génétiques du NB.

Plusieurs protocoles de traitement ont été utilisés dans différentes unités de pédiatrie oncologique durant la période de l'étude (2000-2014). La cure d'induction OPEC/OJEC (carboplatine, cisplatine, étoposide, cyclophosphamide et vincristine) a rapporté le moins de toxicités avec un meilleur taux de rémission métastatique pour le neuroblastome de haut risque. La Ferritine présente une valeur prédictive pour le taux de rémission complet, tandis que la LDH a une valeur prédictive pour la survie globale à deux ans. Ces marqueurs tumoraux sont adéquats comme substitut pour des tests génétiques sophistiqués et des scans-mIBG dans le contexte des ressources limitées. L'âge au diagnostic, spécifiquement la valeur seuil de 18 mois, reste un facteur prognostique important, similaire au HICs.

En raison de l'accès limité à la transplantation de cellules souches autologues, les rôles de la chirurgie et de la radiothérapie dans la gestion des maladies à haut risques ont été évalués. Il a été constaté une amélioration importante de la survie générale à 5 ans avec la chirurgie et marginalement avec la radiothérapie ($p<0.001$, 95% CI). En outre, des disparités de prise en charge du neuroblastome dans les différentes provinces d'Afrique du Sud ont été constatées et doivent être adressées pour assurer à tous les enfants une prestation de soin équitable conformément à la constitution Sud-Africaine.

La mise en place de l'essai clinique national à bras unique portant sur le NB et nouvellement développé en Afrique du Sud, ajusté aux ressources disponibles, était un processus complexe avec des défis majeurs d'ordre bureaucratique. Le processus pourrait servir comme directive aux processus similaires dans les LMICs. Le recrutement des patients pour l'essai clinique du NB s'est avéré être compliqué en raison de deux facteurs, la pandémie du COVID-19 et la réticence à recruter des patients de stade avancé dans l'essai clinique. Cependant, avec une étude approfondie et un esprit collaboratif, les maladies rares tel que le neuroblastome en Afrique du Sud ont pu être gérées dans les protocoles de gestion national, visant à améliorer la survie générale et la guérison.

Stellenbosch University https://scholar.sun.ac.za

TABLE OF CONTENTS

Stellenbosch University https://scholar.sun.ac.za

LIST OF ABBREVIATIONS

AUC	Area under the curve
ASCT	Autologous stem cell transplant
CI	Confidence interval
CR	Complete response
CT	Computed tomography
COG	Children's Oncology Group
EC	Eastern Cape
EFS	Event-free survival
FDG/PET	^{129}Fluorodeoxyglucose positron emission tomography
FH	Favorable histology
FS	Freestate
FNA	Fine-needle aspiration
G-CSF	Granulocyte colony stimulating factor
GFAOP	French-African Paediatric Oncology Group
GTR	Gross total resection
GP	Gauteng Province
HFA	Height for age
HIC	High-income countries
HIV	Human immunodeficiency virus
HR	High risk
HR-NB	High-risk neuroblastoma
INPC	International Neuroblastoma Pathology Classification
IDRF	Image-defined risk factors
INRC	International Neuroblastoma Response Criteria
INRG	International Neuroblastoma Risk Group
INSS	International Neuroblastoma Staging System
IR	Intermediate risk
KZN	Kwa-Zulu Natal
LDH	Lactate dehydrogenase
LMICs	Low- and middle-income countries
LP	Limpopo
LR	Low risk
mCR	Metastatic complete remission
mIBG	meta-Iodobenzylguanidine
MIC	Middle-income country
MP	Mpumalanga
MRI	Magnetic resonance imaging
NA	Non-amplified
NC	Northern Cape
NB	Neuroblastoma
nGTR	Near gross total resection
NPV	Negative predictive value
NW	North West

Stellenbosch University https://scholar.sun.ac.za

OMAS	Opsoclonus myoclonus ataxia syndrome
OR	Odds ratio
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PODC	Paediatric Oncology for Developing Countries
POU	Paediatric oncology units
PPV	Positive predictive value
PTR	Primary tumor site relapse
ROC	Receiver operating characteristic curve
RR	Risk ratio
RT	Radiotherapy
SA	South Africa
SACCSG	South African Children's Cancer Study Group
SACTR	South African Children's Tumour Registry
SA-NCR	South African National Cancer Registry
Se	Sensitivity
SIOP	International Society for Paediatric Oncology
SIOPEN	International Society of Paediatric Oncology European Neuroblastoma Research Network
Sp	Specificity
STR	Subtotal resection
TB	Tuberculosis
UH	Unfavorable histology
US	Ultrasonography
VGPR	Very good partial response
VIP	Vasoactive intestinal peptide
VLR	Very-low-risk
WC	Western Cape
WHO	World Health Organization
WFA	Weight for age

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 1

INTRODUCTION

1.1 Background

Definition

Neuroblastoma (NB), with ganglioblastoma (GB) and ganglioneuroblastoma (GNB), is a heterogeneous group of neural crest malignancies that accounts for the most extracranial solid tumours in childhood[1,2]. It is an aggressive, metabolically active and complex disease that clinically mimics many diseases. The heterogeneous pathophysiological presentation of NB, paired with the diverse prognostic outcome in different stages of the disease, makes the treatment of this disease an oncological challenge[3,4]. Advanced disease is present in both low- and middle-income countries (LMICs) as well as high-income countries (HICs). NB poses both clinical and management challenges and requires numerous resources during treatment[4].

Epidemiology

The epidemiology of NB is not well documented in LMICs due to a lack of tumour registries in the context of limitations on resources[5,6]. Although NB is the most common sympathetic tumour in children, reliable paediatric cancer registries do not exist or are limited to single institutions or studies[7,8]. This limits the interpretation of data in Africa[5]. According to the world-age standardised rate (WSR), the incidence is about 12% across regions but less than 10% in Africa[5]. The WSR of 10.2 per million person-years in children in the USA is in contrast with the WSR of 2.7 per million person-years in Sub-Saharan Africa in populations with similar ethnicities[5]. Data from the South African Children's Tumour Registry published in 2015 reported an age-standardised ratio (ASR) of 3.1/1 000 000 for NB and 3.0/1 000 000 for NB and GNB[9].

Clinical presentation

NB has significant differences in prognosis between stages and biological characteristics. Stage 1 disease has an excellent prognosis versus stage 4 disease with an extremely poor prognosis[1,2,10], even in HIC. Without high-intensity treatment, advanced-stage disease has a poor outcome in LMIC with treatment being palliative rather than curative[3,4].

The clinical presentation can be diverse and therefore challenging to diagnose. Although nearly 65% of tumours present in the abdomen[11], other sites include the anatomical distribution of the sympathetic chain from the brain to the kidneys and pelvic sites[1,3]. Other distinctive features of presentations are often due to metastatic manifestations such as bilateral proptosis, "racoon eyes", bone pain, pancytopenia and constitutional symptoms[11]. Two paraneoplastic syndromes associated with NB include Vaso-intestinal peptide syndrome and Opsoclonus/Myoclonus syndrome[11,12,13]. Other neurological presentations are Horner syndrome, due to tumours in the neck[14], and paraspinal symptomatology. In stage 4s, apart from the primary lesion, infants under 12 months may present with cutaneous lesions, bone marrow infiltration of less than 10% and/or liver lesions[1]. Therefore, NB could be classified as either local disease, metastatic disease or stage 4s disease[11]. The clinical presentation will be dependent on the locality of the tumour with local pressure effects, varying from para-spinal disease to a disseminated toxic presentation[4,11].

Stellenbosch University https://scholar.sun.ac.za

Molecular and genetic characteristics

The basis of clinical presentation can be explained by the molecular, genetic and epigenetic diversity of NB[15]. All NB tumours develop from a common precursor, but the variability of genomic instability influences the development alterations responsible for the heterogeneous presentation[15]. NB has both germline and somatic mutations that are expressed in the clinical phenotype of the disease. Genetic aberrations are present at molecular, genetic and epigenetic levels[15]. The development of NB is dependent on the accumulation of mutations where the pre-malignant cells rapidly replicate in proliferating tissue during embryogenesis in the central nervous system[16,17]. The N-MYC proto-oncogene is overexpressed in neurogenesis for the rapid expansion of progenitor cell populations[16,17]. Two types of somatic mutations are important in the pathogenesis[18,19]. Type 1 is characterised by whole chromosomal gains and losses and few segmental alterations, which predisposes to favourable biology like hyperdiploidy and MYCN-negative tumours[18]. These tumours express high levels of TrkA receptor[19]. Type 2 is characterised by segmental alterations of unbalanced deletions and alterations, with or without whole chromosome changes[18]. A further subtype 2A is mainly the 3p and 11q segmental deletions without MYCN amplification[18]. Subtype 2A is very aggressive[18,20]. Subtype 2B is MYCN amplified with 1p deletion and unbalanced 17q gain whilst expressing TrkB receptor. Tumour genetics is also important to identify genomic characteristics that cause chemotherapy resistance and poor treatment response[21]. At the epigenetic level, DNA methylation of tumour suppressors contributes to refractory disease[22,23].

Diagnosis and treatment

Biological and clinical characteristics at presentation form an important part in the treatment of NB. International management protocols are based on risk stratification[24,25]. These risk classifications are based on the presence of prognostic factors such as age, image-defined risk factors (IDRF) and stage[4,24]. Children younger than 12 to 18 months have favourable outcomes as opposed to children older than 18 months[4,24]. IDRF is determined via x-rays, ultrasound, computer-tomography and magnetic resonance imaging and includes encasement, compression and infiltration of structures in local areas. The more structures involved or amount of metastasis, the more the treatment risks are upscaled[24,26]. Local complete resectable tumours are stage 1 and have a good prognosis whilst distant metastasis to bone, bone marrow, liver and lungs, or stage 4 disease, has a poor prognosis[4,24]. Metastasis is diagnosed by the presence of NB cells in bone marrow aspirates and bone marrow trephine biopsies, tumour-involved organs on advanced imaging or with the positive enhancement of nuclear isotopes of bones on bone scans and bone, bone marrow and soft tissue involvement on Iodine-123-metaiodobenzylguanidine (^{123}I-MIBG) scans[27].

By using the Modified Shimada or International Neuroblastoma Pathology Classification (INPC) classification, histology is classified into favourable and unfavourable prognostic classifications[28]. This is determined by the age of the patient and the morphological differentiation of the NB cells[18]. Undifferentiated or poorly differentiated morphology and age over five years are factors associated with unfavourable histology[18,28].

Other prognostic factors included in risk stratifications that determine the outcome of treatment in NB are translocations, ploidy and the presence of MYCN in molecular studies. MYCN is an adverse prognostic marker in NB, as are 11q aberrations and diploidy[24,25]. In LMIC, where either fluorescent in

Stellenbosch University https://scholar.sun.ac.za

vitro hybridization (FISH) or polymerase chain reaction (PCR) studies to detect the presence of MYCN are not available, ferritin and/or lactate dehydrogenase (LDH) can be used as surrogate markers to indicate an adverse prognosis[4,25]. Although not included into risk stratification failure of the tumour to respond to induction chemotherapy[27], less than 95% tumour resection or debulking[29] and a diffuse pattern of metastatic infiltration on ^{123}I-MIBG scans are poor prognostic factors[27].

Based on these risk factors, treatment is stratified into very low risk (VLR), low risk (LR), intermediate-risk (IR) and high-risk (HR) groups, with each risk group dictating various treatment schedules. Two internationally recognised clinical staging systems include the International Neuroblastoma Staging System/Children's Oncology Group (INSS/COG) and the International Neuroblastoma Risk Group (INRG)[1,2]. The INSS is a surgical outcome-based system that was developed by the North America COG. The risk factors include tumour stage, age and biological characteristics[1]. Stage I is completely resected tumours, stage 2 is incompletely resected tumours with or without lymph node involvement, stage 3 unresectable tumours and stage 4 metastatic disease[1]. Patients younger than 12 months of age have a better outcome than children younger than 18 months[1]. Patients older than 18 months have a poorer prognosis than younger children[1]. Biological characteristics such as the tumour-specific MYCN, poor tumour cell differentiation according to the Shimada classification and DNA ploidy incur a poorer prognosis with higher risk stratification[1].

The INRG system was developed in Europe by the International Society for Paediatric Oncology (SIOP) and also includes age, MYCN and tumour cell differentiation as risk factors. The difference is that this staging system is based mainly on pre-surgical assessment, relying on imaging, bone marrow morphology and nuclear studies. This staging system includes a very low-risk group[1,2]. INRG utilizes IDRF such as the encasement, compression and infiltration of structures in local areas to define the staging[25,26]. The staging is performed by clinical examination and various investigation modalities which include chest X-ray, abdominal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), meta-iodobenzylguanidine (MIBG) scan as well as bilateral bone marrow biopsies[4,25]. Furthermore, it includes 11q aberration as a biological risk factor[1].

Neuroblastoma in low- and middle-income countries

Paediatric Oncology for Developing Countries (PODC), a dedicated group within SIOP, developed an adapted risk group classification based on imaging, availability of resources per setting, and the initial surgical status of the tumour to guide treatment[4]. The classification includes non-specific tumour markers such as LDH and ferritin as risk factors apart from surgical outcome and MYCN[4].

In the LR groups of both staging systems, surgery and observation are the standards of care, with chemotherapy as a second-line treatment modality for progressive disease[1,21]. This strategy obtains a five-year overall survival (OS) of up to 98%[1,21]. In the IR groups, the main treatment modalities are chemotherapy, surgery and to a lesser extent radiotherapy[1,29,30], obtaining a five-year OS of up to 96%. The VLR strategy employs a "wait-and-see" approach[1,29,30] with five-year OS outcomes of nearly 100%[1,31]. Ideally, the treatment for high-risk disease comprises an induction with high-intensity chemotherapy, surgery and radiotherapy, autologous bone marrow transplantation, immunotherapy with anti-GD2 targeted therapy and tumour cell maturation therapy with cis-retinoic acid (CRA)[29,32]. Even with this treatment intensity, OS is only 50 to 60% in HIC[1,31]. In LMIC, advanced NB has a poor prognosis of less than 20% five-year survival[3,4].

Stellenbosch University https://scholar.sun.ac.za

Due to limited resources and limited access to medical care, the full complement of treatment may not be available in resource-limited settings[4]. Many factors play a role in providing the ultimate care to patients with NB, including the stage of the disease, nutritional status at diagnosis, family resources, belief systems and personal autonomy. Resources, medicine security and expertise in institutions influence treatment decisions to a similar extent as treatment adherence and response to treatment. The ability of facilities to provide supportive care in terms of antibiotics, intensive care and granulocyte stimulating factors (G-CSF) determine the intensity of treatment[4,6]. In South Africa, the basic three treatment modalities of chemotherapy, surgery and radiotherapy, as well as good supportive care, are available and are part of the standard of care for children with NB. With the three modalities of chemotherapy, surgery and radiotherapy the prognosis varies from 96% five-year OS for stage 1 to a dismal less than 20% five-year OS for stage 4[1,2,4]. Autologous transplantation and CRA for advanced disease are institution dependant and patients with private medical care[32] mostly have greater access to funds. Immunotherapy with anti-GD2 is currently not available in South Africa. As the full complement of treatment available to HIC for a high-risk disease is not available in resource-constrained countries, a high-risk disease has a poor prognostic outcome of less than 20 to 50% five-year OS[3,4].

The evaluation of treatment response is important for two reasons. The incomplete response to induction treatment carries a poorer prognosis and indicates a very high-risk group of patients in the high-risk classification group[33]. This group includes patients with a metastatic bone disease that displays poor treatment response after induction chemotherapy[33]. To identify risk groups and evaluate the treatment response, basic, advanced and nuclear imaging form the basis for recommendations of criteria based on the International Neuroblastoma Response Criteria (INRC) of 1993, the revised INRC criteria of 2017 and the soft tissue Response Evaluation Criteria in Solid Tumours (RECIST), which are validated tools to evaluate treatment response[34]. All three response evaluation tools grade the decrease or increase of tumour volume in primary and metastatic regions and correlate it to complete response, very good partial response, partial response, stable disease or progressive disease[34]. Treatment responses provide guidelines for possible further treatment actions.

The International Society for Paediatric Oncology European Neuroblastoma group (SIOPEN), a multi-national group which includes Europe, Israel, Japan and Australia, developed the INRG classification system, and are developing protocols together and sharing information to improve outcomes[35]. In rare diseases, such as NB, co-operative working groups promote treatment development and evaluation tools and improve outcomes[36,37]. Implementing clinical trials for new treatment options improves the statistical significance with pooled data[37]. This is evident in the improved outcomes for high-risk disease from the five-year OS of 20% to 57% due to co-operative research over the past 20 years[38].

With the growing population in LMIC, the burden of malignancies in paediatrics will become a challenge for LMIC [4]. Yet most advanced diagnostics and treatment developments are taking place in the HIC [4]. As the field of translational oncology is still in its infancy, the translation of novel diagnostics and treatments at a laboratory level still has far to go concerning the application in the clinical setting. It is vitally important that clinicians research these translational aspects according to the capacity of their settings and population-based research to improve outcomes.

Stellenbosch University https://scholar.sun.ac.za

1.2 Problem Statement and Study Rationale

Historically, the treatment strategies for NB in South Africa have been diverse and are based on the experience of the individual paediatric oncologists. Under the umbrella of the South African Children's Cancer Group's (SACCSG) commitment to developing national management protocols for common childhood cancer [4], the study retrospectively investigated the prevalence and outcome of NB, treated with various treatment protocols, as well as documented the development and initiation of a standard national NB management protocol for children in South Africa.

1.3 Purpose and Objectives

The purpose of the doctoral research

The purpose of the research was to investigate neuroblastoma in South Africa for three purposes. Firstly, a retrospective review was done to evaluate the treatment approaches, morbidity and outcome over 14 years in all paediatric oncology units (POUs) in South Africa. Data were evaluated to compare the OS of different treatment strategies that included either chemotherapy, radiotherapy or surgery. The data were further compared to international data. Secondly, the doctoral study investigated what the associations were between known risk factors and mCR, OS and event-free survival (EFS) as outcomes. Age at diagnosis, specific and non-specific tumour markers and ^{123}I-MIBG scans were evaluated for the respective outcomes. The impact of local treatment on the outcome of NB in South Africa was investigated to formulate recommendations for the prospective study. Thirdly, the prospective standard management protocol for all South African children diagnosed with biopsy-proven neuroblastoma to describe and evaluate the implementation of the protocol was implemented. During the process, the researchers aimed to develop recommendations and resources for other LMICs to initiate similar projects.

The objectives of the research

1. To complete a literature review of the characteristics at diagnosis, prognostic factors and management of NB and OS with a focus on successful management strategies in LMICs with limited resources in comparison to HIC.

2. To perform a retrospective analysis of the pathophysiology, EFS and OS of NB patients treated with various standard treatment protocols in South African POUs from 2000 to 2014.

3. To determine the true incidence of NB in South Africa based on registrations at the South African Children's Tumour Registry (SACTR).

4. To investigate the prognostic value of age at diagnosis in South African patients and the association between the prognostic non-specific tumour markers LDH and Ferritin, specific tumour marker MYCN and the prognostic value of MIBG scan with post-induction remission and OS in children with NB.

5. To evaluate the outcomes between the standard chemotherapy protocols and local management interventions for evidence-based practices in the South African setting to inform recommendations for a prospective protocol.

Stellenbosch University https://scholar.sun.ac.za

6. To describe the development and implementation of a national prospective management protocol to improve the OS of patients diagnosed with NB and evaluate the implementation of a national protocol in the South African setting.

7. To provide an interim analysis of the patients recruited on the South African national neuroblastoma single-arm clinical trial.

1.4 Structure of the Dissertation

This thesis, in a publication format, consists of a series of reviews and individual studies to chronologically document the development of a national prospective management protocol for neuroblastoma, each with the corresponding hypothesis and objectives.

CHAPTER 2: The management of neuroblastoma in limited-resource settings (2020)

In HIC, neuroblastoma is the most common extra-cranial solid tumour. Yet in LMIC, limited knowledge is available about NB beyond single institutional reports and abstracts. The last report on NB in South Africa was in the 1990s by Hesseling et al. From these few reports, patients in LMICs present at a later mean age at diagnosis, more advanced disease and with more high-risk prognostic indicators. The management is limited by the resource-poor settings without standardised approaches to treatment. This narrative review provided an extensive overview of the regional approaches of LMICs towards NB, summarised the presenting symptoms, disease characteristics at diagnosis and the prognostic factors. Due to the non-standardised reporting on the comparison of outcomes between LMICs and with HICs not being feasible, an overview of reported results was analysed. Finally, the review reflected on the barriers to the implementation of evidence-based treatment protocols and socioeconomic variables that influenced the diagnosis, management and follow-up of patients with NB.

Hypothesis: The clinical and pathological characteristics and outcomes in LMIC are comparable to HICs.
Objective: A literature review of the management and OS in the field of NB research in LMIC.

CHAPTER 3: Reporting incidences of neuroblastoma in various resource settings (2020). Ethics no: S18/07/138

Worldwide, NB contributes to 7% of all childhood malignancies that are diagnosed yearly. In sub-Saharan countries, accurate incidences of paediatric malignancies have not been documented for various reasons including inaccurate recording in tumour registries. In South Africa, underreporting of up to 50% has been reported compared to HIC statistics. This heterogeneous spectrum of disease poses challenges to the diagnosis of NB and the subsequent inclusion into tumour registries in limited-resourced settings. This study evaluated the context of NB in LMIC if HIC reporting standards were applied and calculated the expected incidences. South Africa had two tumour registries that include childhood malignancies. The researchers evaluated the NB data from both registries and included the patients from clinical files diagnosed from 2000 to 2016 to compare the NB patients in South Africa who were included in the two registries. The same methodology was applied for the age at diagnosis and sex in patients diagnosed with NB.

Stellenbosch University https://scholar.sun.ac.za

Hypothesis: Neuroblastoma is a rare disease in South Africa.
Objective: To determine the prevalence of NB in South Africa through retrospective analysis and accessing a dedicated paediatric tumour registry.

CHAPTER 4: Overall survival for neuroblastoma in South Africa between 2000 and 2014 (2019). Ethics no: S18/07/138

For the development of a prospective national management protocol for neuroblastoma in South Africa, it was necessary to evaluate the local overall survival against international data to formulate management recommendations. The hypothesis was that the various neuroblastoma treatment strategies had varying outcomes in South Africa. A national multicentre chart review of newly diagnosed neuroblastoma patients over 14 years in nine POUs of South Africa was done. The data were sourced retrospectively where the known risk factors of NB including age, stage, non-specific tumour markers, pathology and biological disease characteristics were obtained. The various applied treatment modalities during management were evaluated. The outcomes, OS and EFS were determined for all patients registered with the SACTR from 2000 to 2014. This information was used to evaluate the various treatment protocols used in South Africa and to facilitate the development of the prospective management protocol aligned with both international guidelines and local resources.

Hypothesis: Various neuroblastoma treatment strategies had varying outcomes in South Africa.
Objective: To determine the management and outcomes of NB in South African POUs.

CHAPTER 5: Age at diagnosis as a prognostic factor in South African children with neuroblastoma (2020). Ethics no: S18/07/138

Age at diagnosis has consistently been an important prognostic factor in determining treatment risk classifications regardless of the advances in treatment options. Observations from LMICs had reported older median ages at presentation for NB compared to HICs. During the retrospective study, a similar delay in the age of diagnosis was reported in South Africa. Internationally, children under the age of 12 months have a favourable prognosis regardless of tumour biology and patients older than five years have poor outcomes. The research hypothesis is that the delayed age of presentation in South Africa would have prognostic implications for the risk stratification and therefore for the treatment. The researchers determined the 18-month cut-point value to be of prognostic significance concerning South African children, which is in keeping with international findings.

Hypothesis: Various age groups had varying outcomes in South Africa.
Objective: To determine the prognostic value of age at diagnosis of patients diagnosed with NB in South Africa.

CHAPTER 6: The correlation of tumour markers and ^{123}I-mIBG-studies in South African children with neuroblastoma (2020). Ethics no: S18/07/138

An mIBG scan is part of the gold standard in the diagnosis and evaluation of NB treatment response, which is not freely available in LMICs. Furthermore, the production of radioisotopes is not reliable. In NB, it is important to administer chemotherapy at the indicated intervals and therefore it is important to be able to perform tests at the correct point of evaluation. Blood-based tests are part of the standard of care, are cheaper and need limited technology to perform. Non-specific tumour markers

Stellenbosch University https://scholar.sun.ac.za

lactate dehydrogenase (LDH) and ferritin have previously been validated in predicting treatment response and overall survival. The MYCN-gene is a specific tumour marker to NB and has been validated in predicting treatment response and overall survival as well. MYCN-amplification can be determined on both tissue from the NB tumour as well as the NB-cells in bone marrow aspirates yet requires lab-based technology that is often centralised in countries and more expensive than LDH and ferritin. LDH and ferritin predict two-year OS, where the modified Curie scores did not. LDH and ferritin may serve as surrogate tumour markers to the gold standard of mIBG-scans to assist in the management of NB in LMICs.

Hypothesis: Non-specific tumour markers LDH and ferritin are as good as specific tumour markers MYCN and ^{123}I-MIBG scans to indicate prognosis.
Objective: To investigate the prognostic value of diagnostic non-specific tumour markers LDH and ferritin in comparison to specific tumour marker MYCN and the prognostic value of MIBG scan in terms of OS in children with NB in the South African context.

CHAPTER 7: Induction chemotherapy for high-risk neuroblastoma in South African children (2019).
Ethics no: S18/07/138

The majority of patients (77%) diagnosed between 2000 and 2016 in South Africa with NB had high-risk tumours. As metastatic remission after induction therapy in high-risk neuroblastoma (HR-NB) was of prognostic importance, this study investigated mCR after induction chemotherapy using three standard neuroblastoma protocols in the South African setting. The purpose was to identify an induction regimen for HR-NB based on remission rate, toxicity and OS that could be administered in al POUs in South Africa. There was no significant difference between the three induction regimens but OPEC/OJEC had the most favourable toxicity profile for the South African setting.

Hypothesis: Various induction chemotherapy groups have varying outcomes in South Africa.
Objective: To determine the outcomes of the three most commonly used induction-chemotherapy regimens in the treatment of NB in South African children.

CHAPTER 8: The importance of local control management in neuroblastoma in South Africa (2020).
Ethics no: S18/07/138

Surgery and radiotherapy are important in the management of neuroblastoma. In patients with low-risk tumours, surgery could be curative, whilst in patients with intermediate-risk tumours, surgery, chemotherapy and, in cases of residual tumours, radiotherapy are curative. HR-NB trimodal therapy (chemotherapy, surgery and radiotherapy) only secures OS of up to 20%. In higher-resourced settings, autologous stem cell transplants, molecular targeted therapies and maturation therapy have secured outcomes of up to 60% OS but decreased the significance of local therapies. The role of surgery in NB in South Africa, especially the degree of resection, has never been evaluated. The relevance of radiotherapy in curative and palliative management as part of trimodal therapy and in the absence of surgery has not been explored in the South African context. The researchers' study concluded that a surgical resection between 90% to 100% has better outcomes than no surgery and that radiotherapy in the absence of surgery increases progression-free survival.

Stellenbosch University https://scholar.sun.ac.za

Hypothesis 1: Different degrees of NB tumour resection have varying outcomes in South Africa.
Objective: To determine the outcomes of the different degrees of tumour resection in the management of NB in South African children.
Hypothesis 2: Radiotherapy has prognostic value in the management of NB in South African children.
Objective: To determine the outcomes of irradiated and non-irradiated tumours during the management of NB in South African children.

CHAPTER 9: The implementation of a national paediatric oncology protocol for neuroblastoma in South Africa (2020). Ethics no: S18/07/138

Collaborative guidelines are important in establishing a standard of care for paediatric oncology in South Africa. The South African Children's Cancer Study Group (SACCSG) has embarked on developing national management protocols for individual childhood malignancies. By evaluating the process of developing the neuroblastoma protocol by the NB-working group of South Africa, valuable resources and methodologies for future protocol development were documented. The SACCSG NB-2017 protocol was an example where multiple international guidelines were incorporated for the local setting. The implementation research of this article may provide insight into the development and implementation of similar protocols in other LMICs.

Hypothesis: International guidelines can be implemented for a national prospective NB management protocol in South Africa.
Objective: To document the strategies in the development of a national prospective NB management protocol for South Africa.

CHAPTER 10: Inequality in paediatric oncology in South Africa – the neuroblastoma case study (2020)

In 1996, South Africa adopted its first democratic Constitution. The Bill of Rights included a section protecting children's rights beyond the constitutional right to life and equality. With the guarantee of access to health services enshrined in the Constitution, lifesaving treatment for neuroblastoma should be included in the right to life-saving medical treatment. With a more equitable population-based distribution of the country's budget and various administrative reforms, the national health services should after 20 years have established equal health care access to children with cancers. Based on the data from the researchers' retrospective NB study between 2000 and 2014, they evaluated the equity of the human resources, the level of paediatric oncology services and the access to these services based on distance and travel duration and found inequity in access to these resources for children with cancer.

Hypothesis: All children diagnosed with NB in South Africa have equal access to paediatric oncology services.
Objective: To determine the resources available to children from different provinces in the management of NB.

CHAPTER 11: An interim assessment of the prospective national neuroblastoma protocol (SACCSG NB-2017) in South Africa

The SACCSG NB-2017 commenced in January 2019. The researchers summarised the initial inclusion of patients into the study and reflected on the challenges of the inclusion process.

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 2

The management of neuroblastoma in limited-resource settings: a narrative review (2020)

Reference: van Heerden J, Kruger M. Management of neuroblastoma in limited-resource settings. World J Clin Oncol 2020; 11(8): 0-0. https://dx.doi.org/10.5306/wjco.v11.i8.0000
(Impact factor 2.81)

A vast base of literature has been published on the clinical characteristics, pathology, genetics and treatment strategies of neuroblastoma. The majority of this knowledge is based on research from high-income countries (HICs). Although research into these aspects of neuroblastoma management in low- and middle-income countries (LMICs) has increased in recent years, there was no single publication that evaluated whether the clinical characteristics, pathology, genetics and treatment strategies of neuroblastoma in LMIC were the same and how these management aspects compared to literature in HICs.

Stellenbosch University https://scholar.sun.ac.za

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2020 August 24; 11(8): 0-0

DOI: 10.5306/wjco.v11.i8.0000

ISSN 2218-4333 (online)

REVIEW

Management of neuroblastoma in limited-resource settings

Jaques van Heerden, Mariana Kruger

ORCID number: Jaques van Heerden 0000-0002-4502-1169; Mariana Kruger 0000-0002-6838-0180.

Author contributions: van Heerden J conceptualised and designed the study, sourced and screened articles, collected the data and performed the data analysis and wrote the manuscript; Kruger M conceptualised and designed the study, evaluated the source articles and critically reviewed and edited the manuscript.

Conflict-of-interest statement: All the authors declare that they have no competing interests.

PRISMA 2009 Checklist statement: The guidelines of the PRISMA 2009 Statement have not been adopted, because the review is a descriptive review.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Jaques van Heerden, Mariana Kruger, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town 8000, South Africa

Jaques van Heerden, Department of Paediatric Haematology and Oncology, Antwerp University Hospital, Edegem 2650, Belgium

Corresponding author: Jaques van Heerden, MD, Doctor, Department of Paediatric Haematology and Oncology, Antwerp University Hospital, No. 10, Wilrijkstreet, Edegem 2650, Belgium. jaques.vanheerden@uza.be

Abstract

BACKGROUND

Neuroblastoma (NB) is a heterogeneous disease with variable outcomes among countries. Little is known about NB in low- and middle-income countries (LMICs).

AIM

The aim of this review was to evaluate regional management protocols and challenges in treating NB in paediatric oncology units in LMICs compared to high-income countries (HICs).

METHODS

PubMed, Global Health, Embase, SciELO, African Index Medicus and Google Scholar were searched for publications with keywords pertaining to NB, LMICs and outcomes. Only English language manuscripts and abstracts were included. A descriptive review was done, and tables illustrating the findings were constructed.

RESULTS

Limited information beyond single-institution experiences regarding NB outcomes in LMICs was available. The disease characteristics varied among countries for the following variables: sex, age at presentation, MYCN amplification, stage and outcome. LMICs were found to be burdened with a higher percentage of stage 4 and high-risk NB compared to HICs. Implementation of evidence-based treatment protocols was still a barrier to care. Many socioeconomic variables also influenced the diagnosis, management and follow-up of patients with NB.

CONCLUSION

Patients presented at a later age with more advanced disease in LMICs. Management was limited by the lack of resources and genetic studies for

Stellenbosch University https://scholar.sun.ac.za

Manuscript source: Unsolicited manuscript

Received: April 28, 2020
Peer-review started: April 28, 2020
First decision: June 20, 2020
Revised: June 23, 2020
Accepted: July 26, 2020
Article in press: July 26, 2020
Published online: August 24, 2020

P-Reviewer: Cenciarelli C
S-Editor: Zhang H
L-Editor: A
E-Editor: Li JH

improved NB classification. Further research is needed to develop modified diagnostic and treatment protocols for LMICs in the face of limited resources.

Key words: Neuroblastoma; Limited resources; Management; Outcomes; Low- and middle-income countries

©The Author(s) 2020. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Neuroblastoma (NB) is a childhood malignancy of the sympathetic system that accounts for a large percentage of the childhood malignancy mortality. The heterogenous presentation contributes to various treatment challenges especially in low- and middle-income countries (LMICs). NB in LMICs has not been investigated beyond single institutions, but the limited reports differ from those in high-income countries (HICs). The incidence of NB in LMICs has been reported to be lower than HICs, but the disease presents with a higher incidence of high-risk and advanced disease. Furthermore, the limited resources in these countries contribute to the challenges in the management of NB that leads to a high mortality rate. The genetic profile of NB in LMICs is also not known due to limited capacity to perform genetic investigations. This article aims to comprehensively describe NB in LMICs.

Citation: van Heerden J, Kruger M. Management of neuroblastoma in limited-resource settings. *World J Clin Oncol* 2020; 11(8): 0-0
URL: https://www.wjgnet.com/2218-4333/full/v11/i8/0.htm
DOI: https://dx.doi.org/10.5306/wjco.v11.i8.0000

INTRODUCTION

The burden of disease in low- and middle-income countries (LMICs) is predominantly infectious in origin[1,2]. Yet, it is shifting towards non-communicable diseases such as congenital diseases, malignancies and road traffic incidents[2,3]. To date, the focus in research has been on communicable paediatric diseases with the World Health Organization's initial integrated management of childhood illness programme being one example[2]. Building the capacity of health care professionals to identify childhood malignancies has not been optimal[4]. This possibly explains the 28%-49% childhood malignancy gap reported between LMICs and high-income countries (HICs)[5].

Neuroblastoma (NB) data from HICs are well documented, whereas data from LMICs are limited. NB, predominantly a childhood malignancy, remains a major contributor to childhood cancer mortality and accounts for up to 15% of paediatric malignancy-related deaths[6]. Even with increased-intensity treatment in HICs, the five-year overall survival (OS) remains approximately 60%[7]. However, there is a major divide between HICs and LMICs due to the advances in diagnostics, treatment options and outcomes of NB in HICs[8].

Because of the variability of NB symptoms, they can easily be misdiagnosed as infections, bone marrow failure, neuropathology and obstructive enteropathies in LMICs by primary health care workers. Nurse-led primary care clinics or general practitioners may not have the expertise to recognise rare diseases in children and are often the first contact versus HICs where the first contact is usually more experienced health care workers[9].

Early diagnosis is crucial and necessitates a high index of suspicion with appropriate risk stratification and treatment[5]. The prognosis of NB is determined by a set of well-described prognostic factors that include patient factors (age at diagnosis), biochemical factors (lactate dehydrogenase and ferritin), tumour-related factors (primary site, tumour histology and stage), biological factors (MYCN amplification, ploidy and loss of chromosome 1p) and management factors (post-induction metastatic remission and degree of resection)[10,11]. NB pathophysiology and biological features, predominantly MYCN status, loss of chromosome 1p and ploidy, determine the spontaneous regression or aggressive growth and spread of metastases but do not explain the international difference in characteristics completely[6]. Similarly, notable differences in outcomes have been reported for risk classifications between LMICs and HICs with similar therapies[12-16]. The aim of this narrative review was to evaluate

Stellenbosch University https://scholar.sun.ac.za

regional variations in the diagnosis and management of NB in LMICs versus HICs.

MATERIALS AND METHODS

A comprehensive literature review of publications on PubMed, Global Health, Embase, SciELO, African Index Medicus and Google Scholar with medical subject headings pertaining to NB and outcomes relating to LMICs was done. Search terms included (but were not limited to) 'neuroblastoma', 'limited resources', 'low-income', 'middle-income' and names of LMICs. The search was conducted from April 2019 to January 2020 with terms adapted according to search engines without limitations on the date or language, provided that English summaries or abstracts were included. Conference proceedings were included. No authors were contacted regarding publications.

Due to the variability in reporting, nonstandard application of definitions in the reported clinical results, heterogeneous data and paucity of information, the authors constructed limited tables to evaluate clinical and/or biological characteristics to report in the descriptive review.

The systemic literature search retrieved 127 articles, abstracts and documents on NB in LMICs. After removing 11 documents for possible duplicated reporting, the 116 remaining documents consisted of 13 cancer registry-based reports and 103 non-registry-based documents. Twenty-three non-registry-based, nonrandomised studies (two prospective studies and 21 retrospective studies) were selected. All 116 articles, including the remaining 83 articles that were not specific to NB but contained epidemiological and non-interventional data on NB, were utilised to draw descriptive conclusions regarding epidemiological elements and outcomes for NB in the respective countries. Despite significant population numbers, certain LMIC regions were underrepresented in this review due to possible publication bias of reports.

RESULTS

Data from Asia (China, India, Pakistan, Thailand and Vietnam)[13,17-22], the Middle East and North Africa (Egypt, Iran, Iraq and Morocco)[23-28] and the Americas (Argentina, Brazil, Chile, Cuba, Mexico and Uruguay)[12,16,29-35] were accessible, but reports from sub-Saharan Africa and the Pacific Ocean were limited to single reports from the French-African Paediatric Oncology Group (GFAOP) and reunion[15,36]. The differences between HICs and LMICs could be evaluated from these reports, but complete management and outcome data for interregional variations among LMIC regions were less robust.

Incidence of neuroblastoma in low- and middle-income countries versus high-income countries according to international cancer registries

In sub-Saharan Africa, the incidence of NB was low, ranging from 0.4 cases per million in Niger to 5.9 cases per million in Kenya[37], compared to HICs such as North America and Europe where the respective incidences were reported as 10.5 and 11.6 cases per million per year in children younger than 15 years [11,38,39]. South Africa reported an incidence of 2.68 cases per million in children under 15 years of age between 1985 and 2007[40]. In Argentina, intraregional variations in incidence were demonstrated with a higher incidence being associated with areas of high socioeconomic status[29]. Yet, the international incidences have remained stable regardless of economic status[41]. As perinatal and low-risk (LR) NB can be asymptomatic and/or spontaneously regress, underdiagnosis of cases is a possible reason[5,37] but the degree of discrepancy is not known.

Epidemiology of neuroblastoma in low- and middle-income countries

Difference in age at presentation: In LMICs, the majority of patients were under the age of 5 years, but the percentages of infants reported for China (16.3%) and India (5.9%) (Table 1) were low. The mean or median age of presentation was delayed in some LMICs. In Thailand, the median was 34.8 mo of age and in India as high as 48 mo of age. The median age of presentation in the 16 paediatric oncology units (POUs) of the GFAOP study was 48 mo as well[15]. The age-standardised rates varied among countries, but the ratio of patients under 12 to 60 mo could be as low as 2.3:1 in Argentina and 1.2: 1 in Brazil compared to an HIC like Germany with a 4:1 ratio

Stellenbosch University https://scholar.sun.ac.za

Table 1 Age distribution at diagnosis

Country	*n*	< 12 mo	< 18 mo	< 60 mo	< 120 mo	< 180 mo	Mean	Median
Asia								
China (2008-2013)[17]	59	44%	56%					24
China (2000-2006)[18]	98	16.3%	4.1%	53%	21.5%	4%		48
India (1990-2004)[19]	103	0%-5.9%	77%-98.1%		1.9%		41	-
Pakistan (2015-2016)[20]	70	30%		63%		7%		36
South America								
Argentina (2000-2012)[29]	753	30%	52.2%		12.9%	45.3%		26.4
Brazil (1991-2012)[30]	258	29%	49%		17%	5%	40.5	28.9
Brazil (1990-2000)[16]	125	26%	13%	41%	20%		38.2	33
Middle East and North Africa								
Egypt (2005-2010)[23]	142	24.2%	75.8%					30
Egypt (2001-2010)[24]	53	22.6%	77.4%					
Iran (1974-2005)[25]	219	21.5%		78.5%			40.5	
Iraq (2008-2014)[26]	62	30.6%	50%		16.1%	3.2%	37	
Sub-Saharan Africa								
Ethiopia (2010-2013)[79]	5	0	40%		40%	20%		
Kenya (1997-2005)[44]	22	31.8%	50%		18.2%			60

(Table 2). However, other LMICs such as Cuba (4.8:1), with a good reputation for health care, and Reunion (2.7:1), a French territory in Africa, compared favourably with the United States of America (2.4:1) in this regard (Table 1). The median age of presentation in HICs was reported to be between 17 and 18 mo of age, of whom approximately 40% were diagnosed under 1 year of age[41]. Many studies have reproduced the 18-mo watershed dividing good prognosis (under the age of 18 mo) and poorer prognosis (over the age of 18 mo). Stage 4 patients were per definition below 12 mo of age with a good prognosis. In HICs, 90% of NB patients were younger than 5 years at diagnosis, with a median age at diagnosis of 19 mo, and 37% of patients had been diagnosed as infants[11]. The ATRX-gene is associated with advanced-age presentations, especially over 9 years of age, conferring a poorer prognosis in adolescents and adults[42]. The paucity of genetic studies in LMICs limited the interpretation of gene mutations related to age at diagnosis.

Gender distribution at diagnosis: The GFAOP reported that the male to female ratio for 16 African POUs was 2: 1[15]. In other LMICs, the male predominance as well as the greater male to female ratio was reproducible (Table 3). The ratios varied from 1.06: 1 to 2: 1. Previous studies from Southern Africa reported a ratio of 1.7:1[43] in keeping with the male predominance, while a Mexican study reported a lower NB incidence of 2.5-4.1 cases per million per year, in keeping with the situation in other LMICs, yet the male to female ratio of 1.1:1 was similar to HICs[32]. Kenya also reported a 1: 1 ratio in an LMIC setting[44]. The incidences based on gender have not been explained by other biological features. These findings were in contrast to the reported surveillance, epidemiology, and end results programme data from North America and European data, according to which a slight male predominance with a ratio of 1.1:1 was noted[38,45].

Population variations: Population variations related to epidemiology and pathophysiology contributed to a difference in the presentation of high-risk (HR) disease but not non-HR disease[46]. Independent from social circumstances, certain ethnicities were diagnosed at an older median age (> 20 mo) and had a higher prevalence of stage 4 disease and unfavourable histology tumours (undifferentiated cells)[46]. Studies amongst Alaskan indigenous ethnicities (a heterogeneous group of Eskimos, Native Indians and Aleuts) reported an incidence of 0.7 cases per million[47]. In Australia, Aboriginal and Torres Strait Island children were 1.83 times more likely to die from neuroblastoma than nonindigenous children while only contributing 3.7%

Stellenbosch University https://scholar.sun.ac.za

Table 2 Incidences of neuroblastoma according to the age at diagnosis

Country	*n*	< 12 mo	< 60 mo	Ratio < 12: < 60	< 120 mo	< 180 mo	Total incidence
South America							
Argentina (2000-2012)[29]	753	32.9	14.6	2.3: 1	2.8	1.0	8.3
Uruguay (2001-2010)[35]	69	63.1	18.1	3.4: 1	2.3	0	9.1
Chile (2007-2012)[31]	88	21.9	6.7	3.2: 1	2.1	0.3	4.7
Brazil (1998-2002)[33]	372	15.3	12.4	1.2: 1	3.8	1.3	5.9
Central America and the Caribbean							
Mexico (1996-2005)[32]	72	18.5	5.4	3.4: 1	1.1	0.2	3.8
Cuba (2001-2003)[34]	46	3.9	0.8	4.8: 1	0.5	0.2	0.1
Sub-Saharan Africa							
Reunion (2005-2011)[36]	12	44.1	15.8	2.7: 1	4.1	0	9.6

of diagnoses[48]. The lower incidence of NB among indigenous ethnicities was not reproduced in LMICs of South America or the Pacific Islands[49,50].

Variations in tumour characteristics

Difference in stage during presentation: Many LMICs reported stage 4 rates upward of 50%, with India and Pakistan reporting 71.8% and 79% stage 4 tumours respectively (Table 4). Egypt, Pakistan and Iran did not report any patients with stage 1 tumours, while China and India reported 3% and 1% stage 1 diagnosis respectively[18-20,25]. The GFAOP reported metastatic disease for up to 80% of patients except Burkina Faso and Morocco, where it varied from 20% to 50%[15]. Kenya reported the highest percentage of metastatic disease at 92.3%[44]. The data suggested that presentation in LMIC was usually metastatic.

Difference in MYCN amplification: Molecular and genetic diagnostics were not available in the greater number of reports and were recorded as a challenge in the literature[13,15,51]. In the GFOAP study, only North African countries could determine MYCN status[15] with Namibia and South Africa reporting MYCN studies in Southern Africa[44]. MYCN is present in about 20% of tumours[51,52]. Limited data are available on biological studies, especially genetic studies, in LMICs mainly due to resource constraints. In Iran, MYCN amplification was reported in 80% of NB patients, while Vietnam, Argentina and Egypt respectively reported rates of 17.8%, 20% and 20.8% (Table 4)[14,17,19].

Intra-risk group classification variability: Age groups, biological information and treatment protocols were not standardised in the literature, due to the development of classifications and changing treatments during the review period. Of note, risk classification was either not possible or was done retrospectively. Management protocols focus on administering risk-based treatments after identification of the classification of each patient yet many patients were treated on the basis of stage[39]. LMICs concluded that optimal treatment was doubtful due to the suboptimal classification of tumours[9,15,19]. The International Neuroblastoma Risk Group classification and the Children's Oncology Group classification rely on histological and genetic information (mitosis-karyorrhexis index, MYCN amplification, 11q aberration and DNA ploidy) to determine classification[11], which is not available in many resource-limited settings. Even when available, the lack of consistent cytogenetic evaluation, as was the case in Argentina, relegated patients in need of high-intensity treatment to LR categories and suboptimal treatment[12]. Due to the aggressive nature of especially HR NB, palliative rather than curative options have been pursued in LMICs[11]. Yet, variability in outcomes has been described within each risk class, highlighting that individual assessment is probably suboptimal Therefore, the International Society for Paediatric Oncology (SIOP)-Paediatric Oncology for Developing Countries (PODC) has adapted the approach to risk stratification with therapy based on available resources and utilising available diagnostic techniques[11]. The classification relies on age, stage and the common available nonspecific tumour markers ferritin and lactate dehydrogenase for risk classification[11]. Morocco has

Stellenbosch University https://scholar.sun.ac.za

Table 3 Distribution of sex at diagnosis

Country	Total	Male	Female	Ratio M: F
Asia				
Pakistan (2015-2016)[20]	70			1.8: 1
India (2000-2017)[64]	85	57 (67%)	28 (33%)	2: 1
India (1990-2004)[19]	103	76 (74%)	27 (26%)	2.8: 1
Thailand (2000-2007)[21]	67	39 (58.2%)	23(34.3%)	1.7: 1
Vietnam (2010-2012)[22]	130	76(58.5%)	54 (41.6%)	1.4: 1
China (2008-2013)[17]	59	35 (59%)	24 (40.1%)	1.5: 1
China (2000-2006)[18]	98			1.3: 1
South America				
Brazil (1991-2012)[30]	258	148 (57%)	110 (43%)	1.3: 1
Brazil (1990-2000)[16]	125	68 (54.4%)	57 (45.6%)	1.2: 1
Argentina (1999-2015)[12]	39	21 (54%)	18 (46%)	1.2: 1
Argentina (2000-2012)[29]	971	509 (52%)	462 (48%)	1.1: 1
Middle East and North Africa				
Iran (1974-2005)[25]	219			1.9: 1
Iraq (2008-2014)[26]	62	37 (59.7%)	25 (40.3%)	1.5: 1
Morocco (2012-2015)[27]	40	26 (65%)	14 (35%)	1.8: 1
Egypt (2005-2010)[23]	142	68 (51.5%)	64 (48.5%)	1.06: 1
Egypt (2001-2010)[24]	53	35 (66%)	18 (35%)	1.9: 1
Egypt (2007-2011)[28]	271	169 (62.4%)	102 (37.6%)	1.65: 1
Sub-Saharan Africa				
Northern Nigeria (2003-2009)[80]	14	10 (71.4%)	4 (28.6%)	2.5: 1
Southern Africa (South Africa and Namibia) (1983-1997)[43]				1.7: 1
Ethiopia (2010-2013)[79]	5	3 (60%)	2 (40%)	1.5: 1
Kenya (1997-2005)[44]	22	11 (50%)	11 (50%)	1: 1

implemented this classification system in the prospective NB protocol and has concluded that it allowed for more accurate diagnosis and systematic treatment[27]. For more accurate comparisons across resource-limited settings, classifications such as the SIOP-PODC classification should be standardly applied.

Variable reporting and treatment priorities

Reports from LMICs were predominantly single-institution reports. A multi-institutional survey by the GFAOP[15] and a review from India including 17 institutions and 11 cities[3] described the epidemiology, heterogeneous management approaches and outcomes of NB in LMICs[5]. Sub-Saharan African countries reported lower incidences of NB (3%-7.5%) among childhood malignancies compared to North-African countries (7%-30%)[15]. The same study identified the limitations of reporting: Plain radiography, ultrasonography, computed tomography and magnetic resonance imaging were available at all centres, but access to imaging studies was variable. None of the sub-Saharan centres had metaiodobenzylguanidine scans. The North African centres had these scans, but only Algeria had consistent access due to government funding[15]. In Honduras and the Philippines, diagnostic resources were available in large cities but were inaccessible to most patients living in rural areas[50]. This is a typical problem in LMICs[53]. An Indian multi-study review concluded that variability in India included treatment protocols, reporting of outcomes and calculation of survival rates[13]. This conclusion could also be applied to other LMICs. Morocco and Argentina were the only LMICs to describe prospective national studies regarding

Stellenbosch University https://scholar.sun.ac.za

Table 4 Disease characteristics of neuroblastoma at diagnosis

Country	*n*	Stage 1	Stage 4	Non-MYCN amplified	MYCN amplified	Non-HR	HR
Asia							
China (2008-2013)[17]	59	6.8%	37.3%	55%	45%	53%	47%
China (2000-2006)[18]	98	3%	50%				
India (1990-2004)[19]	103	1%	71.8%				
Pakistan (2015-2016)[20]	70	0%	79%				> 61.1%
South America							
Argentina (2000-2012)[29]	753	12%	55.5%	80%	20%		
Brazil (1991-2012)[30]	258	15%	46%	75%	25%		
Brazil (1990-2000)[16]	125	7%	64%	53%	47%		
Middle East and North Africa							
Egypt (2005-2010)[23]	142	0%	64.7%			24.2%	75.8%
Egypt (2001-2010)[24]	53	0%	67.9%	79.2%	20.8%	32%	68%
Iran (1974-2005)[25]	219	14.5%	53.8%				
Iraq (2008-2014)[26]	62	1.6%	69.4%			45.2%	54.8%
Sub-Saharan Africa							
Kenya (1997-2005)[44]	26	0%	92.3%				

HR: High-risk.

NB[27,29]. This is representative of the diverse, nonstandardised approach to NB in most LMICs. Most studies found a lack of access to biological tests for stratification (based on HIC-validated data), the presentation of advanced disease, poor socioeconomic circumstances and a significant percentage of patients who absconded from treatment[23,24]. Advanced disease and higher than average percentages of HR disease were described (Table 4). The PODC committee of the SIOP has developed adapted guidelines for the management of NB in LMICs[11]. Yet, in the field of paediatric oncology, especially in sub-Saharan Africa, a prioritised, stepwise approach has been advised in limited-resource settings, prioritising pain management, supportive care, comorbid diseases and malignancies with a higher incidence and relatively uncomplicated treatment regimens above rare childhood malignancies[54]. In Africa, only Morocco has published data from standardised prospective NB protocols from four POUs based on the PODC guidelines[27].

Challenges in improving outcomes

Clinical presentation, index of suspicion and misdiagnosis: Because of its heterogeneous clinical presentation, NB can be challenging to diagnose[30]. The presenting signs of NB can be similar to those of non-malignant diseases and can confound recognition of the disease[10,55]. Symptoms of an NB abdominal mass can be misdiagnosed as more common childhood illnesses such as constipation[56]. In LMICs, similar to HICs, the most common presentation reported in 19%-87% of patients was an abdominal mass (Table 5)[18,19,23,30]. Other common presentations were nonspecific abdominal pain (22%-73.5%)[18,30] and fever (25%-65%)[18,19,23,30], metastatic manifestations such as bilateral proptosis (27%-42.4%)[19,23], bone pain (19%)[30] and pancytopaenia, and constitutional symptoms such as loss of weight[56]. The clinical progression of the tumour involves a spectrum of behaviour from aggressive advancement to metastatic disease or spontaneous regression and mature differentiation of cell types such as ganglioneuroma[29,57]. Health care practitioners must have a high index of suspicion for NB with a varied clinical picture[35]. Misdiagnosing NB from other abdominal tumours prevents accurate registration of the diagnosis[29]. In resource-limited settings, the diagnosis of asymptomatic benign clinical types is less common, possibly due to underdiagnosis. Early detection by screening in HICs neither impacted outcomes nor was it cost-effective[57]. While the incidence was increased during active screening of the disease in the European, North American and Japanese context, surgical

Stellenbosch University https://scholar.sun.ac.za

Table 5 Most common clinical presentations in low- and middle-income countries

Asia				
China (2000-2006)[18]	Abd pain (73.5%)	Abd mass (54.1%)	Fever (45.9%)	Limb pain (25.5%)
India (1990-2004)[19]	Fever (65%)	Abd mass (54%)	Bone pain (31%)	Proptosis (27%)
South America				
Brazil (1991-2012)[30]	Fever (25%)	Abd pain (22%)	Abd mass (19%)	Bone pain (19%)
Middle East and North Africa				
Egypt (2005-2010)[23]	Abd mass (87%)	Pallor (57.6%)	Fever (45.5%)	Proptosis (42.4%)
Sub-Saharan Africa				
Kenya (1997-2005)[44]	Abd mass (53.8%)	Bone pain (50%)	Proptosis (38.5%)	Fever (19.8%)

interventions were increased without improvement of survival[57].

Access to and assignment of treatment: The number and capacities of POUs varied substantially among LMICs, and capacities also varied among POUs in a single country[50]. Basic paediatric oncology components were not available in the Philippines and Senegal[50], while Venezuela and Egypt had adequate intensive care facilities and even transplant services[50]. This is also true of POUs in South Africa[44]. Furthermore, paediatric services may not even exist in certain countries or often compete with adult services for resources[54].

Current treatment protocols are based on risk stratification[11]. The LMIC reports included treatments over four decades[13,30]. Therefore, outcomes were predominantly reported per stage and, subsequently, as classification systems evolved, research describing the treatment of LR and intermediate-risk (IR) patients but focussing primarily on HR disease as the greatest NB burden was reported.

In many LMICs, NB treatment choices are limited to mainly chemotherapy, surgery and radiotherapy[1]. In HR NB, multimodal therapy is of vital importance for cure and five-year OS of up to 60% (Figure 1).

Due to advanced disease at diagnosis, palliative treatment is often the only plausible option (Figure 1). Other challenges for the management of NB include lack of surgical and radiotherapy skills or equipment as well as lack of chemotherapy[1,11]. Poor outcomes have necessitated the development of palliative strategies, yet many LMICs where drug insecurity is high do not have even basic medicines for palliation[58]. Resources, drug security and expertise in institutions influence treatment decisions to a similar extent as treatment adherence and response to treatment. The ability of facilities to provide supportive care, in terms of antibiotics, intensive care and granulocyte-stimulating factors, influences decision making regarding the intensity of treatment that patients receive[10,11].

Treatment protocols utilised in low- and medium-income countries and outcomes: Over the past decades, guidelines for the treatment of NB have changed as a result of an improved understanding of biological prognostic factors and changing classification systems yet chemotherapy remains based on etoposide and platinum (cisplatin and/or carboplatin) backbones plus dose- and time-intensive administration of chemotherapy[11]. Some approaches include doxorubicin in the regimens, while the SIOP-PODC treatment guidelines for NB are based on settings relating to the level of supportive care and resources available in a POU[11]. Indicators for reporting outcomes were not consistent over the same period. Some studies reported according to stage, while others reported according to risk classification.

The GFAOP administered various local and international protocols based on the standard backbone including doxorubicin[15]. Individual POUs reported a long-term OS of less than 10% for metastatic disease. Tunisia reported an OS of 78% for non-metastatic disease, while Senegal reported an OS (metastatic plus non-metastatic) of 38.9%. The report concluded that with all countries having access to surgical options, the outcomes were 'generally poor' and standardised protocols were being developed for multicentre use[15]. In Morocco, a GFAOP member, a national prospective protocol divided into an HR protocol and a non-HR protocol based on the risk-adapted SIOP-PODC treatment guidelines was studied[11,15,27]. Long-term outcomes were not reported, but 60.6% of HR patients experienced a partial or very good partial response, receiving local control with surgery or consolidation therapy[27]. The study concluded that risk

Stellenbosch University https://scholar.sun.ac.za

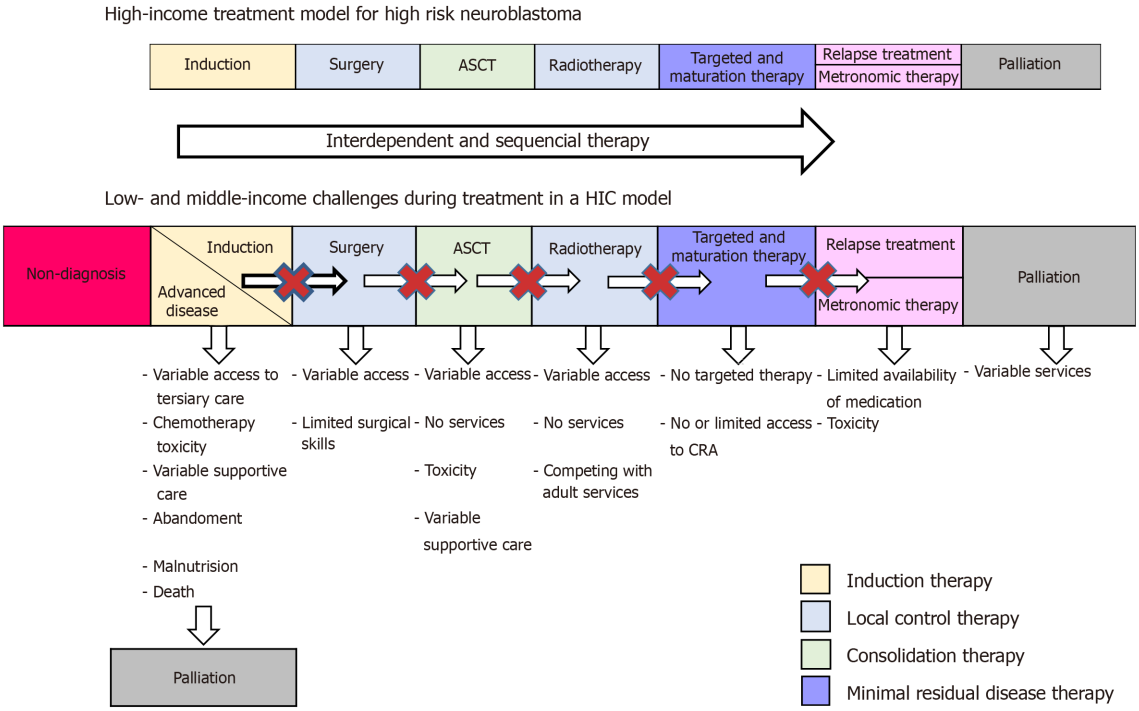


Figure 1 Challenges of non-tumour-related factors during the treatment of high-risk neuroblastoma in low- and middle-income countries.
ASCT: Autologous stem-cell transplant; HIC: High-income country; CRA: Cis-retinoic acid.

stratification and treatment guidelines adapted for LMICs improved the accuracy of diagnosis and access to systematic treatment[27]. The protocol was also suitable for multicentre use[27].

A Chinese study administered OPEC by modifying the Japanese study group protocol[18]. The five-year OS was 80% for stages 1 and 2 and 48.3% and 20% for stages 3 and 4 respectively, which was less than the Japanese outcomes[18].

Egyptian and Indian centres based their HR treatment on the North American CCG-3891 protocols, while other LMIC centres administered chemotherapy according the European protocols from France and the International Society of Paediatric Oncology European Neuroblastoma Research Network (SIOPEN)[59]. Indian institutions followed a non-standardised approach including OPEC/OJEC, doxorubicin-containing and Ifosfamide-containing regimens[13]. Iran and Egypt used OPEC/OJEC regimens[23-25], while Brazil, Thailand and China followed doxorubicin-based regimens[16-18,21,30]. Stage 1 disease had a five-year OS of 100% in Brazil[16], China[17,18] and Thailand[21], while stage 4 OS was under 20%[16,18]. The three-year OS for stage 4 disease in Thailand and China was less than 35%[17,21]. While the outcomes for stage 1 disease were comparable to HICs, the poorer stage 4 outcomes were less optimal than in HICs[10]. The same conclusion was reached in an Indian study with three-year OS and event-free survival for non-metastatic disease of 77% and 54% respectively[60].

Argentina alternated between rapid COJEC and the modified N7 for HR disease according to the SIOPEN HR NBL-1 protocol[12]. The five-year OS was 24%. The study concluded that improved supportive care, optimal treatment and maximising available resources were needed[12]. A second Argentinian study associated lower socioeconomic status with poorer outcomes independent of treatment[29].

In LMICs, no conformity was found in the management of NB amongst regions within countries. Failing to complete one aspect of the sequential treatment protocol relegates the outcome to being suboptimal. This is often the case in LMICs with limited access to health care and limited resources for optimal treatment[61]. It is possible that without genetic factors to distinguish more clearly between IR and HR disease, the IR cohorts in LMICs contain a number of HR patients, thereby affecting outcomes[11].

Main factors affecting outcomes: LMICs have identified treatment-related, tumour-related and social factors that affect the outcomes of children with NB. Delayed diagnosis[30] and inaccurate diagnosis of tumours due to limited radiologic and pathology resources were cited as major obstacles[25,27,60]. The limited ability to perform

Stellenbosch University https://scholar.sun.ac.za

biological testing impaired accurate risk stratification[25,27,30,62]. Centres with higher levels of supportive care reported the inability to perform bone marrow transplants as a limitation to improving outcomes[24,60]. The variability of tumours and nonspecific presentation contributed to late diagnosis and the incidence of advanced disease[12,25,27,30,62]. Yet, the greatest problems were the abandonment of treatment and patients lost to follow-up of up to 50%[11,70,62], which were linked to social factors and the distance from treatment centres[12].

Social circumstances and outcomes: A Brazilian study reported intraregional variation in the incidence of NB based on socioeconomic status[33]. The study concluded that patients from regions with a lower socioeconomic status had poorer outcomes[33]. In South African populations, socioeconomic and/or cultural factors related to access to or utilisation of health care services are a possible contributing factor to poorer outcomes[1]. A large proportion of rural inhabitants have restricted access to medical facilities and thus experience a delay in treatment[1,63,64]. A Harvard study concluded that in the United States of America, NB diagnosis was influenced by social circumstances[65]. According to the study, the Human Development Index showed a direct relationship between socioeconomic status and the incidence of NB[65].

Factors influencing health-seeking behaviour: The heterogeneous and aggressive pathophysiology of NB demands prompt response and immediate medical intervention for nonspecific symptoms[66,67]. The economic structure of LMICs influences the affordability of healthcare and parental education[68-70]. These factors determine the promptness of the response to and the action taken with regard to nonspecific symptoms associated with the initial phases of childhood malignancies. The steadfast belief in traditional medicine as a first treatment option and cultural systems in which elders or a single authority figure decide about seeking medical intervention may delay action towards directed care[71,72]. Political stability and government policies have a direct impact on the availability, accessibility and quality of health care systems in treating childhood cancer[73,74].

Research priorities

The focus of research for LMICs should be on creating greater awareness in the diagnosis of NB, improving diagnostics and establishing social support strategies for successful, harmonised management protocols and homogenous treatment facilities to improve outcomes[55,75]. The main priority should be accurate tumour registries to document not only the most common or treatable childhood malignancies but also the rarer tumours such as NB[37]. In resource-limited settings, the need for genetic markers to develop more accurate risk classifications exists, especially to distinguish clearly between IR and HR patients. This is important in the case of stage 2 and stage 4 patients with adverse biology tumours who have in a higher risk classification compared to patients with non-adverse biology tumours[11,25,29]. Genome and exome sequencing have improved the understanding of the pathophysiology of NB in HICs[76]. However, knowledge regarding genetics of NB in the diverse ethnicities in LMICs is limited. A further challenge would be to make treatments and advanced diagnostics, such as liquid biopsies and biological tests, more widely available to all countries, whether HICs or LMICs, to improve diagnostic capacities and outcomes[75]. In advanced disease, palliative research could contribute to a greater understanding of the role of metronomic therapies and disease control in the context of NB[77].

DISCUSSION

Childhood malignancy awareness and advocacy still face great challenges, especially in LMICs, notably countries with large rural populations and great geographical divides, in accurately diagnosing malignancies, especially heterogeneous tumours such as NB. The lack of uniform treatment protocols for this variable disease is still a barrier to care. Epidemiological data are reproducible in different international studies, but data from across the world are not uniform. More research regarding tumour biology, specifically genomics, is needed not only in HICs but also in LMICs to determine underlying differences in molecular biology of the tumours, genetic targets and drug processing of NB patients, especially in heterogeneous populations. This information must then be made available to treatment centres where biological investigation is not possible, ready for clinical application to achieve improved outcomes for NB worldwide.

Stellenbosch University https://scholar.sun.ac.za

ARTICLE HIGHLIGHTS

Research background

Neuroblastoma (NB) is a well-documented childhood malignancy with the greatest source of knowledge originating from high-income countries. The management of NB in low- and middle countries (LMIC) is less robust due to various social and resource limitations.

Research motivation

The outcomes of various LMIC during the same period like South America, Francophone/North African countries, Asia and South Pacific Islands was evaluated.

Research objectives

This literature review was to evaluate regional development of management protocols, the challenges in treating NB in paediatric oncology units in LMIC as compared to high-income countries, new laboratory and clinical developments in the treatment of NB.

Research methods

A literature review of publications searched on PubMed, Medline, Global Health, Embase, SciELO and Google Scholar with keywords in keeping with NB and outcomes. Due to the variability in reporting, nonstandard application of definitions in the reported clinical results, heterogeneous data and paucity of information, the authors constructed limited tables to evaluate clinical and/or biological characteristics to report in the descriptive review.

Research results

Childhood malignancy awareness and advocacy still face great challenges, especially in LMICs, in accurately diagnosing malignancies, especially heterogeneous tumours such as NB. The lack of uniform treatment protocols for this variable disease is still a barrier to care. Epidemiological data are reproducible in different international studies, but data from across the world are not uniform.

Research conclusions

More research regarding tumour biology, specifically genomics, is needed not only in high-income countries but also in LMICs to determine underlying differences in molecular biology of the tumours, genetic targets and drug processing of NB patients, especially in heterogeneous populations.

Research perspectives

The focus of research for LMICs should be on creating greater awareness in the diagnosis of NB, improving diagnostics and establishing social support strategies for successful, harmonised management protocols and homogenous treatment facilities to improve outcomes. In resource-limited settings, the need for genetic markers to develop more accurate risk classifications exists. A further challenge would be to make treatments and advanced diagnostics, such as liquid biopsies and biological tests, more widely available to all countries. With advanced disease, palliative research could contribute to a greater understanding of the role of metronomic therapies and disease control in the context of NB.

ACKNOWLEDGEMENTS

Dr. van Heerden, as staff member of the Department of Paediatric Haematology and Oncology, Antwerp University Hospital, University of Antwerp, acknowledges the department for research support. Our gratitude to Annamarie du Preez for language editing of the article.

REFERENCES

1 **Magrath I**, Steliarova-Foucher E, Epelman S, Ribeiro RC, Harif M, Li CK, Kebudi R, Macfarlane SD, Howard SC. Paediatric cancer in low-income and middle-income countries. *Lancet Oncol* 2013; **14**: e104-

Stellenbosch University https://scholar.sun.ac.za

e116 [PMID: 23434340 DOI: 10.1016/S1470-2045(13)70008-1]
2 **Global Burden of Disease Pediatrics Collaboration**, Kyu HH, Pinho C, Wagner JA, Brown JC, Bertozzi-Villa A, Charlson FJ, Coffeng LE, Dandona L, Erskine HE, Ferrari AJ, Fitzmaurice C, Fleming TD, Forouzanfar MH, Graetz N, Guinovart C, Haagsma J, Higashi H, Kassebaum NJ, Larson HJ, Lim SS, Mokdad AH, Moradi-Lakeh M, Odell SV, Roth GA, Serina PT, Stanaway JD, Misganaw A, Whiteford HA, Wolock TM, Wulf Hanson S, Abd-Allah F, Abera SF, Abu-Raddad LJ, AlBuhairan FS, Amare AT, Antonio CA, Artaman A, Barker-Collo SL, Barrero LH, Benjet C, Bensenor IM, Bhutta ZA, Bikbov B, Brazinova A, Campos-Nonato I, Castañeda-Orjuela CA, Catalá-López F, Chowdhury R, Cooper C, Crump JA, Dandona R, Degenhardt L, Dellavalle RP, Dharmaratne SD, Faraon EJ, Feigin VL, Fürst T, Geleijnse JM, Gessner BD, Gibney KB, Goto A, Gunnell D, Hankey GJ, Hay RJ, Hornberger JC, Hosgood HD, Hu G, Jacobsen KH, Jayaraman SP, Jeemon P, Jonas JB, Karch A, Kim D, Kim S, Kokubo Y, Kuate Defo B, Kucuk Bicer B, Kumar GA, Larsson A, Leasher JL, Leung R, Li Y, Lipshultz SE, Lopez AD, Lotufo PA, Lunevicius R, Lyons RA, Majdan M, Malekzadeh R, Mashal T, Mason-Jones AJ, Melaku YA, Memish ZA, Mendoza W, Miller TR, Mock CN, Murray J, Nolte S, Oh IH, Olusanya BO, Ortblad KF, Park EK, Paternina Caicedo AJ, Patten SB, Patton GC, Pereira DM, Perico N, Piel FB, Polinder S, Popova S, Pourmalek F, Quistberg DA, Remuzzi G, Rodriguez A, Rojas-Rueda D, Rothenbacher D, Rothstein DH, Sanabria J, Santos IS, Schwebel DC, Sepanlou SG, Shaheen A, Shiri R, Shiue I, Skirbekk V, Sliwa K, Sreeramareddy CT, Stein DJ, Steiner TJ, Stovner LJ, Sykes BL, Tabb KM, Terkawi AS, Thomson AJ, Thorne-Lyman AL, Towbin JA, Ukwaja KN, Vasankari T, Venketasubramanian N, Vlassov VV, Vollset SE, Weiderpass E, Weintraub RG, Werdecker A, Wilkinson JD, Woldeyohannes SM, Wolfe CD, Yano Y, Yip P, Yonemoto N, Yoon SJ, Younis MZ, Yu C, El Sayed Zaki M, Naghavi M, Murray CJ, Vos T. Global and National Burden of Diseases and Injuries Among Children and Adolescents Between 1990 and 2013: Findings From the Global Burden of Disease 2013 Study. *JAMA Pediatr* 2016; **170**: 267-287 [PMID: 26810619 DOI: 10.1001/jamapediatrics.2015.4276]
3 **Bollyky TJ**, Templin T, Cohen M, Dieleman JL. Lower-Income Countries That Face The Most Rapid Shift In Noncommunicable Disease Burden Are Also The Least Prepared. *Health Aff (Millwood)* 2017; **36**: 1866-1875 [PMID: 29137514 DOI: 10.1377/hlthaff.2017.0708]
4 **Geel JA**, Stevenson BT, Jennings RB, Krook LE, Winnan SJ, Katz BT, Fox TJ, Nyati L. Enough is not enough: Medical students' knowledge of early warning signs of childhood cancer. *S Afr Med J* 2017; **107**: 585-589 [PMID: 29025447 DOI: 10.7196/SAMJ.2017.v107i7.12211]
5 **Howard SC**, Lam CG, Arora RS. Cancer epidemiology and the “incidence gap” from non-diagnosis. *Pediatr Hematol Oncol J* 2018; **3**: 75-78 [DOI: 10.1016/j.phoj.2019.02.001]
6 **Cao Y**, Jin Y, Yu J, Wang J, Yan J, Zhao Q. Research progress of neuroblastoma related gene variations. *Oncotarget* 2017; **8**: 18444-18455 [PMID: 28055978 DOI: 10.18632/oncotarget.14408]
7 **Whittle SB**, Smith V, Doherty E, Zhao S, McCarty S, Zage PE. Overview and recent advances in the treatment of neuroblastoma. *Expert Rev Anticancer Ther* 2017; **17**: 369-386 [PMID: 28142287 DOI: 10.1080/14737140.2017.1285230]
8 **Knaul FM**, Arreola-Ornelas H, Rodriguez NM, Méndez-Carniado O, Kwete XJ, Puentes-Rosas E, Bhadelia A. Avoidable Mortality: The Core of the Global Cancer Divide. *J Glob Oncol* 2018; **4**: 1-12 [PMID: 30096010 DOI: 10.1200/JGO.17.00190]
9 **Erdmann F**, Feychting M, Mogensen H, Schmiegelow K, Zeeb H. Social Inequalities Along the Childhood Cancer Continuum: An Overview of Evidence and a Conceptual Framework to Identify Underlying Mechanisms and Pathways. *Front Public Health* 2019; **7**: 84 [PMID: 31106186 DOI: 10.3389/fpubh.2019.00084]
10 **National Cancer Institute**. Neuroblastoma. [cited 22 July 2019]. Available from: https://www.cancer.gov/types/neuroblastoma
11 **Parikh NS**, Howard SC, Chantada G, Israels T, Khattab M, Alcasabas P, Lam CG, Faulkner L, Park JR, London WB, Matthay KK; International Society of Pediatric Oncology. SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for neuroblastoma in low- and middle-income settings. *Pediatr Blood Cancer* 2015; **62**: 1305-1316 [PMID: 25810263 DOI: 10.1002/pbc.25501]
12 **Easton JC**, Gomez S, Asdahl PH, Conner JM, Fynn AB, Ruiz C, Ojha RP. Survival of high-risk pediatric neuroblastoma patients in a developing country. *Pediatr Transplant* 2016; **20**: 825-830 [PMID: 27235336 DOI: 10.1111/petr.12731]
13 **Kulkarni KP**, Marwaha RK. Outcome of neuroblastoma in India. *Indian J Pediatr* 2013; **80**: 832-837 [PMID: 23340984 DOI: 10.1007/s12098-012-0948-9]
14 **Greengard E**, Hill-Kayser C, Bagatell R. Treatment of high-risk neuroblastoma in children: recent clinic trial results. *Clin Invest* 2013; **3**: 1071–1081 [DOI: 10.4155/cli.13.90]
15 **Traoré F**, Eshun F, Togo B, Yao JJA, Lukamba MR. Neuroblastoma in Africa: A Survey by the Franco-African Pediatric Oncology Group. *J Glob Oncol* 2016; **2**: 169-173 [PMID: 28717698 DOI: 10.1200/JGO.2015.001214]
16 **Parise IZ**, Haddad BR, Cavalli LR, Pianovski MA, Maggio EM, Parise GA, Watanabe FM, Ioshii SO, Rone JD, Caleffe LG, Odone Filho V, Figueiredo BC. Neuroblastoma in southern Brazil: an 11-year study. *J Pediatr Hematol Oncol* 2006; **28**: 82-87 [PMID: 16462579 DOI: 10.1097/01.mph.0000199601.35010.52]
17 **Shao JB**, Lu ZH, Huang WY, Lv ZB, Jiang H. A single center clinical analysis of children with neuroblastoma. *Oncol Lett* 2015; **10**: 2311-2318 [PMID: 26622841 DOI: 10.3892/ol.2015.3588]
18 **Li K**, Dong K, Gao J, Yao W, Xiao X, Zheng S. Neuroblastoma management in Chinese children. *J Invest Surg* 2012; **25**: 86-92 [PMID: 22439835 DOI: 10.3109/08941939.2011.605203]
19 **Bansal D**, Marwaha RK, Trehan A, Rao KL, Gupta V. Profile and outcome of neuroblastoma with convertional chemotherapy in children older than one year: a 15-years experience. *Indian Pediatr* 2008; **45**: 135-139 [PMID: 18310793]
20 **Ahmad A**, Asghar N, Masood N, Najamuddin, Khan FS, Rathore Z, Rathore AW. Clinical spectrum of Advanced Neuroblastoma. *Journal of Rawalpindi Medical College (JRMC)* 2017; **21**: 229-232
21 **Shuangshoti S**, Shuangshoti S, Nuchprayoon I, Kanjanapongkul S, Marrano P, Irwin MS, Thorner PS. Natural course of low risk neuroblastoma. *Pediatr Blood Cancer* 2012; **58**: 690-694 [PMID: 21922650 DOI: 10.1002/pbc.23325]

Stellenbosch University https://scholar.sun.ac.za

22 **Bui C**, Nguyen U, Trong D, Vo N. Neuroblastoma in Vietnam: A retrospective analysis of MYCN status and clinical features to inform prognosis and improve outcome. *Pediatr Dimensions* 2019; **4**: 1-6 [DOI: 10.15761/PD.1000195]

23 **Al-Tonbary Y**, Badr M, Mansour A, El Safy U, Saeed S, Hassan T, Elashery R, Nofal R, Darwish A. Clinico-epidemiology of neuroblastoma in north east Egypt: A 5-year multicenter study. *Oncol Lett* 2015; **10**: 1054-1062 [PMID: 26622625 DOI: 10.3892/ol.2015.3335]

24 **El-Sayed MI**, Ali AM, Sayed HA, Zaky EM. Treatment results and prognostic factors of pediatric neuroblastoma: a retrospective study. *Int Arch Med* 2010; **3**: 37 [PMID: 21182799 DOI: 10.1186/1755-7682-3-37]

25 **Mehdiabadi GB**, Arab E, Rafsanjani KA, Ansari S, Moinzadeh AM. Neuroblastoma in Iran: an experience of 32 years at a referral childrens hospital. *Asian Pac J Cancer Prev* 2013; **14**: 2739-2742 [PMID: 23803024 DOI: 10.7314/apjcp.2013.14.5.2739]

26 **Abdallah BK**, Rashid NG, Tawfiq SA. Neuroblastoma in Iraq-KRG-Sulaimani. *J Cancer Sci Clin Ther* 2018; **2**: 001-008

27 **Salman Z**, Kababri M, Hessissen L, Khattab M. An Intensive Induction Protocol for High Risk Neuroblastoma in Morocco. *J Glob Oncol* 2016; **2** Suppl: 80s-81s [DOI: 10.1200/JGO.2016.004259]

28 **Moussa E**, Fawzy F, Younis A, Shafei M. Combined Treatment Strategy and Outcome of High-Risk Neuroblastoma: Experience of the Children's Cancer Hospital-Egypt. *J Cancer Ther* 2013; **4**: 1435-1442 [DOI: 10.4236/jct.2013.49171]

29 **Moreno F**, Marti JL, Palladino M, Lobos P, Gaultieri A, Cacciavillano W. Childhood Neuroblastoma: Incidence and Survival in Argentina. Report from the National Pediatric Cancer Registry, ROHA Network 2000–2012. *Pediatr Blood Cancer* 2016; **63**: 1362-1367 [PMID: 27135302 DOI: 10.1002/pbc.25987]

30 **Lucena JN**, Alves MTS, Abib SCV, Souza GO, Neves RPC, Caran EMM. Clinical and epidemiological characteristics and survival outcomes of children with neuroblastoma: 21 years of experience of the Instituto du Oncologia Pediatrica, Sao Paulo, Brazil. *Rev Paul Pediatrics* 2018; **36**: 254-260 [PMID: 29995142 DOI: 10.1590/1984-0462/;2018;36;3;00007]

31 **Rice HE**, Englum BR, Gulack BC, Adibe OO, Tracy ET, Kreissman SG, Routh JC. Use of patient registries and administrative datasets for the study of pediatric cancer. *Pediatr Blood Cancer* 2015; **62**: 1495-500 [PMID: 25807938 DOI: 10.1002/pbc.25506]

32 **Juárez-Ocaña S**, Palma-Padilla V, González-Miranda G, Siordia-Reyes AG, López-Aguilar E, Aguilar-Martínez M, Mejía-Aranguré JM, Carreón-Cruz R, Rendón-Macías ME, Fajardo-Gutiérrez A. Epidemiological and some clinical characteristics of neuroblastoma in Mexican children (1996-2005). *BMC Cancer* 2009; **9**: 266 [PMID: 19650918 DOI: 10.1186/1471-2407-9-266]

33 **de Camargo B**, de Oliveira Ferreira JM, de Souza Reis R, Ferman S, de Oliveira Santos M, Pombo-de-Oliveira MS. Socioeconomic status and the incidence of non-central nervous system childhood embryonic tumours in Brazil. *BMC Cancer* 2011; **11**: 160 [PMID: 21545722 DOI: 10.1186/1471-2407-11-160]

34 **Torres P**, Galán Y, Lence J, García M, Lezcano M, Fernández L. Childhood cancer incidence in Cuba, 2001 to 2003. *MEDICC Rev* 2010; **12**: 19-25 [PMID: 20486410]

35 **Howard SC**, Metzger ML, Wilimas JA, Quintana Y, Pui CH, Robison LL, Ribeiro RC. Childhood cancer epidemiology in low-income countries. *Cancer* 2008; **112**: 461-472 [PMID: 18072274 DOI: 10.1002/cncr.23205]

36 **Ramiandrisoa J**, Jehanne M, Sauvat F, Reguerre Y, Chamouine A, Chirpaz E. Incidence and survival of childhood cancer in the French islands of Reunion and Mayotte (2005-2011). *Cancer Epidemiol* 2017; **49**: 61-65 [PMID: 28575782 DOI: 10.1016/j.canep.2017.05.009]

37 **Stefan C**, Bray F, Ferlay J, Liu B, Maxwell Parkin D. Cancer of childhood in sub-Saharan Africa. *Ecancermedicalscience* 2017; **11**: 755 [PMID: 28900468 DOI: 10.3332/ecancer.2017.755]

38 **Spix C**, Pastore G, Sankila R, Stiller CA, Steliarova-Foucher E. Neuroblastoma incidence and survival in European children (1978-1997): report from the Automated Childhood Cancer Information System project. *Eur J Cancer* 2006; **42**: 2081-2091 [PMID: 16919772 DOI: 10.1016/j.ejca.2006.05.008]

39 **Ward E**, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 83-103 [PMID: 24488779 DOI: 10.3322/caac.21219]

40 **Stefan DC**, Stones DK, Wainwright RD, Kruger M, Davidson A, Poole J, Hadley GP, Forman D, Colombet M, Steliarova-Foucher E. Childhood cancer incidence in South Africa, 1987-2007. *S Afr Med J* 2015; **105**: 939-947 [PMID: 26632323 DOI: 10.7196/SAMJ.2015.v105i11.9780]

41 **Johnsen JI**, Dyberg C, Wickström M. Neuroblastoma-A Neural Crest Derived Embryonal Malignancy. *Front Mol Neurosci* 2019; **12**: 9 [PMID: 30760980 DOI: 10.3389/fnmol.2019.00009]

42 **Cheung NK**, Zhang J, Lu C, Parker M, Bahrami A, Tickoo SK, Heguy A, Pappo AS, Federico S, Dalton J, Cheung IY, Ding L, Fulton R, Wang J, Chen X, Becksfort J, Wu J, Billups CA, Ellison D, Mardis ER, Wilson RK, Downing JR, Dyer MA; St Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project. Association of age at diagnosis and genetic mutations in patients with neuroblastoma. *JAMA* 2012; **307**: 1062-1071 [PMID: 22416102 DOI: 10.1001/jama.2012.228]

43 **Hesseling PB**, Ankone K, Wessels G, Schneider JW, Du Plessis L, Moore S. Neuroblastoma in southern Africa: epidemiological features, prognostic factors and outcome. *Ann Trop Paediatr* 1999; **19**: 357-363 [PMID: 10716030 DOI: 10.1080/02724939992202]

44 **Kitonyi GW**, Macharia WM, Mwanda OW, Pamnani R. Clinico-pathologic characteristics and treatment outcomes in children with neuroblastoma at the Kenyatta National Hospital, Nairobi. *East Afr Med J* 2009; **86**: S39-S46 [PMID: 21591508 DOI: 10.4314/eamj.v86i12.62899]

45 **Panagopoulou P**, Georgakis MK, Baka M, Moschovi M, Papadakis V, Polychronopoulou S, Kourti M, Hatzipantelis E, Stiakaki E, Dana H, Tragiannidis A, Bouka E, Antunes L, Bastos J, Coza D, Demetriou A, Agius D, Eser S, Gheorghiu R, Šekerija M, Trojanowski M, Žagar T, Zborovskaya A, Ryzhov A, Dessypris N, Morgenstern D, Petridou ET. Persisting inequalities in survival patterns of childhood neuroblastoma in Southern and Eastern Europe and the effect of socio-economic development compared with those of the US. *Eur J Cancer* 2018; **96**: 44-53 [PMID: 29673989 DOI: 10.1016/j.ejca.2018.03.003]

46 **Henderson TO**, Bhatia S, Pinto N, London WB, McGrady P, Crotty C, Sun CL, Cohn SL. Racial and ethnic

Stellenbosch University https://scholar.sun.ac.za

disparities in risk and survival in children with neuroblastoma: a Children's Oncology Group study. *J Clin Oncol* 2011; **29**: 76-82 [PMID: 21098321 DOI: 10.1200/JCO.2010.29.6103]

47 **Lanier AP**, Holck P, Ehrsam Day G, Key C. Childhood cancer among Alaska Natives. *Pediatrics* 2003; **112**: e396 [PMID: 14595083 DOI: 10.1542/peds.112.5.e396]

48 **Valery PC**, Youlden DR, Baade PD, Ward LJ, Green AC, Aitken JF. Cancer survival in Indigenous and non-Indigenous Australian children: what is the difference? *Cancer Causes Control* 2013; **24**: 2099-2106 [PMID: 24036890 DOI: 10.1007/s10552-013-0287-9]

49 **Chatenoud L**, Bertuccio P, Bosetti C, Levi F, Negri E, La Vecchia C. Childhood cancer mortality in America, Asia, and Oceania, 1970 through 2007. *Cancer* 2010; **116**: 5063-5074 [PMID: 20629033 DOI: 10.1002/cncr.25406]

50 **Ribeiro RC**, Steliarova-Foucher E, Magrath I, Lemerle J, Eden T, Forget C, Mortara I, Tabah-Fisch I, Divino JJ, Miklavec T, Howard SC, Cavalli F. Baseline status of paediatric oncology care in ten low-income or mid-income countries receiving My Child Matters support: a descriptive study. *Lancet Oncol* 2008; **9**: 721-729 [PMID: 18672210 DOI: 10.1016/S1470-2045(08)70194-3]

51 **Maris JM**. Recent advances in neuroblastoma. *N Engl J Med* 2010; **362**: 2202-2211 [PMID: 20558371 DOI: 10.1056/NEJMra0804577]

52 **Louis CU**, Shohet JM. Neuroblastoma: molecular pathogenesis and therapy. *Annu Rev Med* 2015; **66**: 49-63 [PMID: 25386934 DOI: 10.1146/annurev-med-011514-023121]

53 **Usmani GN**. Pediatric oncology in the third world. *Curr Opin Pediatr* 2001; **13**: 1-9 [PMID: 11176236 DOI: 10.1097/00008480-200102000-00001]

54 **Israels T**, Ribeiro RC, Molyneux EM. Strategies to improve care for children with cancer in Sub-Saharan Africa. *Eur J Cancer* 2010; **46**: 1960-1966 [PMID: 20403685 DOI: 10.1016/j.ejca.2010.03.027]

55 **Smith MA**, Altekruse SF, Adamson PC, Reaman GH, Seibel NL. Declining childhood and adolescent cancer mortality. *Cancer* 2014; **120**: 2497-2506 [PMID: 24853691 DOI: 10.1002/cncr.28748]

56 **Heck JE**, Ritz B, Hung RJ, Hashibe M, Boffetta P. The epidemiology of neuroblastoma: a review. *Paediatr Perinat Epidemiol* 2009; **23**: 125-143 [PMID: 19159399 DOI: 10.1111/j.1365-3016.2008.00983.x]

57 **Brodeur GM**. Spontaneous regression of neuroblastoma. *Cell Tissue Res* 2018; **372**: 277-286 [PMID: 29305654 DOI: 10.1007/s00441-017-2761-2]

58 **Logie DE**, Harding R. An evaluation of a morphine public health programme for cancer and AIDS pain relief in Sub-Saharan Africa. *BMC Public Health* 2005; **5**: 82 [PMID: 16092958 DOI: 10.1186/1471-2458-5-82]

59 **SIOPEN**. European association involved in the research and care of children with neuroblastoma. [cited 28 October 2019]. Available from: https://www.siopen.net

60 **Radhakrishnan V**, Raja A, Dhanushkodi M, Ganesan TS, Selvaluxmy G, Sagar TG. Real World Experience of Treating Neuroblastoma: Experience from a Tertiary Cancer Centre in India. *Indian J Pediatr* 2019; **86**: 417-426 [PMID: 30778950 DOI: 10.1007/s12098-018-2834-6]

61 **Friedman DL**. High-risk neuroblastoma: challenges in management in low- and middle-income countries. *Pediatr Transplant* 2016; **20**: 742-743 [PMID: 27501322 DOI: 10.1111/petr.12775]

62 **Bhatnagar SN**, Sarin YK. Neuroblastoma: a review of management and outcome. *Indian J Pediatr* 2012; **79**: 787-792 [PMID: 22528697 DOI: 10.1007/s12098-012-0748-2]

63 **Statistics South Africa**. General household survey 2010. In: stats sa. [cited 22 July 2019]. Available from: http://www.statssa.gov.za

64 **Swaminathan R**, Sankaranarayanan R. Under-diagnosis and under-ascertainment of cases may be the reasons for low childhood cancer incidence in rural India. *Cancer Epidemiol* 2010; **34**: 107-108 [PMID: 20022839 DOI: 10.1016/j.canep.2009.11.006]

65 **Kamihara J**, Ma C, Fuentes Alabi SL, Garrido C, Frazier AL, Rodriguez-Galindo C, Orjuela MA. Socioeconomic status and global variations in the incidence of neuroblastoma: call for support of population-based cancer registries in low-middle-income countries. *Pediatr Blood Cancer* 2017; **64**: 321-323 [PMID: 27734570 DOI: 10.1002/pbc.26244]

66 **Stones DK**. Childhood cancer: early warning signs. *CME* 2010; **28**: 314–316

67 **Poyiadjis S**, Tuyisenge L. (2014) Early Warning Signs of Cancer in Children/Models for Early Diagnosis. Stefan D. *Rodriguez-Galindo C. Pediatric Hematology-Oncology in Countries with Limited Resources*. New York: Springer 2014; pp 65

68 **Viana MB**, Fernandes RA, de Oliveira BM, Murao M, de Andrade Paes C, Duarte AA. Nutritional and socio-economic status in the prognosis of childhood acute lymphoblastic leukemia. *Haematologica* 2001; **86**: 113-120 [PMID: 11224478]

69 **Louie DS**, Liang JP, Owyang C. Characterization of a new CCK antagonist, L364, 718: in vitro and in vivo studies. *Am J Physiol* 1988; **255**: G261-G266 [PMID: 2458681 DOI: 10.1371/journal.pone.0089482]

70 **Mostert S**, Sitaresmi MN, Gundy CM, Janes V, Sutaryo, Veerman AJ. Comparing childhood leukaemia treatment before and after the introduction of a parental education programme in Indonesia. *Arch Dis Child* 2010; **95**: 20-25 [PMID: 19679573 DOI: 10.1136/adc.2008.154138]

71 **Arora RS**, Pizer B, Eden T. Understanding refusal and abandonment in the treatment of childhood cancer. *Indian Pediatr* 2010; **47**: 1005-1010 [PMID: 21220796 DOI: 10.1007/s13312-010-0172-5]

72 **Peltzer K**. Utilization and practice of traditional/complementary/alternative medicine (TM/CAM) in South Africa. *Afr J Tradit Complement Altern Med* 2009; **6**: 175-185 [PMID: 20209010]

73 **Hiatt RA**, Breen N. The social determinants of cancer: a challenge for transdisciplinary science. *Am J Prev Med* 2008; **35**: S141-S150 [PMID: 18619394 DOI: 10.1016/j.amepre.2008.05.006]

74 **Coovadia H**, Jewkes R, Barron P, Sanders D, McIntyre D. The health and health system of South Africa: historical roots of current public health challenges. *Lancet* 2009; **374**: 817-834 [PMID: 19709728 DOI: 10.1016/S0140-6736(09)60951-X]

75 **Carter NH**, Avery AH, Libes J, Lovvorn HN 3rd, Hansen EN. Pediatric Solid Tumors in Resource-Constrained Settings: A Review of Available Evidence on Management, Outcomes, and Barriers to Care. *Children (Basel)* 2018; **5** [PMID: 30360527 DOI: 10.3390/children5110143]

76 **Liu Z**, Thiele CJ. Molecular Genetics of Neuroblastoma. Diagnostic and Therapeutic Nuclear Medicine for Neuroendocrine Tumors. In: Pacak K, Taïeb D. Diagnostic and Therapeutic Nuclear Medicine for

Stellenbosch University https://scholar.sun.ac.za

Neuroendocrine Tumors, Contemporary Endocrinology. Switzerland: Springer International Publishing, Cham, 2017: 83-125

77 **Pasquier E**, Street J, Pouchy C, Carre M, Gifford AJ, Murray J, Norris MD, Trahair T, Andre N, Kavallaris M. β-blockers increase response to chemotherapy via direct antitumour and anti-angiogenic mechanisms in neuroblastoma. *Br J Cancer* 2013; **108**: 2485-2494 [PMID: 23695022 DOI: 10.1038/bjc.2013.205]

78 **Yifru S**, Muluye D. Childhood cancer in Gondar University Hospital, Northwest Ethiopia. *BMC Res Notes* 2015; **8**: 474 [PMID: 26404043 DOI: 10.1186/s13104-015-1440-1]

79 **Ibrahim M**, Abdullahi SU, Hassan-Hanga F, Atanda A. Pattern of childhood malignant tumors at a teaching hospital in Kano, Northern Nigeria: A prospective study. *Indian J Cancer* 2014; **51**: 259-261 [PMID: 25494118 DOI: 10.4103/0019-509X.146765]

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 3

Reporting incidences of neuroblastoma in various resource settings (2020)
Ethics no: S18/07/138

For submission to Journal of Global Oncology

van Heerden, Jaques[1,2]; Kruger, Mariana [1].

Affiliations:

[1] Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

[2] Paediatric Haematology and Oncology, Department of Paediatrics Antwerp University Hospital, Antwerp, Belgium

Correspondence

Dr Jaques van Heerden, Department of Paediatric Haematology and Oncology, University Hospital of Antwerp, Drie Eikenstraat 655, Edegem, 2650, Belgium. Email: Jaques.vanheerden@uza.be

Contributors' statement

Jaques van Heerden and Mariana Kruger conceptualised and designed the study, collected data, performed the data analysis and wrote the manuscript.

Keywords:
Neuroblastoma, registries, incidences, South Africa

Abstract

Background

The incidences of neuroblastoma (NB) differ significantly between countries. The heterogeneous presentation, varying quality of cancer registries and underdiagnoses of NB complicate the recording of incidences in various resource settings.

Purpose

The aim was to evaluate current regional variations of NB as reported by international cancer registries. The primary objective was to evaluate the theoretical and reported differences in international incidences. The secondary objective was to evaluate South Africa as a case for variable reporting.

Method

A comprehensive literature review on registries reporting on NB was done to construct incidence tables. The Surveillance, Epidemiology, and End Results (SEER) Program incidence of 10.5/ million

Stellenbosch University https://scholar.sun.ac.za

children for NB was used to calculate the expected number of cases for each country. Registry data of NB cases between 2000 and 2015 were requested from The South African National Cancer registry (SA – NCR) and the South African Children's Tumour Registry (SACTR) for comparison.

Results

Internationally incidences varied between -97.1% to +80% compared to the SEER program. Between 2000-2015 the SA-NCR reported between 23 – 51 cases/ year whilst the SACTR reported between 18 – 57 cases/ year for the same period. Both registries reported incidences less than HIC.

Conclusion

Underdiagnosis of NB and undiagnosed cases due to spontaneous regression alone cannot explain the lower incidence of NB and further and improvement in the South African tumour registries are required to determine actual incidence.

Article

Introduction

According to The Surveillance, Epidemiology, and End Results (SEER) program the incidence of childhood malignancies between 2011 and 2015 for children under 15-years were 16/ 100 000 children compared to 953 malignancies per 100 000 adults [1]. Internationally neuroblastoma (NB) is the second most common solid tumour of childhood, after central nervous tumours, but only contributes to 7% of childhood malignancies [2]. NB has no single clinical presentation underlined by heterogeneous pathophysiologies which challenges surveillance and diagnosis in variable resourced settings challenging [2]. The incidence of neuroblastoma (NB) is well described in HIC. The SEER program of the National Cancer Institute of the United States of America reported an incidence of 10.5 cases/ million for NB, but a lower incidence has been recorded in LMIC [3]. According to the International Agency for Research on Cancer (IARC) the HICs Canada and the USA reported age standardised rates (ASR) of 12.5/ million children under 15 years and 14.0/ million children under 15 years for respectively [4]. In Europe the ASR varied between 8.3/ million children under 15 years (The Netherlands) and 19.0/ million children (Italy) [4]. The South American countries reported ASRs between 2.0/ million children under 15 years in Ecuador and 11.2/ million children under 15 years in Uruguay [4]. There is very little known about the epidemiology of the disease in Sub-Saharan Africa. In a region where communicable diseases, neonatal deaths, malnutrition and the HIV-epidemic contribute the greatest burden to health care systems, the incidences of rare diseases, such as NB, even in the presence of disease specific registers, are inaccurately recorded [5]. According to the IARC's International Incidence of Childhood Cancer program the African region reported ASRs for NB of between 0.8/ million children under 15 years in Uganda to 10.4/ million children under 15 years in Egypt [4]. Due to the inaccuracies of cancer registers and lower than average diagnoses of childhood malignancies in these regions, it is not understood whether these incidences are false low values or a true incidence of NB in these regions [5,6].

Stellenbosch University https://scholar.sun.ac.za

South Africa (SA) is a country with a youthful age structure where 29.2% of the population is under 15-years of age [4]. According to the IACR reports South Africa reported an ASR of 2.7/ million children under 15 years for NB, which is much lower than reports from HICs. The South African Children's Tumour Registry (SACTR) is a clinical base registry compiled by data submitted from physicians treating children with childhood malignancies established in 1987 by the South African Children's Cancer Study Group (SACCSG) [6]. The registry complies with international quality standards for cancer registries. Cases with incomplete data are not included in reported data [6]. The South African National Cancer Registry (SA – NCR) is the main cancer surveillance system in SA. Although it was established in 1986 as a voluntary, pathology-based cancer reporting system, the register was mandated through legislation in 2011 to monitor and report the national cancer burden [7]. These two registries serve as the main sources of information regarding the incidence of NB in South Africa.

The aim of the study was to evaluate current regional variations of NB as reflected by internal registries and incidence reports. The first objective was to evaluate the theoretical and reported differences in NB incidences around the world. The second objective was to determine the expected incidences of NB per country if the incidence were the same of SEER program. The third objective was to evaluate South Africa as a case study and describe the differences in the two local South African registries.

Methodology

African Index Medicus, ScieLo, Pubmed, Global Health, Embase, and Google Scholar were searched to perform a comprehensive literature review of publications with medical subject headings (MESH) in keeping with registries reporting on NB such as "registries"," neuroblastoma"," children" and country specific names. The search was conducted from April 2019 to January 2020. No limitations were set on the date or language provided English summaries or abstracts were included. Reports of tumour registries were used to construct incidence tables. If no reports were found data was requested electronically from relevant cancer registries of each country.
The NB incidence of 10.5/ million children reported by the SEER Program was used to calculate the expected number of cases for each country. The percentage children under 15 years old per population and the population under 15 years old in each country were sourced or calculated from data from the World Bank [8] and the World Factbook website [9].
Registry data were requested from The South African National cancer registry (SA – NCR) and the South African Children's Tumour Registry (SACTR) for registered NB cases between 2000 and 2016. Patient data from the two registries were compared across the two data sets according to the number of cases per year, sex and age. Incidences were calculated with data sourced from Statistics South Africa [4].

Results

International registries

The systemic literature search retrieved 127 articles, abstracts and documents on NB which included 13 cancer registry-based reports. The national incidence of NB varied between 0.2 – 18.9/million children under 15 years/ year (average 7.9/million) which varied between -97.1% to 80.0% according to the 10.5/ million reported by SEER data.

Stellenbosch University https://scholar.sun.ac.za

South African registries

The SA-NCR reported between 23 – 51 cases/ year between 2000 and 2015, whilst the SACTR reported between 18 – 57 cases/ year for the same period (see Graph 1).
The SA-NCR reported 11-27 males/year and 7-24 females/year whilst the SACTR reported 5-21 males and 7-19 females per year respectively (see Graph 2).
The SA-NCR reported 1-48 children aged 0-18 months/year, 1-30 aged 19-60 months/year and 0-1 children older than 60 months/year whilst the SACTR reported 3-15 children aged 0-18 months/year, 6-22 aged 19-60 months/year and 3-11 children older than 60 months/year (see Graph 3).
The incidence reported by the SA-NCR varied between 1.5-2.8/ million children under 15-years per year whilst the SACTR reported 1.74-2.6 cases/million children. This varied between 0.3-0.92 cases/million (see Table 1). The incidence of males reported by the SA-NCR varied between 1.8-3.5/ million males under 15-years per year whilst the SACTR reported 1.0-2.76 cases/million males. This varied between 0.4-0.89 males/million (see Table 1). The incidence of females reported by the SA-NCR varied between 1.6-2.3/ million females under 15-years per year whilst the SACTR reported 1.25-3.69 cases/million females. This varied between 0.14-2.09 females/million (see Table 1).

Discussion

Neuroblastoma is a significant cause of childhood cancer deaths, and a burden on resources regardless of the country of diagnosis. Based on mortality estimates to calculate disability adjusted life years the burden disproportionately affects populations in resource-limited settings [10]. Yet since these calculations are in part based on population-based cancer registries, the burden might be underrepresented [10]. With the heterogeneous presentation of NB, the impact cannot be reliably determined as the national incidences are not accurately recorded.

Availability of robust tumour registries

The paucity of robust tumour registries contributes to the limited epidemiological data in LMIC [11,12]. Although NB is the most common extra cranial solid tumour in children, in Africa it is less common than nephroblastoma and retinoblastoma [13]. However, reliable paediatric cancer registries are variable or are limited to single institutions in LMICs [14]. This undermines the optimal interpretation of data to reflect the true burden of NB [11]. According to world-age standardised rate (WSR) the incidence is about 12% across regions but less than 10% in Africa [11]. The WSR of 10.5 per million person-years in children in the USA is in contrast with the WSR of 2.7 per million person-years in Sub-Saharan Africa [15]. In figure 1 the current reported number of cases are reflected with figure 2 reflecting the countries that obtain the 10.5/ million incidence that is reported in the SEER database. The figures indicate a predominance of NB in westernised countries. Yet, in Figure 3 the expected number of NB cases for each country are reflected based on a HIC incidence (*see Table 1*). Each country's total expected number of diagnoses was calculated as a value equal to a WSR 10.5 per million incidences based on the 0-15-year population figures sourced from the World Bank [16].

Stellenbosch University https://scholar.sun.ac.za

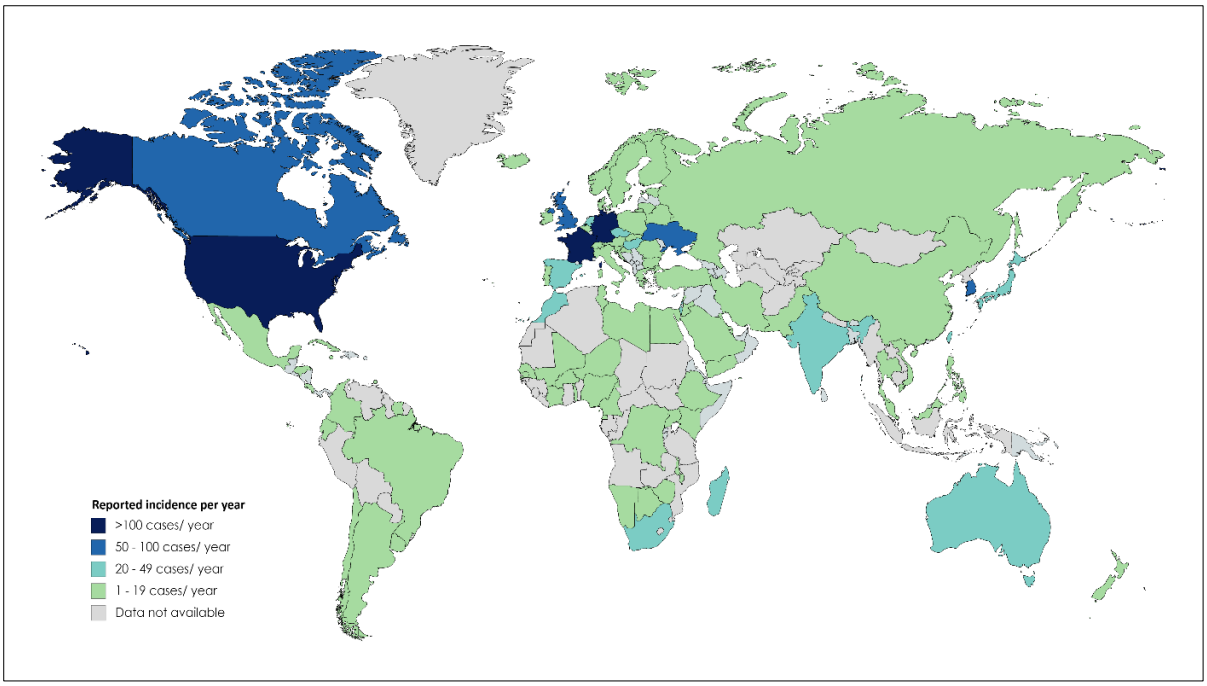


Fig. 1. Reported incidence of NB cases in children under 15 years of age

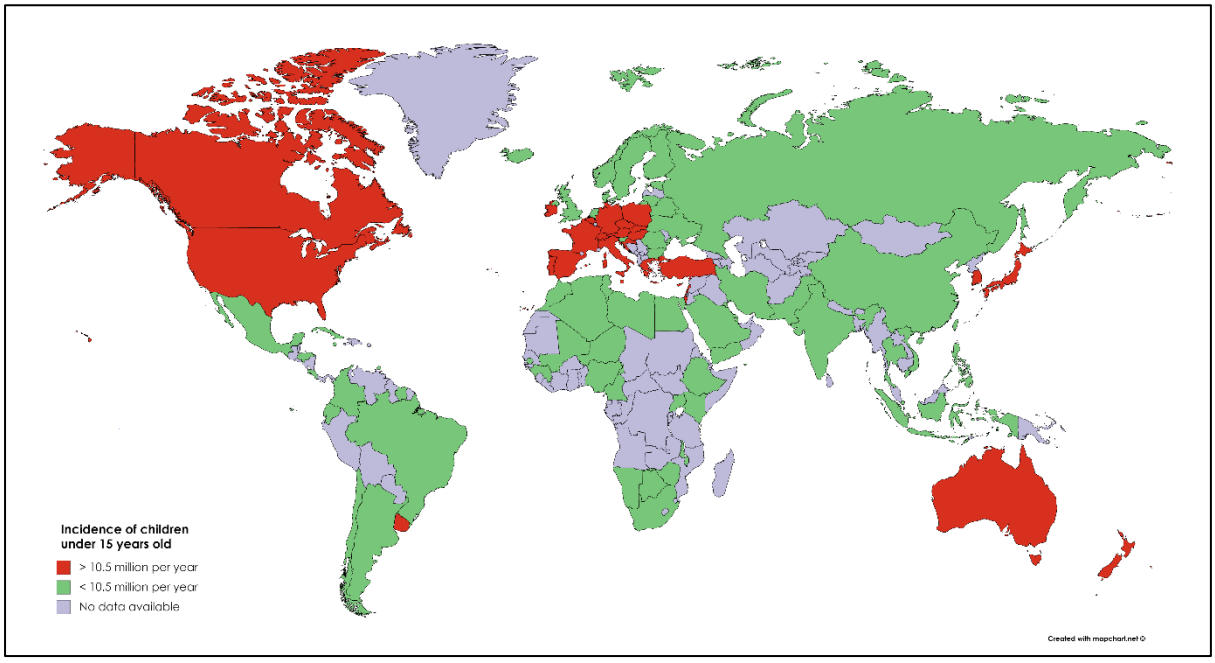


Fig. 2. Countries that obtain an incidence equal to the HIC rate of 10.5/ million children

Stellenbosch University https://scholar.sun.ac.za

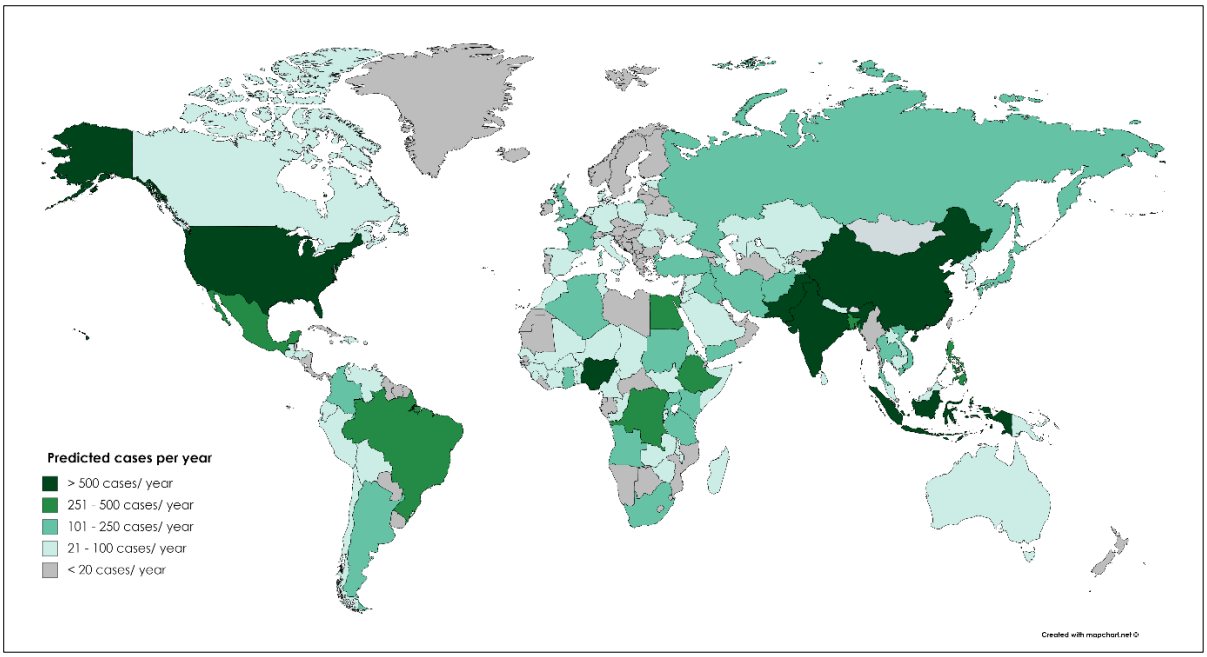


Fig. 3. Expected number of NB cases for each country based on an incidence of 10.5/ million children

The limitations of surrogate inter-regional incidence rates

In *Table 1* the calculated incidence gaps as a percentage between the reported incidence and the SEER incidence are indicated. It is generally stated that the incidence of NB is lower in resource limited settings [10,11]. Yet HICs Singapore and Qatar have incidences of 5.9 and 6.6 respectively, whilst Reunion, a French territory in sub-Saharan Africa, has an incidence of 11.1/ million children. This is higher than the 10.5/million children reported for the United States [17].

South Africa as a case study

Population based cancer registries report on the occurrence of cancer in a population, during a specific time period and is a tool to evaluate the impact of cancer on the community [18]. The medical system in South Africa is a dual public and private medical system that serves 85% and 15% of the population respectively [19]. To evaluate the incidence of neuroblastoma in the country both medical systems should be surveilled. Registration of cancers in both sectors are overseen by the SA-NCR based on voluntary pathology reporting that became compulsory in 2012. The limitation of this system is that neuroblastoma can be diagnosed on clinical signs in conjunction with radiological images and confirmed with urine catecholamine levels. Thereby no confirmatory biopsy is done.
The SACTR is a clinical registry compiled by mainly paediatric oncologists in both the public and private sector. The limitation of this registry with regards to registering neuroblastoma is that it excludes patients not treated by paediatric oncologists, patients that died before referral for treatment and patients that was misdiagnosed. Together the two registries should account for nearly all patients that were biopsied and started with treatment in a healthcare facility with minimal impact on the incidence when diagnosed patients are not reported. As there are no common unique identifiers in both sets record linkage should be done using probabilistic record linkage techniques with variables such as name(s), surname, date-of-birth, date of diagnosis, sex and province of diagnosis to link the patients from the two sets. The record linkage was not done due to limitations in ethical consent.

Stellenbosch University https://scholar.sun.ac.za

In South Africa non-diagnosis of childhood malignancies have led to a significant underreporting of childhood malignancies [20]. In relation to the patho-physiology of NB the non-diagnosed cases could either be explained by tumours that underwent maturation and remained undetected, neuroblastoma was misdiagnosed as non-malignant diseases or patients died undiagnosed.

NB screening in infants for urine vanillylmandelic acid and homovanillic acid have identified cases that would have undergone spontaneous regression [21,22]. Screening studies only proved to identify more tumours with favourable histology, but not advanced disease, nor did it improve overall survival outcomes [21,22]. By adopting a "wait and see" management strategy, tumour regression in untreated patients have been seen in up to 47% of patients with localised stage 1 and 2 neuroblastomas [21,22]. This represents 0.7 cases/ million infants screened [23].

Sudden unexpected death due to neoplastic disease in infancy and childhood is rare [24,25]. Autopsy case series have demonstrated that a variety of neoplasms including cardiac neoplasms and central nervous system (CNS) tumours account for the largest number of cases [24,25]. There are limited published paediatric autopsy registers available in South Africa to determine undiagnosed NB deaths, yet international reports concluded that neuroblastoma contributed less than 8% of autopsy cases [26].

Conclusion

Even with meticulous registration of pathological and clinical cases of NB, undiagnosed and misdiagnosed cases will lead to underreporting of cases in many countries. Yet by calculating the percentage of underdiagnosis statistically, evaluating possible additional cases by autopsies and screening procedures, South Africa will still not have an incidence reported in Europe and North America. The paucity of genetic information regarding NB in LMIC may be a factor to understanding the difference in incidences.

References:

1. Cancer in children and adolescents. National Cancer Institute. https://www.cancer.gov/types/childhood-cancers/child-adolescent-cancers-fact-sheet#r7 Accessed March 2020.
2. Whittle, SB; Smith, V; Doherty, E; Zhao, S; et al. Overview and recent advances in the treatment of neuroblastoma. Expert Review of Anticancer Therapy. 2017;17(4):369–386.
3. Steliarova-Foucher E, Stiller CA, Hesselinge P, Moreno F, Ries L, Shin H. International Incidence of Childhood Cancer Volume III Chapter IVa. Neuroblastoma and ganglioneuroblastoma. http://iicc.iarc.fr/includes/results/comparative/0401_Neuroblastoma.pdf Accessed April 2019.
4. World Health Organisation International Agency for Research on Cancer. International Incidence of Childhood Cancer Volume 3: IV. Neuroblastoma and other peripheral nervous cell tumours. https://iicc.iarc.fr/includes/results/comparative/04_Neuroblastoma.pdf Accessed September 2020.
5. Stefan, CS; Bray, F; Ferlay, J et al. Cancer of childhood in sub-Saharan Africa. *e*cancer 2017, 11:755 https://doi.org/10.3332/ecancer.2017.755

Stellenbosch University https://scholar.sun.ac.za

6. Stefan DC, Stones DK, Wainwright D, Kruger M, et al. Childhood cancer incidence in South Africa, 1987 - 2007. S Afr Med J. 2015; 105(11): 939.
7. National Department of Health. National Health Act (Act No. 61 of 2003): Regulations Relating to Cancer Registration. No. R 380. Republic of South Africa, 2011. http://www.nioh.ac.za/assets/files/ Gazetted/Versio/2026_4_2011/20(2).pdf Accessed January 2020.
8. World Bank. World Bank Country and Lending Groups. Based on the statistics reported by the World Bank for 2019. https://data.worldbank.org/indicator. Accessed April 2019.
9. Central Intelligence Agency, United States of America. The World Factbook – Guide to country comparisons. www.cia.gov/library/publications Accessed July 2019.
10. Ward Z, Yeh JN, Bhakta N, Frazier AL, Atun R. Estimating the total incidence of global childhood cancer: a simulation-based analysis. The Lancet Oncology 2019;20(4):483-493.
11. Steliarova-Foucher E, et al.: International incidence of childhood cancer, 2001–10: a population-based registry study. Lancet Oncol 2017; 18: 719–31.
12. Kruger M, Hendricks M, Davidson A, Stefan CD, van Eyssen AL, Uys R, van Zyl A, Hesseling P. Childhood cancer in Africa. Pediatr Blood Cancer 2014; 61(4): 587-92.
13. Stefan, CS; Bray, F; Ferlay, J et al. Cancer of childhood in sub-Saharan Africa. *e*cancer 2017, 11:755 https://doi.org/10.3332/ecancer.2017.755
14. Hadley G, Van Heerden J. High-risk neuroblastoma in a sub-Saharan African country: telling it like it is. Trop Doct. 2017; 47(4):370-374.
15. Bollyky TJ, Templin T, Cohen M, Dieleman JL. Lower-Income Countries That Face the Most Rapid Shift in Noncommunicable Disease Burden Are Also the Least Prepared. Health Affairs. 2017; 36(11):1866-1875.
16. World bank. Population: all countries and economies. Based on the statistics reported by the World Bank for 2019. https://data.worldbank.org/indicator. Accessed April 2019.
17. National Cancer Institute: Neuroblastoma http://www.cancer.gov/types/neuroblastoma Accessed July 2019.
18. Curado MP, Voti L, Sortino-Rachou AM. Cancer registration data and quality indicators in low- and middle-income countries: their interpretation and potential use for the improvement of cancer care. Cancer Causes & Control. 2008; 20(5): 751–756.
19. Singh E, Underwood JM, Natty C, Babb C, et al. South African National Cancer Registry: Effect of withheld data from private health systems on cancer incidence estimates. S Afr Med J 2015; 105(2): 107-109.
20. Singh E, Ruff P, Babb C, et al. Establishment of a cancer surveillance programme: the South African experience. Lancet Oncol. 2015;16(8):e414-e421.
21. Localized Infant Neuroblastomas Often Show Spontaneous Regression: Results of the Prospective Trials NB95-S and NB97. Barbara Hero, Thorsten Simon, Ruediger Spitz, Karen Ernestus, Astrid K. Gnekow, Hans-Guenther Scheel-Walter, Dirk Schwabe, Freimut H. Schilling, Gabriele Benz-Bohm, and Frank Berthold Journal of Clinical Oncology 2008 26:9, 1504-1510
22. Yamamoto K, Hanada R, Kikuchi A, et al.: Spontaneous regression of localized neuroblastoma detected by mass screening. J Clin Oncol 16 (4): 1265-9, 1998.
23. Neuroblastoma screening. National Cancer Institute. https://www.cancer.gov/types/neuroblastoma/hp/neuroblastoma-screening-pdq Accessed March 2020.
24. Bryant VA, Booth J, Palm L, Ashworth M ; et al. Childhood neoplasms presenting at autopsy: A 20-year experience. Pediatric Blood & Cancer. 2017; 64(9): e26474. doi:10.1002/pbc.26474.

Stellenbosch University https://scholar.sun.ac.za

25. Taggart MW, Craver R. Causes of Death, Determined by Autopsy, in Previously Healthy (or Near-Healthy) Children Presenting to a Children's Hospital. Arch Pathol Lab Med. 2006; 130: 1780–1785.
26. Park KU, Prahlow JA. Sudden death due to adrenal neuroblastoma: child abuse mimic? Forensic SciMed Pathol. 2011; 7(1): 47–52.

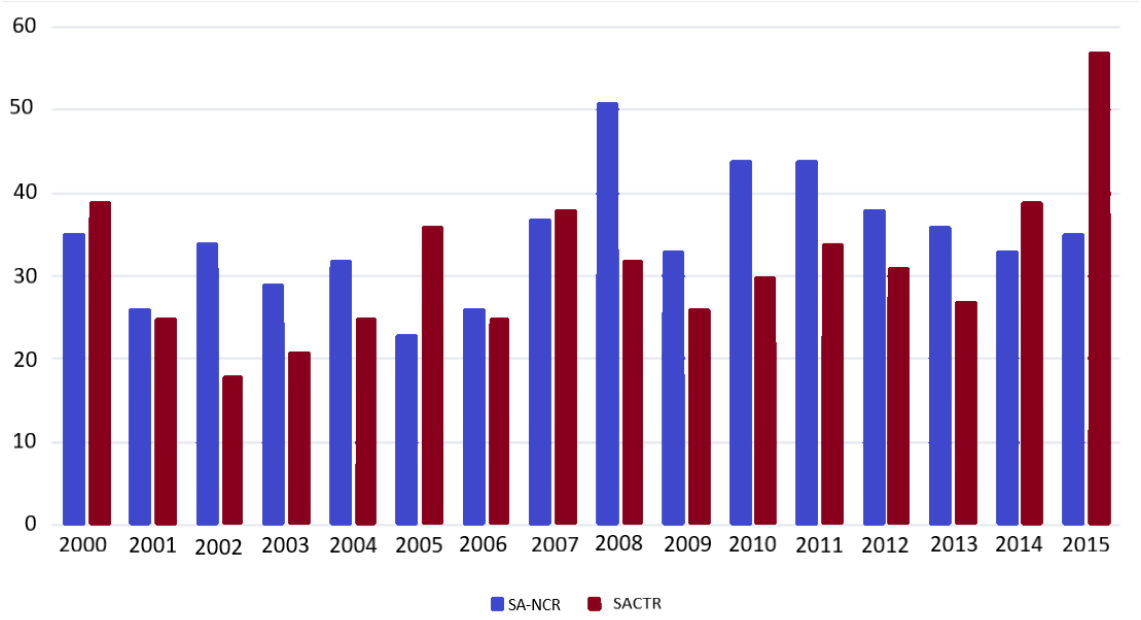
Tables and graphs:

Graphs:

Tables:

Graphs:

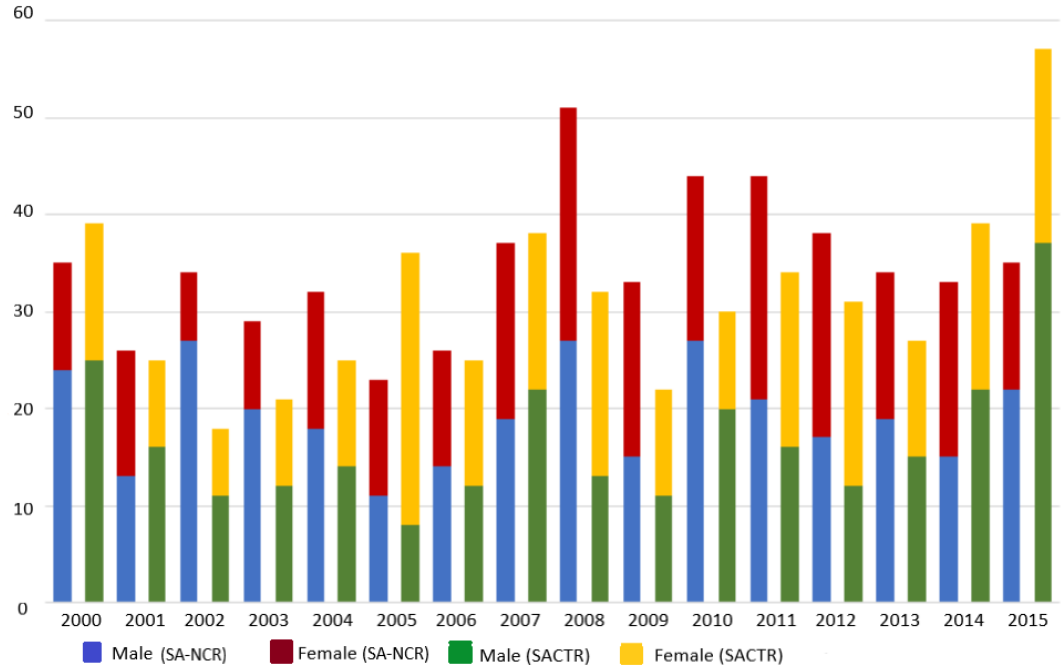
Graph 1. The discordant number of registered neuroblastoma cases in the SA-NCR and SACTR



SACTR – South African Children's tumour registry; SA-NCR – South African National Cancer Registry

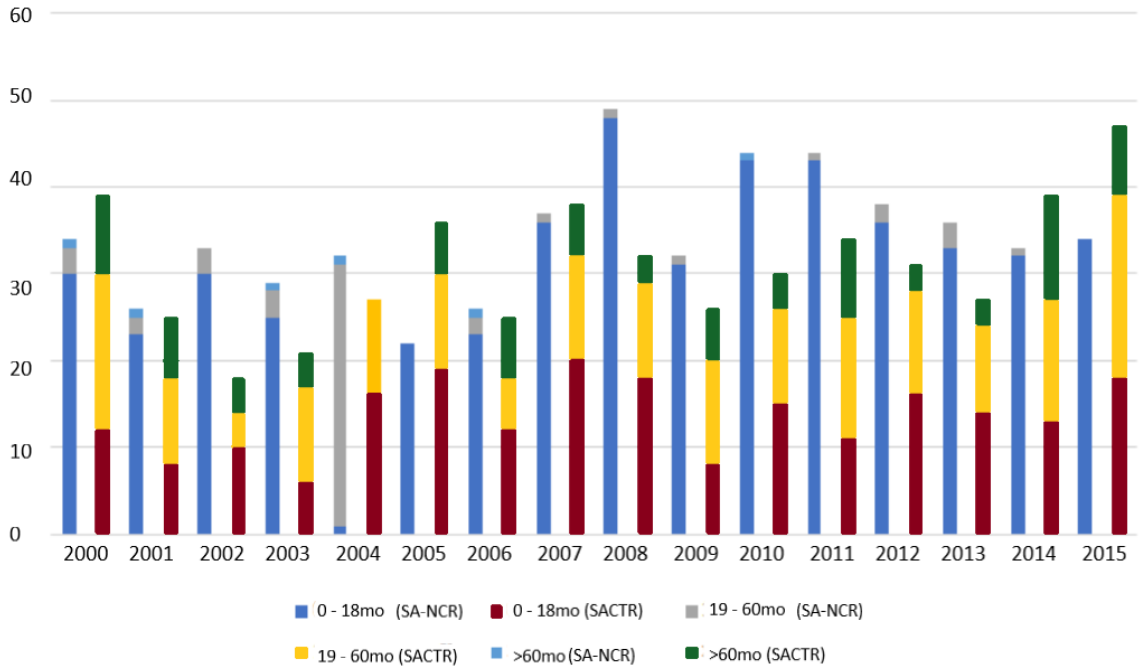
Stellenbosch University https://scholar.sun.ac.za

Graph 2. The discordant number of neuroblastoma cases in the SA-NCR and SACTR per sex



SACTR – South African Children's tumour registry; SA-NCR – South African National Cancer Registry

Graph 3. The discordant number of neuroblastoma cases in the SA-NCR and SACTR per age group



SACTR – South African Children's tumour registry; SA-NCR – South African National Cancer Registry

Stellenbosch University https://scholar.sun.ac.za

Table 1: Epidemiological characteristics of neuroblastoma per country

Country	% Children 0-14 yrs per population (2017)[1,2]	Population <0-14 yrs[1,2]	Expected number of cases (10.5 per million children)	Reported number of cases	Cases per year	AIR/ASR/CIR (% incidence difference)
Afghanistan	43	15.27 million	143	-	-	ND
Albania	17	0.49 million	4	-	-	ND
Algeria	29	11.98 million	111	112 (1996-2014)	6.2	7.2 (-31.4%)
American Samoa	30	55 641	<1	-	-	ND
Andorra	14	76 965	<1	-	-	ND
Angola	47	13.99 million	130	-	-	ND
Antigua and Barbuda	24	24 483	<1	-	-	ND
Argentina	25	11.06 million	103	164 (1991-2013)	7.5	8.6 (-18.0%)
Armenia	20	586 000	5	-	-	ND
Aruba	18	18 947	<1	-	-	ND
Australia	19	4.67 million	44	895 (1992-2014)	38.9	11.6 (+10.5%)
Austria	14	1.22 million	11	345 (1990-2012)	15.6	13.3 (+26.6%)
Azerbaijan	23	2.27 million	21	-	-	ND
Bahamas, The	20	79 072	<1	-	-	ND
Bahrain	20	298 600	3	28 (1998-2012)	2	9.6 (-8.5%)
Bangladesh	28	46.1 million	430	-	-	ND
Barbados	19	54286	<1	-	-	ND
Belarus	17	1.61 million	15	355 (1990-2015)	14.2	9.3 (-11.4%)
Belgium	17	1.92 million	18	216 (2004-2013)	11.3	13.4 (+27.6%)
Belize	31	116 151	<1	-	-	ND
Benin	43	4.8 million	44	-	-	ND
Bermuda	17	65 441	<1	0 (2013-2018)	0	0
Bhutan	27	218 054	<1	-	-	ND
Bolivia	32	3.53 million	33	-	-	ND
Bosnia and Herzegovina	14	490 980	4	-	-	ND
Botswana	31	710 520	7	9 (2008-2012)	2.25	2.7 (-74.3%)
Brazil	22	46.04 million	430	134 (1995-2012)	7.9	8.4 (-20%)
British Virgin Islands	16	31 196	<1	-	-	ND
Brunei Darussalam	23	98 600	<1	-	-	ND
Bulgaria	14	994 280	9	178 (1990-2013)	7.7	7.1 (-32.3%)
Burkina Faso	45	8.635 million	81	-	<5	ND

Stellenbosch University https://scholar.sun.ac.za

Country	% Children 0-14 yrs per population (2017)[1,2]	Population <0-14 yrs[1,2]	Expected number of cases (10.5 per million children)	Reported number of cases	Cases per year	AIR/ASR/CIR (% incidence difference)
Burundi	45	4.88 million	45	-	-	ND
Cape Verde	30	163 916	1	-	-	ND
Cambodia	31	4.96 million	46	-	-	ND
Cameroon	43	10.34 million	96	1 (2004-2006)	<1	0.4 (-96.1%)
Canada	16	5.85 million	55	1359 (1992-2013)	61.7	13.8 (+31.4%)
Cayman Islands	18	61 559	<1	-	-	ND
Central African Republic	43	2.0 million	19	-	-	ND
Chad	47	5.4 million	50	-	-	ND
Channel Islands	15	24 681	<1	-	-	ND
Chile	20	3.61 million	34	59 (1993-2013)	2.9	4.2 (-60.0%)
China	18	249.48 million	2331	389 (1990-2013)	16.9	8.6 (-18.1%)
Colombia	3	11.29 million	105	59 (1992-2003)	5.3	4.0 (-61.9%)
Comoros	40	325 564	3	-	-	ND
Congo, Dem. Rep.	46	37.41 million	350	-	<5	ND
Congo, Rep.	42	2.2 million	20	-	-	ND
Costa Rica	22	1.079 million	10	82 (1993-2012)	4	4.0 (-61.9%)
Cote d'Ivoire	42	10.2 million	95	-	<5	ND
Croatia	15	623 100	6	113 (2000-2014)	8	13.2 (+25.7%)
Cuba	16	1.84 million	17	193 (2000-2012)	16	8.5 (-19.4%)
Curacao	19	30 593	<1	-	-	ND
Cyprus	17	145 316	1	27 (1998-2013)	1.8	13.9 (+32.3%)
Czech Republic	15	1.58 million	15	467 (1990-2012)	21.2	14.1 (+34.2%)
Denmark	16	919 840	9	160 (1981-2000)	8	9.6 (-8.6%)
Djibouti	31	296 665	3	-	-	ND
Dominica	22	73925	<1	-	-	ND
Dominican Republic	29	3.12 million	29	-	-	ND
Ecuador	28	4.65 million	43	39 (1993-2013)	1.9	1.9 (-81.9%)
Egypt, Arab Rep.	33	32.19 million	300	133 (1999-2010)	12	10.1 (-3.8%)
El Salvador	27	1.722 million	16	-	-	ND
Equatorial Guinea	37	469 160	4	-	-	ND
Eritrea	49	2.45 million	23	-	-	ND
Estonia	16	1.32 million	21	50 (1990-2012)	2.27	10.0 (-4.7%)
Eswatini (Swaziland)	37	505 790	5	-	-	ND

Stellenbosch University https://scholar.sun.ac.za

Country	% Children 0-14 yrs per population (2017) [1,2]	Population <0-14 yrs[1,2]	Expected number of cases (10.5 per million children)	Reported number of cases	Cases per year	AIR/ASR/CIR (% incidence difference)
Ethiopia	41	43.05 million	402	5 (2011-2013)	1.6	3.1 (-70.4%)
Faroe Islands	20	51 095	<1	-	-	ND
Fiji	28	253 540	2	-	-	ND
Finland	16	880 480	8	26 (1987-2003)	1.6	2.9 (-72.3%)
France	18	12.09 million	113	1847 (2000-2012)	142	14.2 (+35.2%)
French Polynesia	23	65 091	<1	-	-	ND
Gabon	36	729 000	7	-	-	ND
Gambia, The	45	945 450	9	3 (2002-2011)	0.33	0.4 (-96.2%)
Georgia	19	706 230	7	-	-	ND
Germany	13	10.76 million	100	2314 (1996-2012)	136.1	13.7 (+30.5%)
Ghana	39	11.24 million	105	-	-	ND
Gibraltar	20	34 571	<1	-	-	ND
Greece	14	1.5 million	14	160 (2009-2016)	9.4	14.4 (+37.1%)
Greenland	21	56 171	<1	-	-	ND
Grenada	26	28 034	<1	-	-	ND
Guam	25	41 057	<1	-	-	ND
Guatemala	35	5.9 million	55	-	-	ND
Guinea	42	5.3 million	50	0 (2001-2010)	0	0
Guinea-Bissau	41	763 010	7	-	-	ND
Guyana	29	225 579	2	-	-	ND
Haiti	33	3.62 million	34	-	-	ND
Honduras	32	2.96 million	28	12 (2002-2012)	1.1	1.6 (-84.8%)
Hong Kong	11	813 120	7	-	-	ND
Hungary	14	1.37 million	13	563 (1991-2014)	24.4	17.0 (+61.9%)
Iceland	20	67 669	<1	9 (1990-2014)	0.38	6.2 (-40.9%)
India	28	374.9 million	3503	526 (1990-2013)	22.9	3.6 (+65.7%)
Indonesia	27	71.28 million	666	-	-	ND
Iran, Islamic Rep.	24	16.9 million	157	8 (2004-2011)	1	2.6 (-75.2%)
Iraq	40	11.04 million	103	-	-	ND
Ireland	22	1.05 million	10	159 (1994-2012)	9.3	10.9 (+3.8%)
Isle of Man	16	84 287	<1	-	-	ND
Israel	28	1.94 million	18	568 (1990-2012)	25.8	14.6 (+39%)
Italy	14	8.48 million	79	142 (1998-2011)	10.9	18.9 (+80%)

Stellenbosch University https://scholar.sun.ac.za

Country	% Children 0-14 yrs per population (2017) [1,2]	Population <0-14 yrs[1,2]	Expected number of cases (10.5 per million children)	Reported number of cases	Cases per year	AIR/ASR/CIR (% incidence difference)
Jamaica	23	664 700	6	36 (1982-2012)	1.8	6.8 (-35.2%)
Japan	13	16.5 million	154	795 (1990-2013)	34.6	15.7 (+49.5%)
Jordan	36	3.49 million	33	222 (2000-2012)	18.5	9.0 (-14.2%)
Kazakhstan	28	5.05 million	47	-	-	ND
Kenya	40	19.88 million	186	19 (2007-2012)	3.8	1.7-2.8 (-73.3% - - 83.8%)
Kiribati	35	40 739	<1	-	-	ND
Dem. People's Rep of Korea	21	5.35 million	50	1130 (1999-2012)	86.9	11.3 (+6.7%)
Rep. of Korea	13	6.69 million	62	-	-	ND
Kosovo	25	1.831	<1	-	-	ND
Kuwait	21	868 770	8	61 (1994-2012)	3.4	9.8 (-6.7%)
Kyrgyz Republic	32	1.98 million	19	-	-	ND
Laos	33	2.26 million	21	-	-	ND
Latvia	15	292 500	3	-	-	ND
Lebanon	23	1.4 million	13	33 (2008-2010)	11	10.6 (+0.01%)
Lesotho	35	781 550	7	-	-	ND
Liberia	42	1.99 million	19	-	-	ND
Libya	28	1.78 million	17	22 (2003-2008)	3.7	8.1 (-22.6%)
Liechtenstein	15	37 810	<1	-	-	ND
Lithuania	15	427 200	4	55 (2000-2012)	4.2	9.8 (-6.7%)
Luxembourg	16	94 506	<1	-	-	ND
Madagascar	41	10.48 million	98	-	20 - 30	ND
Malawi	44	5.58 million	52	9 (2003-2010)	1.125	2.8 (-73.3%)
Malaysia	24	7.59 million	70	47 (2007-2011)	9.4	6.1 (-41.9%)
Maldives	23	100 355	1	-	-	ND
Mali	48	6.12 million	57	15 (2006-2014)	1.875	2.2 (-79.0%)
Malta	14	64 441	<1	17 (1995-2015)	0.85	14.2 (+35.2%)
Marshall Islands	34	53 127	<1	-	-	ND
Mauritania	40	1.77 million	17	-	-	ND
Mauritius	18	227 700	2	10 (2003-2012)	1.11	4.1 (-60.9%)
Mexico	27	34.88 million	326	36 (1997-2013)	2.25	3.5 (-66.7%)
Micronesia, Fed. States.	33	34 829	<1	-	-	ND
Moldova	16	568 000	5	-	-	ND
Monaco	10	38 695	<1	-	-	ND

Stellenbosch University https://scholar.sun.ac.za

Country	% Children 0-14 yrs per population (2017) [1,2]	Population <0-14 yrs[1,2]	Expected number of cases (10.5 per million children)	Reported number of cases	Cases per year	AIR/ASR/CIR (% incidence difference)
Mongolia	30	922 800	9	-	-	ND
Montenegro	18	112 044	1	-	-	ND
Morocco	27	9.64 million	90	146 (2005-2012)	20.8	9.1-9.6 (- 8.6% - -13.3%)
Mozambique	45	13.35 million	124	-	-	ND
Myanmar (Burma)	27	14.4 million	134	-	-	ND
Namibia	37	937 580	9	12 (2003-2011)	1.3	1.2 (-88.6%)
Nauru	31	13 649	<1	-	-	ND
Nepal	31	9.08 million	84	-	-	ND
Netherlands	16	2.73 million	26	435 (1993-2013)	20.7	8.1 (-22.9%)
New Caledonia	23	64 505	<1	16 (1990-2013)	0.6	12.9 (+22.9%)
New Zealand	20	958 800	9	178 (1993-2012)	9.3	11.3 (+7.6%)
Nicaragua	29	1.8 million	17	-	-	ND
Niger	50	10.74 million	100	1 (2001-2009)	0.125	0.3 (-97.1%)
Nigeria	44	83.99 million	785	9 (2003-2012)	1	1.9 (-81.9%)
North Macedonia	17	350 000	3	-	-	ND
Northern Mariana Islands	26	55 144	<1	-	-	ND
Norway	18	945 000	9	173 (1990-2013)	7.5	9.2 (-12.4%)
Oman	22	1.01 million	10	-	-	ND
Pakistan	35	68.95 million	644	38 (1995-2012)	2.2	1.7 (-83.8%)
Palau	19	21 729	<1	-	-	ND
Panama	27	1.106 million	10	-	-	ND
Papua New Guinea	36	2.97 million	28	-	-	ND
Paraguay	29	1.98 million	18	-	-	ND
Peru	27	8.69 million	81	-	-	ND
Philippines	32	33.57 million	313	205 (1993-2012)	10.7	2.8 (-73.3%)
Poland	15	5.76 million	53	111 (1999-2014)	7.4	13.9 (+32.4%)
Portugal	14	1.44 million	13	227 (1990-2010)	11.35	10.9-16.5 (+3.8% - 57.1%)
Puerto Rico	18	598 500	6	-	-	ND
Qatar	14	369 460	3	19 (2002-2014)	1.6	6.6 (-37.1%)
Reunion	26	227 906	2	16 (2002-2008, 2011)	2.28	11.1 (+5.7%)
Romania	15	2.95 million	27	25 (2008-2012)	6.25	9.0 (-14.3%)
Russian Federation	18	26.01 million	243	163 (1998-2015)	9.5	9.3-9.8 (-6.6 - 11.4%)
Rwanda	40	4.8 million	45	-	-	ND

Stellenbosch University https://scholar.sun.ac.za

Country	% Children 0-14 yrs per population (2017) [1,2]	Population <0-14 yrs[1,2]	Expected number of cases (10.5 per million children)	Reported number of cases	Cases per year	AIR/ASR/CIR (% incidence difference)
Samoa	37	72 682	<1	-	-	ND
San Marino	15	33 400	<1	-	-	ND
Sao Tome and Principe	43	87 860	<1	-	-	ND
Saudi Arabia	25	4.78 million	44	198 (1994-2012)	11	6.3 (-40%)
Senegal	43	6.81 million	63	-	5 - 20	ND
Serbia	16	1.12 million	10.5	-	-	ND
Seychelles	22	21 085	<1	-	-	ND
Sierra Leone	42	3.17 million	29	-	-	ND
Singapore	15	841 800	8	22 (2003-2007)	4.4	5.9 (-43.8%)
Slovak Republic	15	815 250	7	122 (2000-2012)	9.3	12.8 (+21.9%)
Slovenia	15	309 900	3	57 (1990-2013)	2.1	9.0 (-14.2%)
Solomon Islands	39	238 423	2	-	-	ND
Somalia	46	6.78 million	63	-	-	ND
South Africa	29	16.4 million	153	197 (2008-2012)	49.25	2.7 (-74.2%)
South Sudan	42	5.28 million	49	-	-	ND
Spain	15	6.98 million	65	1011 (1990-2013)	42.1	13.8-14.6 (+31.4%– 39%)
Sri Lanka	24	5.15 million	48	-	-	ND
St. Kitts and Nevis	20	55 345	<1	-	-	ND
St. Lucia	19	33 980	<1	-	-	ND
St. Martin	26	32 125	<1	-	-	ND
St. Vincent and the Grenadines	24	26 375	<1	-	-	ND
Sudan	41	16.6 million	155	-	-	ND
Suriname	26	146 484	1	2 (1980-2008)	0.1	0.2 (-98.1%)
Sweden	18	1.8 million	16	291 (1990-2011)	13.2	9.4 (-10.4%)
Switzerland	15	1.26 million	12	292 (1990-2013)	12.1	11.7 (+11.4%)
Syrian Arab Republic	37	6.76 million	63	-	-	ND
Taiwan	13	3.0 million	286	463 (1996-2010)	30.8	1.3 (-87.6%)
Tajikistan	35	3.12 million	29	-	-	ND
Tanzania	45	25.8 million	241	-	-	ND
Thailand	17	11.73 million	109	156 (1993-2013)	7.8	4.6 (-52.1%)
Timor-Leste	44	570 240	53	-	-	ND
Togo	42	3.27 million	31	-	<5	ND
Tonga	36	38 887	<1	-	-	ND

Stellenbosch University https://scholar.sun.ac.za

Country	% Children 0-14 yrs per population (2017) [1,2]	Population <0-14 yrs[1,2]	Expected number of cases (10.5 per million children)	Reported number of cases	Cases per year	AIR/ASR/CIR (% incidence difference)
Trinidad and Tobago	21	287 490	2	11 (2001-2006)	1.8	0.6 (-94.2%)
Tunisia	24	2.76 million	25	121 (1993-2007)	8.6	7.7 (-26.7%)
Turkey	25	19.9 million	186	134 (1993-2013)	6.7	10.6 (+0.01%)
Turkmenistan	31	1.78 million	17	-	-	ND
Turks and Caicos Islands	22	35 446	<1	-	-	ND
Tuvalu	29	11 192	<1	-	-	ND
Uganda	48	20.86 million	192	8 (2003-2012)	1	1.0 (-90.5%)
Ukraine	15	6.724 million	62	633 (2000-2012)	52.7	8.2 (-21.9%)
United Arab Emirates	14	1.316 million	12	-	-	ND
United Kingdom	18	11.89 million	111	1099 (2000-2011)	91.9	9.6 (-8.6%)
United States	19	61.88 million	578	9709 (1993-2012)	511	12.4 (+18.1%)
Uruguay	21	725 970	7	148 (1993-2012)	7.4	11.2 (+6.7%)
Uzbekistan	28	9.06 million	85	-	-	ND
Vanuatu	36	99 447	1	-	-	ND
Venezuela, RB	28	8.95 million	83	-	-	ND
Vietnam	23	21.97 million	205	170 (1995-2013)	9.4	7.7 (-26.7%)
Virgin Islands (U.S.)	20	21 453	<1	-	-	ND
West Bank and Gaza	40	1.86 million	17	-	-	ND
Yemen, Rep.	40	11.3 million	105	9 (1997-2006)	0.9	1.9 (-81.9%)
Zambia	45	7.69 million	71	-	-	ND
Zimbabwe	41	6.77 million	63	7 (2003-2012)	0.78	1.4 (-86.7%)

Abbreviations: AIR- age specific incidence rate; ASR - age standardized incidence rate; CIR - crude incidence rate; ND – no data

Table sources:

1. Based on the statistics reported by the World Bank for 2017. https://data.worldbank.org/indicator. Accessed April 2019.
2. The World Factbook, Central Intelligence Agency, United States of America. www.cia.gov/library/publications Accessed July 2019
3. Georgakis, MK; Dessypris, N; Baka, M; et al. Neuroblastoma among children in Southern and Eastern European cancer registries: Variations in incidence and temporal trends compared to US. Int. J. Cancer 2018, 142(10): 1977-1985.
4. Stefan, CS; Bray, F; Ferlay, J et al. Cancer of childhood in sub-Saharan Africa. *e*cancer 2017, 11:755 https://doi.org/10.3332/ecancer.2017.755
5. Ba-Saddik IA, Childhood cancer in Aden, Yemen. Cancer Epidemiology 2013, 37(6): 803-806.
6. Lui Y, Lo W, Chiang C, Yang Y, et al. Incidence of cancer in children aged 0–14 years in Taiwan, 1996–2010. Cancer Epidemiology 2015; 39(1): 21-28.

Stellenbosch University https://scholar.sun.ac.za

7. Steliarova-Foucher E, Stiller CA, Hesselinge P, Moreno F, Ries L, Shin H. International Incidence of Childhood Cancer Volume III Chapter IVa. Neuroblastoma and ganglioneuroblastoma. http://iicc.iarc.fr/includes/results/comparative/0401_Neuroblastoma.pdf Accessed April 2019.
8. Schroeder H, Wacher J ,Larsson H, Rosthoej S , et al. Unchanged incidence and increased survival in children with neuroblastoma in Denmark 1981–2000: a population-based study. British Journal of Cancer (2009) 100, 853–857.
9. Cancer in Finland, Finnish Cancer Registry https://cancerregistry.fi/statistics/cancer-statistics Accessed April 2019.
10. Mans D, Zijlmans W. Childhood Cancer in the Republic of Suriname (1980 Through 2008) The Open Epidemiology Journal, 2014, 7, 27-3.
11. D. Cristina Stefan, Patterns of Distribution of Childhood Cancer in Africa, *Journal of Tropical Pediatrics*, 2015; 61(3): 165–173.
12. Horton-Perinchief K. Bermuda National Tumour Registry. www.bermudahospitals.bm/cancer/tumour-registry.asp Accessed April 2019.
13. Azizah Ab M, Nor Saleha IT, Noor Hashimah A, Asmah ZA, Mastulu W. Malaysian National Cancer Registry Report 2007-2011. https://nci.moh.gov.my/index.php/ms/pengumuman/340-national-cancer-registry-report Accessed April 2019.
14. Moy TA, Ha C. Singapore Childhood Cancer Registry 2003-2007. https://www.nrdo.gov.sg/docs/librariesprovider3/Publications-Cancer Accessed April 2019.
15. Bodkyn C, Lalchandani S. Incidence of childhood cancer in Trinidad and Tobago. West Indian med. j. 2010; 59 (5): 465-468.
16. Traoré, F; Eshun, F ; Togo, B ; et al. Neuroblastoma in Africa: A Survey by the Franco-African Pediatric Oncology Group. Journal of Global Oncology 2016; 2(4): 169-173.

Stellenbosch University https://scholar.sun.ac.za

Table 2: South African Neuroblastoma crude Incidence rates for children under the age of 15 years

South African Neuroblastoma crude Incidence rates for children under the age of 15 years										
		Total U15			Male			Female		
Year	**Subject**	**SACTR**	**SA-NCR**	**Difference**	**SACTR**	**SA-NCR**	**Difference**	**SACTR**	**SA-NCR**	**Difference**
2000	NB	39	35		25	24		14	11	
	SA	15,084,120	15,084,120							
	Incidence	2.6	2.3	0.3						
2001	NB	25	26		16	13		9	13	
	SA	14,365,288	14,365,288		7,168,491	7,168,491		7,196,767	7,196,767	
	Incidence	1.74	2.5	0.76	2.23	1.8	0.43	1.25	1.8	0.55
2005	NB	36	23		8	11		28	12	
	SA	15,150,381	15,150,381		7,624,900	7,624,900		7,568,700	7,568,700	
	Incidence	2.4	1.5	0.9	1.0	1.4	0.4	3.69	1.6	2.09
2010	NB	30	44		20	27		10	17	
	SA	15,100,089	15,100,089		7,637,041	7,637,041		7,463,048	7,463,048	
	Incidence	1.98	2.9	0.92	2.61	3.5	0.89	1.4	2.2	0.8
2011	NB	34	44		16	21		18	23	
	SA	15,812,268	15,812,268							
	Incidence	2.2	2.8	0.6						
2013	NB	27	36		15	19		12	15	
	SA	15,454,742	15,454,742							
	Incidence	1.74	2.3	0.56						
2014	NB	39	33		22	15		17	18	
	SA	15,812,268	15,812,268		7,969,880	7,969,880		7,842,388	7,842,388	
	Incidence	2.4	2.0	0.4	2.76	1.9	0.86	2.16	2.3	0.14

SACTR – South African Children's tumour registry; SA-NCR – South African National Cancer Registry

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 4

Overall survival for neuroblastoma in South Africa between 2000 and 2014 (2019)

Ethics no: S18/07/138

Reference: Van Heerden J, Hendricks M, Geel J, et al. Overall survival for neuroblastoma in South Africa between 2000 and 2014. Pediatr Blood Cancer. 2019; 66: e27944
https://doi.org/10.1002/pbc.27944
(Impact factor 2.442)

At the onset of developing a national prospective protocol, the South African Children's Cancer Study Group (SACCSG) set out to conduct a retrospective study to source baseline data and establish management strategies used in the local setting as possible recommendations in the treatment of NB in South Africa. With 10 paediatric oncology units in the public setting with varying levels of services, a retrospective study provided the knowledge for the prospective protocol development that would align with the resources of South African Paediatric Oncology units.

Stellenbosch University https://scholar.sun.ac.za

Received: 17 April 2019 | Revised: 27 June 2019 | Accepted: 1 July 2019

DOI: 10.1002/pbc.27944

Pediatric Blood & Cancer | SIOP International Society of Paediatric Oncology | aspho The American Society of Pediatric Hematology/Oncology | WILEY

RESEARCH ARTICLE

Overall survival for neuroblastoma in South Africa between 2000 and 2014

Jaques Van Heerden[1] | Marc Hendricks[2] | Jennifer Geel[3] | Benn Sartorius[4,5] | G. P. Hadley[6] | Jan Du Plessis[7] | Ané Büchner[8] | Gita Naidu[9] | Barry Van Emmenes[10] | Anel Van Zyl[1] | Mariana Kruger[1]

[1]Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa

[2]Haematology Oncology Service, Red Cross War Memorial Children's Hospital, Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Cape Town, South Africa

[3]Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa

[4]Discipline of Public Health Medicine, School of Nursing and Public Health, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

[5]Department of Health and Medical Sciences, University of Washington, Washington, Seattle

[6]Department of Paediatric Surgery, Faculty of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa

[7]Department of Paediatrics, Faculty of Health Sciences, University of the Free State, Division of Paediatric Haematology and Oncology, Universitas Hospital, Bloemfontein, South Africa

[8]Paediatric Haematology and Oncology, Department of Paediatrics, University of Pretoria, Steve Biko Academic Hospital, Pretoria, South Africa

[9]Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa

[10]Division of Paediatric Haematology and Oncology, Department of Paediatrics, Frere Hospital, East London, South Africa

Correspondence
Jaques Van Heerden, Department of Paediatric Haematology and Oncology, University Hospital of Antwerp, Wilrijkstreet 10, Edegem 2650, Belgium.
Email: Jaques.vanheerden@uza.be

Abstract

Background: Outcome data for neuroblastoma in sub-Saharan Africa are minimal, whereas poor outcome is reported in low- and middle-income countries. A multi-institutional retrospective study across South Africa was undertaken to determine outcome.

Methods: Patients treated between January 2000 and December 2014 in nine South African pediatric oncology units were included. Kaplan–Meier curves and Cox regression models were employed to determine two-year survival rates and to identify prognostic factors.

Results: Data from 390 patients were analyzed. The median age was 39.9 months (range, 0–201 months). The majority presented with stage 4 disease (70%). The main chemotherapy regimens were OPEC/OJEC (44.8%), St Jude NB84 protocol (28.96%), and Rapid COJEC (22.17%). Only 44.4% had surgery across all risk groups, whereas only 16.5% of high-risk patients received radiotherapy. The two-year overall survival (OS) for the whole cohort was 37.6%: 94.1%, 81.6%, and 66.7%, respectively, for the very-low-risk, low-risk, and intermediate-risk groups and 27.6% for the high-risk group ($P < 0.001$, 95% CI). The median survival time for the whole group was

Abbreviations: CI, confidence interval; COG, Children's Oncology Group; CR, complete response; CT, computed tomography; FDG/PET, ^{129}Fluorodeoxyglucose positron emission tomography; FNA, fine-needle aspiration; HFA, height for age; HIC, high-income countries; HIV, human immunodeficiency virus; HR, high-risk; IDRF, image-defined risk factors; INPC, International Neuroblastoma Pathology Classification; INRC, International Neuroblastoma Response Criteria; INRG, International Neuroblastoma Risk Group; INSS, International Neuroblastoma Staging System; IR, intermediate risk; LDH, lactate hydrogenase; LR, low risk; MIC, middle-income country; MRI, magnetic resonance imaging; NB, neuroblastoma; OMAS, opsoclonus myoclonus ataxia syndrome; OS, overall survival; PD, progressive disease; POU, pediatric oncology units; SACCSG, South African Children's Cancer Study Group; SACTR, South African Children's Tumour Registry; TB, tuberculosis; US, ultrasonography; VIP, vasoactive intestinal peptide; VLR, very low risk; WFA, weight for age.

JVH is a PhD student at Stellenbosch University, South Africa, but a permanent staff member of the Antwerp University Hospital, Antwerp, Belgium.

© 2019 Wiley Periodicals, Inc.

Stellenbosch University https://scholar.sun.ac.za

13 months (mean, 41.9 months; range, 0.1–209 months). MYCN-nonamplified patients had a superior two-year OS of 51.3% in comparison with MYCN-amplified patients at 37.3% ($P = 0.002$, 95% CI).

Conclusions: Limited disease had an OS comparable with high-income countries, but advanced disease had a poor OS. South Africa should focus on early diagnosis and implementation of a national protocol with equitable access to treatment.

KEYWORDS
neuroblastoma, outcomes, overall survival, South Africa

1 | INTRODUCTION

Neuroblastoma (NB) is a malignancy of the sympathetic nervous system during early childhood with a worsening prognosis with increasing age.[1] NB presents various management challenges in high-income countries (HICs) and more so in resource-limited settings.[2] The varied pathophysiological, biological, and genetic characteristics contribute to differing treatment approaches for low-risk (LR) disease (local disease, favorable histology, no raised tumor markers, MYCN nonamplification, and no adverse genetics), with relatively little intervention required beyond surgery. Intermediate-risk (IR) disease has LR features combined with a single genetic aberration such as 11q and diploidy or undifferentiated pathology.[3] IR disease is treated with chemotherapy and surgery with radiotherapy indicated for limited indications.[3] Multimodal therapy (chemotherapy, surgery, radiotherapy, myeloablative stem cell transplantation, immunotherapy, and maturation therapy) is vital for improved outcomes in high-risk (HR) disease (metastatic disease, unfavorable histology, raised tumor markers, MYCN amplification, and adverse genetics).[3] Despite the use of high-intensity treatment for HR disease, the outcomes for LR and HR disease are vastly dissimilar.[3,4] Epigenetics is emerging as a factor influencing outcomes.[5]

South Africa is an upper-middle-income country with a heterogeneous healthcare system, consisting of public and private care facilities.[6,7] Most children with malignancies are treated in the public sector where the level of care varies according to provincial resources and institutional preference.[6] The treatment of NB in South Africa, like elsewhere in sub-Saharan Africa, is further complicated by several factors, including an HIV epidemic and sociocultural differences in disease interpretation, contributing to late presentation, advanced disease, and increased abandonment rates.[8,9] This retrospective study aimed to review NB management and outcomes in South Africa with the overarching aim to develop a standardized national NB treatment protocol.

2 | METHODS

2.1 | Patient population

There were 564 newly diagnosed registered cases of NB between January 2000 and December 2014 from nine dedicated pediatric oncology units (POUs) and the private health care sector in South Africa (see flow diagram) in the South African Children's Cancer Study Group (SACCSG) South African Children's Tumour Registry (SACTR).[10] Data from 174 (30.9%) patients' files were not available. Access to files from most private hospital facilities was not available, and incomplete or loss of files from archives had to be omitted from data analysis.

2.2 | Setting

The study was conducted in nine POUs, servicing both rural and urban populations from varying socioeconomic backgrounds.[11] Only four POUs had access to stem cell transplant services and seven had nuclear imaging services. There is still a disparity in resource allocation between different POUs in the nine provinces in South Africa.[11,12] This impacted on both the available human and physical resources availability. During the study period, autologous stem cell transplantation and cis-retinoic acid were largely limited to patients with private health care insurance. Immunotherapy with anti-GD2 was and is not available in South Africa.

2.3 | Data collection

Age was defined in months. Endpoint was defined as two-year overall survival (OS). Treatment failure was calculated from date of diagnosis until date of disease progression, or relapse or death due to NB. Refractory disease was defined as disease in which a complete response (CR) with initial induction was not obtained. Early and late relapse were respectively defined as relapse within or more than six months after completion of therapy. Treatment abandonment was defined as failure to initiate or complete treatment with a curative intent, except when the treating physician elected to opt for palliative management. If a patient did not attend follow-up appointments and if contact with family or caregivers was unsuccessful for a duration of two years, the patient was considered lost to follow-up.

2.4 | Staging and imaging

Investigations included ultrasonography (US), computed tomography (CT), or magnetic resonance imaging (MRI) of the neck, chest, and abdomen as well as bone marrow aspiration and trephine biopsy. Patients were clinically and radiologically staged according

Stellenbosch University https://scholar.sun.ac.za

to the International Neuroblastoma Staging System (INSS) or image-defined risk factors (IDRFs).[3] The INSS included unfavorable histology or favorable histology and MYCN amplification as part of the risk classification. Depending on institutional availability, staging and skeletal screening were done by ^{123}I-MIBG scan and/or bone scan. In ^{123}I-MIBG scan-negative patients, when available, staging with ^{129}fluorodeoxyglucose positron emission tomography/CT (FDG PET/CT) was performed. Primary tumor and metastatic response evaluations were performed according to institutional availability based on the International Neuroblastoma Response Criteria (INRC).[13–15] The treatment response evaluations were done according to the most recent version of the criteria pertaining to the specific investigations used to determine the response.

2.5 | Risk classification

Risk classification was based on the Children's Oncology Group (COG) classification system (Appendix A) and the International Neuroblastoma Risk Group (INRG) Consensus Pre-treatment Classification Scheme (Appendix B).[3] Lactate hydrogenase (LDH) and ferritin were used as nonspecific tumor markers as per the SIOP-PODC adapted risk stratification and treatment guidelines[2] that specify that a level of >750 UI/L for LDH and >120 ng/mL for ferritin is indicative of a poor prognosis. INRG was used for statistical purposes.

2.6 | Treatment

During the study period, different treatment protocols were used according to either institutional preference or disease severity. Regimens consisted of a surgical approach in very-low-risk (VLR), LR, IR and HR disease, and chemotherapy in IR and HR disease (employed as neoadjuvant or adjuvant chemotherapy) and with progression in LR disease. Conventional radiotherapy was included according to protocols in IR and HR disease. Surgical intervention was dictated by the resectability of the tumor, response to chemotherapy, and surgical expertise in the local institution.

2.6.1 | Chemotherapy regimens

Patients were, regardless of risk group, treated with a spectrum of regimens ranging from observation to platin- and etoposide-based regimens, which included OPEC/OJEC (carboplatin, cisplatin, etoposide, cyclophosphamide, and vincristine),[16] the St Jude NB84 protocol (cisplatin, etoposide, doxorubicin, and cyclophosphamide),[17] and Rapid COJEC (carboplatin, cisplatin, etoposide, cyclophosphamide, and vincristine).[16] CCG-321-P2 (cyclophosphamide, doxorubicin, cisplatin, and etoposide)[18] and VACEpi (vincristine, actinomycin, cyclophosphamide, and epirubicin) were used to a lesser degree. CADO (cyclophosphamide, adriamycin, and vincristine)[2,19] or single-agent oral cyclophosphamide was used as a palliative chemotherapy option in certain centers.

2.6.2 | Surgery

Surgery was performed for diagnostic reasons or as primary treatment in patients with LR and IR disease with curative intent with adequate neoadjuvant treatment. Other indications were palliative or emergency purposes. Emergencies included spinal cord compression or bowel, airway, and urinary tract obstructive symptoms. In patients with HR disease, surgery was performed based on upfront resectability of the primary tumor or after demonstrable cytoreduction with neoadjuvant chemotherapy.

2.6.3 | Radiotherapy

Radiotherapy was given according to the indications and doses of either of the following protocols: St Jude NB84,[17] CCG-321-P2[18], or International Society of Paediatric Oncology European Neuroblastoma Group (SIOPEN) OPEC/OJEC or Rapid COJEC.[2,16] Only six institutions offered maturation therapy of cis-retinoic acid after completion of primary treatment per the abovementioned protocols.[17]

2.6.4 | Autologous stem cell transplant

Autologous stem cell transplantation was available to four POUs, mainly for patients with private health care insurance.

2.7 | Statistical analysis

Data were analyzed using Stata 15.0. Differences in medians and means were assessed using the Mann–Whitney *U* test or Student *t* test. In cohorts of fewer than five observations, the Fisher exact test was applied. The Pearson chi-square (χ^2) test was used to assess the categorical association among covariates. OS as well as two-year survival, with associated 95% confidence intervals, was calculated and described using Kaplan–Meier curves. Differences between bivariate and multivariate survival curves by group were assessed using a log-rank test. Multiple Cox regression modeling to assess statistical significance of various prognostic factors was employed. The proportional hazards assumption was also confirmed for the final multivariable model. A *P* value of less than 0.05 was considered significant for all calculations.

3 | RESULTS

3.1 | Presentation and comorbid diseases

The final analysis included 390 patient records (see Supporting Information Figure S1), after 174 patients' files (30.9%) had been excluded as data were lacking or the diagnosis had not been confirmed. There was a male predominance with a male-to-female ratio of 1.08:1, and patients had been most frequently diagnosed in the 18- to 60-month category (50.7%). The median age was 39.9 months (range, 0–16.7 years; mean, 30.5 months). The diagnosis had been established by core biopsy in 208 patients (53.3%), fine-needle aspiration (FNA) in 62 patients (15.9%), and bone marrow biopsy in 120 patients (24.4%) with bone marrow involvement, with bone marrow infiltration by NB in association with increased urine catecholamine levels or high index of radiological suspicion. Chemotherapy data on 10 patients (2.7%) were insufficient for analysis, and these records were excluded. In 168

Stellenbosch University https://scholar.sun.ac.za

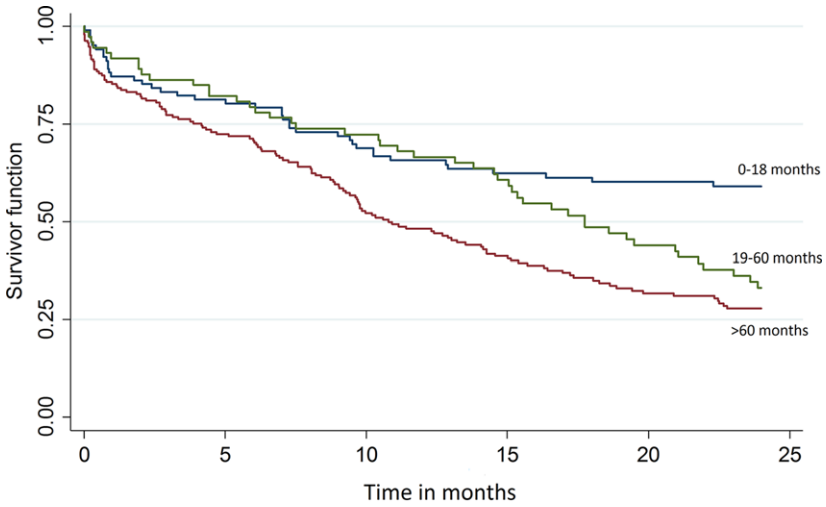


FIGURE 1 Kaplan–Meier curves of the two-year OS for age ($P = 0.049$)

patients (47.5%), chemotherapy had to be interrupted or adjusted due to comorbid disease, advanced disease, severe toxicity, or absconding.

The most common presentation was a clinically palpable mass (43.3%) followed by metastatic skull lesions (18.4%), bone pain (17.9%), and loss of weight or anorexia (13.3%). A unilateral adrenal mass (75.1%) was the most frequently detected primary mass, followed by a thoracic mass (11.2%). A primary tumor could not be identified in eight (2.1%) patients. Only 4.6% of the patients presented with spinal symptoms. Bone metastases were present in 47.4%, and the bone marrow was infiltrated in 44.6% of patients.

Comorbid disease was present in 21 patients with 11 (2.8%) being HIV positive: nine were on antiretroviral therapy at diagnosis. Ten patients had tuberculosis (TB) (2.5%), with five (1.25%) on TB treatment and five (1.25%) newly diagnosed. Opsoclonus myoclonus ataxia syndrome (OMAS) and vasoactive intestinal peptide (VIP) were present in 0.51% ($n = 2$) and 0.26% ($n = 1$) of the patients, respectively.

3.2 | Diagnostics

Diagnostic investigations included chest X-rays (163, 23.1%), plain-film skeletal surveys (45, 11.5%), and skull X-rays (41, 10.5%) (see Supporting Information Table S2). Abdominal ultrasounds were done in 205 children (52.6%). CT/MRI scans of chest and abdomen were done in 299 (76.7%) patients, and 136 (34.6%) of CT/MRI scans included screening for skeletal metastasis. Nuclear studies included 157 (40.3%) scintigraphy investigations and 143 (41.8%) ^{123}I-MIBG studies. Three hundred and twenty-one (82.3%) staging bone marrow aspirates were done, of which 204 (63.6%) identified infiltration by NB cells. Only 163 (41.8%) had complete staging, which included biopsy proven disease, a ^{123}I-MIBG scan, and bone marrow aspirate.

3.3 | Age, sex, stage, and risk classifications

Age at diagnosis was prognostic with a better two-year OS of 58.9% for age less than 18 months compared with older than 18 months (25.8%; $P < 0.049$, 95% CI) (see Figure 1; see Supporting Information Table S3). Sex ($P = 0.3$, 95% CI) was not significant in either univariate or multivariate analysis (see Tables 1 and 2).

According to the INSS, 273 (70%) patients had stage 4 disease and 14 (3.6%) had stage 4S disease. Only 17 (4.3%) patients were classified as VLR, 26 (6.6%) as LR, 30 (7.7%) as IR, and 275 (75.6%) as HR. The most common metastatic site was bone ($n = 185$, 47.4%) followed by bone marrow at 44.6% ($n = 174$). Lung metastases were present in 3.8% ($n = 15$). The two-year OS for the cohort was 37.6%, with stage being statistically significant for OS ($P < 0.01$, 95% CI). According to the INSS, patients with stage 1 and 2A disease had a two-year OS of 100%, those with stage 2B disease an OS of 74%, those with stage 3 disease an OS of 63.4%, those with stage 4 disease an OS of 23.8%, and those with stage 4S disease an OS of 64.4% ($P < 0.001$, 95% CI) (see Figure 2). According to risk classification, patients with VLR disease had a two-year OS of 94.1%, those with LR disease an OS of 81.6%, those with IR disease an OS of 66.7%, and those with HR disease an OS of 27.6% ($P < 0.001$, 95% CI) (see Figure 3).

3.4 | Histology and tumor markers

NB was confirmed by core biopsy in 270 patients (53.3%), with 128 (31.8%) classified as unfavorable histology and 80 (20.5%) as favorable histology (see Table 1 and Supporting Information Table S4). Sixty-two (15.9%) patients were diagnosed by FNA without sufficient information to distinguish between favorable and unfavorable histology. The diagnosis in the remaining 120 (30.8%) patients was made through bone marrow cytology and/or imaging with raised urine catecholamine levels. Patients with favorable histology, according to the International Neuroblastoma Pathology Classification (INPC), had a superior two-year OS of 66.6% while those with unfavorable histology fared worse at 32.8% ($P = 0.002$, 95% CI).

In patients with HR disease, the mean LDH level ($n = 251$, 78.7%) was 2364 UI/L (median 1197, 255–11 520), and for those with IR disease ($n = 27$, 8.4%) and LR disease ($n = 20$, 6.3%), it was 930 UI/L (median 595, 184–1787) and 498 UI/L (median 407, 241–806), respectively ($P < 0.01$, 95% CI). In patients with HR disease, the serum ferritin ($n = 189$, 80.4%) had a mean of 483 ng/dL (median 263, 18–1650), and in those with IR disease ($n = 18$, 7.7%) and LR disease ($n = 15$, 6.4%), it had mean values of 97 ng/dL (median 239, range, 37–342) and

Stellenbosch University https://scholar.sun.ac.za

TABLE 1 Prognostic factors and overall outcomes (univariate analysis)

Prognostic factor	*n*	Percentage	2-year OS	*P* value CI = 95%
Age at diagnosis (months)				
Range (months)	0–201			
Average (months)	30.5			
Median (months)	39.9			
0–18	123	31.5%	58.9%	< 0.049
18–60	191	49%	25.8%	
>60	76	19.5%	33%	
LDH				
<750 U/L	137	42.9%	54.8%	< 0.0001
>750 U/L	182	57.1%	25.2%	
Ferritin				
<120 ng/dL	75	31.9%	66.3%	0.0004
>120 ng/dL	159	69.1%	25.6%	
MYCN				
Total	145			
Not amplified	64	44%	51.3%	0.002
Amplified	78	54%	37.3%	
Extra copies	3	2%	0%	
U-catecholamine levels				
Total	271			
Normal	50	18.4%	63.4%	< 0.049
Raised	221	81.6%	34.9%	
Pathology				
Total	390			
Favorable histology	80	20.5%	66.6%	0.002
Unfavorable histology	128	32.8%	32.8%	
FNA	62	15.9%		
Bone marrow cytology	120	30.8%	24.4%	
INSS				
1	16	4%	100%	< 0.0001
2A	8	2.1%	100%	
2B	10	2.6%	74%	
3	68	17.4%	63.8%	
4	273	70%	23.8%	
4S	14	3.6%	64.6%	
Risk classification				
VLR	17	4.3%	94.1%	< 0.0001
LR	26	6.6%	81.6%	
IR	30	7.7%	66.7%	
HR	295	75.6%	27.6%	

Abbreviations: FNA, fine-needle aspiration; HR, high risk; INSS, International Neuroblastoma Staging System; IR, intermediate risk; LDH, lactate hydrogenase; LR, low risk; VLR, very low risk.

64 ng/dL (median 244, range, 15–440), respectively ($P < 0.01$, 95% CI). Patients with an LDH value of <750 U/L at diagnosis had a more favorable two-year OS of 54.8% versus 25.2% for an LDH value of >750 U/L ($P < 0.0001$, 95% CI), and those with ferritin levels <120 ng/dL at diagnosis had a more favorable two-year OS of 66.3% compared with levels >120 ng/dL (25.6%) ($P = 0.0004$, 95% CI).

MYCN was amplified in 54% ($n = 78$) of patients. Patients with MYCN-nonamplified tumors ($n = 64$, 44%) had a two-year OS of 51.3% compared with 37.3% for patients with MYCN-amplified tumors, whereas patients with a copy-number gain (1–9 copies, $n = 3$, 2%) of MYCN had the best two-year OS of 66.6% ($P = 0.002$, 95% CI). Raised urine catecholamine levels were detected in 81.6% ($n = 221$) of patients. Patients with raised catecholamine levels at diagnosis had a poorer two-year OS of 34.9% in comparison with 63.4% in patients with normal urine values ($P = 0.05$, 95% CI). Patients with raised urine catecholamine levels at diagnosis had a poorer two-year OS of 34.9% as opposed to 63.4% in patients with normal levels ($P < 0.049$).

3.5 | Nutritional status at diagnosis and outcome

Nutritional status was significant as patients with a weight for age (WFA) above the −1 SD z-score ($n = 162$, 60%) had a better two-year OS of 86.6% than wasted patients (WFA < −2 SD z-score, $n = 55$, 20.4%) at 79.3% ($P = 0.04$) (see Supporting Information Table S5). Patients with a height for age (HFA) above the −1 SD z-score ($n = 129$, 49.8%) had a better two-year OS of 88.1% than stunted patients (HFA < −2 SD z-score, $n = 83$, 32%) at 84.1% ($P = 0.05$).

3.6 | Multivariate analysis

On multivariate analysis, age >18 months ($P = 0.03$, HR 0.96; 95% CI, 1.01–1.3), LDH > 750 U/L ($P < 0.0001$, HR 0.96; 95% CI, 1.3–1.9), ferritin > 120 ng/dL ($P = 0.02$, HR 0.96; 95% CI, 1.01–1.5), and MYCN gene amplification ($P = 0.01$, HR 0.96; 95% CI, 1.03–1.3) were significant covariates (see Table 2). Stage ($P < 0.0001$, HR 0.89; 95% CI, 1.6–3.5) and risk classification ($P < 0.0001$, HR 0.96; 95% CI, 1.4–2.1) were also significant. Pathology according to the INPC was not a significant covariate ($P = 0.37$, HR 0.96, 95% CI, 0.8–1.3).

3.7 | Management protocols

Two patients died before treatment was started (see flow diagram) (Table 3). Only 253 (64.9%) patients were treated with curative intent, although 354 (90.8%) patients received chemotherapy. Surgery as only treatment was received by 24 patients (6.2%) for LR disease. Complete excisions or debulking procedures were performed only in 174 (44.4%) patients (including 23 LR patients). In the IR, LR, and VLR groups, 11 (36.6%), 4 (16.6%), and 2 (11.8%) patients, respectively, were not operated on. VLR patients had a two-year OS of 100% independent of surgical status. Tumors completely resected for IR and LR patients, respectively, had an improved two-year OS of 83.3% and 84.4% versus a two-year OS of 42.8% and 42.8% ($P < 0.01$, 95% CI) for nonoperated tumors. In the HR group, 185 (62.8%) patients were assessed as

Stellenbosch University https://scholar.sun.ac.za

TABLE 2 Multivariate analysis

Covariate	Coefficient	Standard error	Wald	*P* value	Exp	95% CI	
						Min	Max
Age	0.1490	0.07038	4.4817	0.0343	1.1607	1.0111	1.3323
Race	0.09036	0.1066	0.7183	0.3967	1.0946	0.8882	1.3489
Sex	−0.1366	0.1361	1.0084	0.3153	0.8723	0.6681	1.1389
MYCN	0.1622	0.06318	6.5943	0.0102	1.1761	1.0392	1.3312
LDH	0.4862	0.09801	24.6057	< 0.0001	1.6261	1.3419	1.9704
Ferritin	0.2230	0.09526	5.4786	0.0193	1.2498	1.0369	1.5063
INPC	0.09132	0.1025	0.7941	0.3729	1.0956	0.8962	1.3393
INSS	0.8876	0.1959	20.5212	< 0.0001	2.4293	1.6546	3.5667
Risk classification	0.5555	0.1106	25.2176	< 0.0001	1.7428	1.4031	2.1648

Abbreviations: INPC, International Neuroblastoma Pathology Classification; INSS, International Neuroblastoma Staging System; LDH, lactate hydrogenase.

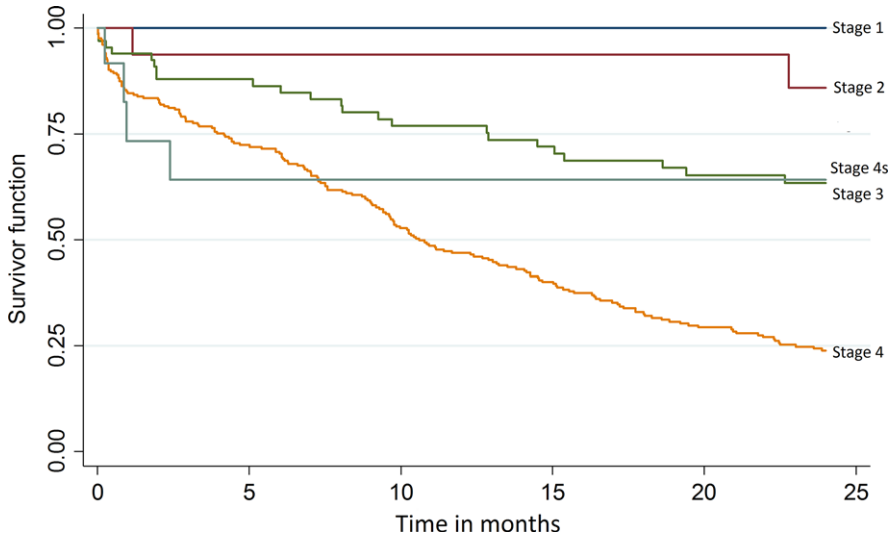


FIGURE 2 Kaplan–Meier curves of the two-year OS for INSS ($P < 0.001$)

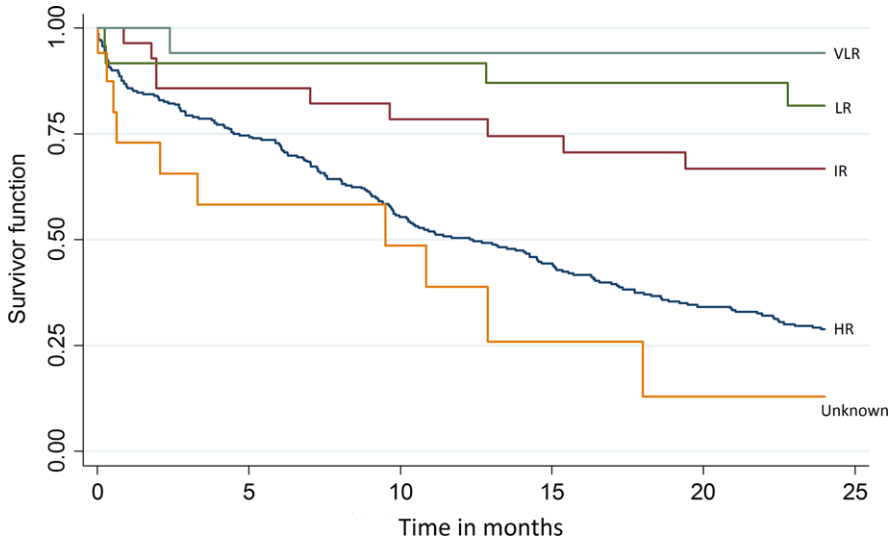


FIGURE 3 Kaplan–Meier curves of the two-year OS for risk classification ($P < 0.001$)
VLR, very low risk; LR, low risk; IR, intermediate risk; HR, high risk

Stellenbosch University https://scholar.sun.ac.za

TABLE 3 Treatment (univariate analysis)

Chemotherapy				
Chemotherapy interruptions	**Number of patients**	**Percentage**	**Two-year OS**	***P* value CI = 95%**
Never started	2	< 1%		
Surgery only	24	6.2%		
Insufficient data	10	2.7%		
Chemotherapy received	354	90.8%		
No interruptions	186	52.4%		
Interrupted	168	47.5%		
High-risk protocols				
Rapid COJEC	49	22.17%	24.8%	*P* = 0.01
OPEC/OJEC	99	44.8%	32.5%	
St Jude NB84	64	28.96%	41%	
Remission rate post induction				
Total evaluated	221			
Remission	68	30.8%		
Not in remission	153	69.2%		
Surgery				
Total	390			
No	216	55.6%		
Yes	174	44.4%		
Complete	98	56.3%	60.4%	< 0.01
Incomplete	76	43.6%	57.3%	
HR				
Total	294			
No surgery	185	62.8%	16.23%	< 0.01
Surgery	109	37.7%		
Incomplete	49	60%	51.4%	
Complete	60	40%%	46.4%	
IR				
Total	30			
No surgery	9	30%	42.8%	< 0.01
Surgery	21	70%		
Incomplete	14	66.6%	70.1%	
Complete	7	33.3%	83.3%	
LR				
Total	26			
No surgery	4	16.6%	42.8%	< 0.01
Surgery	22	83.4%		
VLR				
Total	17			
No surgery	2	11.8%	100%	< 0.01
Surgery	15	88.2%		
Radiotherapy				
Total	390			
Yes	61	15.6%	44.8%	< 0.01
No	329	84.4%	36.2%	
HR				
Total	224			
Radiotherapy	37	16.5%	42.4%%	< 0.01
No radiotherapy	187	83.5%	26.1%	
IR				
Total	11			
Radiotherapy	1	9%	75%	< 0.01
No radiotherapy	10	91%	63.3%	
Surgery and radiotherapy				
Total HR	167			
No surgery without radiotherapy	153	91.6%	17.3%	< 0.01
No surgery with radiotherapy	14	8.4%	25.8%	< 0.01

Abbreviations: HR, high risk; IR, intermediate risk; LR, low risk; OS, overall survival; VLR, very low risk

inoperable. Not all HR patients were operated on, and complete resection in HR patients ($n = 60$, 40%) had a two-year OS of 46.4% versus nonoperated tumors ($n = 185$, 62.8%) at 16.2% ($P < 0.01$, 95% CI).

Different institutions used different chemotherapy protocols in HR disease. OPEC/OJEC ($n = 99$, 44.8%), St Jude NB84 ($n = 64$, 28.9%), and Rapid COJEC ($n = 49$, 22.2%) were the three main protocols, and 28.1% of the patients were treated with several other protocols. The post-induction remission rate for HR patients was 30.8%. The two-year OS for OPEC/OJEC was 32.5% compared with 41% for the St Jude protocol and 24.8% for Rapid COJEC ($P = 0.05$, 95% CI).

Patients who received radiotherapy to the primary tumor site had an improved outcome with a two-year OS of 44.8% compared with those without radiotherapy treatment with an OS of 36.2% ($P < 0.01$, 95% CI). HR patients who received radiotherapy treatment had a two-year OS of 42.4% compared with those without radiotherapy treatment with an OS of 26.1% ($P < 0.01$, 95% CI). Patients with HR disease who never came for surgery but who did receive radiotherapy had a superior two-year OS of 25.8% as opposed to 17.3% for those who did not receive either surgery or radiotherapy ($P < 0.01$, 95% CI).

Of the 295 HR patients, only 11 (3.7%) (see Supporting Information Table S6) received a single autologous stem cell transplant with a significant two-year OS of 72.7% ($P = 0.029$, 95% CI). Only 13 (33.3%) patients had therapeutic MIBG treatment for refractory disease ($n = 12$) or relapse ($n = 1$) with a nonsignificant two-year OS of

Stellenbosch University https://scholar.sun.ac.za

38.4% ($P = 0.4917$, 95% CI). Cessation of all treatment occurred in 168 (47.5%) patients due to either disease progression or refractory disease.

3.8 | Follow-up

The median survival time of all patients was 13 months (0.1–209 months): for HR patients it was 7.5 months (0.1–87 months), for IR patients it was 116 months (36–193 months), for LR patients it was 140 months (76–199 months), and for VLR patients it was 123 months (36–209 months) (see Supporting Information Tables S7 and S8).

The reason for death was documented in 260 patients, of whom 246 (94.6%) were disease related. The majority of HR patients ($n = 153$; 58.9%) had progressive disease (PD) while nine (3.1%) relapsed. For those with IR disease, 11 (36.7%) had PD and three (10%) relapsed, whereas for those with LR disease, three (11.5%) had PD and three (11.5%) relapsed. Only one patient with VLR disease had PD.

Treatment toxicity contributed to 3.2% ($n = 10$) of the deaths, whereas surgical complications contributed to 0.6% ($n = 2$) of the deaths. Ten patients (2.6%) died secondary to neutropenic sepsis and two (0.6%) to cardiotoxicity.

The time of death was documented in 56.7% of patients, with 95 deaths (24.4%) occurring within six months after diagnosis. A total of 88.7% of deaths occurred within two years from diagnosis.

Of the total cohort, 50 (12.8%) patients were lost to follow-up, constituting 40% of surviving patients ($P < 0.001$, 95% CI), with 54% being HR, 10% being IR, 10% being LR, and 6% being VLR patients.

4 | DISCUSSION

This study represented the first documented multicenter retrospective cohort study of NB in sub-Saharan Africa. Although the majority of cases were diagnosed in the 18- to 60-month age group, similar to international trends, the median age was higher at 39.9 months compared with 19 months in the United States (HIC) or 33.2 months in Egypt (MIC).[20]

South Africa's healthcare system suffers from various resource challenges in the management of NB. Diagnostic imaging and staging were done in the patients under study, ranging from less sensitive to specific imaging. Plain X-rays are nonspecific with limited diagnostic value beyond the silhouette of primary tumours[21] or mixed sclerotic and lytic lesions of metastasis.[22] Skeletal screening was performed by means of various imaging techniques, but only 41.8% of the study group were surveyed by MIBG scan. Nonnuclear techniques such as plain-film X-rays can be utilized at diagnosis but are not sufficiently sensitive to evaluate skeletal treatment response as healing lesions can present as sclerotic lesions, similar to primary lesions.[22] The metabolic response to therapy, demonstrated with MIBG scans, has a higher sensitivity and proven prognostic value.[23]

In our study, stage 4 patients constituted 70% of the total cohort, which is higher than the 40% reported by the INRG[24] or the 41.9% in European studies.[25] Yet, the incidence of stage 4 disease was comparable with studies in Indian (71.4%)[26] and Kenyan (92.8%)[27] hospitals. Although lung metastases were present in 3.8% of patients, which is marginally higher than those reported in North America,[28] only 76.7% of patients had CT or MR imaging and 41.8% a ^{123}I-MIBG scan to confirm metastasis. The percentage of lung metastasis could be underestimated. Unknown origin of the primary tumor occurred only in 2.1% of patients, as opposed to the 10% reported internationally.[23]

Only 53.3% of the diagnoses in this cohort were confirmed by biopsy, similar to MICs such as Egypt with 40% to 60%[20] but less than Argentina with up to 96%.[29] Diagnoses were made through FNA in 15.9% of patients due to institutional expertise and its being a less invasive technique in emaciated patients with advanced disease. Quite a number of patients were diagnosed through disease-associated imaging, bone marrow aspirates, and raised catecholamine levels, which limited the necessary testing for MYCN in 37.1% ($n = 245$) patients.

The SIOP-PODC guidelines for LDH and ferritin in NB advise 750 UI/L and 120 ng/dL, respectively, as the threshold values for poor and good prognosis,[2] which was confirmed by our study. Our study indicated that 54% of patients were MYCN amplified, which is higher than the 20% reported internationally.[30] MYCN copy-number gains produced conflicting reports with regard to prognosis,[31,32] but in this study these were associated with a good prognosis. Even with a limited number of tumors' MYCN status known ($n = 145$, 37%), a two-year OS advantage was demonstrated between MYCN-amplified and MYCN-nonamplified patients ($P = 0.002$). This compares with international trends.[2]

All major historical HR induction chemotherapy regimens were used in South Africa, according to institutional preference. The South African doxorubicin-containing post-induction CR rate of 38.5% in HR patients was less than that in the doxorubicin-containing COG A3973 studies with up to a 66.1% CR rate.[33] The South African OPEC/OJEC post-induction CR rate of 29.7% was less than that in European studies with a 38% CR rate.[16] The South African Rapid COJEC post-induction CR rate of 16.3% was considerably less than the 44% CR rate for Rapid COJEC in Europe.[10]

Optimizing local control is important for improved survival and cure.[34] International definitions of complete resection were based on more than 95% or more than 90%.[34,35] In the COG A3973 studies, resection of >90% had a better five-year OS of 57.3% compared with 49.4% for resection of <90% ($P = 0.3$), and in the HR-NBL1/SIOPEN trial, complete resection had a better five-year OS compared with incomplete resection (39% vs 30%).[36] In our cohort, 62.8% of the HR group had no surgery. Outcomes in resected patients in all risk groups, regardless of the volume of tumor resection, had a better two-year OS (46.4%) compared with those who had no resection at all (two-year OS 16.23%). Various factors contribute to resectability of tumors, but in this multicenter study where surgical skills and experience among surgeons differed, it has been shown that, as in international studies, surgery improved survival in patients with undetectable metastatic disease confirmed on bone marrow aspirate and trephine as well as ^{123}MIBG scan.[34]

Stellenbosch University https://scholar.sun.ac.za

In keeping with expert opinion in the SIOP-PODC guidelines,[2] in the absence of a surgical resection, radiotherapy to the primary tumor bed, with or without metastatic sites, had a short-term benefit in patients with a superior two-year OS in unresected patients receiving radiotherapy versus patients not receiving radiotherapy.

European studies reported chemotherapy toxicity-related mortality of 3% for OPEC/OJEC and 4% for Rapid COJEC,[37] which is comparable to the 3.2% toxicity-related mortality in this cohort, keeping in mind that a less toxic OPEC/OJEC regimen was used locally. Yet, it was higher than the 2% to 2.5% toxicity reported in St Jude studies.[38]

The risk-based two-year OS of the South African cohort was lower when compared with the outcomes reported in HICs (95% for LR disease, 90%–95% for IR disease and 50%–60% for HR disease)[3] and an MIC such as Argentina.[29] The median survival of 12.2 months was lower than reported in other MICs, such as India, China, and Brazil.[26,39] Patients with HR disease dominated in this cohort at 75.6%, which was higher in comparison with INRG Task Force studies at 40%.[24] A North American study concluded that biology was related to race with HR disease more prevalent in black patients, possibly explaining the finding.[40] A combination of late presentation and poor disease biology possibly explains the median time to death of six months from diagnosis for 42% of patients. Malnutrition was a significant risk factor in our cohort, as reported in previous international studies.[41]

Limitations included the retrospective data analysis of prospectively collected data, where collection might not have been uniform. There might also be a lack of documentation of crucial findings. The limited access to genetic assessments such as DNA ploidy, MYCN gene amplification, and 1p36 deletion status might have impacted negatively on risk stratification determination. Nonstandard documentation of management information, investigation reporting definitions, surgical classifications, and treatment outcomes limits comparative studies due to the heterogeneous nature of the data. During the study period, the availability of investigations, such as isotopes for nuclear imaging for staging and risk classification, was not always guaranteed. This could have contributed to staging and risk classification being inaccurate, resulting in suboptimum treatment and poorer outcomes. The treatment protocols were not standardized nationally. Treatment was adapted with limitations of accurate risk classification according to the clinical presentation and with palliative or curative intent. No accurate longitudinal comparisons could therefore be made.

In resource-constrained settings, cost-effective management options are of great importance.[42] Even with limitations in diagnostic procedures, referral and treatment protocols, and the variable availability of expertise in all fields, management should be optimized to obtain the best possible outcomes. Our study indicated that non-doxorubicin–containing and doxorubicin-containing chemotherapy produced comparable outcomes. OPEC/OJEC is an acceptable primary protocol, especially considering the favorable toxicity profiles, while attempting surgery. Even incomplete surgical resections have a survival advantage versus no surgical intervention.

5 | CONCLUSIONS

The outcomes for NB patients varied according to different treatment protocols used in South Africa. A uniform, nationally standardized treatment strategy might ensure better risk classification, ensure the appropriate intensity of chemotherapy, especially in LR and IR disease. Harmonizing treatment might improve OS by creating equitable access to all treatment modalities, including surgical expertise, autologous stem cell transplant, and maturation therapy. To this end, a standardized protocol, incorporating risk-based therapy, should be implemented.

ACKNOWLEDGMENTS

Dr Van Heerden acknowledges the Department of Paediatric Haematology and Oncology, Antwerp University Hospital, University of Antwerp, for research support with financial contributions from the VZW Kinderkankerfonds, Belgium. The authors acknowledge the SACCSG for supporting the study and the SACTR for providing statistical data.

AUTHOR CONTRIBUTIONS

JVH conceptualized and designed the study, collected the data, and performed the data analysis. MK assisted with concept development and design of the study, supervised the data analysis, and critically reviewed and revised the manuscript. BS performed the statistical analysis and critically reviewed the manuscript. All the other authors collected data in their respective pediatric oncology units and critically reviewed and contributed toward revision of the manuscript.

CONFLICTS OF INTEREST

The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Jaques Van Heerden https://orcid.org/0000-0002-4502-1169
Jennifer Geel https://orcid.org/0000-0001-8792-3251
G. P. Hadley https://orcid.org/0000-0002-2459-7118
Mariana Kruger https://orcid.org/0000-0002-6838-0180

REFERENCES

1. Matthay K, Maris J, Schleiermacher G, et al. Neuroblastoma. *Nat Rev Dis Primers*. 2016;2:16078.
2. Parikh N, Howard S, Chantada G, et al. SIOP-PODC adapted risk stratification and treatment guidelines: recommendations for neuroblastoma in low- and middle-income settings. *Pediatr Blood Cancer*. 2015;62(8):1305-1316.

Stellenbosch University https://scholar.sun.ac.za

3. Pinto N, Applebaum M, Volchenboum S, et al. Advances in risk classification and treatment strategies for neuroblastoma. *J Clin Oncol*. 2015;33(27):3008-3017.
4. Whittle S, Smith V, Doherty E, et al. Overview and recent advances in the treatment of neuroblastoma. *Expert Rev Anticancer Ther*. 2017;17(4):369-386.
5. Yáñez Y, Grau E, Rodríguez-Cortez VC, et al. Two independent epigenetic biomarkers predict survival in neuroblastoma. *Clin Epigenetics*. 2015;7(1):16.
6. Stuckler D, Basu S, McKee M. Health care capacity and allocations among South Africa's provinces: infrastructure-inequality traps after the end of apartheid. *Am J Public Health*. 2011;101(1):165-172.
7. [World Bank Group Web site] 2018. https://data.worldbank.org/income-level/low-and-middle-income. Accessed Nov 12, 2018.
8. Israels T, Ribeiro RC, Molyneux EM. Strategies to improve care for children with cancer in sub-Saharan Africa. *Eur J Cancer*. 2010;46(11):1960-1966.
9. Mayosi B, Lawn J, Van Niekerk A, et al. Health in South Africa: changes and challenges since 2009. *Lancet*. 2012;380(9858):2029-2043.
10. Stefan D, Stones D, Wainright R, et al. Childhood cancer incidence in South Africa, 1987–2007. *S Afr Med J*. 2015;105(11):939-947.
11. Mayosi B, Benatar S. Health and health care in South Africa — 20 years after Mandela. *New Engl J Med*. 2014;371(14):1344-1353.
12. Benatar S. The challenges of health disparities in South Africa. *S Afr Med J*. 2013;103(3):154-155.
13. Brodeur G, Pritchard J, Berthold, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *J Clin Oncol*. 1993;11(8):1466-1477.
14. Park J, Bagatell R, Cohn S, et al. Revisions to the International Neuroblastoma Response Criteria: a consensus statement from the National Cancer Institute Clinical Trials Planning Meeting. *J Clin Oncol*. 2017;35(22):2580-2587.
15. Eisenhauer E, Therasse P, Bogaert J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247.
16. Peinemann F, Tushabe D, Berthold F. Rapid COJEC versus standard induction therapies for high-risk neuroblastoma. *Cochrane Database Syst Rev*;201519(5):CD010774.
17. Bowman L, Hancock M, Santana V, et al. Impact of intensified therapy on clinical outcome in infants and children with neuroblastoma: the St Jude Children's Research Hospital Experience, 1962 to 1988. *J Clin Onco*. 1991;9(9):1599-1608.
18. Stram D, Matthay K, O'Leary M, et al. Consolidation chemoradiotherapy and autologous bone marrow transplantation versus continued chemotherapy for metastatic neuroblastoma: a report of two concurrent Children's Cancer Group studies. *J Clin Oncol*. 1996;14(9):2417-2426.
19. Coze C, Hartmann O, Michon J, et al. NB87 induction protocol for stage 4 neuroblastoma in children over 1 year of age: a report from the French Society of Pediatric Oncology. *J Clin Oncol*. 1997;15(12):3433-3440.
20. Al-Tonbary Y, Badr M, Mansour A, et al. Clinico-epidemiology of neuroblastoma in north east Egypt: a 5-year multicenter study. *Oncol Lett*. 2015;10(2):1054-1062.
21. Duarte M, Gelmini A, Marques de Souza G. Neuroblastoma: a correlation between radiologic methods. *Urol Nephrol Open Access J*. 2018;6(2):65-67.
22. David R, Lamki N, Fan S, et al. The many faces of neuroblastoma. *RadioGraphics*. 1989;9(5):859-882.
23. Decarolis B, Hero B, Simon T, et al. Iodine-^{123}meta-iodobenzylguanidine scintigraphy scoring allows prediction of outcome in patients with stage 4 neuroblastoma: results of the Cologne Interscore Comparison Study. *J Clin Oncol*;31(7):944-951.
24. Cohn L, Pearson A, London W, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task force report. *J Clin Oncol*. 2009;27(2):289-297.
25. Haupt R, Garaventa A, Gambini C, et al. Improved survival of children with neuroblastoma between 1979 and 2005: a report of the Italian Neuroblastoma Registry. *J Clin Oncol*. 2010;28(14):2331-2338.
26. Bansal D, Marwaha R, Trehan A, et al. Profile and outcome of neuroblastoma with conventional chemotherapy in children older than one year: a 15-years' experience. *Indian Pediatrics*. 2008;45:135-139.
27. Kitonyi G, Macharia W, Mwanda O, Pamnani R. Clinico-pathologic characteristics and treatment outcomes in children with neuroblastoma at the Kenyatta National Hospital, Nairobi. *East Afr Med J*. 2009;86(12 suppl):S39-S46.
28. DuBois S, London W, Zhang Y, et al. Lung metastases in neuroblastoma at initial diagnosis: a report from the International Neuroblastoma Risk Group (INRG) Project. *Pediatr Blood Cancer*. 2008;51(5):589-592.
29. Moreno F, Lopez Marti J, Palladino M, et al. Childhood neuroblastoma: incidence and survival in Argentina. Report from the National Pediatric Cancer Registry, ROHA Network 2000–2012. *Pediatr Blood Cancer*. 2016;63(8):1362-1367.
30. Smith V, Foster J. High-risk neuroblastoma treatment review. *Children*. 2018;5(9):114.
31. Wang M, Zhou C, Cai R, et al. Copy number gain of MYCN gene is a recurrent genetic aberration and favorable prognostic factor in Chinese pediatric neuroblastoma patients. *Diagnostic Pathology*. 2013;8(5):1-6.
32. Seeger R, Brodeur G, Sather H, et al. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N Engl J Med*. 1985;313(18):1111-1116.
33. Yanik G, Parisi M, Shulkin B, et al. Semiquantitative mIBG scoring as a prognostic indicator in patients with stage 4 neuroblastoma: a report from the Children's Oncology Group. *J Nucl Med*. 2013;54(4):541-548.
34. Von Allmen D, Davidoff A, London W, et al. Impact of extent of resection on local control and survival in patients from the COG A3973 study with high-risk neuroblastoma. *J Clin Oncol*. 2017;35(2):208-216.
35. Kiely E. A technique for excision of abdominal and pelvic neuroblastoma. *Ann R Coll Surg Engl*. 2007;98(4):342-348.
36. Holmes K, Poetschger U, Sarnacki S, et al. The influence of surgical excision on survival in high-risk neuroblastoma revisited after introduction of ch14.18/CHO immunotherapy in the HR-NBL1/SIOPEN trial. *J Clin Oncol*. 2018;36(15 suppl):10521-10521.
37. Pearson A, Pinkerton C, Lewis I, et al. High-dose rapid and standard induction chemotherapy for patients aged over 1 year with stage 4 neuroblastoma: a randomised trial. *Lancet Oncology*. 2008;9(3):247-256.
38. Bowmen L, Hancock M, Santana V, et al. Impact of intensified therapy on clinical outcome in infants and children with neuroblastoma: the St Jude Children's Research Hospital Experience, 1962 to 1988. *J Clin Oncol*. 1999;9:1599-1608.
39. Parize I, Haddad B, Cavalli L, et al. Neuroblastoma in southern Brazil: an 11-year study. *J Pediatr Hematol Onco*. 2006;28(2):82-87.
40. Henderson TO, Bhatia S, Pinto N, et al. Racial and ethnic disparities in risk and survival in children with neuroblastoma: a Children's Oncology Group study. *J Clin Oncol*. 2011;29(1):76-82.
41. Bauer J, Jürgens H, Frühwald M. Important aspects of nutrition in children with cancer. *Adv Nutr*. 2011;2(2):67-77.
42. Bhakta N, Martiniuk A, Gupta S, et al. The cost effectiveness of treating paediatric cancer in low-income and middle-income countries: a case-study approach using acute lymphocytic leukaemia in Brazil and Burkitt lymphoma in Malawi. *Arch Dis Child*. 2012;98(2):155-160.

Stellenbosch University https://scholar.sun.ac.za

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Van Heerden J, Hendricks M, Geel J, et al. Overall survival for neuroblastoma in South Africa between 2000 and 2014. *Pediatr Blood Cancer*. 2019;e27944. https://doi.org/10.1002/pbc.27944

Stellenbosch University https://scholar.sun.ac.za

APPENDIX A: COG CLASSIFICATION

INSS	Age	MYCN	Shimada	DNA ploidy	Risk group
1	Any	Any	Any	Any	Low
2A/2B	< 365 d	Any	Any	Any	Low
	> 365 d	Nonamplified	Any	—	Low
	> 365 d	Amplified	Favorable histology	—	Low
	> 365 d	Amplified	Unfavorable histology	—	High
3	< 365 d	Nonamplified	Any	Any	Intermediate
	< 365 d	Amplified	Any	Any	High
	> 365 d	Nonamplified	Favorable histology	—	Intermediate
	> 365 d	Nonamplified	Unfavorable histology	—	High
	> 365 d	Amplified	Any	—	High
4	< 548 d	Nonamplified	Any	Any	Intermediate
	< 548 d	Amplified	Any	Any	High
	> 548 d	Any	Any	—	High
4S	< 365 d	Nonamplified	Favorable histology	> 1	Low
	< 365 d	Nonamplified	Any	1	Intermediate
	< 365 d	Nonamplified	Unfavorable histology	Any	Intermediate
	< 365 d	Amplified	Any	Any	High

APPENDIX B: INRG CLASSIFICATION

INRG stage	Age (months)	Histologic category	Grade differentiation	MYCN	11 q aberration	Ploidy	Pretreatment risk group
L1/L2	Any	GN maturing GNB intermittent	Any	Any	Any	Any	A: Very low risk
L1	Any	Any, except GN maturing	Any	nAmp	Any	Any	B: Very low risk
		GNB intermittent		Amp	Any	Any	K: High risk
L2	< 18	Any, except GN maturing	Any	nAmp	No	Any	D: Low risk
		GNB intermittent			Yes	Any	G: Intermediate risk
	≥ 18	GNB nodular, neuroblastoma	Differentiated	nAmp	No	Any	E: Low risk
					Yes		H: Intermediate risk
			Poorly differentiated or undifferentiated	nAmp	Any		
			Any	Amp	Any	Any	H: High risk
M	< 18	Any	Any	nAmp	Any	Hyperdiploid	F: Low risk
	< 12	Any	Any	nAmp	Any	Diploid	I: Intermediate risk
	12 to <18	Any	Any	nAmp	Any	Diploid	J: Intermediate risk
	< 18	Any	Any	Amp	Any	Any	O: High risk
	≥ 18	Any	Any	Any	Any	Any	P: High risk
MS	< 18	Any	Any	nAmp	No	Any	C: Very low risk
					Yes	Any	O: High risk
				Amp	Any	Any	R: High risk

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 5

Age at diagnosis as prognostic factor in South African children with neuroblastoma (2020)

Ethics no: S18/07/138

Accepted by Pediatric Blood and Cancer December 2020

Jaques van Heerden[1,2], Tonya M Esterhuizen[3], Marc Hendricks[4], Janet Poole[5], Ané Büchner[6], Gita Naidu[7], Jan du Plessis[8], Barry van Emmenes[9], Ronelle Uys[1], GP Hadley[10], Mariana Kruger[1]

Affiliations

[1,2] Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa

[2] Paediatric Haematology and Oncology, Department of Paediatrics Antwerp University Hospital, Antwerp, Belgium

[3] Division of Epidemiology and Biostatistics, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

[4] Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Cape Town, Paediatric Haematology and Oncology Service, Red Cross War Memorial Children's Hospital, South Africa

[5] Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Charlotte Maxeke Johannesburg Academic Hospital, South Africa

[6] Paediatric Haematology and Oncology, Department of Paediatrics University of Pretoria, Steve Biko Academic Hospital, South Africa

[7] Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Chris Hani Baragwanath Academic Hospital, South Africa

[8] Department of Paediatrics Faculty of Health Sciences, University of the Free State, Division of Paediatric Haematology and Oncology, Universitas Hospital, South Africa

[9] Division of Paediatric Haematology and Oncology Hospital, Department of Paediatrics, Frere Hospital, South Africa

[10] Department of Paediatric Surgery, Faculty of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, South Africa

For the South African Children's Cancer Study Group

E-mail address: Jaques.vanheerden@uza.be
Department of Paediatric Haematology and Oncology, University Hospital of Antwerp, Wilrijkstreet 10, Edegem, 2650, Belgium.

Stellenbosch University https://scholar.sun.ac.za

Contributors' Statement

Jaques van Heerden conceptualized and designed the study, collected data, performed the data analysis and wrote the manuscript.
Mariana Kruger assisted with concept development, as well as design of the study, supervised data analysis, critically reviewed and revised the manuscript.
Tonya Esterhuizen performed the statistical analysis.
All other authors collected data in their respective pediatric oncology units and contributed significantly to the manuscript.

Keywords

Neuroblastoma, age of diagnosis, prognostic factor, low- and middle-income country

Abstract:

Purpose

Low-and middle-income countries (LMICs) reported a higher median age at diagnosis compared to high-income countries. The aim was to determine if the optimal age at diagnosis, which maximizes the difference in overall survival between younger versus older patients in the South African population was similar to the internationally validated 18 months age cut-point.

Methods

Four hundred and sixty NB patients diagnosed between 2000-2016 were included. Receiver operating characteristic (ROC) curves were used to predict potential age cut-point values for overall survival in all risk group classifications. Risk ratios, sensitivity, specificity, positive and negative predictive values at the specific cut points were estimated with 95% confidence intervals, and time to mortality by age at the specific cut points was shown with Kaplan-Meier curves and compared using log-rank tests.

Results

The median age at diagnosis for the total cohort was 31.9 months (range 0.2-204.7). For high-risk (HR), intermediate-risk, low-risk, and very low-risk the median age at diagnosis was respectively 36 months (range 0.4-204.7), 16.8 months (range 0.7-145.1), 14.2 months (range 2.0-143.5) and 8.7 months (range 0.2-75.6). The ROC curves for the total NB cohort (area under the curve (AUC) 0.696; $p<0.001$) and HR (AUC 0.682; $p<0.001$) were analyzed further. The optimal cut-point value for the total cohort was at 19.1 months (sensitivity 59%; specificity 78%). The HR cohort had potential cut-point values identified at 18.4 months age at diagnosis (sensitivity 45%; specificity 87%) and 31.1 months (sensitivity 67%; specificity 62%). The 19.1 months cut-point value in the total cohort and the 18.4 months cut-point value in HR were as useful in predicting overall survival as 18 months age at diagnosis.

Conclusion

The 18 months cut-point value appears to be the appropriate age for prognostic determination despite the higher median age at diagnosis in South Africa.

Stellenbosch University https://scholar.sun.ac.za

Introduction

Neuroblastoma (NB) is a sympathetic tumor presenting mainly in childhood with a median age at diagnosis of 19 months [1,2]. The majority of children are diagnosed under 5-years of age [1]. Age at diagnosis is an important risk factor in all international NB risk classification systems that predict the prognosis and influences the intensity of treatment. These include the Children's Oncology Group (COG)-risk classification system, International Neuroblastoma Risk Group (INRG) staging system and the International Society for Paediatric Oncology – Paediatric Oncology for Developing Countries (SIOP-PODC) guidelines for the treatment of neuroblastoma in low- and middle-income countries (LMIC) [2,3,4]. The prognostic effect of age at diagnosis is evident by an almost 90% five-year overall survival in children diagnosed under one year of age compared to 52% in children diagnosed older than five years of age at diagnosis [5].

Although the prognostic contribution of age is a continuum, the original age cut-off that predicted a binary outcome was 1 year of age [6]. While Shimada et al introduced an 18-month age cut point in the definition of International Neuroblastoma Pathology classification (INPC) to predict unfavorable histology [7], it was Breslow et al. who first proposed using age as a prognostic factor with an 18-month cut point [6]. Subsequently multivariate analysis with clinically significant factors including stage, histology and MYCN-amplification, a cut-point value of 18 months remained of prognostic significance and 18 to 20 months were determined as an acceptable range [8].

These established binary cut-point values were based on studies that were conducted in high-income countries (HIC) [8]. There is limited data available from LMIC, where the median age at diagnosis ranged from 24 to 48 months in Thailand, Iran and Egypt [9,10,11] with five-year overall survival that ranged from less than 10% to 48% [9,10,11]. We hypothesized that the South African population would follow similar LMIC trends and that the delayed median age at diagnosis (compared to HIC) would have prognostic significance. The aims of this study were threefold: the first aim was to estimate the median age at diagnosis in South Africa. The second aim was to identify a potential cut-point value for the age of diagnosis with overall survival (OS) as a primary endpoint in the South African population. The third aim was to evaluate whether the determined potential cut-point value and median age at diagnosis in the South African population was similar to the established 18 months international age cut-point value for prognosis. Thereby a possible optimal cut-point value for the age at diagnosis could be identified for the South African population.

Materials and Methods

A total of 460 children were diagnosed with NB in nine dedicated pediatric oncology units (POUs) in South Africa between January 2000 and December 2016. POUs were invited to participate in the study on a voluntary basis and the nine POUs represented all the regions of South Africa. The documented date of birth for each patient corresponded to the age stated on their birth certificates. Age at diagnosis was calculated as the period between the date of birth and the date of tumor biopsy, bone marrow aspiration, or raised urinary catecholamine levels, if biopsy was not possible. Patients were clinically and radiologically restaged according to the International Neuroblastoma Risk Group (INRG) classification system (*Appendix A*) [12]. The overall survival (OS) time was defined as the period from diagnosis to death or date last seen. The potential cut-point values were defined as the points that classified most of the individuals correctly with the "point closest-to-(0.1) corner" method in the ROC

Stellenbosch University https://scholar.sun.ac.za

plane or the point with the smallest Euclidean distance between the ROC curve and the (0.1) point [13]. The sensitivity refers to the proportion of patients diagnosed under the cut-point age who were still alive. The specificity refers to the portion of patients over the cut-point age who were dead [14]. For the study a high sensitivity was prioritized in the evaluation for the OS. An optimal cut-point value was defined as a statistically significant cut-point value with the highest sensitivity as determined by the "point closest-to-(0.1)" method on the ROC curve.

The risk ratio or relative risk (RR) was interpreted as the ratio of the risk of death in those diagnosed above the cut point age to the risk of death of those diagnosed under the cut point age on the ROC-curves [15]. The hazard ratio (HR) was defined as the instantaneous event rates of older patients (diagnosed above the cut-point age) compared to younger patients (diagnosed below the cut-point age) [15]. The Faculty of Health Sciences Research Ethics Committee of Stellenbosch University (S18/07/138) approved the study.

Statistical analysis

Data were analyzed using IBM SPSS version 25 (IBM Corporation, USA) statistical software and EpiCalc was used to determine predictive values for survival [16,17]. Since the high-risk cohort constituted a significant proportion of the total cohort, the study aims were applied to both the total study cohort and the high-risk cohort. The median age and age range for the cohorts were calculated from demographic data. Receiver operating characteristic (ROC) curves, with age as a continuous variable, were constructed for the purpose of identifying optimal cut-point values and estimating the sensitivities (true positive rate) and specificities (false positive rate) of the age at diagnosis against overall survival at several cut-points values [18]. The usefulness of the ROC curves was evaluated by the size of the area under the curve. AUC values between 0.7 and 0.8 identified age cut-points that were deemed acceptable to be able to discriminate between patients who died and those who did not, while AUCs of 0.5 or less were unable to perform this discrimination [19]. The "point closest-to-(0.1) corner" method was used to identify the potential cut-point values for both the total cohort and the HR-cohort. A Cox Proportional Hazard model was used estimate HR and 95% confidence interval (CI) at each potential cut point age. Kaplan-Meier curves and log-rank tests in the total cohort (all risk groups) and the high-risk cohort separately visualize time to event for different age cut-points. There was survival data available for 442 patients in the total cohort and 346 patients in the HR-cohort. The 95% CIs of both the sensitivities and specificities for the potential cut point values were compared to those of the internationally validated 18-months age at diagnosis to evaluate if the optimal cut-point values had similar prognostic value to the 18-month cut point.

Cut-point values for the age of diagnosis were determined at the sensitivity increments of 10%. Thereafter the cut-point values were evaluated by using log-rank tests. The sensitivities, specificities, risk ratios, positive and negative predictive values for all potential cut-point values were determined to evaluate the overlap of the confidence intervals. The chi-squared test was used to test the association between age at different cut points and the occurrence of death as a binary endpoint. The log rank test was used to compare time-to-mortality at different cut-points for OS. P-values of less than 0.05 indicated statistical significance.

Results

There was a male predominance with a male to female ratio of 1:0.92 for the 460 included patients. The median age at diagnosis for the total cohort was 31.9 months (range 0.2-204.7) (*Table 1 and Figure*

Stellenbosch University https://scholar.sun.ac.za

1). There were 179 patients (38.9%) diagnosed before two years and 369 (80.0%) within five years. The remaining 19.9% was diagnosed older than age five years. The high-risk (HR) group contributed 354 (77.0%) patients with a median age of 36 months (range 0.4-204.7). Intermediate-risk (n=36; 7.8%), low-risk (n= 30; 6.5%) and very low-risk (n=18; 3.9%) had median ages of 16.8 months (range 0.7-145.1), 14.2 months (range 2.0-143.5) and 8.7 months (range 0.2-75.6) respectively. Twenty-two (4.8%) patients could not be classified. This group had a median age of 15.6 months and a range of 0.4-108.

Using age at diagnosis as a continuous variable to predict the OS, the area under the ROC curve for the total cohort was 0.696 (0.633 – 0.759; p<0.001) and 0.682 (0.594 – 0.770; p<0.001) for the HR cohort (*Table 2*) thus acceptable for evaluation purposes. The cohorts for IR, LR and VLR were too small for valid ROC curve analysis. When evaluating the sensitivity and specificity coordinates for both the total cohort and the HR cohort, the sensitivities increased with increasing age at diagnosis, whilst the specificity decreased (*Table 3*).

Determining cut-point values with ROC curves

The ROC curves for the total cohort (all risk classifications) are presented in Figure 2 and the HR cohort in Figure 3. The sensitivity of each age was determined at several specificity levels. Selected sensitivities at the current international standardized prognostic age at diagnosis of 18 months and sensitivities at selected age of diagnosis representing increments of 10% were determined. These cut-point values on the ROC curves in Figure 2 and Figure 3 are given in Table 3.

The total cohort had an optimal age at diagnosis cut-point value at 19.1 months, which yielded a sensitivity of 59% and specificity of 78% (Fig 2, point A). The HR cohort had two optimal cut-point values at 18.4 months, sensitivity of 45%; specificity of 87% (Fig 3, point A) and 31.1 months, sensitivity of 67%; specificity of 62% (Fig 3, point B).

The risk ratios and predictive values of the cut-point values

When considering the risk ratios (RR) for the determined ROC curve cut-point values for the total cohort and HR cohort, the RR was the highest at 19.1 months for the total cohort (RR = 4.7). In the HR cohort the highest RR was at 18 months (RR = 4.2) (*Table 3*). For the 18 months cut-off the positive predictive value (PPV, interpreted as the percentage of those who were diagnosed at an age younger than the cut point who survived) for survival and the negative predictive value (NPV, interpreted as the percentage of those who were diagnosed at an age older than the cut point who died) were the highest for both the total cohort (PPV 36%; NPV 92%) and the HR cohort (PPV 36%; NPV 92%) (*Table 3*).

Determining the overall survival at the optimal cut-point value

We determined the OS outcomes at the potential ROC curve cut-point values for both the total cohort (Fig 2, point A) and HR cohort (Fig 3 points A and B). To determine the significance of the OS we estimated the p-values and quantified the effect by determining hazard ratios for the relevant cut-point values.

For the total cohort: At the ROC curve potential cut-point value point A (sensitivity 59%; specificity 78%; age at diagnosis of 19.1 months) (*Figure 2*) the difference in OS between the two age groups

Stellenbosch University https://scholar.sun.ac.za

(Hazard ratio 2.0), as illustrated by the Kaplan-Meier curve at 18 months, appears to be similar to the potential cut-point value (p<0.001) (*Figure 4*). Therefore, point A conforms to our definition of an optimal cut-point value.

For the HR cohort: At the ROC curve potential cut-point value A (sensitivity 45%; specificity 87%; age at diagnosis at 18.4 months) the difference in OS between the two age groups (Hazard ratio 1.9), as illustrated by the Kaplan-Meier curve at 18 months, appears to be similar to the 18.4 months potential cut-point value (p <0.001) (*Figure 5*). Therefore, point A conforms to our definition of an optimal cut-point value. At the ROC curve potential cut-point value point B (sensitivity 67%; specificity 62%; age at diagnosis at 31.1 months) was not statistically significant (p=0.178) and therefore did not meet our definition for an optimal cut-point value in the South African HR-cohort.

For both the total cohort and HR cohort: The sensitivities, specificities, RR, PPV and NPV including their respective 95% CIs of the 19.1 months cut-point value of the total cohort as well as the 18.4 months cut-point value of the HR appeared to be similar to those of the international validated 18 month cut-point values. (*Table 3*). There is large degree of overlap of the 95% CIs for the 18.0 months and 19.1 months cut-points values of the total cohort as well as the 18.0 months and 18.4 months cut-points values of the HR cohort.

Discussion

In various studies age, stage and biological factors have individually and in multivariate analysis been shown to have prognostic significance in NB [2]. North American studies reported the median age at diagnosis as 19 months (range 12–20 months), while German studies reported median ages as low as 15 months (range 10-23 months) [2,20]. Familial neuroblastoma often presents younger at a median of 9 months of age [21]. The median age at diagnosis for LR disease in the SIOPEN trial was 11 months [22] and varied between 5.4 to 18 months according to stage for IR in COG studies [23,24]. In two North American studies Kreismann et al. and Park et al. respectively concluded that the median age at diagnosis for patients with HR disease were 37.0 months (range 2.4 – 349.2 months) and 37.2 months (range 23.0 – 53.6 months) [25,26]. The SIOPEN HR trial had a median age of 36 months (range 26.4 – 52.8 months) [27]. An Indian review, representative of LIMC, reported median age at diagnosis between 30-42 months for NB [28], while a Chinese study reported a median age at diagnosis of 42 months [29]. The total South African cohort had a median age at diagnosis of 31 months comparable to LMICs. When the South African median age at diagnosis for LR (11 months), IR (16 months) and HR (36 months) are individually evaluated, the median ages at diagnosis per risk group were comparable to HICs. In North America up to 36% of patients with NB were diagnosed before the age of two years, while 90% were diagnosed by five years [2]. In this South African cohort, we found that 38.9% was diagnosed before two years, while only 80.1% of the cohort was diagnosed by five years and the remaining 19.9% was diagnosed after five years, which differ from HICs (*see Figure 1*) [1].

A possible explanation for older median age at diagnosis in the South African study is that a greater percentage of the cohort (77% in South Africa versus <70% in HIC) [30] comprised of HR patients older than 18 months. HICs have superior diagnostic capacities to diagnose children at a younger age, which included the NB infant screening studies in Japan and Germany [30,31]. A German study concluded there was a substantial overdiagnosis of non-metastatic NB estimated at a rate of 7/100 000 children (95%CI, 4.6 to 9.2), while screening did not identify more metastatic NB [31]. Similarly, Japanese studies screening only benefited the younger age groups, including those tumors that would otherwise

Stellenbosch University https://scholar.sun.ac.za

have spontaneously regressed [30]. Therefore, the median age at diagnosis for the entire South African cohort was predominantly determined by the HR cohort with a higher median age at diagnosis.

In the Children's Oncology Group study, a range of significant ages at diagnosis between 12.2 and 20 months were reported to be potential cut-point values [2]. The same was true for the total and HR-cohort in the South African study (*Table 3*). When determining a potential cut-point value, or binary value, we prioritized a higher sensitivity. The range between 12.2 months and 20 months in the North American study [2] would include the South African 19.1 months potential cut point value for the total cohort. The HR cohort represented 77.0% of the South African cohort and had an optimal cut point value of 18.4 months. In the South African cohorts both the 18.4 and 19.1 months cut-point values were significant predicting OS according to the Kaplan-Meier curves. In the North American study, even adjusted for stage and MYCN status, it was found that the optimal adjusted age cut-off for a decreased risk of an event was at 19.7 months [2].

Although the INRG risk classification incorporates the 18 months international age cut-point, the stratification was developed from an overall cohort [2]. Therefore, the South African HR cohort was not compared to the 18 months international age cut-point. Although the total cohort from the South African study was evaluated in terms of the total cohort of the North American study the South African study define OS as the end point and the North American study event free survival [2]. The South African OS is poor compared to HICs mostly due to high incidences of advanced disease at diagnosis and limited access to autologous stem cell transplantation, *cis*-Retinoic acid and no access to immune therapy. The effect of the difference in OS on the age cut-point values was not evaluated.

Limitations

Data collection was retrospective and treatment in the various POUs were not standardized. The first international studies to determine an optimal cut-point value for the age of diagnosis predates the 2000s. Our own cohort includes patients between 2000 and 2016. During these periods the diagnostic strategies have changed and may possibly affect the South African age estimates and comparisons. We acknowledge the relative bias in determining optimal cut-point values innate to the analysis of ROC-curves. In the determination of cut-point values the data were not adjusted for other prognostic factors such as stage and biological features. The INRG criteria was used for risk stratification and depend on a pre-determined age cut-point value. This possibly affected the analysis of the age cut point in the high-risk group.

Conclusion

Age is one of the most important prognostic factors in the management of neuroblastoma. This South African cohort for the age at diagnosis had a wide range of cut-point values up to 25 months with the possibility of prognostic significance for OS. The 18 months cut-point value appears to be the appropriate age for prognostic determination despite the higher median age at diagnosis in South Africa.

Conflict of interest

There is no conflict of interest to disclose.

Stellenbosch University https://scholar.sun.ac.za

Acknowledgements

Dr van Heerden, as staff member of the Department of Paediatric Haematology and Oncology, Antwerp University Hospital, University of Antwerp, acknowledges the Department for research support.

The authors acknowledge the SACCSG, for supporting the study, and the South African Children's Tumour Registry (SACTR), for providing statistical data.

Permissions to reproduce the figure from London et al. and the INRG classification system from Cohn et al. were obtained from The Journal of Clinical Oncology for academic and manuscript purposes in July 2020.

References:

1. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations), National Cancer Institute. Bethesda, MD, USA.
2. London WB, Castleberry RP, Matthay KK, et al.: Evidence for an age cutoff greater than 365 days for neuroblastoma risk group stratification in the Children's Oncology Group. J Clin Oncol. 2005, 23(27): 6459-65.
3. Pinto N, Applebaum M, Volchenboum S, Matthay K, et al.: Advances in Risk Classification and Treatment Strategies for Neuroblastoma. J Clin Oncol. 2015; 33(27): 3008-17.
4. Parikh N, Howard S, Chantada G, Israels T, et al.: SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for neuroblastoma in low- and middle-income settings. Pediatr Blood Cancer 2015; 62(8): 1305-1316.
5. Horner MJ, Ries LA, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2006 (Vintage 2006 Populations), National Cancer Institute. Bethesda, MD, USA.
6. Breslow N, McCann B: Statistical estimation of prognosis for children with neuroblastoma. Cancer Res. 1971; 31(12): 2098-2103.
7. Brodeur G, Seeger R, Barrett A, et al.: International criteria for diagnosis, staging, and response to treatment in patients with neuroblastoma. J Clin Oncol 6 (12): 1874-81, 1988.
8. Schmidt ML, Lal A, Seeger R, Maris J, et. al.: Favorable prognosis for patients 12 to 18 months of age with stage 4 nonamplified MYCN neuroblastoma: A children's cancer group study. J Clin Oncol. 2005, 23(27): 6474-80.
9. Shuangshoti S, Shuangshoti S, Nuchprayoon I, Kanjanapongkul S, et al.: Natural course of low risk neuroblastoma. Pediatr Blood Cancer. 2012; 58(5): 690-694.
10. Al-Tonbary Y, Badr M, Mansour A, El Safy U, Saeed S, et al. Clinico-epidemiology of neuroblastoma in north east Egypt: A 5-year multicenter study. Oncol Lett. 2015; 10(2): 1054-1064.
11. Mehdiabadi GB, Arab E, Rafsanjani KA, Ansari S, et al.: Neuroblastoma in Iran: An Experience of 32 years at a referral Childrens Hospital. Asian Pacific J Cancer Prev. 2013; 14 (5), 2739-2742.
12. Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. *J Clin Oncol*. 2009;27(2):289-297
13. Perkins NJ, Schisterman EF. The inconsistency of "optimal" cut-points using two ROC based criteria. Am J of Epidem. 2006; 163(7): 670–675.

Stellenbosch University https://scholar.sun.ac.za

14. Florkowski CM. Sensitivity, specificity, receiver-operating characteristic (ROC) curves and likelihood ratios: communicating the performance of diagnostic tests. Clin Biochem Rev. 2008;29 Suppl 1(Suppl 1):S83-S87.
15. Case LD, Kimmick G, Paskett ED, Lohman K, Tucker R. Interpreting Measures of Treatment Effect in Cancer Clinical Trials. The Oncologist. 2002; 7: 181-187.
16. SPSS citation: IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.
17. Epicalc citation: EpiCalc 2000 version 1.02. 1998. Joe Gilman and Mark Myatt. Brixton Books. http://www.brixtonhealth.com/epicalc.html.
18. Kamarudin AN, Cox T, Kolamunnage-Dona R. Time-dependent ROC curve analysis in medical research: current methods and applications. BMC Med Res Methodol. 2017; 17, 53. DOI 10.1186/s12874-017-0332-6
19. Hosmer DW, Lemeshow S. Applied Logistic Regression, 2nd Ed. Chapter 5, John Wiley and Sons, New York, NY (2000), pp. 160-164.
20. Berthold F, Spix C, Kaatsch P. et al.: Incidence, Survival, and Treatment of Localized and Metastatic Neuroblastoma in Germany 1979–2015. Pediatr Drugs. 2017; 19(6): 577–593.
21. Mosse YP, Laudenslager M, Khazi D, et al.: Germline PHOX2B mutation in hereditary neuroblastoma. Am J Hum Genet. 2004; 75(4): 727–730.
22. De Bernardi B, Mosseri V, Rubie H, Castel V, et al.: Treatment of localised resectable neuroblastoma. Results of the LNESG1 study by the SIOP Europe Neuroblastoma Group. Br J Cancer. 2008; 99(7): 1027–1033.
23. Matthay KK, Perez C, Seeger RC, Brodeur G.M, et. al.: Successful treatment of stage III neuroblastoma based on prospective biologic staging: A Children's Cancer Group study. J Clin Oncol. 1998; 16(4): 1256–1264.
24. Supplement to: Baker DL, Schmidt ML, Cohn SL, et al. Outcome after reduced chemotherapy for intermediaterisk neuroblastoma. N Engl J Med 2010; 363:1313-23.
25. Kreismann SG, Seeger RC, Matthey KK, London WB, et al. Purged versus non-purged peripheral blood stem-cell transplantation for high-risk neuroblastoma (COG A3973): a randomised phase 3 trial. Lancet Oncol. 2013; 14(7): 999–1008.
26. Park JR, Kreissman SG, London WB, et al. Effect of Tandem Autologous Stem Cell Transplant vs Single Transplant on Event-Free Survival in Patients with High-Risk Neuroblastoma: A Randomized Clinical Trial. *JAMA.* 2019;322(8):746–755.
27. Ladenstein R, Pötschger U, Pearson ADJ, Brock P, et al. Busulfan and melphalan versus carboplatin, etoposide, and melphalan as high-dose chemotherapy for high-risk neuroblastoma (HR-NBL1/SIOPEN): an international, randomised, multi-arm, open-label, phase 3 trial. The Lancet Oncology. 2017; 18(4), 500–514.
28. Kulkarni KP, Marwaha RK. Outcome of Neuroblastoma in India. Indian J Pediatr.2013; 80(10): 832–837.
29. Kai L, Kuiran D, Jiechun G, Wei Y, Xianmin X, Shan Z. Neuroblastoma Management in Chinese Children. J Invest Surg. 2012; 25(2): 86–92.
30. Bessho F. Effects of mass screening on age-specific incidence of neuroblastoma. Int J Cancer. 1996; 67(4), 520–522.
31. Schilling FH, Spix C, Berthold F, Erttmann R, et al.: Neuroblastoma Screening at One Year of Age. N Eng J Med. 2002; 346(14), 1047–1053.

Stellenbosch University https://scholar.sun.ac.za

Tables

Figures

Appendices

Stellenbosch University https://scholar.sun.ac.za

Tables

Table 1: The age at diagnosis according to risk groups of the International neuroblastoma risk group (INRG) classification system in a cohort of 460 children with neuroblastoma in South Africa.

	n (%)	Median (months)	Range (months)
Total cohort	460	31.9	0.2-204.7
HR cohort	354 (77.0%)	36.0	0.4-204.7
IR cohort	36 (7.8%)	16.8	0.7-145.1
LR cohort	30 (6.5%)	14.2	2.0-143.5
VLR cohort	18 (3.9%)	8.7	0.2-75.6
Unclassified	22 (4.8%)	15.6	0.4-108

Abbreviations: HR – high-risk, IR – intermediate-risk, LR – low-risk, VLR – very low risk

Table 2: Receiver operating characteristic curve's area under the curve (AUC) for the age at diagnosis as a continuous variable [18].

	N (%)	AUC	Std. Error	p-value	95% CI on the AUC	
					Lower limit	Upper limit
Total cohort	460	0.696	0.032	<0.001	0.633	0.759
HR cohort	354 (77.0%)	0.682	0.045	<0.001	0.594	0.770

References for the usefulness of the ROC curves: AUC 0.5 – no discrimination/ inability to use as measure; 0.7 to 0.8 – acceptable; 0.8 to 0.9 – excellent; > 0.9 – outstanding.

Abbreviations: HR – high-risk

Stellenbosch University https://scholar.sun.ac.za

Table 3: Cut-points for age at diagnosis, sensitivity and specificity determined on the ROC-curves

Total cohort (All risk groups) n = 460 (100%)						
Age at diagnosis (months)	**Se% (95%CI)**	**Sp% (95% CI)**	**RR (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**p-value***
18.0	55% (45%, 65%)	81% (77%, 85%)	3.41 (2.44, 4.76)	0.46 (0.37, 0.55)	0.87 (0.82, 0.90)	<0.001
19.1	59% (49%, 69%)	80% (76%, 84%)	3.64 (2.59, 5.12)	0.47 (0.38, 0.55)	0.87 (0.83, 0.91)	<0.001
27.3	70% (60%, 79%)	61% (56%, 66%)	2.82 (1.92, 4.14)	0.34 (0.28, 0.41)	0.88 (0.83, 0.92)	<0.001
43.9	80% (71%, 87%)	35% (30%, 40%)	1.85 (1.18, 2.89)	0.26 (0.21, 0.31)	0.86 (0.79, 0.91)	0.005
67.7	90% (82%, 95%)	18% (14%, 23%	1.77 (0.97, 3.24)	0.24 (0.20, 0.28)	0.87 (0.76, 0.93)	0.049
HR cohort n= 354 (77.0%)						
Age at diagnosis (months)	**Se% (95%CI)**	**Sp% (95% CI)**	**RR (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**p-value***
18.0	46% (31%, 61%)	88% (83%, 91%)	4.2 (2.53, 6.98)	0.36 (0.24, 0.49)	0.92 (0.88, 0.94)	<0.001
18.4	48% (33%, 63%)	86% (82%, 90%)	4.08 (2.44, 6.80)	0.34 (0.23, 0.47)	0.92 (0.88, 0.94)	<0.001
27.1	61% (45%, 75%)	66% (60%, 71%)	2.58 (1.49, 4.49)	0.21 (0.15, 0.29)	0.92 (0.87, 0.95)	0.001
31.1	71% (61%, 80%)	58% (52%, 63%)	2.62 (1.77, 3.86)	0.32 (0.26, 0.39)	0.88 (0.83, 0.91)	0.001
38.3	72% (56%, 84%)	48% (43%, 54%)	2.14 (1.17, 3.93)	0.17 (0.12, 0.23)	0.92 (0.86, 0.95)	0.011
43.8	78% (63%, 89%)	37% (32%, 43%)	1.94 (1.00, 3.78)	0.16 (0.11, 0.21)	0.92 (0.85, 0.96)	0.047

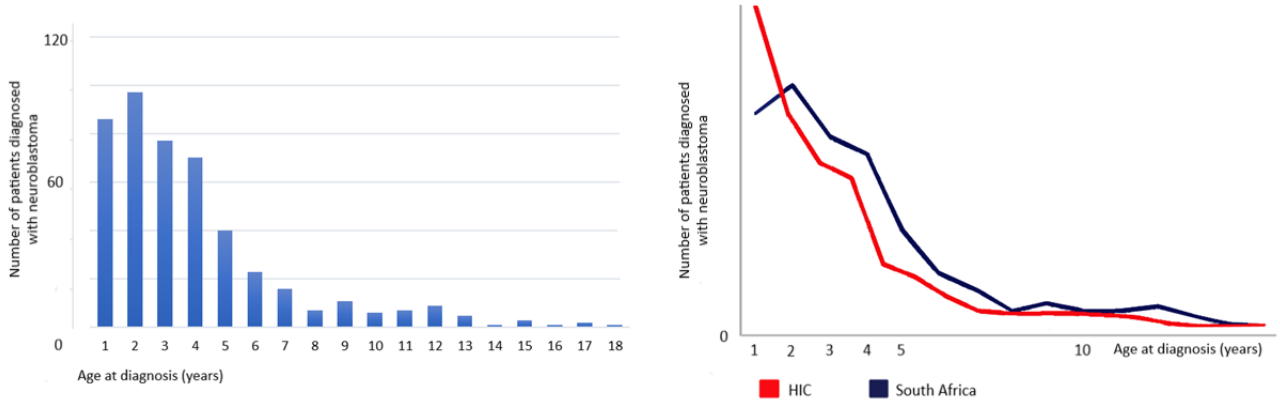
* p-values for sensitivity and specificity calculations at the cut points for age at diagnosis were assessed by chi-squared test.

Abbreviations: HR – high-risk, Se – sensitivity, Sp – specificity, RR – risk ratio, PPV – positive predictive value, NPV – negative predictive value

Stellenbosch University https://scholar.sun.ac.za

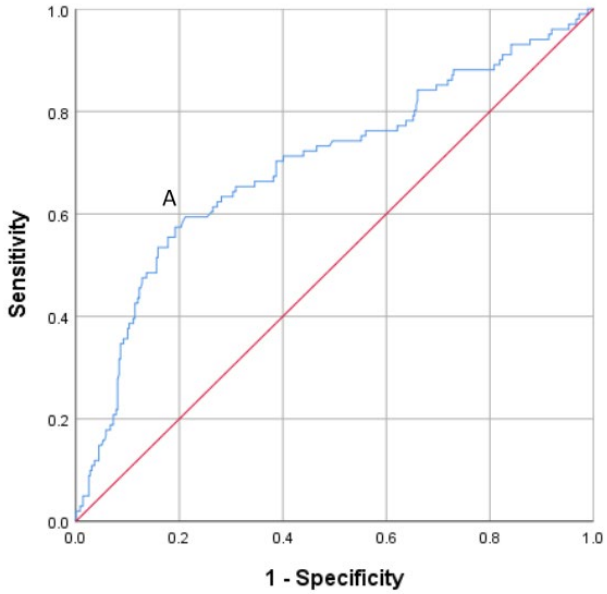
Graphs

Figure 1: The distribution of the total South African NB age at diagnosis cohort (n = 460) compared to high income countries (HIC).



HIC curve based on the "Distribution of age at diagnosis" data by London et. al. JCO. 2005, 23(27): 6461.

Figure 2: ROC curve for the age at diagnosis in all neuroblastoma risk groups (total cohort) in South Africa between 2000 and 2016 ($p<0.001$)

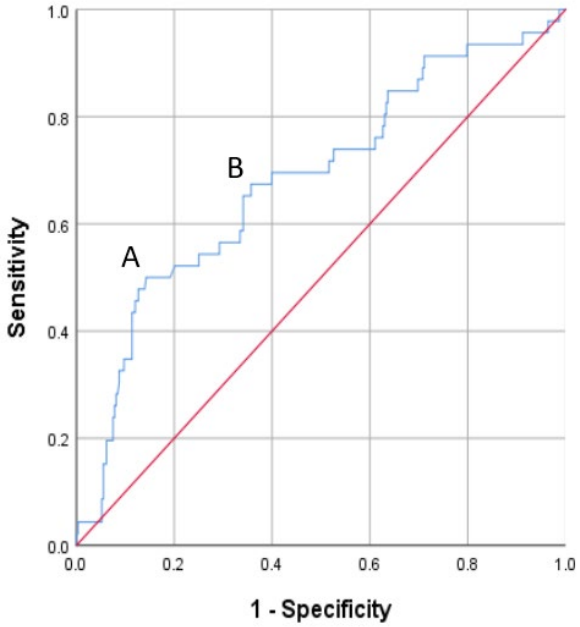


Total cohort n = 460

A – sensitivity of 59%; specificity of 78%; age of diagnosis 19.1 months

Stellenbosch University https://scholar.sun.ac.za

Figure 3: ROC curve for the age at diagnosis in the HR neuroblastoma group in South Africa between 2000 and 2016 (p<0.001)



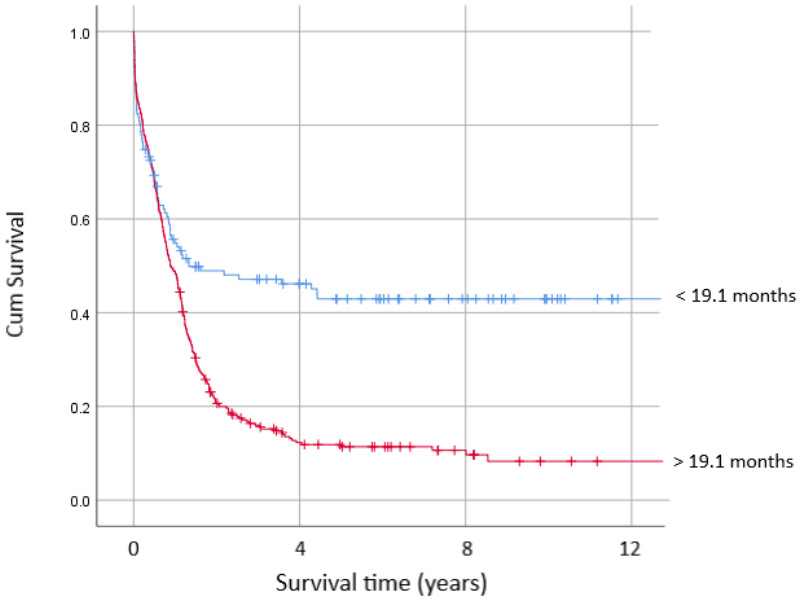
HR-cohort n = 354/442 (77.0%)

A – sensitivity of 45%; specificity of 87%; age of diagnosis 18.4 months

B – sensitivity of 67%; specificity of 62%; age of diagnosis 31.1 months

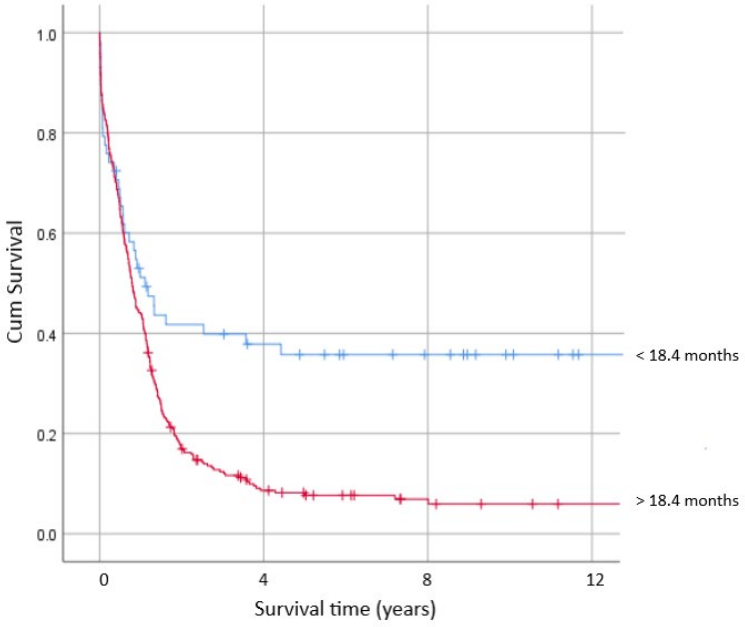
Stellenbosch University https://scholar.sun.ac.za

Figure 4: Kaplan Meier curves for OS of age at diagnosis of 19.1 months cut-point value for the total neuroblastoma cohort (p<0.001, Hazard ratio 2.0)



Total (n) 442; ≤ 19.1 months n (%) = 131 (29.6%); > 19.1 months n (%) = 311 (61.4%)

Figure 5: Kaplan Meier curves for OS of age at diagnosis of 18.4 months cut-point value in HR neuroblastoma (p<0.001, Hazard ratio 1.9)



Total (n) 346; ≤ 18.4 months n (%) = 58 (16.8%); > 18.4 months n (%) = 288 (83.2%)

Stellenbosch University https://scholar.sun.ac.za

Appendices:

Appendix A: International Neuroblastoma Risk Group (INRG) classification system [12]

INRG stage	Age (months)	Histologic category	Grade differentiation	MYCN	11q aberration	Ploidy	Pretreatment risk group
L1/L2	Any	GN maturing GNB intermittend	Any	Any	Any	Any	A – very low risk
L1	Any	Any, except GN maturing GNB intermittend	Any	nAmp Amp	Any Any	Any Any	B – very low risk K – High
L2	<18	Any, except GN maturing GNB intermittend	Any	nAmp	No	Any	D – Low
					Yes	Any	G – Intermediate
	≥18	GNB nodular, neuroblastoma	Differentiating	nAmp	No	Any	E - Low
					Yes		H – Intermediate
			Poorly differentiated or undifferentiated	nAmp	Any		
			Any	Amp	Any	Any	H - High
M	<18	Any	Any	nAmp	Any	Hyperdiploid	F – Low
	<12	Any	Any	nAmp	Any	Diploid	I - Intermediate
	12 to <18	Any	Any	nAmp	Any	Diploid	J - Intermediate
	<18	Any	Any	Amp	Any	Any	O - High
	≥18	Any	Any	Any	Any	Any	P - High
MS	<18	Any	Any	nAmp	No	Any	C - Very low risk
					Yes	Any	O - High
				Amp	Any	Any	R - High

Abbreviations: GN – ganglioneuroma, GNB – ganglioneuroblastoma, Amp – Amplified, nAmp – non-amplified

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 6

The correlation of tumour markers and MIBG-studies in South African children with neuroblastoma (2020)

Ethics no: S18/07/138

Submitted to Clinical Oncology October 2020

Van Heerden, Jaques[1,2]; Kruger, Mariana[1]; Esterhuizen, Tonya Marianne[3]; Hendricks, Marc[4]; Du Plessis, Jan[5]; Engelbrecht, Gert[6]; Janse van Vuuren, Magritha[7]; van Emmenes, Barry[8]; Uys, Ronelle[1]; Burger, Celeste[9]; Nyakale, Nozipho[10]; More, Stuart[11]; Brink, Anita[11].

Affiliations

[1] Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa

[2] Paediatric Haematology and Oncology, Department of Paediatrics, Antwerp University Hospital, Antwerp, Belgium

[3] Division of Epidemiology and Biostatistics, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

[4] Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Cape Town, Paediatric Haematology and Oncology Service, Red Cross War Memorial Children's Hospital, South Africa

[5] Department of Paediatrics, Faculty of Health Sciences, University of the Free State, Division of Paediatric Haematology and Oncology, Universitas Hospital, Bloemfontein, South Africa

[6] Department of Nuclear Medicine, University of the Free State, Universitas Hospital, Bloemfontein, South Africa

[7] Drs B Vorster and M Janse van Vuuren Incorporated, Nuclear Physicians, Bloemfontein, South Africa

[8] Division of Paediatric Haematology and Oncology Hospital, Department of Paediatrics, Frere Hospital, East London, South Africa

[9] Department of Nuclear Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa

[10] Department of Nuclear Medicine, Faculty of Health Sciences, University of KwaZulu-Natal, Inkosi Albert Luthuli Academic Hospital, Durban, South Africa

[11] Department of Paediatrics and Child Health, Division of Nuclear Medicine, Faculty of Health Sciences, University of Cape Town, Red Cross War Memorial Children's Hospital, South Africa

Correspondence:

Dr Jaques van Heerden, Department of Paediatric Haematology and Oncology, University Hospital of Antwerp, Wilrijkstraat 10, Edegem, 2650, Belgium. E-mail: Jaques.vanheerden@uza.be

Stellenbosch University https://scholar.sun.ac.za

Contributors' statement

Jaques van Heerden conceptualized and designed the study, collected data, performed the data analysis and wrote the manuscript.
Anita Brink assisted with conceptualization and design of the study, did all the control scores for the mIBG studies, and critically reviewed and revised the manuscript.
Mariana Kruger assisted with concept development as well as design of the study, supervised data analysis, and critically reviewed and revised the manuscript.
Celeste Burgers, Gerrit Engelbrecht, Stuart More, Nozipho Nyakale and Magritha van Vuuren were the hospital mIBG evaluators.
Tonya Esterhuizen performed the statistical analysis.
All other authors collected or generated data in their respective paediatric oncology units and contributed significantly to the manuscript.

Keywords

Neuroblastoma; mIBG; modified Curie scores; tumor markers; lactate dehydrogenase; ferritin; MYCN; South Africa

Abstract

Background

Diagnostic and post induction ^{123}I-mIBG-scans have prognostic significance in the treatment of neuroblastoma (NB), but data from low- and middle-income (LMICs) countries are limited due to resource constraints.

Objectives

The objective was to determine the association between NB associated tumours markers (lactate dehydrogenase (LDH), ferritin and MYCN-amplification) and ^{123}I-mIBG-scans (modified Curie scores and metastatic disease patterns) in predicting complete metastatic response rates (mCR) and overall survival (OS).

Patients and Methods

Ninety-eight patients diagnosed with NB between January 2000 and May 2018 in South Africa with diagnostic ^{123}I-mIBG-scans were included. Data collection included LDH, ferritin and MYCN-amplification at diagnosis. Two nuclear physicians independently determined the modified Curie scores and pattern of distribution for each diagnostic and post induction ^{123}I-mIBG-scans with high interrater agreement (r=0.952) and reliability (K=0.805). The cut-point values for the diagnostic and post induction modified Curie scores of ≥7.0 (p=0.657) and 3 (p=0.182) were generated respectively. The association between the tumour markers and modified Curie score of the ^{123}I-mIBG-scans, using post induction mCR and OS were determined.

Results

Only the diagnostic modified Curie scores predicted mCR (p=0.009). LDH (p=0.004), ferritin (p=0.005), diagnostic modified Curie scores (p=0.004) and post induction modified curie scores (p=0.05)

Stellenbosch University https://scholar.sun.ac.za

predicted 2-yr OS. Only ferritin correlated with diagnostic modified Curie scores (p=0.003) but had a low correlation coefficient of 0.353. On multivariable analysis the only significant co-variate for 2-yr OS at diagnosis was LDH <750U/L (p=0.003). A post induction chemotherapy modified Curie score ≤ 3.0 had a 2-yr OS of 41.0% compared to 31.2% for a score >3.0 (p=0.05).

Conclusion

LDH, ferritin and the diagnostic ^{123}I-mIBG scans significantly predicted 2-year OS, but only ferritin and the modified Curie-scores correlated. MYCN-amplification neither correlated with any aspect of the ^{123}I-mIBG scans nor significantly predicted mCR or 2-year OS. LDH and ferritin are therefore appropriate neuroblastoma tumour markers to be used in LMICs with limited or no access to MIBG scans and/or MYCN amplification studies.

Introduction

Neuroblastoma (NB) is a metabolically active neuro-endocrine malignancy of the adrenal medulla and sympathetic nervous system with a spectrum of histopathological behaviour ranging between spontaneous regression, local maturation or aggressive proliferation of immature cells with metastases [1]. Tumour markers and mIBG-scans facilitate the diagnosis of the primary tumour and metastases, as well as the prognostic classification of the disease [2,3]. The tumour or the body produce non-specific NB tumour markers as part of the pathophysiological hallmarks of the malignancy [4]. Lactate dehydrogenase (LDH), especially subunit A, reduces pyruvate to lactate thereby bypassing oxidative phosphorylation; diverts pyruvate precursors into the pentose phosphate pathway to supply metabolic substrate for cancerous cell growth [5]. Elevated extracellular lactate levels promote a conducive microenvironment for tumour angiogenesis and immune evasion [5]. The acute phase inflammatory marker ferritin is present in both glycosylated and non-glycosylated forms, secreted by active and necrotic tumour cells respectively [6]. Clinically the presence of these two non-specific tumour markers signals increased cell activity and have independently been proven to correlate with management outcomes in NB [7]. Both serum LDH and ferritin can be determined by blood tests accessible in resource limited settings.

Amplification of the MYCN-gene, a specific NB tumour marker, plays a central role in regulating transcription and binding to DNA of other genes [8]. MYCN regulates the proliferation, self-renewal and angiogenesis of the primary tumour while promoting metastases by supressing differentiation and limit immune surveillance [8]. On an epigenetic level MYCN has a regulating role in histone methylation and acetylation [8]. MYCN amplification is associated with more aggressive features at diagnosis, an increased progression rate during induction chemotherapy and poorer survival due to relapse and disease progression [9]. MYCN-amplification of a tumour can be determined on a tumour biopsy sample as well as on NB-cells in a bone marrow aspirate [7]. The fluorescent in situ hybridization test for MYCN-amplification availability is resource dependant but are mostly available in high income countries (HICs) [7].

The response of NB to induction chemotherapy is an important prognostic factor [10]. Although bilateral bone marrow aspiration and trephine biopsy remain important for the detection of metastases, meta-iodobenzylguanidine (mIBG)-scans improve the accuracy of detecting and staging neuroendocrine tumours, such as neuroblastoma and pheochromocytoma [1,2,7]. Radio-labelled

Stellenbosch University https://scholar.sun.ac.za

meta-iodobenzylguanidine is actively taken up by the norepinephrine transporters of the sympathetic neurons of NB thereby increasing the diagnostic sensitivity [2]. The metastatic pattern of distribution as demonstrated with mIBG-scan at diagnosis, has proven to have prognostic significance with a diffuse spread denoting a poorer outcome [10]. There is an association reported between MYCN-amplification and a diffuse metastatic spread [10]. MIBG-scans scored according to the modified Curie score is a validated scoring system, developed by the Children's Oncology Group (COG), which correlates with prognosis and management outcomes [11]. The score divides the body into nine segments, with four degrees of nuclear differentiation for the extent of involvement [11]. The modified Curie score also assesses soft tissue involvement of NB [11]. Limitation in the use of MIBG scans include limited access in LMICs, the need for thyroid blockade pre-testing with potassium iodate or Lugol's iodine solution and controlled timing in relation to chemotherapy for optimal evaluation [12]. In South Africa radio-isotope availability is not guaranteed due to trade embargoes, plant closures and production subject to national holidays, research guidelines and the lack of manpower [13].

The literature relating to mIBG-studies and the prognostic relationship between tumour markers and ^{123}I-mIBG-scans in NB are limited. The primary objective of this study was to determine the association between NB associated tumours markers (LDH, ferritin and MYCN-amplification) and modified Curie scores using post induction metastatic complete response (mCR) and 2-year overall survival (OS) as end points. The secondary objective was to evaluate the association of these tumour markers with metastatic disease pattern on a diagnostic ^{123}I-mIBG-scan and mCR and 2-yr OS.

Materials and Methods

Patient population

Only 98 children with NB diagnosed between January 2000 and May 2018 from five paediatric oncology units met inclusion criteria namely a diagnostic ^{123}I-mIBG-scans and serial LDH, ferritin and MYCN-amplification. Exclusion criteria included incomplete or lost files, no or paper-based images only mIBG-scans and participation refusal.

Data collection

Data collection included demographic data (age at diagnosis, sex, disease stage), LDH, ferritin and MYCN at diagnosis and test results (radiological and nuclear imaging as well as bone marrow aspiration) to evaluate remission status after induction chemotherapy Primary tumour and metastatic evaluations were performed subject to availability of institutional resources, based on the International Neuroblastoma Response Criteria (INRC) [14,15,16]. Diagnostic and post induction ^{123}I-mIBG-scans from each participating hospital were scored according to the modified Curie score (*Appendix A*) by two nuclear physicians who were blinded to each other's scores. The metastatic pattern of nuclear radio-isotope distribution was reported according to the research done by Bleeker et.al [10]. The mCR was defined as the disappearance of all demonstrable signs of neuroblastoma cells in response to treatment, excluding the primary tumour. The end points were defined as mCR and 2-yr OS.

Stellenbosch University https://scholar.sun.ac.za

Interrater agreement and reliability

Interrater agreement was evaluated for both the pattern of distribution (focal versus diffuse) and the continuous variables of the modified Curie scores (0 to 30) for each ^{123}I-mIBG scan. The modified Curie score interrater agreement was 0.952 (High interrater agreement) ($p<0.001$) [17] and the level of reliability on the Cohen's Kappa measure of agreement was strong (K=0.805) [18].

Statistical analysis

The differences in means or medians were assessed using the Mann-Whitney U test or Student's t-test, using IBM SPSS version 25 (IBM Corporation, USA) statistical software [19]. After continuous variables of LDH and Ferritin did not show significant association with the continuous variables of the diagnostic and post induction modified Curie scores, receiver operating characteristic (ROC) curves were constructed for the purpose of identifying an optimal binary cut-point value for the both the diagnostic and post induction modified Curie scores to predict post-induction complete metastatic remission (mCR) [20]. The "point closest-to-(0.1) corner" method was used to identify the potential cut-point values for the modified Curie scores. We prioritized a higher sensitivity to be able to include the greatest number of patients in possible metastatic remission. Sensitivity was defined as the proportion of patients without residual metastatic disease (in remission) who had modified Curie scores below the cut-point value. Specificity was defined as the proportion of patients with residual metastatic disease (not in remission) who had modified Curie scores above the cut-point value. The modified Curie score as a binary variable was then used to evaluate the association with the tumour markers: LDH, ferritin and MYCN. For statistical analysis the internationally validated prognostic cut point values of 750U/L and 120g/dL for LDH and ferritin were used respectively [7]. The cut point values for LDH and ferritin were validated in the same population of South African children by the SACCSG neuroblastoma tumour working group [21]. Categorical association between independent variables such as tumour marker categories and mIBG score categories, and overall survival (OS) was assessed using the Pearson Chi-square (χ^2) test or, when appropriate, the Fishers exact test. OS and associated 95% confidence intervals (CI) were calculated and described using Kaplan-Meier curves with differences evaluated using log rank tests. To estimate the effect of prognostic factors on OS, univariate and multivariable Cox regression modelling approach was employed. The proportional hazards assumption was also confirmed for the final multivariable model. An area under the curve (AUC) of 0.7 and higher was defined as acceptable to discriminate between values [22]. For all calculations a *p*-value less than 0.05 was considered significant.
Ethical approval (S18/07/138) for the study was obtained from The Faculty of Health Sciences Research Ethics Committee of Stellenbosch University, South Africa.

Results

Diagnostic epidemiology and disease profile

Final analysis included 98 patients with diagnostic ^{123}I-mIBG scans (*Supplemental table 1)*. There was a male: female ratio of 1:1 with the majority in the 18.1 to 60 months age group (n=51, 52.0%). The median age was 28.9 months (range 1.6 to 196.3 months, mean 43.2 months). The most frequent origin was the adrenal gland (67.3%), followed by a non-adrenal abdominal mass (14.3%) and paraspinal masses in eight (8.2%) patients. LDH-values were available in 97 (99.0%) patients with 57.1% (n=52) greater than 750 U/L. Ferritin values were available in 79 (81.6%) patients with 62.2%

Stellenbosch University https://scholar.sun.ac.za

(n=61) greater than 120 ng/dl. MYCN-amplification was determined in only 61 (62.3%) tumours of which 33 (33.7%) were MYCN-amplified. Ninety-eight diagnostic modified Curie scores were determined with a mean of 10 (median 5, range 0 – 29), while 42 (42.9%) post induction chemotherapy modified Curie scores were determined with a mean of 7 (median 1, range 0 – 20). Of the diagnostic ^{123}I-mIBG scans, 50 (51.0%) had focal and 43 (43.9%) diffuse metastatic patterns, while 5 (5.1%) were limited to the primary tumour.

Association of continuous variables

When evaluating continuous variables of LDH, ferritin and the diagnostic modified Curie scores the only statistically significant correlation coefficients were LDH: ferritin (p=0.004) and ferritin: diagnostic modified Curie score (p=0.001), with low correlation coefficients (r) of 0.324 and 0.353 respectively (*Table 1*). Since no clear linear trend could be distinguished between ferritin and the modified Curie score, associations between categories of variables were performed. Comparing categorical values of LDH (≤/> 750 U/L), ferritin (≤/> 120 g/dL) and MYCN-amplification (non-amplified vs amplified) with the continuous variables of the diagnostic modified Curie scores, LDH (p=0.029) and ferritin (p<0.001) were significantly associated with the continuous distribution of the diagnostic mIBG-scores, but not MYCN-amplification (p=0.518 (*Table 2 and Figure 1*).

Clinical data analysis and binary cut-point value determination

The ROC curve for the diagnostic modified Curie scores (n=98) against remission is presented in Table 3 and Figure 2. The AUC was 0.657 (p=0.012). The sensitivity of each score was determined at several specificity levels. The optimal cut point value A was at a modified Curie score of ≥7.0 with a sensitivity of 71.9% and specificity of 56.1%. The ROC curve for the post induction modified Curie scores (n=42) (*Table 3 and Figure 3*) had an AUC of 0.661 (p=0.182). The optimal cut point value A was at a modified Curie score of 3.0 with a sensitivity of 92.6% and specificity of 38.6%.

Associations between categorical variables

Only the categorical ferritin values (≤/> 120 g/dL) were associated with the categorical 7.0 cut point values of the diagnostic modified Curie scores (p=0.003) (*Table 4*) in predicting post induction mCR. The categorical ferritin and LDH values (≤/> 750 U/L) were associated with the metastatic pattern of distribution (p=0.008 and p=0.026 respectively) in predicting post induction mCR (*Table 4*). The probability that a ferritin level ≤120ng/dl was determined when the diagnostic modified Curie score was <7.0 was 63.3% and a ferritin level >120ng/dl when a diagnostic modified Curie score was ≥7.0 was 77.8% (p=0.003). The probability that a ferritin level ≤120ng/dl was determined when the mIBG scan pattern was focal was 80.0% and a ferritin level >120ng/dl when the pattern was diffuse was 58.3% (p=0.008). MYCN-amplification was not associated with either the categorical 7.0 cut-point value for the diagnostic MIBG scores (p=0.554) or pattern of distribution (p=0.887).

Univariate and multivariate analysis predicting post induction mCR and 2-yr OS (Table 5, 6 and 7)

Diagnostic LDH (p=0.072), ferritin (p=0.372), MYCN-amplification (p=0.202) and the mIBG pattern of distribution (p=0.527) were not significant predictors of remission. Patients with an LDH value at diagnosis <750U/L had a more favourable 2-yr OS of 40.0% versus 17.5% ≥750U/L (p<0.004) and those with ferritin levels at diagnosis of <120ng/dl had a more favourable 2-yr OS of 56.0% compared to 19.1% for levels ≥120ng/dl (p=0.005) (*Figure 4)*. A diagnostic modified Curie score <7.0 had a 2-yr OS

Stellenbosch University https://scholar.sun.ac.za

of 36.0% compared to 23.0% for a score ≥7.0 (p=0.004). MYCN-amplification and the distribution of the mIBG-scan pattern at diagnosis did not reach significance in predicting 2-yr OS. On multivariable analysis the only significant co-variate for 2-yr OS at diagnosis was LDH <750U/L (p=0.003, HR 0.39, 95% CI, 0.21-0.73) (*Figure 5*). Ferritin <120 ng/dl (p=0.293, HR 0.66, 95% CI, 0.3-1.44) and the diagnostic modified Curie score > 6.5 (p=0.837, HR 0.94, 95% CI, 0.53-1.66) did not remain significant covariates. A post induction chemotherapy modified Curie score ≤ 3.0 had a 2-yr OS of 41.0% compared to 31.2% for a score >3.0 (p=0.052).

Discussion

Neuroblastoma is a heterogenous tumour, which present challenges in accurate diagnosis and management [7]. To be able to predict the possibility of remission in NB is of value win the context of settings with limited resources [7]. A wide range of investigations can be utilised, but the availability varies according to health care resources in countries and POUs [7]. Therefore, evaluating the correlation of more affordable, readily available tests with specialised, costly, but less accessible tests could assist in more robust neuroblastoma management in LMICs.

In the current study ≥7.0 was determined to be the cut-point value for diagnostic mIBG-scans and 3 for post induction mIBG-scans. Various international studies have determined prognostic cut-point values for diagnostic mIBG-scores to be between 2 and 10 and values between 0 and 6 for post induction mIBG-scores [11,23]. In the SIOPEN/HR-NBL1 dataset a diagnostic Curie score was of prognostic significance for 5-year OS at a cut-point value of 12 (p=0.013) [26], but Yanik et al. reported a diagnostic Curie score of 9 [11]. This is higher than the 7.0 cut-point value in our study, yet in our cohort 7.0 did carry prognostic significance at 2-yr OS (p=0.004). For the post induction the prognostic significance at a cut-point value of 3.0 (p=0.052) was comparable to the results by Katzenstein et al. [23] and close to the prognostic cut-point value of 2 for the post induction Curie score in the SIOPEN/HR-NBL1 dataset [24]. The main conclusion in both studies were that patients with residual mIBG-avid sites after induction had inferior OS even after further treatment which included an autologous stem cell transplant [24]. Schmidt et al. reported that the ^{123}I-mIBG status of the primary tumour site had no influence on outcomes while the metastatic burden identified by an mIBG-scan significantly determined prognostication [11,23,25,29].

Bleeker et al described two distinct metastatic patterns of ^{123}I-mIBG distribution in stage 4 NB [10]. Patients with MYCN amplified tumours were associated with focal metastatic lesions and had an improved event free survival (EFS) and OS [10]. The diffuse metastatic groups were associated with MYCN single copy numbers [10]. This correlation between the pattern of the ^{123}I-mIBG-scans and MYCN amplification could not be reproduced in our study, nor were the two diagnostic tests of prognostic significance.

LDH and/or ferritin can be used as surrogate markers to predict both treatment response and overall survival in LMIC where the detection of MYCN with either polymerase chain reaction (PCR) studies or fluorescent in vitro hybridization (FISH) is not available, [7, 27]. Pang et al. concluded that a higher LDH-value at diagnosis predicted a poorer treatment response with a poorer outcome [27]. We reproduced the prognostic significance of LDH in 2-year OS (p=0.004), but not in predicting mCR (p=0.072) although the effect of a small cohort size cannot be excluded. In our study the diagnostic ^{123}I-mIBG-scan did significantly predict outcomes for both mCR (p=0.009) and 2-year OS (p=0.004).

Stellenbosch University https://scholar.sun.ac.za

Yet the correlation of LDH and the diagnostic modified Curie scores did not significantly correlate (p=0.114). Morgenstern et al. reported that higher diagnostic ferritin predicted poorer treatment response and outcomes in NB [28]. This finding was reproduced in this study and the correlation between ferritin and modified Curie scores were significant (p=0.003). Metastatic neuroblastomas have higher infiltration of tumour associated macrophages than loco-regional tumours [29]. Patients with a higher metastatic burden will have an increase in the tumour associated inflammatory cells [29]. The higher metastatic burden will be reflected in the higher modified Curie score where the radio-isotopes are stored in NB-cells and imaged during an mIBG-scan [30]. Morgenstern et al. demonstrated that LDH and ferritin were of prognostic significance when an autologous stem cell transplant (ASCT) was part of the management [28]. The significance was not evaluated in the South African context, because only 3.7% of high-risk patients received an ASCT [21].

The study results prove the prognostic value of LDH and ferritin in risk stratification and can be used in in LMIC in the absence of access to mIBG-scans and MYCN studies. Ferritin can be used as a surrogate predictor for the modified Curie scores in predicting metastatic remission. Although ^{123}I-mIBG-scans are more sensitive and specific in evaluating metastatic response [11], ferritin is more affordable and readily available in LMIC. LDH and ferritin must be used in in conjunction with imaging and bone marrow aspiration to evaluate the degree of remission status. MYCN-amplification neither correlated with any aspect of the ^{123}I-mIBG scans nor did it significantly predict mCR or 2-year OS in our study. Yet non-secreting tumours are less likely to have an adrenal primary, bone metastasis or secreted catecholamine, but are more likely to be *MYCN*-amplified [31]. The utilisation of MYCN-amplification is used to identify a patient with higher risk in Stage 2 and 4s tumours, which normally are low-risk tumours, but have increase poor outcome if MYCN is amplified [7]. In the absence of MYCN-testing, LDH and ferritin can be used to determine risk stratification, but our findings should be validated in larger cohorts.

Limitations included retrospective data collection and a small cohort. Although the statistical analysis was based on the assumption of small cohorts, the significance of results may still be affected. This speaks to the urgent need for LMICs to initiate prospective data collection. The data were not adjusted for other prognostic factors when determining cut-point values. The modified Curie score is a validated system, but a small possibility of subjectivity in score allocations exists.

Conclusion

High levels of LDH, ferritin and the ^{123}I-mIBG scans were significantly associated with each other but did not predict mCR. On univariate analysis LDH, ferritin and the diagnostic ^{123}I-mIBG scans significantly predicted 2-yr OS. When adjusting for LDH and ferritin, the modified Curie-score was not a significant predictor for 2-yr OS. However, LDH remained the only significant predictor of 2-yr OS. MYCN-amplification neither correlated with any aspect of the ^{123}I-mIBG scans nor significantly predicted mCR or 2-yr OS. LMICs should use LDH and Ferritin in the management of neuroblastoma when there is no access to MIBG or MYCN studies.

References

1. Bhatnagar, S; Sarin, Y. Neuroblastoma: A Review of Management and Outcome. Indian J Pediatr. 2012; 79 (6):787-792.

Stellenbosch University https://scholar.sun.ac.za

2. Vik TA, Pfluger T, Kadota R, Castel V, Tulchinsky M, Farto JCA., et al. 123I-mIBG scintigraphy in patients with known or suspected neuroblastoma: Results from a prospective multicenter trial. Pediatric Blood & Cancer. 2009; 52(7): 784–790.
3. Papaioannou G. Neuroblastoma in childhood: review and radiological findings. Cancer Imaging. 2005; 5(1): 116–127.
4. Cangemi G, Reggiardo G, Barco S, et al. Prognostic value of ferritin, neuron-specific enolase, lactate dehydrogenase, and urinary and plasmatic catecholamine metabolites in children with neuroblastoma. Onco Targets Ther. 2012; 5:417-423.
5. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009; 324:1029–33.
6. Hann HW, Stahlhut MW, Evans AE. Source of increased ferritin in neuroblastoma: studies with concanavalin A-sepharose binding. Journal of the National Cancer Institute. 1986 Jun;76(6):1031-1033.
7. Parikh, N; Howard, S; Chantada, G; Israels, T; Khattab, M; Alcasabas, P; Lam, C; Faulkner, L; Park, J; London, W; Matthay, K: SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for neuroblastoma in low- and middle-income settings. Pediatr Blood Cancer 2015; 62(8): 1305-1316.
8. Huang M, Weiss WA. Neuroblastoma and MYCN. Cold Spring Harbor Perspectives in Medicine. 2013; 3(10): a014415–a014415. doi:10.1101/cshperspect. a014415.
9. Lee JW, Son MH, Cho HW, Ma YE, Yoo KH, et al. Clinical significance of MYCN amplification in patients with high-risk neuroblastoma. Pediatric Blood & Cancer. 2018; 65(10): e27257.
10. Bleeker G, van Eck-Smit BL, Zwinderman KH, Versteeg R, et al. MIBG scans in patients with stage 4 neuroblastoma reveal two metastatic patterns, one is associated with MYCN amplification and in MYCN-amplified tumours correlates with a better prognosis. European Journal of Nuclear Medicine and Molecular Imaging. 2014; 42(2): 222–230.
11. Yanik G, Parisi M, Shulkin B, et al.: Semiquantitative MIBG scoring as a prognostic indicator in patients with stage 4 neuroblastoma: a report from the Children's oncology group. J Nucl Med. 2013; 54 (4): 541-8.
12. Bar-Sever Z, Biassoni L, Shulkin B. et al. Guidelines on nuclear medicine imaging in neuroblastoma. Eur J Nucl Med Mol Imaging. 2018; 45(11), 2009–2024.
13. Iturralde MP. Production of radionuclides at the National Accelerator Centre Facility at Faure, Western Cape, South Africa. European Journal of Nuclear Medicine. 1996; 23(12): 1685–1686.
14. Brodeur G, Pritchard J, Berthold, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol. 1993;11(8):1466-1477.
15. Park J, Bagatell R, Cohn S, et al. Revisions to the International Neuroblastoma Response Criteria: A consensus statement from the National Cancer Institute Clinical Trials Planning Meeting. J Clin Oncol. 2017;35(22):2580-2587.
16. Eisenhauer E, Therasse P, Bogaert J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-247.
17. Tinsley HEA; Weiss DJ. Inter-rater reliability and agreement of subjective judgments. Journal of Counseling Psychology. 1975; 22, 358-376.
18. McHugh M. Interrater reliability: the kappa statistic. Biochem Med 2012; 22(3): 276-82.
19. SPSS citation: IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.

Stellenbosch University https://scholar.sun.ac.za

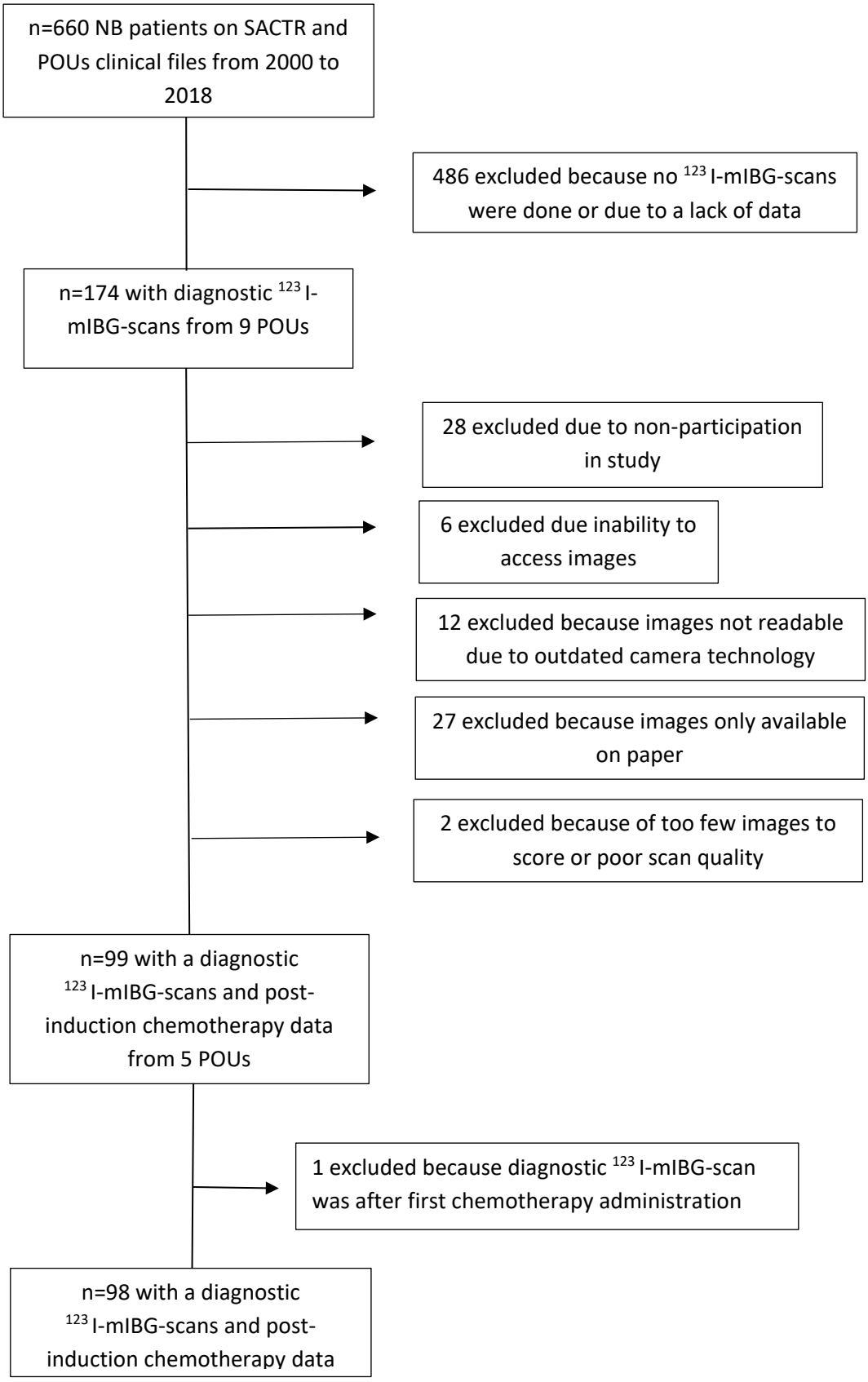
20. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993; 39(4): 561-577.
21. Van Heerden J, Hendricks M, Geel J, et al: Overall survival for neuroblastoma in South Africa between 2000 and 2014. Pediatr Blood Cancer 66: e27944, 2019.
22. Hosmer DW, Lemeshow S. Applied Logistic Regression, 2nd Ed. Chapter 5, John Wiley and Sons, New York, NY (2000), pp. 160-164.
23. Katzenstein H, Cohn S, Shore R et al. Scintigraphic Response by ^{123}I-Metaiodobenzylguanidine Scan Correlates with Event-Free Survival in High-Risk Neuroblastoma. J Clin Oncol. 2004; 22: 3909-3915.
24. Yanik GA, Parisi MT, Naranjo A, et al. Validation of Postinduction Curie Scores in High-Risk Neuroblastoma: A Children's Oncology Group and SIOPEN Group Report on SIOPEN/HR-NBL1. J Nucl Med. 2018; 59(3): 502-508.
25. Schmidt M, Simon T, Hero B, Schicha H, Berthold F. The prognostic impact of functional imaging with ^{123}I-mIBG in patients with stage 4 neuroblastoma >1 year of age on a high-risk treatment protocol: results of the German Neuroblastoma Trial NB97. Eur J Cancer. 2008; 44(11): 1552-1558.
26. Decarolis B, Schneider C, Hero B, et al.: Iodine-123 metaiodobenzylguanidine scintigraphy scoring allows prediction of outcome in patients with stage 4 neuroblastoma: results of the Cologne interscore comparison study. J Clin Oncol. 2013; 31 (7): 944-51.
27. Ladenstein R, Philip T, Lasset C, et al. Multivariate analysis of risk factors in stage 4 neuroblastoma patients over the age of one year treated with megatherapy and stem-cell transplantation: a report from the European Bone Marrow Transplantation Solid Tumor Registry. J Clin Oncol. 1998;16:953–965.
28. Pang QM, Li K, Ma LJ, Sun RP. Clinical research on neuroblastoma based on serum lactate dehydrogenase. J Biol Regul Homeost Agents. 2015;29(1):131-134.
29. Morgenstern DA, London WB, Stephens D, Volchenboum SL, et al. Prognostic significance of pattern and burden of metastatic disease in patients with stage 4 neuroblastoma: A study from the International Neuroblastoma Risk Group database. Eur J of Cancer. 2016; 65(9): 1–10.
30. Asgharzadeh S, Salo JA, Ji L, et al. Clinical significance of tumor-associated inflammatory cells in metastatic neuroblastoma. J Clin Oncol. 2012; 30(28): 3525–3532.
31. Pandit-Taskar N, Modak S. Norepinephrine Transporter as a Target for Imaging and Therapy. J Nucl Med. 2017; 58(Suppl 2): 39S-53S.
32. DuBois SG, Mody R, Naranjo A, et al. MIBG avidity correlates with clinical features, tumor biology, and outcomes in neuroblastoma: A report from the Children's Oncology Group. Pediatr Blood Cancer. 2017;64(11):10.1002/pbc.26545.

Stellenbosch University https://scholar.sun.ac.za

Figures and tables

Stellenbosch University https://scholar.sun.ac.za

Flow diagram: Cohort of patients diagnosed with neuroblastoma in South Africa between 2000-2018



Abbreviations: NB – neuroblastoma; SACTR – South African Children's Tumour Registry; POUs – Paediatric Oncology Units

Stellenbosch University https://scholar.sun.ac.za

Tables

Table 1: Spearman's rho correlation between tumour markers with continuous variables and diagnostic modified Curie scores with continuous variables

		LDH	Ferritin	Diagnostic modified Curie score
LDH	Correlation Coefficient	1.000	0.324	0.167
	P-value		0.004	0.103
	N	97	78	97
Ferritin	Correlation Coefficient	0.324	1.000	0.353
	P-value	0.004		0.001
	N	78	79	79
Diagnostic modified Curie score	Correlation Coefficient	0.167	0.353	1.000
	P-value	0.103	0.001	
	N	97	79	98

Table 2: Association between tumour markers (categorical variables) and diagnostic modified Curie scores (continuous variables) predicting post induction remission and survival

				95% CI		
	LDH	**N (%)**	**Median**	**Lower**	**Upper**	**P-value**
Diagnostic modified Curie score	<750	45 (46.4%)	3	1	17	0.029
	≥750	52 (53.6%)	7	3	22	
	Total	97				
	Ferritin	**N (%)**				
Diagnostic modified Curie score	<120	18 (18.4%)	3	1	3	<0.001
	≥120	61 (62.3%)	10	4	23	
	Total	98				
	MYCN	**N (%)**				
Diagnostic modified Curie score	Non-amplified	28 (45.9%)	7	3	21	0.518
	Amplified	33 (54.1%)	9	3	24	
	Total	61				

Table 3: Receiver operating characteristic curve's area under the curve (AUC) for the diagnostic and post induction modified Curie scores with mCR as outcome

				95% CI		
	N (%)	**AUC**	**Std. Error**	**Lower Bound**	**Upper Bound**	**P-value**
Diagnostic modified Curie scores	98 (100%)	0.658	0.055	0.549	0.766	0.012
Post induction chemotherapy modified Curie scores	42 (42.9%)	0.661	0.097	0.471	0.852	0.182

Abbreviations: CI – confidence interval

Stellenbosch University https://scholar.sun.ac.za

Table 4: Cross tabulation of tumour markers (categorical variables) and diagnostic modified MIBG scans (categorical variables) and the pattern of distribution to predict post induction complete metastatic remission.

			LDH			
			<750	**>750**	**Total**	**P-value**
Diagnostic modified Curie scores	≥7.0	N (%)	17 (37.8%)	28 (53.8%)	45 (46.4%)	0.114
	<7.0	N (%)	28 (62.2%)	24 (46.2%)	52 (53.6%)	
Total		N (%)	45 (100%)	52 (100%)	97 (100%)	
			LDH			
			<750	**>750**	**Total**	**P-value**
Pattern of distribution	Diffuse	N (%)	13 (32.5%)	29 (55.8%)	42 (45.7%)	0.026
	Focal	N (%)	27 (67.5%)	23 (44.2%)	50 (54.3%)	
Total		N (%)	40 (100%)	52 (100%)	92 (100%)	
			Ferritin			
			<120	**≥120**	**Total**	
Diagnostic modified Curie scores	≥7.0	N (%)	4 (22.2%)	38 (63.3%)	42 (53.2%)	0.003
	<7.0	N (%)	14 (77.8%)	23 (37.7%)	37 (46.8%)	
Total		N (%)	18 (100%)	61(100%)	79 (100%)	
			Ferritin			
			<120	**≥120**	**Total**	**P-value**
Pattern of distribution	Diffuse	N (%)	3 (20%)	35 (58.3%)	38 (50.7%)	0.008
	Focal	N (%)	12 (80%)	25 (41.7%)	37 (49.3%)	
Total		N (%)	15 (100%)	60 (100%)	75 (100.0%)	
			MYCN			
			NA	**Amplified**	**Total**	**p-value**
Diagnostic modified Curie scores	≥7.0	N (%)	14 (51.9%)	16 (50.0%)	30 (50.8%)	0.554
	<7.0	N (%)	13 (49.1%)	16 (50.0%)	29 (49.2%)	
Total		N (%)	27 (100%)	32 (100%)	59 (100%)	
			MYCN			
			NA	**Amplified**	**Total**	**P-value**
Pattern of distribution	Diffuse	N (%)	14 (50.0%)	19 (57.6%)	33 (54.1%)	0.887
	Focal	N (%)	14 (50.0%)	14 (42.4%)	28 (46.9%)	
Total		N (%)	28 (100%)	33 (100%)	61 (100%)	

Stellenbosch University https://scholar.sun.ac.za

Table 5: Univariate analysis of the categorical variables predicting post induction complete metastatic remission (mCR)

			mCR		Total	Pearson χ^2 P-value
			Yes	No		
LDH	<750	N (%)	19(42.2%)	26 (57.8%)	45 (100.0%)	0.072
	≥750	N (%)	13 (25.0%)	39 (75.0%)	52 (100.0%)	
	Total	N (%)	32 (33.0%)	65 (67.0%)	97 (100.0%)	
Ferritin	<120	N (%)	7 (38.9%)	11 (61.1%)	18 (100.0%)	0.372
	≥120	N (%)	17 (27.9%)	44 (72.1%)	61 (100.0%)	
	Total	N (%)	24 (30.4%)	55 (69.6%)	79 (100.0%)	
MYCN	NA	N (%)	12 (42.9%)	16 (57.1%)	28 (100.0%)	0.202
	Amplified	N (%)	9 (27.3%)	24 (72.7%)	33 (100.0%)	
	Unknown	N (%)	11 (29.7%)	26 (70.3%)	37 (100.0%)	
	Total	N (%)	32 (32.7%)	66 (67.3%)	98 (100.0%)	
Diagnostic modified Curie scores	≥7.0	N (%)	9 (19.6%)	37 (80.4%)	46 (100.0%)	0.009
	<7.0	N (%)	23 (44.2%)	29 (55.8%)	52 (100.0%)	
	Total	N (%)	32 (32.7%)	66 (67.3%)	98 (100.0%)	
Pattern of distribution	Diffuse	N (%)	12 (27.9%)	31 (72.1%)	43 (100.0%)	0.527
	Focal	N (%)	17 (34.0%)	33 (66.0%)	50 (100.0%)	
	Total	N (%)	29 (31.2%)	64 (68.8%)	93 (100.0%)	

Stellenbosch University https://scholar.sun.ac.za

Table 6: Univariate analysis categorical variables predicting 2-year overall survival (OS)

			Survival		Total	2-year OS	P-value
			Alive	Death			
LDH	<750	N (%)	12 (27.3%)	32 (72.7%)	44 (100.0%)	40.0%	0.004
	≥750	N (%)	3 (5.9%)	48 (94.1%)	51 (100.0%)	17.5%	
	Total	N (%)	15 (15.8%)	80 (84.2%)	95 (100.0%)		
Ferritin	<120	N (%)	6 (35.3%)	11 (64.7%)	17 (100.0%)	56.0%	0.005
	≥120	N (%)	5 (8.3%)	55 (91.7%)	60 (100.0%)	19.1%	
	Total	N (%)	11 (14.3%)	66 (85.7%)	77 (100.0%)		
MYCN	NA	N (%)	6 (22.2%)	21 (77.8%)	27 (100.0%)	25.0%	0.156
	Amplified	N (%)	3 (9.1%)	30 (90.9%)	33 (100.0%)	21.3%	
	Total	N (%)	9 (15.0%)	51 (85.0%)	60 (100.0%)		
Diagnostic modified Curie scores	≥7.0	N (%)	2 (4.4%)	43 (95.6%)	45 (100.0%)	23.0%	0.004
	<7.0	N (%)	13 (25.5%)	38 (74.5%)	51 (100.0%)	36.0%	
	Total	N (%)	15 (15.6%)	81 (84.4%)	96 (100.0%)		
Pattern of distribution	Diffuse	N (%)	3 (7.0%)	40 (93.0%)	43 (100.0%)	29.9%	0.595
	Focal	N (%)	11 (22.0%)	39 (78.0%)	50 (100.0%)	31.1%	
	Total	N (%)	14 (15.1%)	79 (84.9%)	93 (100.0%)		
Post induction modified Curie scores	>3.0	N (%)	0 (0.0%)	13 (100.0%)	13 (100.0%)	31.2%	0.052
	≤3.0	N (%)	7 (24.1%)	22 (75.9%)	29 (100.0%)	41.0%	
	Total	N (%)	7 (16.7%)	35 (83.3%)	42 (100.0%)		

NA – non-Amplified

Table 7: Multivariate Cox regression analysis for predicting time to mortality

	HR	95% CI		P-value
		Lower	Upper	
LDH < 750U/dl	0.39	0.21	0.73	0.003
Ferritin < 120g/dL	0.66	0.30	1.44	0.293
Diagnostic modified Curie scores > 6.5	0.94	0.53	1.66	0.837

Stellenbosch University https://scholar.sun.ac.za

Supplemental tables

Supplemental table 1: Diagnostic epidemiology and disease profile

Prognostic factor	**N (%)**
Total	98
Sex	
Male	49 (50.0%)
Female	49 (50.0%)
Age at diagnosis	
Range (months)	1.6 – 196.3
Mean (months)	43.2
Median (months)	28.9
0 -18 months	26 (27.0%)
18.1 - 60 months	51 (52.0%)
>60 months	21 (21.4%)
Primary tumour	
Adrenal	66 (67.3%)
Abdominal	14 (14.3%)
Pelvic	2 (2.0%)
Paraspinal	8 (8.2%)
Cervical	6 (6.2%)
Other sites	2 (2.0%)
LDH	
<750 U/L	45 (42.9%)
≥750 U/L	52 (57.1%)
Unknown	1 (1.0%)
Ferritin	
<120 ng/dl	18 (18.4%)
≥120 ng/dl	61 (62.2%)
Unknown	19 (19.4%)
MYCN	
Not amplified	28 (28.6%)
Amplified	33 (33.7%)
Unknown	37 (37.3%)
Pathology	
Favourable histology	13 (13.3%)
Unfavourable histology	31 (31.6%)
Bone marrow cytology	54 (55.1%)
INSS	
1, 2, 4s	2 (2.0%)
3	14 (14.3%)
4	82 (83.7%)
Risk classification	
VLR, LR	7 (7.1%)
IR	5 (5.1%)
HR	84 (85.7%)
Unknown	2 (2.0%)
Hospital investigator diagnostic modified Curie scores	

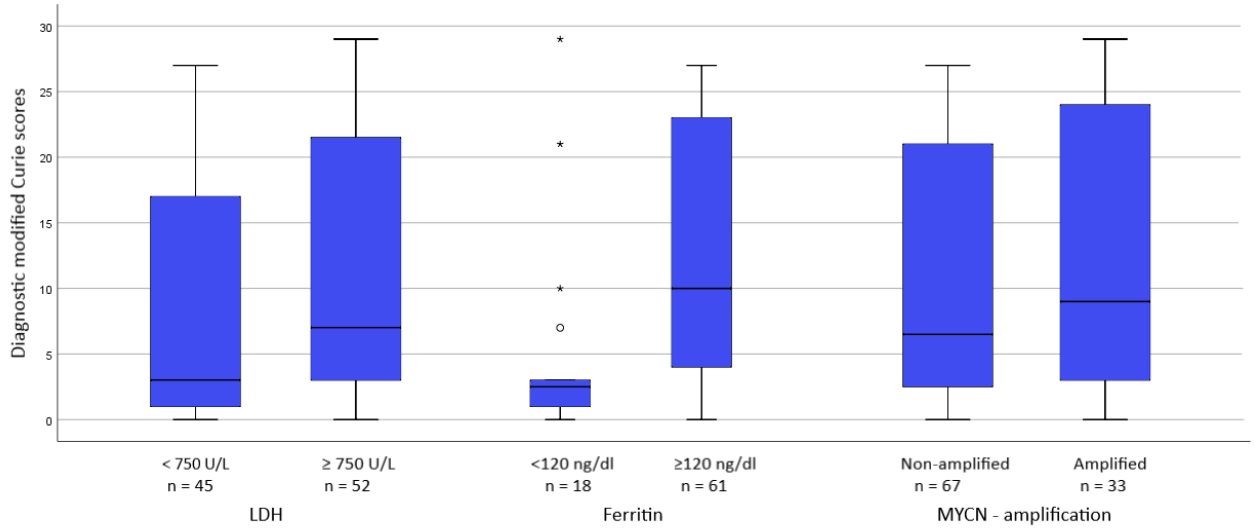
Stellenbosch University https://scholar.sun.ac.za

Total	98 (100%)
Range	0 – 29
Mean	10
Median	5
Hospital investigator post induction modified Curie scores	
Total	42 (42.9%)
Range	0 – 20
Mean	7
Median	1
Hospital investigator diagnostic ^{123}I-mIBG pattern of distribution	
Focal	50 (51.0%)
Diffuse	43 (43.9%)
N/A	5 (5.1%)
Post induction remission	
Remission	32 (32.7%)
Not in remission	66 (67.3%)

Abbreviations: INSS – International neuroblastoma staging system; VLR – very low risk; LR – low risk; IR – intermediate risk; HR – high risk

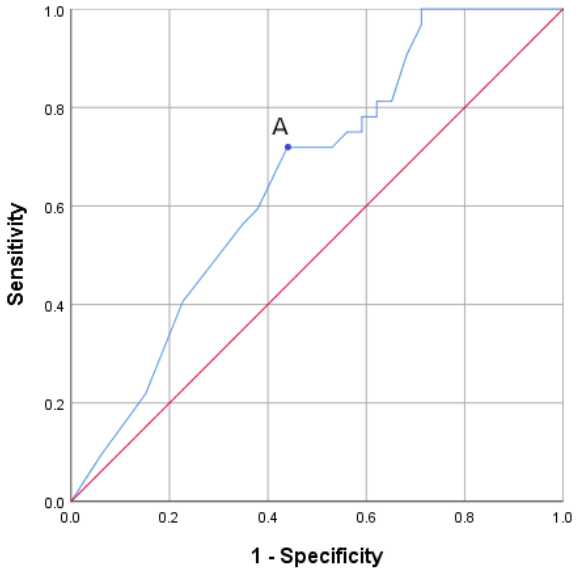
Figures

Figure 1: Box and Whisker plots of the correlation between categorical variables of tumours markers associated with neuroblastoma and the continuous variables of the diagnostic modified Curie scores



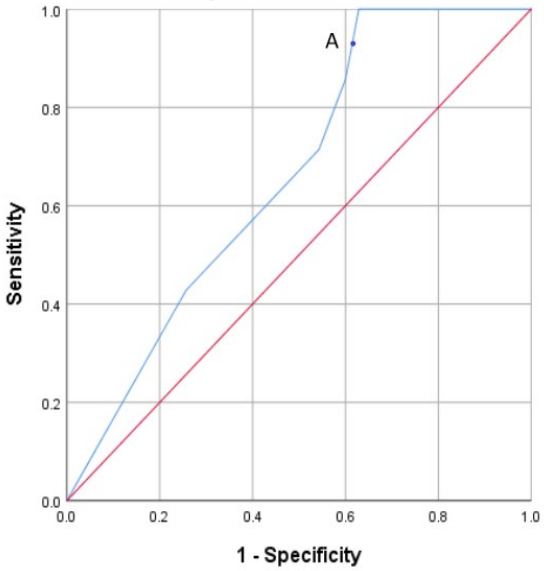
Stellenbosch University https://scholar.sun.ac.za

Figure 2: ROC curve for the hospital investigators modified Curie scores (AUC = 0.657; p=0.012)



A – sensitivity of 71.9%; specificity of 56.1%; modified Curie score of 7.0

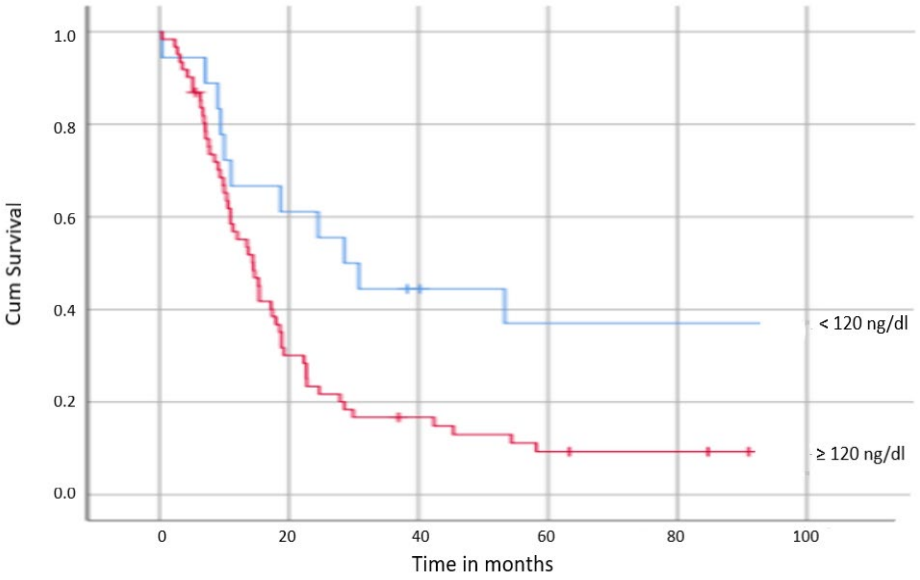
Figure 3: ROC curve for the hospital investigators post induction chemotherapy modified Curie scores (AUC = 0.661; p=0.182)



A – sensitivity of 92.6%; specificity of 38.6%; modified Curie score of 3.0

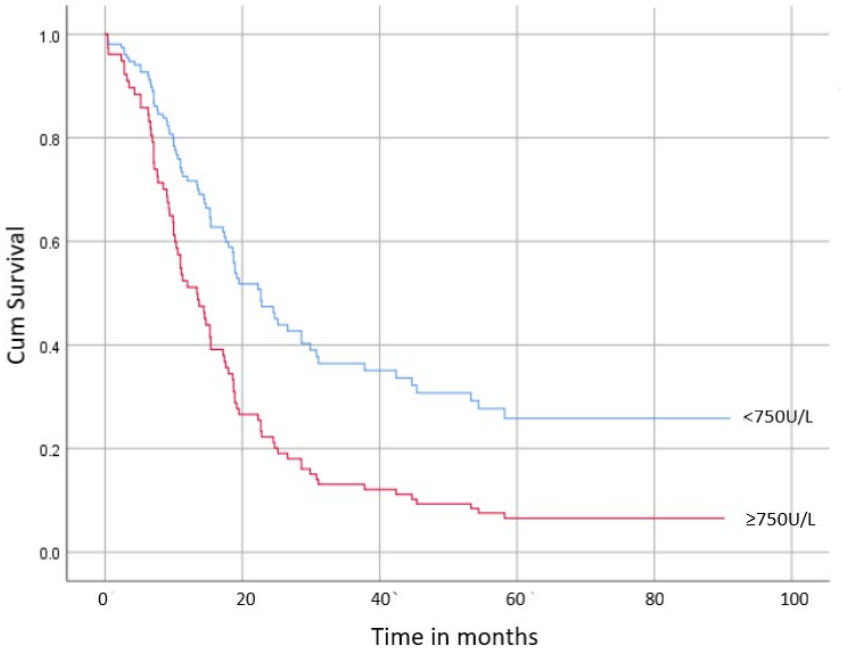
Stellenbosch University https://scholar.sun.ac.za

Figure 4: The Kaplan-Meier curve for ferritin in predicting 2-year overall survival on univariate analysis (p=0.005)



Total (n) 79; < 120 ng/dl n (%) = 18 (37.8%); ≥ 120 ng/dl n (%) = 61 (62.2%)

Figure 5: The Kaplan-Meier curve for LDH in predicting 2-year overall survival on multivariate analysis (p=0.003)



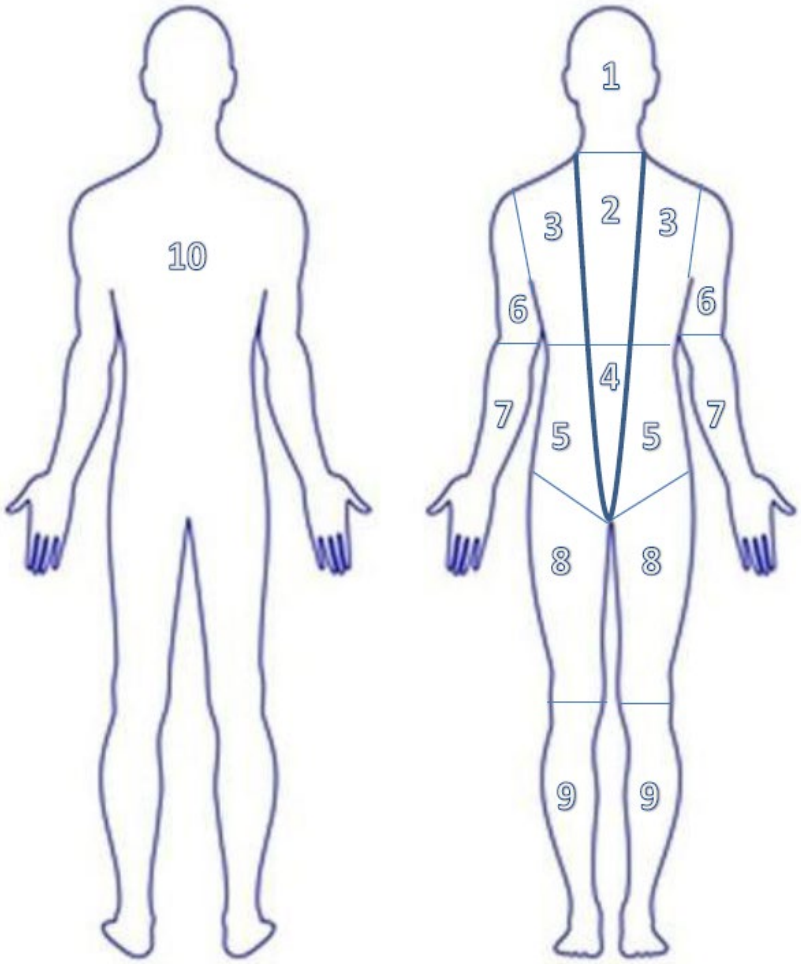
Total (n) 97; < 750 U/L n (%) = 45 (42.9%); ≥ 750 U/L n (%) = 52 (57.1%)

Stellenbosch University https://scholar.sun.ac.za

Appendices

Appendix 1: Modified Curie score

The scoring system divides the skeleton into 9 compartments.
A 10th compartment represents the soft tissue lesions.



Scoring of MIBG scan
Level of lesion certainty:

0 = unknown
1 = possible
2 = probable
3 = definite

Extent of uptake:

0 = no uptake/ no foci per segment
1 = one focal lesion per segment
2 = more than one focal lesion per segment
3 = diffuse involvement (≥50% of segment involved)

Intensity of uptake:

0 = no sites of uptake
1 = doubtful uptake
2 = obvious but mild uptake
3 = obvious and intense uptake

Soft-tissue lesions scoring system:

0 = no MIBG involvement
1 = one MIBG-avid soft-tissue lesion present
2 = more than one MIBG-avid soft-tissue lesion present
3 = MIBG avidity in a soft-tissue lesion that occupies 50% of the chest or abdomen

*Maximum attainable score is 30
*Focal uptake: uptake with clearly defined margins
* Diffuse uptake: is indistinguishable from background

Stellenbosch University https://scholar.sun.ac.za

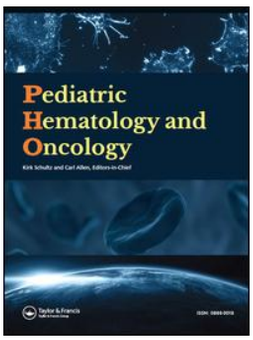
CHAPTER 7

Induction chemotherapy for high-risk Neuroblastoma in South African children (2019)
Ethics no: S18/07/138

Reference: Van Heerden J, Geel J, Hendricks M, et al. The evaluation of induction chemotherapy regimens for high-risk neuroblastoma in South African children. Pediatric Hematology and Oncology. 2020; 37: 4(1), 300-313. https://doi.org/10.1080/08880018.2020.1717698
(Impact factor 1.232)

The development of induction chemotherapy recommendations for the treatment of high-risk neuroblastoma has been characterised by increased time and toxicity intensity. The second factor related to the inclusion of doxorubicin in regimens. In South Africa all POUs differ in the level of supportive care that can be provided, the bed capacity for longer admissions of chemotherapy and malnutrition co-morbidities. With data over 14 years and several different induction regimens that were administrated in various POUs, we evaluated the data to formulate recommendations on the induction regimen for a national protocol.

Stellenbosch University https://scholar.sun.ac.za



Pediatric Hematology and Oncology

ISSN: 0888-0018 (Print) 1521-0669 (Online) Journal homepage: https://www.tandfonline.com/loi/ipho20

The evaluation of induction chemotherapy regimens for high-risk neuroblastoma in South African children*

Jaques Van Heerden, Jennifer Geel, Marc Hendricks, Kristien Wouters, Ané Büchner, Gita Naidu, G. P. Hadley, Jan Du Plessis, Barry Van Emmenes, Anel Van Zyl, Johani Vermeulen & Mariana Kruger

To cite this article: Jaques Van Heerden, Jennifer Geel, Marc Hendricks, Kristien Wouters, Ané Büchner, Gita Naidu, G. P. Hadley, Jan Du Plessis, Barry Van Emmenes, Anel Van Zyl, Johani Vermeulen & Mariana Kruger (2020): The evaluation of induction chemotherapy regimens for high-risk neuroblastoma in South African children*, Pediatric Hematology and Oncology, DOI: 10.1080/08880018.2020.1717698

To link to this article: https://doi.org/10.1080/08880018.2020.1717698

Published online: 19 Feb 2020.

Submit your article to this journal

Article views: 21

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ipho20

Stellenbosch University https://scholar.sun.ac.za

PEDIATRIC HEMATOLOGY AND ONCOLOGY
https://doi.org/10.1080/08880018.2020.1717698

Taylor & Francis
Taylor & Francis Group

Check for updates

The evaluation of induction chemotherapy regimens for high-risk neuroblastoma in South African children*

Jaques Van Heerden[a]†, Jennifer Geel[b], Marc Hendricks[c], Kristien Wouters[d], Ané Büchner[e], Gita Naidu[f], G. P. Hadley[g], Jan Du Plessis[h], Barry Van Emmenes[i], Anel Van Zyl[a], Johani Vermeulen[j], and Mariana Kruger[a]

[a]Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa; [b]Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa; [c]Haematology Oncology Service, Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Cape Town, Red Cross War Memorial Children's Hospital, Cape Town, South Africa; [d]Clinical Trial Center, CRC Antwerp, Antwerp University Hospital, University of Antwerp, Edegem, Belgium; [e]Paediatric Haematology and Oncology, Department of Paediatrics, University of Pretoria, Steve Biko Academic Hospital, Pretoria, South Africa; [f]Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Chris Hani Baragwanath Academic Hospital, Johannesburg, South Africa; [g]Department of Paediatric Surgery, Faculty of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa; [h]Department of Paediatrics, Faculty of Health Sciences, University of the Free State, Division of Paediatric Haematology and Oncology, Universitas Hospital, Bloemfontein, South Africa; [i]Division of Paediatric Haematology and Oncology, Department of Paediatrics, Frere Hospital, East London, South Africa; [j]Paediatric Haematology Oncology, Department of Paediatrics and Child Health, Port Elizabeth Provincial Hospital, Walter Sisulu University, Port Elizabeth, South Africa

ABSTRACT

Achieving remission after induction therapy in high-risk neuroblastoma (HR-NB) is of significant prognostic importance. This study investigated remission after induction-chemotherapy using three standard neuroblastoma protocols in the South African (SA) setting. Retrospective data of 261 patients with HR-NB diagnosed between January 2000 and December 2016, who completed induction chemotherapy with standard treatment protocols were evaluated. The treatment protocols were either OPEC/OJEC or the St Jude NB84 protocol (NB84) or rapid COJEC (rCOJEC). The postinduction metastatic complete remission (mCR) rate, 2-year overall survival (OS) and 2-year event free survival (EFS) were determined as comparative denominators. The majority (48.3%; $n = 126$) received OPEC/OJEC, while 70 patients received (26.8%) rCOJEC and 65 (24.9%) NB84. Treatment with NB84 had the best mCR rate (36.9%), followed by OPEC/OJEC (32.5%) and rCOJEC (21.4%). The 2-year OS of treatment with NB84 was 41% compared to OPEC/OJEC (35%) and rCOJEC (24%) ($p = 0.010$). The 2-year EFS of treatment with NB84 was 37% compared to OPEC/OJEC (35%) and rCOJEC (18%) ($p = 0.008$). OPEC/OJEC had the least treatment-related deaths (1.6%) compared to rCOJEC

ARTICLE HISTORY
Received 31 October 2019
Revised 30 December 2019
Accepted 14 January 2020

KEYWORDS
High risk; induction chemotherapy; neuroblastoma; South Africa

CONTACT Jaques van Heerden Jaques.vanheerden@uza.be Department of Paediatric Haematology and Oncology, University Hospital of Antwerp, Wilrijkstreet 10, Edegem 2650, Belgium.
*For the South African Children's Cancer Study Group
†The first author is a PhD student at Stellenbosch University, South Africa, but a permanent staff member of the Antwerp University Hospital, Antwerp, Belgium.

Supplemental data for this article can be accessed at https://doi.org/10.1080/08880018.2020.1717698
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ipho.

© 2020 Taylor & Francis Group, LLC

Stellenbosch University https://scholar.sun.ac.za

(7.1%) and NB84 (7.5%) ($p = 0.037$). On multivariate analysis LDH ($p = 0.023$), ferritin ($p = 0.002$) and INSS stage ($p = 0.006$) were identified as significant prognostic factors for OS. The induction chemotherapy was not significant for OS ($p = 0.18$), but significant for EFS ($p = 0.08$) Treatment with NB84 achieved better mCR, OS and EFS, while OPEC/OJEC had the least treatment-related deaths. In resource-constrained settings, OPEC/OJEC is advised as induction chemotherapy in HR-NB due to less toxicity as reflected in less treatment-related deaths.

Introduction

Achieving metastatic remission after induction chemotherapy in high-risk (HR) neuroblastoma (NB) is of prognostic significance, even though the treatment is of high intensity with potentially severe toxicity.[1,2] NB induction chemotherapy is characterized by the delivery of increasingly intensive multimodal therapies. European approaches under the EU-20592 or CCLGNB-1990-11 protocols have studied decreasing time by reducing administration of OPEC/OJEC from 28-day cycles to 21-day cycles.[3] Rapid COJEC (rCOJEC) chemotherapy is highly intensive therapy, administered every 10 days regardless of toxicity.[3] Although this ensures that a patient reaches the consolidation treatment phase sooner, there is a greater likelihood of chemotherapy-induced toxicity.[4]

South African doxorubicin-containing protocols approaches are based on North American protocols such as the Memorial Sloan Kettering Hospital N6 protocol,[5] and the St Jude NB84 protocol.[5] In the N6 protocol, seven cycles have resulted in either complete remission (CR) or very good partial response (VGPR) in 87% of patients. In the N7 and N8 protocols, a reduction from seven to five cycles of chemotherapy have resulted in outcomes of 79% CR or VGPR and less toxicity in 87 patients.[5]

When comparing the European induction protocols OPEC/OJEC and rCOJEC, a Cochrane review has concluded there is no clear difference regarding the efficacy obtaining a complete response, treatment-related mortality, overall survival and event-free survival.[4] It was concluded that prospective trials are needed as there is low level of evidence and due to the changes in risk classifications during the trial periods.[4] The review relied mainly on the CCLG-ENSG-5 randomized controlled trial of 262 patients with HR-NB.[6] In this study, 132 patients were randomized to receive OPEC/OJEC and 130 patients to receive rCOJEC induction chemotherapy.[6] Pearson et al. concluded that there was an increasing difference in EFS after three years with the rCOJEC induction protocol.[6] With the same cumulative dose, rCOJEC at 10-day cycles had better 10-year EFS (27%) versus a 21-day cycle (18%) ($p = 0.085$). The hypothesis was that earlier initiation of myeloablative therapy contributed to improved outcome[6] and could benefit patients in countries with access to autologous stem cell transplant services.

Limited information is available comparing doxorubicin-containing and nondoxorubicin-containing regimens. The objective of the SIOPEN randomization of modified N5-MSKCC induction protocol with rCOJEC in the HR-NBL-1.7/SIOPEN study was to compare these regimens.[7] The study concluded that rCOJEC was less toxic and there was no difference in the postinduction remission rates, OS and EFS.[7]

In resource-limited settings, with suboptimal supportive care, high-intensity chemotherapy poses a risk of increased morbidity and mortality.[8] Challenges include

Stellenbosch University https://scholar.sun.ac.za

inconsistent access to blood products, limited antimicrobial agents, access to intensive care units and the high chemotherapy costs.[8] Malnutrition and advanced disease also impact on the choice of regimen as they both may independently contribute to poorer outcomes.[8] It is important to select a regimen with the highest remission rate and the most favorable toxicity profile for the local setting.[8]

The treatment of HR-NB in SA has been administered according to various protocols and institutional experience.[9] This study investigated remission after standard induction NB chemotherapy in SA POUs as the South African Children's Cancer Study Group (SACCSG) aims to create a standardized prospective treatment protocol based on evidence generated by retrospective studies regarding management of NB in SA.

Materials and methods

Patient population

South Africa is a middle-income country with marked disparities between the resources of POUs. Patients with HR-NB were identified retrospectively from 10 dedicated pediatric oncology units (POUs). The study population included new cases of HR-NB diagnosed from January 2000 to December 2016. High-risk disease was defined by the presence of metastatic disease, unfavorable histology, a raised serum LDH and ferritin, amplified MYCN and adverse genetics based on the Children's Oncology Group (COG) classification system (supporting information Appendix A) and the International Neuroblastoma Risk Group (INRG) consensus pretreatment classification scheme (supporting information Appendix B). Cases were confirmed by core biopsy, fine needle aspirate or NB-defining imaging with NB infiltration of the bone marrow and elevated urine catecholamine levels.

Method

There were 354 patients included with high risk (HR) disease, of whom 93 were excluded, either because of treatment abandonment during induction, or because they were palliated from diagnosis, or the regimen cohorts were too small to be representative and/or because they were treated with a nonstandard induction regimen for HR-NB. The 261 remaining patients received either St Jude NB84 protocol, OPEC/OJEC or rCOJEC as induction regimens (flow diagram Figure 1).

Data collection and analysis

Researchers from participating centers conducted retrospective chart reviews and entered data on Excel spreadsheets after ethical approval was granted. The data were linked to a unique study number and data analysis was anonymous.

Staging was determined according to the International Neuroblastoma Staging System (INSS). Metastatic remission was defined as complete remission (mCR) according to the International Neuroblastoma Response Criteria (INRC) revised in 1993 and 2012 (supporting information Appendix C).[10,11] Death before the end of induction was defined as induction failure. Nutritional parameters were applied according to WHO definitions.[12]

Stellenbosch University https://scholar.sun.ac.za

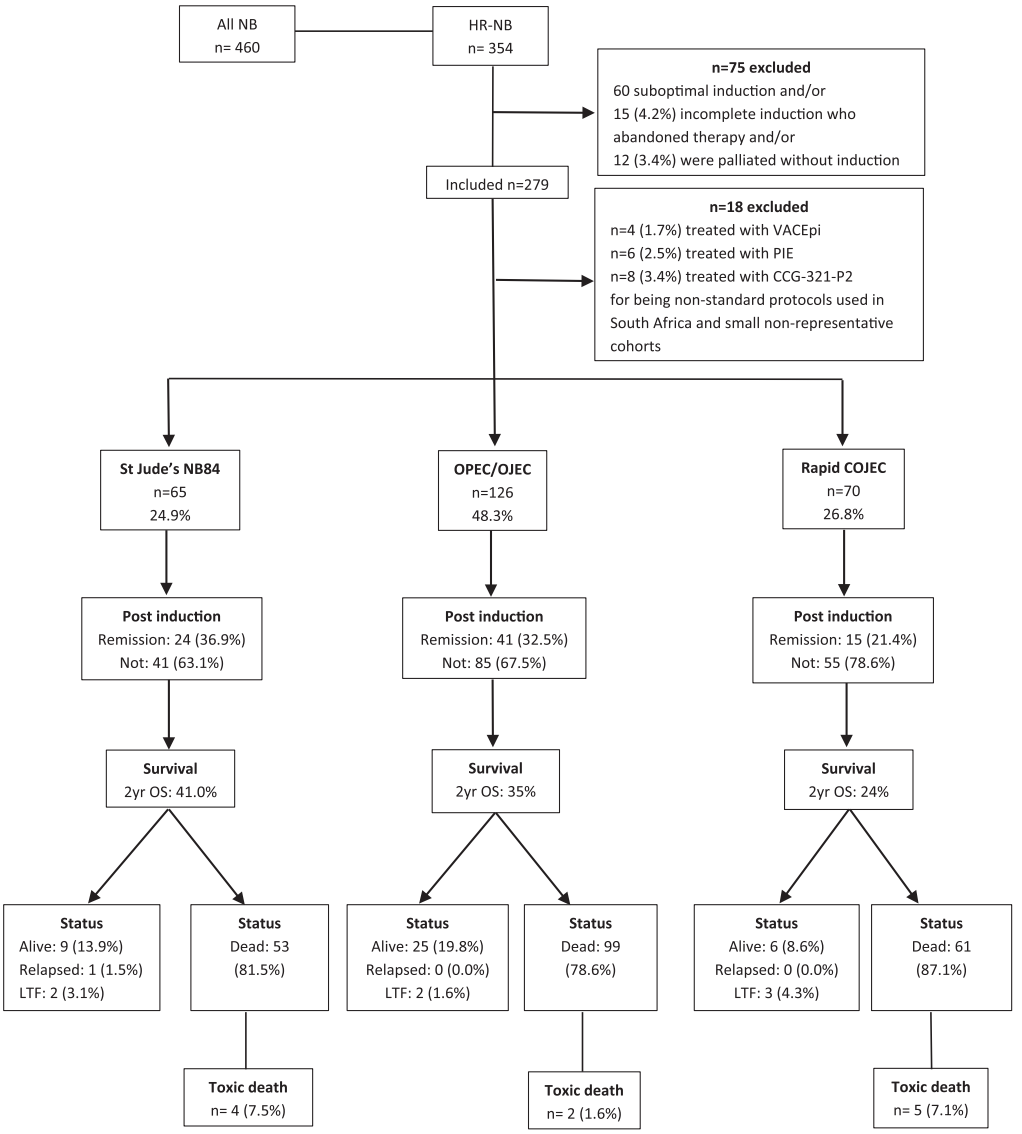


Figure 1. Flow diagram 1: Induction chemotherapy for high-risk neuroblastoma (HR-NB) in South Africa 2000–2016.

Abandonment of treatment was defined as the failure to start therapy or complete curative therapy after missing therapy for four or more consecutive weeks. Patients considered to be lost-to-follow-up were defined as those who did not return for follow-up after curative treatment for one year and who were unreachable despite efforts to contact them.

Chemotherapy regimens

Induction regimens included OPEC/OJEC (Carboplatin, Cisplatin, Etoposide, Cyclophosphamide and Vincristine),[4] the St Jude NB84 protocol (Cisplatin, Etoposide, Doxorubicin

Stellenbosch University https://scholar.sun.ac.za

and Cyclophosphamide)[13] and rCOJEC (Carboplatin, Cisplatin, Etoposide, Cyclophosphamide and Vincristine).[4]

Statistical analysis

All analyses were performed in R version 3.4.4 (R foundation for Statistical Computing, Vienna, Austria).[14] Data were summarized as percentages and compared between subgroups using the chi-squared test (χ^2). EFS and OS were computed and visualized by Kaplan-Meier curves. Median EFS and OS, together with 95% confidence intervals (CI) were reported.

Differences between survival curves by chemotherapy group were assessed by log-rank test. Additionally, simple and multiple Cox regression models were built to determine the effect of various prognostic factors. A full multiple Cox regression model demonstrated the effect of chemotherapy on EFS and OS, respectively, after correction for the other prognostic factors. Similarly, simple and multiple logistic regression models were constructed to assess the effect of chemotherapy on metastatic complete remission while correcting for other factors.

Due to the retrospective methodology, numerous data on prognostic factors were not available and stated per category. Assuming data were missing at random, multiple imputation with chained equations were performed to correct for missing data. The imputation models included all variables in the analysis. Ten imputed data sets were generated and analyzed in Cox and logistic regression models as described above. Results of the imputed data analyses were pooled to obtain parameter estimates and confidence intervals.

Descriptive and simple comparisons of patients with or without complete metastatic remission were on the raw un-imputed data, while all models were based on multiple imputation, as stated in each table.

Results

Two hundred and sixty-one patients were eligible for inclusion in the analysis: 126 (48.3%) treated with OPEC/OJEC, 70 (26.8%) with rCOJEC and 65 (24.9%) were treated with the NB84 protocol. Table 1 provides the demographic data, the high-risk factors per treatment protocol and disease profile of the groups. There was a slight male predominance with a male:female ratio of 1:0.89. The majority (58.2%) of patients were in the 18–60 months category and median age was 36.1 months (interquartile range 20.2–55.7).

Outcomes

A total of 80 (30.7%) HR patients were in mCR post-induction chemotherapy. The highest mCR was obtained by treatment with NB84 (36.9%), followed by OPEC/OJEC (32.5%) and rCOJEC with 21.4% ($p = 0.12$). Treatment with NB84 had the best 2-year OS of 41% (95% CI, 30%–56%) compared to OPEC/OJEC with 35% (95% CI, 27.0%–45.0%), while OJEC/OPEC performed better than rCOJEC with 24% (95% CI, 16.0%–38.0%, $p = 0.010$) (see Figure 2). Treatment with NB84 had superior 2-year EFS

Stellenbosch University https://scholar.sun.ac.za

Table 1. Disease profiles per cohort.

	Total N (%)	St Jude NB84 N (%)	OPEC/OJEC N (%)	Rapid COJEC N (%)
Sex				
Male	138 (52.9%)	31/65 (47.7%)	66/126 (52.4%)	41/70 (58.6%)
Female	123 (47.1%)	34/65 (52.3%)	60/126 (47.6%)	29/70 (41.4%)
Age				
0 – 18 m	51 (19.5%)	14/65 (21.5%)	28/126 (22.2%)	9/70 (12.9%)
18.1 – 60 m	152 (58.2%)	31/65 (47.7%)	76/126 (60.3%)	45/70 (64.3%)
> 60 m	58 (22.2%)	20/65 (30.8%)	22/126 (17.5%)	16/70 (22.9%)
INSS				
Stage 4	223 (85.4%)	48/65 (73.8%)	111/126 (88.1%)	64/70 (91.4%)
Other stage	38 (14.6%)	17/65 (26.2%)	15/126 (11.9%)	6/70 (8.6%)
LDH				
< 750	87 (33.3%)	34/65 (52.3%)	33/122 (27.0%)	20/62 (32.3%)
> 750	162 (62.1%)	31/65 (47.7%)	89/122 (73.0%)	42/62 (67.7%)
Unknown	12 (4.6%)			
Ferritin				
< 120	50 (19.2%)	18/52 (34.6%)	25/88 (28.4%)	7/49 (14.3%)
> 120	139 (53.3%)	34/52 (65.4%)	63/88 (71.6%)	42/49 (85.7%)
Unknown	72 (27.6%)			
INPC				
Favorable	27 (10.3%)	1/8 (12.5%)	23/85 (27.1%)	3/38 (7.9%)
Unfavorable	104 (39.8%)	7/8 (87.5%)	62/85 (72.9%)	35/38 (92.1%)
Unknown	130 (49.8%)			
MYCN				
Nonamplified	38 (14.6%)	4/11 (36.4%)	26/81 (32.1%)	8/25 (32.0%)
Amplified/extra copies	79 (30.3%)	7/11 (63.6%)	55/81 (67.9%)	17/25 (68.0%)
Unknown	144 (55.2%)			
WFA				
> -1 SD	116 (44.4%)	20/45 (44.4%)	64/94 (68.1%)	32/55 (58.2%)
-1SD to -2 SD	41 (15.7%)	8/45 (17.8%)	19/94 (20.2%)	14/55 (25.5%)
< -2SD	37 (14.2%)	17/45 (37.8%)	11/94 (11.7%)	9/55 (16.4%)
Unknown	67 (25.7%)			
HFA				
> -1 SD	97 (37.2%)	16/45 (35.6%)	59/93 (63.4%)	22/52 (42.3%)
-1SD to -2 SD	30 (11.5%)	6/45 (13.3%)	14/93 (15.1%)	10/52 (19.2%)
< -2SD	63 (24.1%)	23/45 (51.1%)	20/93 (21.5%)	20/52 (38.5%)
Unknown	71 (27.0%)			

LDH – lactate dehydrogenase; INPC – International neuroblastoma pathology classification; INSS – International neuroblastoma staging system; WFA – weight for age (z-score); HFA – height for age (z-score); SD – standard deviations.

of 37% (95% CI, 26%–52%) compared to OPEC/OJEC with 35% (27–45%) and rCOJEC at 18% (95% CI, 10%–32%) ($p = 0.008$) (see Figure 3) (see flow diagram Figure 1).

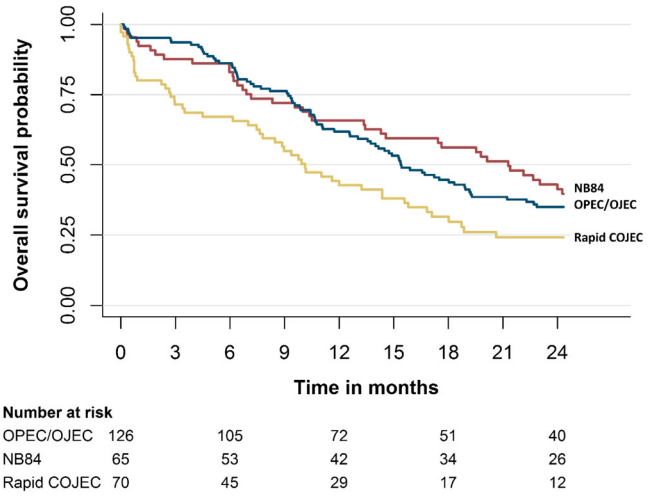
Toxicity

The least treatment-related deaths were seen in patients treated with OPEC/OJEC (1.6%), compared to the St Jude NB84 protocol with 7.5% and rCOJEC with 7.1% ($p < 0.05$). There were no documented doxorubicin cardiac-related deaths in the St Jude NB84 protocol group (see flow diagram 1).

Prognostic factors

With multivariate analysis (see Table 2), statistically significant differences were demonstrated in the remission rates associated with age > 18 months ($p = 0.038$), INSS stage 4 ($p < 0.001$), ferritin > 120 ng/dl ($p < 0.001$), and unfavorable pathology according to the

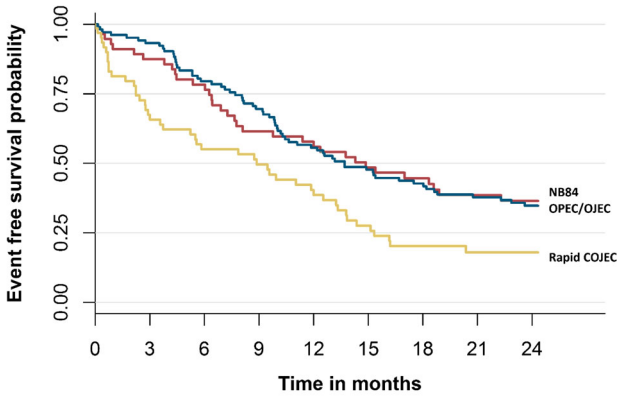
Stellenbosch University https://scholar.sun.ac.za



Number at risk

OPEC/OJEC	126	105	72	51	40
NB84	65	53	42	34	26
Rapid COJEC	70	45	29	17	12

Figure 2. Kaplan-Meier curves of the two-year OS of the induction regimens ($p = 0.010$).



Number at risk

OPEC/OJEC	126	81	56	43	35
NB84	65	42	31	22	18
Rapid COJEC	70	31	22	9	8

Figure 3. Kaplan-Meier curves of the two-year EFS of the induction regimens ($p = 0.008$).

International Neuroblastoma Pathology Classification (INPC) ($p = 0.023$). Nonsignificant differences were demonstrated between induction chemotherapy regimens with better remission achieved with NB84 than OPEC/OJEC ($p = 0.12$). rCOJEC had the worst outcomes ($p = 0.12$). Multivariate risk factor analysis confirmed that ferritin >120 ng/dL ($p = 0.051$) and INSS stage 4 ($p < 0.002$) were significant predictors of achieving mCR. The choice of induction chemotherapy regimen was not significant to achieve remission ($p = 0.65$). Significant prognostic factors associated with OS (see Table 4) were INSS stage 4 ($p = 0.006$) and ferritin > 120 ng/dl ($p = 0.002$), LDH >750 ($p = 0.023$) and low WFA ($p = 0.081$). The particular induction chemotherapy protocol was not shown to influence survival ($p = 0.18$). Significant prognostic factors, on univariate and multivariate analyses, associated with EFS (see Table 5) were INSS stage 4 ($p = 0.017$), ferritin > 120 ng/dl ($p < 0.001$) and the induction chemotherapy regimen ($p = 0.08$).

Stellenbosch University https://scholar.sun.ac.za

Table 2. Multivariate analyses with complete metastatic remission as main denominator.

	Total N (%)	Remission N (%)	No remission N (%)	*p*-Value
Sex				0.20
Male	138 (52.9%)	37/138 (26.8%)	101/138 (73.2%)	
Female	123 (47.1%)	43/123 (35.0%)	80/123 (65.0%)	
Age				0.038
0 – 18 m	51 (19.5%)	23/51 (45.1%)	28/51 (54.9%)	
18.1 m – 5 y	152 (58.2%)	43/152 (28.3%)	109/152 (71.7%)	
> 5 y	58 (22.2%)	14/58 (24.1%)	44/58 (75.9%)	
INSS				<0.001
Stage 4	223 (85.4%)	56/223 (25.1%)	167/223 (74.9%)	
Other stage	38 (14.6%)	24/38 (63.2%)	14/38 (36.8%)	
LDH				0.16
< 750	87 (33.3%)	33/87 (37.9%)	54/87 (62.1%)	
> 750	162 (62.1%)	46/162 (28.4%)	116/162 (71.6%)	
Unknown	12 (4.6%)			
Ferritin				<0.001
< 120	50 (19.2%)	27/50 (54.0%)	23/50 (46.0%)	
> 120	139 (53.3%)	36/139 (25.9%)	103/139 (74.1%)	
Unknown	72 (27.6%)			
INPC				0.023
Favorable	27 (10.3%)	15/27 (55.6%)	12/27 (44.4%)	
Unfavorable	104 (39.8%)	31/104 (29.8%)	73/104 (70.2%)	
Unknown	130 (49.8%)			
MYCN				0.37
Nonamplified	38 (14.6%)	17/38 (44.7%)	21/38 (55.3%)	
Amplified/extra copies	79 (30.3%)	27/79 (34.2%)	52/79 (65.8%)	
Unknown	144 (55.2%)			
WFA				0.47
> -1 SD	116 (44.4%)	39/116 (33.6%)	77/116 (66.4%)	
-1SD to -2 SD	41 (15.7%)	15/41 (36.6%)	26/41 (63.4%)	
< -2SD	37 (14.2%)	9/37 (24.3%)	28/37 (75.7%)	
Unknown	67 (25.7%)			
HFA				0.76
> -1 SD	97 (37.2%)	29/97 (29.9%)	68/97 (70.1%)	
-1SD to -2 SD	30 (11.5%)	11/30 (36.7%)	19/30 (63.3%)	
< -2SD	63 (24.1%)	21/63 (33.3%)	42/63 (66.7%)	
Unknown	71 (27.0%)			
Chemo regimen				0.12
St Jude NB84	65 (24.9%)	24/65 (36.9%)	41/65 (63.1%)	
OPEC/OJEC	126 (48.3%)	41/126 (32.5%)	85/126 (67.5%)	
Rapid COJEC	70 (26.8%)	15/70 (21.4%)	55/70 (78.6%)	

LDH – lactate dehydrogenase; INPC – International neuroblastoma pathology classification; INSS – International neuroblastoma staging system; WFA – weight for age (z-score); HFA – height for age (z-score); SD – standard deviations.

Nutrition

Patients with a weight for age (WFA) above the -2SD z-score had a slightly improved, though nonsignificant remission rate (>-1SD with 33.6%; -1SD to -2 SD with 36.6%) compared to wasted patients (24.3% for WFA below -2SD z-score) ($p = 0.47$). Body mass index for age and weight for height were not statistically significant as prognostic factors for survival (see Table 2).

Limitations of the study

The retrospective nature of the data limits the study because of the absence of randomization. Treatment was not standardized as regimens were based on institutional preference and disease severity. Different POUs administered similar regimens for either

Stellenbosch University https://scholar.sun.ac.za

Table 3. Simple and multiple logistic regression model for Remission (with multiple imputation).

	Simple logistic model		Multiple logistic model	
	OR (95%CI)	*p*-Value	OR (95%CI)	*p*-Value
Sex: Female	1.47 (0.86 – 2.49)	0.16	1.36 (0.74 – 2.49)	0.33
Age[a]		0.041		0.64
18 m – 5 y	0.48 (0.25 – 0.92)	0.029	0.69 (0.32 – 1.49)	0.35
> 5 y	0.39 (0.17 – 0.88)	0.024	0.73 (0.27 – 1.99)	0.54
INSS: Stage 4	0.20 (0.09 – 0.40)	<0.001	0.26 (0.11 – 0.61)	0.002
LDH: > 750	0.62 (0.36 – 1.08)	0.095	1.12 (0.53 – 2.36)	0.76
Ferritin: > 120	0.29 (0.15 – 0.57)	<0.001	0.45 (0.20 – 1.00)	0.051
INPC: Unfavorable	0.41 (0.18 – 0.93)	0.041	0.45 (0.17 – 1.17)	0.10
MYCN: Amplified	0.75 (0.35 – 1.59)	0.46	0.72 (0.28 – 1.83)	0.49
WFA: < -2SD	0.64 (0.29 – 1.41)	0.27	0.42 (0.14 – 1.28)	0.13
HFA: < -2SD	1.11 (0.57 – 2.15)	0.77	1.52 (0.59 – 3.90)	0.38
Chemo regimen[b]		0.13 33		0.65
O/O	0.82 (0.44 – 1.54)	0.55	0.98 (0.45 – 2.13)	0.95
Rapid COJEC	0.47 (0.22 – 1.00)	0.050	0.70 (0.29 – 1.71)	0.44

[a]Reference category < 18 m.
[b]Reference category St Jude NB84.

Table 4. Simple and multiple Cox regression model for Overall survival (OS) (with multiple imputation).

	Simple Cox model		Multiple Cox model	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Sex: Female	0.63 (0.47 – 0.84)	0.002	0.67 (0.50 – 0.91)	0.011
Age[a]		0.030		0.42
18 m – 5 y	1.73 (1.15 – 2.60)	0.008	1.11 (0.71 – 1.75)	0.64
> 5 y	1.63 (1.02 – 2.60)	0.040	0.86 (0.49 – 1.51)	0.60
INSS: Stage 4	2.75 (1.70 – 4.43)	<0.001	2.11 (1.24 – 3.57)	0.006
LDH: > 750	1.87 (1.37 – 2.54)	0.001	1.51 (1.06 – 2.16)	0.023
Ferritin: > 120	2.82 (1.82 – 4.39)	<0.001	2.25 (1.35 – 3.75)	0.002
INPC: Unfavorable	1.61 (1.04 – 2.49)	0.035	1.33 (0.83 – 2.13)	0.23
MYCN: Amplified	1.19 (0.79 – 1.77)	0.41	0.97 (0.62 – 1.54)	0.91
WFA: < -2SD	1.40 (0.96 – 2.06)	0.082	1.60 (0.94 – 2.70)	0.081
HFA: < -2SD	1.16 (0.84 – 1.59)	0.37	1.13 (0.74 – 1.71)	0.57
Chemo regimen[b]		0.010		0.18
O/O	1.13 (0.79 – 1.61)	0.50	0.91 (0.60 – 1.38)	0.65
Rapid COJEC	1.75 (1.18 – 2.60)	0.005	1.28 (0.82 – 2.01)	0.28

[a]Reference category < 18 m.
[b]Reference category St Jude NB84.

curative or palliative intent. The prognosis and OS were influenced by nonstandard management indications related to surgery and radiotherapy administration. Risk stratification was limited by the availability of genetic testing especially with regard to MYCN status, deletions and mutations. The degree of supportive care differs among European, North American and South African POUs as well as between different South African POUs. The curative and palliative treatment intent differed between South African POUs with the degree of supportive care dictated by the treatment intent.

Discussion

This study compared the most commonly used induction chemotherapy protocols for HR-NB in South African POUs to identify the most effective, least toxic mCR induction

Stellenbosch University https://scholar.sun.ac.za

Table 5. Simple and multiple Cox regression model for Event-free survival (EFS) (with multiple imputation).

	Simple Cox model		Multiple Cox model	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Sex: Female	0.62 (0.46 – 0.85)	0.003	0.66 (0.47 – 0.92)	0.016
Age[a]		0.007		0.49
18m – 5yr	1.94 (1.21 – 3.13)	0.006	1.23 (0.72 – 2.10)	0.46
> 5y	2.31 (1.36 – 3.91)	0.002	0.99 (0.51 – 1.90)	0.97
INSS: Stage 4	2.70 (1.59 – 4.61)	<0.001	2.09 (1.14 – 3.83)	0.017
LDH: > 750	1.63 (1.18 – 2.26)	0.003	1.16 (0.79 – 1.70)	0.46
Ferritin: > 120	4.46 (2.42 – 8.24)	<0.001	3.52 (1.76 – 7.06)	<0.001
INPC: Unfavorable	1.61 (0.93 – 2.77)	0.095	1.32 (0.76 – 2.30)	0.32
MYCN: Amplified	1.12 (0.76 – 1.67)	0.57	1.02 (0.64 – 1.63)	0.92
WFA: < -2SD	1.40 (0.92 – 2.13)	0.12	1.43 (0.79 – 2.62)	0.24
HFA: < -2SD	1.24 (0.88 – 1.75)	0.22	1.22 (0.79 – 1.87)	0.38
Chemo regimen[b]		0.008		0.08
O/O	0.95 (0.65 – 1.39)	0.81	0.72 (0.46 – 1.13)	0.15
Rapid COJEC	1.66 (1.09 – 2.52)	0.018	1.10 (0.67 – 1.79)	0.70

[a]Reference category < 18 m.
[b]Reference category St Jude NB84.

chemotherapy regimen. In European studies mCR rates of 26.6–36.0% have been reported in patients treated on rCOJEC and 31.6–41.0% on OPEC/OJEC.[4,7] While the SA mCR of 32.5% on OPEC/OJEC is comparable to European studies, the mCR of 21.4% on rCOJEC is poorer than that described in European studies. The reasons were not clear, but nutritional status and available supportive care may have been different for studies in Europe.

The St Jude NB84 protocol and Memorial Sloan Kettering's (MSK) N6 protocol, doxorubicin-containing protocols from North America, achieved postinduction mCR of 77.0% ($n = 154$)[13] and 62.5% ($n = 24$),[15] respectively. The 36.9% mCR achieved in the St Jude NB84 protocol in SA is lower than remission rates in North America, but is the same as the 37% of the doxorubicin containing N5-MSKCC randomization reported during the HRNBL1.5/SIOPEN trial from 2013 to 2017.[7] The patients in the two North American studies were staged according to the Pediatric Oncology Group (POG)[13] and Children's Cancer Group (CCG).[13] The POG staging included stage B patients over the age of 12 months in the cohort,[13] while MSK staged without factoring in MYCN,[13] which was different from the SA POUs where the INRG classifications were used. The varied regional risk classifications create an unequal staging and biological profile for a standardized comparison.

The 2-year OS of the St Jude NB84 protocol (1984–1988) was 68.0%[13] while the 3-year EFS was 24.2% for patients in the OPEC/OJEC group and 31.0% for those in the rCOJEC group during European studies between 1990 and 1999.[4] More recent 2-year OS was 69.0% for rCOJEC,[7] but the protocol included an extended induction with topotecan, vincristine and doxorubicin. In our study the 2-year OS was 39.1% for the St Jude NB84 protocol, 30.7% for OPEC/OJEC and 16.3% for rCOJEC. Whereas the OPEC/OJEC outcomes compared well to the European studies, both rCOJEC and the doxorubicin-containing protocol performed worse. Although the differences in 2-year EFS of our study did not reach significance, the values were comparable to the 3-year EFS of the international studies. In a similar low- and middle income countries setting in India, doxorubicin-containing induction regimens achieved a long-term OS of 35.7% and CR of 17.6%.[16]

Stellenbosch University https://scholar.sun.ac.za

In North America, the treatment related mortality rate (TRM) during the St Jude protocol was reported to be 2.5%.[13] No patients were reported to have developed anthracycline-related cardiac failure.[13] Although there were no cardiac-related deaths in the SA study, the TRM was (7.5%) mainly due to myelosuppression. In Europe, the OPEC/OJEC and COJEC studies reported TRM rates of 3.2% and 4.1%,[4] respectively, whereas in the SA study they were 1.6% and 7.1%, respectively.

The doxorubicin-containing modified POG 9341 induction regimen has been used in Morocco with no toxicity-related deaths.[17] Limiting the use of doxorubicin in favor of optimizing the platinum backbone results in a superior response rate to the CCG3891 protocol, with improved intensity compared to CAdO/PE,[18] and has a more favorable acute toxicity profile than N7[19] or rCOJEC.[1]

In general, there is limited data comparing doxorubicin and nondoxorubicin containing regimens. SIOPEN randomized rCOJEC and N5-MSKCC regimens with the aim of comparing the two protocols.[7] The study concluded that rCOJEC was less toxic and no differences in postinduction remission, OS and EFS.[7] In our study, rCOJEC accounted for more toxicity-related deaths than the doxorubicin-containing St Jude NB84 protocol. OPEC/OJEC accounted for less toxicity-related deaths than both the other two protocols. The St Jude NB84 protocol had the best remission rate and 2-year OS and EFS but had a higher toxicity than OPEC/OJEC.

As in the North American studies,[13] LDH >750 U/L, ferritin >120 ng/dl, stage 4 disease and the choice of induction chemotherapy were significant prognostic denominators in the SA study. In keeping with international trends[7] and as in other middle-income countries (MIC),[20] more male patients were diagnosed with high-risk disease, yet sex was not a significant factor on multivariable analysis. HR-NB was mostly diagnosed in the 18–60 months age group, as in other LMIC,[21] and the median age of 36.1 months is higher compared to 33.2 months reported in Egypt, another MIC.[20] The greatest proportion of patients in the cohort had stage 4 disease and a larger proportion of tumors were MYCN amplified. The rate of malnutrition at diagnosis was marginally higher than in high income countries,[8] which is a cause for concern and suggests a need for more aggressive nutritional support.

The SA study reproduced the findings of both European and North American studies that various NB induction regimens were comparable to each other despite different therapeutic approaches.[4,6,7] A Cochrane review concluded that the only benefit of rCOJEC above OJEC/OPEC was the more rapid progress to autologous transplant.[4] In a MIC with limited access to transplant facilities, this benefit has little relevance. In randomized trials, rCOJEC had improved EFS compared to OPEC/OJEC through dose intensification, but was not superior to North American and other European regimens.[6]

The results of this study should be viewed in the South African context with comparisons between different HR-NB induction protocols among different centers with different supportive care guidelines, nonstandardized treatment and different treatment intents of management. The application in other countries should be evaluated according to local POUs and resources.

Conclusion

Reduction of the primary tumor size and complete remission of metastases by the end of induction chemotherapy are important prognostic factors in the treatment of NB.

Stellenbosch University https://scholar.sun.ac.za

With a marginal difference in outcomes with the various standard NB treatment protocols, the more favorable toxicity profile of OPEC/OJEC, especially considering malnourished children, is the most suitable induction regimen for SA. The ease of administration, favorable toxicity profile and rendering G-CSF support unnecessary are also important considerations for the future implementation of OPEC/OJEC as the standard induction regimen in HR-NB in South Africa.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, JvH. The data are not publicly available due to ethical restrictions.

Contributors' statement

Jaques van Heerden conceptualized and designed the study, collected data, performed the data analysis and wrote the manuscript. Mariana Kruger assisted with conceptualization and design of the study, supervised data analysis, critically reviewed and revised the manuscript. Kristien Wouters performed the statistical analysis. All other authors collected data in their respective pediatric oncology units and contributed significantly to the manuscript.

Acknowledgments

The authors acknowledge the SACCSG for supporting the study and the South African Children's Tumor Registry (SACTR) for providing statistical data. Dr Van Heerden, as staff member of the department of Pediatric Hematology and Oncology, Antwerp University Hospital, University of Antwerp, acknowledges the department for research support with financial contributions from the vzw Kinderkankerfonds.

Disclosure statement

The authors report no conflict of interest.

Funding

vzw Kinderkankerfonds, Belgium

ORCID

Jaques Van Heerden https://orcid.org/0000-0002-4502-1169
Jennifer Geel https://orcid.org/0000-0001-8792-3251
Marc Hendricks https://orcid.org/0000-0002-3636-0994
Gita Naidu https://orcid.org/0000-0002-4412-3825
G. P. Hadley https://orcid.org/0000-0002-2459-7118
Barry Van Emmenes https://orcid.org/0000-0002-0217-347X
Mariana Kruger http://orcid.org/0000-0002-6838-0180

Stellenbosch University https://scholar.sun.ac.za

References

1. Parikh N, Howard S, Chantada G, et al. SIOP-PODC adapted risk stratification and treatment guidelines: recommendations for neuroblastoma in low- and middle-income settings. *Pediatr Blood Cancer*. 2015;62(8):1305–1316. doi:10.1002/pbc.25501.
2. Bochennek K, Esser R, Lehrnbecher T, et al. Impact of minimal residual disease detection prior to autologous stem cell transplantation for post-transplant outcome in high-risk neuroblastoma. *Klin Padiatr*. 2012;224(3):139–142. doi:10.1055/s-0031-1301334.
3. Pinto N, Applebaum M, Volchenboum S, et al. Advances in risk classification and treatment strategies for neuroblastoma. *JCO*. 2015;33(27):3008–3017. doi:10.1200/JCO.2014.59.4648.
4. Peinemann F, Tushabe D, Berthold F. Rapid COJEC versus standard induction therapies for high-risk Neuroblastoma. *Cochrane Database Syst Rev*. 2015;5:CD010774.
5. Greengard E, Hill-Kayser C, Bagatell R. Treatment of high-risk neuroblastoma in children: recent clinical trials. *Clin. Invest.* 2013;3(11):1071–1081. doi:10.4155/cli.13.90.
6. Pearson AD, Pinkerton CR, Lewis IJ, et al. High-dose rapid and standard induction chemotherapy for patients aged over 1 year with stage 4 neuroblastoma: a randomised trial. *Lancet Onco*. 2008;9(3):247–256. doi:10.1016/S1470-2045(08)70069-X.
7. Garaventa A, Poetschger U, Valteau-Couanet D, et al. The randomised induction for high-risk neuroblastoma comparing COJEC and N5-MSKCC regimens: Early results from the HR-NBL1.5/SIOPEN trial. *JCO*. 2018;36(15_suppl):10507–10507. doi:10.1200/JCO.2018.36.15_suppl.10507.
8. Israels T, Ribeiro RC, Molyneux EM. Strategies to improve care for children with cancer in SubSaharan Africa. *Eur J Cancer*. 2010;46(11):1960–1966. doi:10.1016/j.ejca.2010.03.027.
9. Van Heerden J, Hendricks M, Geel J, et al. Overall survival for neuroblastoma in South Africa between 2000 and 2014. *Pediatr Blood Cancer*. 2019:e27944. doi:10.1002/pbc.27944.
10. Brodeur G, Pritchard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *JCO*. 1993;11(8):1466–1477. doi:10.1200/JCO.1993.11.8.1466.
11. Park J, Bagatell R, Cohn S, et al. Revisions to the international neuroblastoma response criteria: a consensus statement from the national cancer institute clinical trials planning meeting. *J Clin Oncol*. 2017;35(22):2580–2587. doi:10.1200/JCO.2016.72.0177.
12. The World Health Organization. Nutrition Landscape Information System (NLIS) country profile indicators: interpretation guide. 2010. https://www.who.int/nutrition/nlis_interpretation_guide.pdf Accessed August 2019.
13. Bowmen L, Hancock M, Santana V, et al. Impact of intensified therapy on clinical outcome in infants and children with neuroblastoma: the St Jude Children's Research Hospital Experience, 1962 to 1988. *J Clin Oncol*. 1999; 9:1599–1608. doi:10.1200/JCO.1991.9.9.1599.
14. R Core Team 2017. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.
15. Kushner B, LaQuaglia M, Bonilla M, et al. Highly effective induction therapy for stage 4 neuroblastoma in children over 1 year of age. *J Clin Oncol*. 1994;12(12):2607–2613. doi:10.1200/JCO.1994.12.12.2607.
16. Agarwala S, Mandelia A, Bakhshi S, et al. Neuroblastoma: outcome over a 14-year period from a tertiary care referral centre in India. *J Pediatr Surg*. 2014;49(8):1280–1285. doi:10.1016/j.jpedsurg.2014.03.017.
17. Khattab M, Cherkaoui S, Benmiloud S, et al. *Results of an Intensive Induction Protocol for High-Risk Neuroblastoma in Morocco (HR-NBL-MA-10)*. Toronto, CA: SIOP; 2014.
18. Coze C, Hartmann O, Michon J, et al. NB87 induction protocol for stage 4 neuroblastoma in children over 1 year of age: A report from the French society of pediatric oncology. *J Clin Oncol*. 1997;15(12):3433–3440. doi:10.1200/JCO.1997.15.12.3433.
19. Cheung N, Kushner B, LaQuaglia M, et al. N7: A novel multi-modality therapy of high-risk neuroblastoma (NB) in children diagnosed over 1 year of age. *Med Pediatr Oncol*. 2001;36(1):227–230. doi:10.1002/1096-911X(20010101)36:1<227::AID-MPO1055>3.0.CO;2-U.

Stellenbosch University https://scholar.sun.ac.za

20. Al-Tonbary Y, Badr M, Mansour A, et al. Clinico-epidemiology of neuroblastoma in north east Egypt: a 5-year multicenter study. *Oncol Lett*. 2015;10(2):1054–1062. doi:10.3892/ol.2015.3335.
21. Stefan C, Bray F, Ferlay J, et al. Cancer of childhood in sub-Saharan Africa. *ecancer*. 2017; 11:755. doi:10.3332/ecancer.2017.755.

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 8

The importance of local control management in neuroblastoma in South Africa (2020)

Ethics no: S18/07/138

Reference: van Heerden J, Kruger M, Esterhuizen T; et al. The importance of local control management in high-risk neuroblastoma in South Africa. Pediatr Surg Int. 2020; 36(1), 457–469. https://doi.org/10.1007/s00383-020-04627-x
(Impact factor 1.668)

The debate on the contribution of the degree of resection of a NB tumour has confirmed that obtaining a complete resection has a better prognostis compared to an incomplete resection. The resection of the tumour is not always feasible due to the encasing nature of the tumour biology. In the increasing multi-modal treatment modalities in the management of NB, the contribution of each modality has become less defined. Yet in countries where only trimodal (chemotherapy, surgery and radiotherapy) therapy is available, each individual modality has significance. The degree of resection that ensures the same outcome as a complete resection is less clear, because the studies were done in high income countries with access to all possible treatment modalities. Therefore, it was important to study the prognostic value of the degree of surgery and radiotherapy in the South African setting with access only to mainly trimodal therapy. The recommendations would guide paediatric surgeons to establish favourable surgical outcomes without mordidity in low- and middle income countries.

Stellenbosch University https://scholar.sun.ac.za

Pediatric Surgery International
https://doi.org/10.1007/s00383-020-04627-x

ORIGINAL ARTICLE

The importance of local control management in high-risk neuroblastoma in South Africa

Jaques van Heerden[1] · Mariana Kruger[1] · Tonya Esterhuizen[2] · Marc Hendricks[3] · Jennifer Geel[4] · Ané Büchner[5] · Gita Naidu[6] · Jan du Plessis[7] · Barry Vanemmenes[8] · Ronelle Uys[1] · G. P. Hadley[9] · For the South African Children's Cancer Study Group

Accepted: 7 February 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Purpose To investigate the impact of local therapies on high-risk neuroblastoma (HR-NB) outcomes in South Africa.

Methods Data from 295 patients with HR-NB from nine pediatric oncology units between 2000 and 2014 were analysed. All patients received chemotherapy. Five-year overall (OS) and event free survival (EFS) were determined for patients who had received local therapy, either surgery or radiotherapy or both.

Results Surgery was performed in only 35.9% ($n=106/295$) patients. Surgical excision was done for 34.8% ($n=85/244$) of abdominal primaries, 50.0% ($n=11/22$) of thoracic primaries; 22.2% ($n=2/9$) neck primaries and 66.7% ($n=8/12$) of the paraspinal primaries. Only 15.9% ($n=47/295$) of all patients received radiotherapy. Children, who had surgery, had an improved five-year OS of 32.1% versus 5.9% without surgery ($p<0.001$). Completely resected disease had a five-year OS of 30.5%, incomplete resections 31.4% versus no surgery 6.0% ($p<0.001$). Radiated patients had a five-year OS of 21.3% versus 14.2% without radiotherapy ($p<0.001$). Patients who received radiotherapy without surgical interventions, had a marginally better five-year OS of 12.5% as opposed to 5.4% ($p<0.001$). Patients who underwent surgery had a longer mean overall survival of 60.9 months, while patients, who were irradiated, had a longer mean overall survival of 7.9 months ($p<0.001$). On multivariate analysis, complete metastatic remission ($p<0.001$), surgical status ($p=0.027$), and radiotherapy status ($p=0.040$) were significant predictive factors in abdominal primaries.

Conclusion Surgery and radiotherapy significantly improve outcomes regardless of the primary tumor site, emphasizing the importance of local control in neuroblastoma.

Keywords Neuroblastoma · Surgery · Radiotherapy · South Africa · Local therapies · High-risk · Intermediate-risk

Abbreviations

COG	Children's Oncology Group
EFS	Event-free survival
FH	Favorable histology
GTR	Gross total resection
HIC	High-income countries
HR	High risk
IDRF	Image define risk factors
INSS	International Neuroblastoma Staging System
LMIC	Low- and middle-income countries
NA	Not amplified
NB	Neuroblastoma
nGTR	Near gross total resection
OS	Overall survival
PFS	Progression-free survival
PTR	Primary tumor site relapse
POUs	Pediatric oncology units
RT	Radiotherapy
STR	Subtotal resection
UH	Unfavorable histology

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00383-020-04627-x) contains supplementary material, which is available to authorized users.

✉ Jaques van Heerden
Jaques.vanheerden@uza.be

Extended author information available on the last page of the article

Introduction

Neuroblastoma is a solid tumor of the sympathetic nervous system and contributes to 7–10% of all childhood cancer deaths [1]. Outcomes are poor in the absence of local

Published online: 28 February 2020

Stellenbosch University https://scholar.sun.ac.za

therapies, such as surgery and radiotherapy, especially in low and middle income countries [1, 2]. The tumor is chemotherapy and radiotherapy sensitive [1], but can develop significant treatment resistance without local treatment interventions [1]. Cure in high-risk (HR) neuroblastoma is determined by the stage, tumor biology, chemotherapy response, and local treatment [1, 2]. Therefore, in HR disease (metastatic disease, unfavorable histology, raised tumor markers, MYCN amplification and adverse genetics), surgery and radiotherapy are mandatory for cure as components of multimodal management [1, 2]. Local therapies have increased importance in resource limited settings, where access to autologous transplant and molecular targeted therapies as part of standard of care, are limited [2].

Resection of the tumor is not always a feasible therapeutic modality due to the macroscopic encasing of vital structures, such as significant blood vessels, including the aorta and inferior vena cava [3]. With encasement, surgery becomes challenging, with a high surgical morbidity and mortality. Encasement of the abdominal arteries is the single worst prognostic indicator during surgery [3]. IDRF are a set of anatomical site-specific signs that confer a poorer prognosis and assists in surgical decisions toward resection in NB [4]. Before the standardization of image defined risk factors (IDRF) to assess operability post-induction chemotherapy, decisions regarding surgery were made by multi-disciplinary teams (MDTs).

Radiotherapy is used for local control, focal metastatic spread, and symptom control in curative and palliative settings [2]. In HR metastatic disease, the role of radiotherapy for local control and survival in the era of supplementary multimodal therapies remains unclear. Yet complementary radiotherapy, when used as a component of trimodal therapy alongside chemotherapy and surgery, has survival advantages [5]. By utilizing radiotherapy, the same response rates can be achieved in the primary tumor bed, as well as at metastatic sites, for both large and minimal residual tumors [5].

In HR disease, where autologous transplant and molecular targeted therapies are not available, the extent of resection has an impact on the overall (OS) and event-free survival (EFS) [3]. In high-income countries (HIC), a favorable surgical outcome is mandatory for autologous transplant, radiotherapy and targeted therapy, securing an overall survival of up to 60% [2]. Historically, in HIC, chemotherapy, surgery and radiotherapy obtained an OS of approximately 20%, compared to less than 20% in resource-limited settings [2]. In combination with curative care options, local therapies contribute to the prolongation of progression free survival (PFS) and quality of life [6, 7].

A retrospective review was done to determine the value of local therapies on the outcomes of children diagnosed with HR-NB in South Africa, where autologous bone marrow transplants and molecular targeted therapies are readily not available. The objective was to develop standardized national treatment protocols for local therapies, including identifying limitations in the current management of NB in South Africa.

Materials and methods

A total of 295 children with HR-NB were diagnosed in nine dedicated pediatric oncology units (POUs) in South Africa from January 2000 to December 2014 (see Supplementary Figure 1). Diagnosis was confirmed through either a biopsy, bone marrow investigations, or raised urinary catecholamines if biopsy was not possible.

Patients were clinically and radiologically staged according to the International Neuroblastoma Staging System (INSS), using ultrasonography, computed tomography or magnetic resonance imaging of the neck, chest and abdomen, as well as bone marrow aspirate and trephine biopsy. Depending upon the available facilities, staging, and skeletal screening were done by ^{123}I-MIBG scan and/or bone scan. Where available, staging with ^{129}fluorodeoxyglucose-positron emission tomography was done in patients with ^{123}I-MIBG scan non-avid tumors. Risk classification was based on the International Neuroblastoma Risk Groups (INRG) classification system (see Appendix), to identify patients with HR disease [1].

Univariate and multivariate analysis for five-year OS were only calculated for abdominal primaries due to insignificant results in the small cohorts of other primary sites.

Guidelines for local therapies

Data was not robust enough to retrospectively restage patients post induction chemotherapy (pre-operatively) according to IDRF. Most patient records and imaging were stored in non-electronic formats, with incomplete or lost IDRF information. The indication for surgery and external beam radiotherapy treatment was based on risk-based treatment protocols according to the Children's Oncology Group (COG) [8, 9] or the International Society of Paediatric Oncology European Neuroblastoma group (SIOPEN) approaches [10]. In South Africa, the choice of protocol was according to institutional preference and the application decided by MDTs and guided by institutional expertise. Emergency surgery was performed for the alleviation of neurological or obstructive symptoms. It was not

Stellenbosch University https://scholar.sun.ac.za

standard practice in South Africa to perform post resection imaging to assess the completeness of resections.

Definitions

Metastatic remission was defined as complete remission according to the International Neuroblastoma Response Criteria (INRC), revised in 1993 and 2012 [11, 12]. Complete resection was defined as greater than 90% resection as reported by surgeons. Incomplete resection was defined as resection less than 90%, or surgical reports stating an incomplete resection. No surgery was defined as the absence of any surgical excision of the primary tumor, and/or if only a biopsy was done. Radiotherapy treatment was defined as any radiotherapy to the local tumor bed or to metastatic sites for curative or palliative indications.

The overall survival (OS) time was defined as the period from diagnosis to death or date last seen, and event-free survival (EFS) was calculated from the date of diagnosis until disease progression on chemotherapy, or relapse, or treatment abandonment, death or date last seen. Progression-free survival (PFS) was calculated from the date of diagnosis until disease progression on chemotherapy, or relapse or death. Lost to follow-up was defined as any patient who did not return for follow-up appointments for one year and was unreachable despite efforts to contact the family.

Ethical approval was obtained from The Faculty of Health Sciences Research Ethics Committee of Stellenbosch University and the National Department of Health.

Statistical analysis

Data were analyzed using IBM SPSS version 25 (IBM Corporation, USA) statistical software [13]. Medians were used to determine estimations using Student's *t* test. Where estimations could not be determined by median values, the mean values were used. In cohorts of fewer than five observations, Fisher's exact test was applied. The Pearson chi-square test (χ^2) was employed to assess the categorical associations among covariates. Two-year and five-year overall survival (OS), event-free (EFS) survival, and progression-free survival (PFS), with associated 95% confidence intervals, were calculated using Kaplan–Meier curves. Multiple Cox regression modelling was employed to assess the statistical

Table 1 Surgical and radiological interventions according to primary site

Local therapies according to anatomical site of primary				
Primary site	Both local therapies *n* (%)	Surgery only *n* (%)	Radiotherapy only *n* (%)	No therapy *n* (%)
Abdominal	28 (11.5%)	60 (24.6%)	15 (6.1%)	141 (57.8%)
Thoracic	2 (9.0%)	10 (45.5%)	0 (0%)	10 (45.5%)
Neck	1 (11.1%)	1 (11.1%)	0 (0%)	7 (77.8%)
Paraspinal	0 (0%)	8 (66.7%)	0 (0%)	4 (33.3%)
Other	0 (0%)	0 (0%)	1 (12.5%)	7 (87.5%)
Total	31 (10.5%)	79 (26.8%)	16 (5.4%)	169 (57.3%)
Surgical intervention according to anatomical site of primary				
Primary site	Operated *n* (%)	Not operated *n* (%)	Total	
Abdominal	85 (34.8%)	159 (65.2%)	244	
Thoracic	11 (50.0%)	11 (50.0%)	22	
Neck	2 (22.2%)	7 (77.8%)	9	
Paraspinal	8 (66.7%)	4 (33.3%)	12	
Other	0 (0.0%)	8 (100.0%)	8	
Total	106 (35.9%)	189 (64.1%)	295	
Radiotherapy according to anatomical site of primary				
Primary site	Radiated *n* (%)	Not radiated *n* (%)	Total	
Abdominal	43 (17.6%)	201 (82.4%)	244	
Thoracic	2 (9.1%)	20 (90.9%)	22	
Neck	1 (11.1%)	8 (88.9%)	9	
Paraspinal	0 (0%)	12 (100.0%)	12	
Other	1 (12.5%)	7 (87.5%)	8	
Total	47 (15.9%)	248 (84.1%)	295	

Stellenbosch University https://scholar.sun.ac.za

significance of various prognostic factors. The proportional hazards assumption was also confirmed for the final multivariable model. A p value of less than 0.05 was considered significant for all calculations. Some cohorts were too small (less than five) for meaningful calculations and were not included in the final tables.

Results

The majority of patients were diagnosed with abdominal primaries (adrenal and extra-adrenal) (82.7%; $n=244/295$) followed by thoracic primaries (7.5%; $n=22/295$), paraspinal (4.0%; $n=12/295$), neck (3.0%; $n=9/295$); primary not found (2.0%; $n=/295$); bone only (0.4%; $n=1/295$) and one in the soft tissue of the cheek (0.4%) (see Table 1, Supplementary Figure 1).

The majority of patients were not operated (64.1%; $n=189/295$). Surgery excision was done for 34.8% ($n=85/244$) of the abdominal primaries, 50.0% ($n=11/22$) of thoracic primaries; 22.2% ($n=2/9$) of neck primaries and 66.7% ($n=8/12$) of the paraspinal primaries. The majority of patients did not receive radiotherapy (84.1%; $n=248/295$). Irradiation to the tumour bed was done for only 17.6% ($n=43/244$) of the abdominal primaries, 9.1% ($n=11/22$) of thoracic primaries; 11.1% ($n=1/9$) of neck primaries and none of the paraspinal primaries. Eleven patients (3.8%) received an autologous bone marrow transplant after induction chemotherapy, surgery, and radiotherapy.

The main reasons why patients were not operated were the failure to achieve metastatic remission after induction chemotherapy (49.2%; $n=93/189$), local progression of disease (26.5%; $n=50/189$); metastatic progression of disease (6.3%; 12/189) or death during induction chemotherapy (4.2%; $n=8/189$) (see Supplementary table 1). Nine (4.7%) patients were palliated from diagnosis and five (2.6%) abandoned induction chemotherapy.

Patients, who underwent surgery, had a better five-year OS of 32.1% and EFS of 30.2%, compared to patients not operated with a five-year OS of 5.9% and a five-year EFS

Table 2 Surgical outcomes

Outcomes for surgical intervention according to site of primary tumour						
Primary site	5 years OS			5 years EFS		
	Operated	Not operated	p value	Operated	Not operated	p value
Abdominal	28.2%	5.7%	<0.001	25.9%	5.1%	<0.001
Thoracic	63.6%	9.1%	0.001	63.6%	9.1%	0.003
Neck	50.0%	0.0%	0.225	50.0%	0.0%	0.030
Paraspinal	25.0%	0.0%	<0.001	25.0%	0.0%	0.001
Other	12.5%	12.5%	NS	12.5%	12.5%	NS
Total	32.1%	5.9%	<0.001	30.2%	5.3%	<0.001

Outcomes for the extent of surgical resection according to site of primary tumour				
Primary site	5 years OS			
	Complete resection	Incomplete resection	No resection	p values
Abdominal	28.6%	25.6%	5.8%	<0.001
Thoracic	57.1%	60.0%	10.0%	0.017
Neck	0.0%	100.0%	0.0%	0.271
Paraspinal	0.0%	33.3%	0.0%	<0.001
Other	–	–	12.5%	NS
Total	30.5%	31.4%	6.0%	<0.001
Primary site	5 years EFS			
	Complete resection	Incomplete resection	No resection	p values
Abdominal	26.5%	23.1%	5.2%	<0.001
Thoracic	57.1%	60.0%	10.0%	0.030
Neck	100.0%	0.0%	0.0%	0.095
Paraspinal	0.0%	33.3%	0.0%	0.003
Other	–	–	12.5%	NS
Total	30.5%	27.5%	5.5%	0.001

OS overall survival, *EFS* event free survival

Stellenbosch University https://scholar.sun.ac.za

of 5.3% ($p<0.001$) (see Table 2). There was also a significant improved five-year OS per anatomical site for patients operated versus patients not operated: abdominal primary 28.2% compared to 5.7% ($p<0.001$) (see Fig. 1); thoracic primary 63.6% compared to 9.1% ($p=0.001$) and paraspinal 25.0% compared to 0.0% ($p<0.001$). The significant five-year EFS per anatomical site for patients that could be operated and patients not operated were: abdominal primary 25.9% compared to 5.1% ($p<0.001$); thoracic primary 63.6% compared to 9.1% ($p=0.003$); neck 50.0% compared to 0.0% ($p=0.03$) and paraspinal 25.0% compared to 0.0% ($p=0.001$).

The five-year OS was significantly improved for patients regardless of primary site with either complete resection or incomplete resection, at 30.5% and 31.4% respectively, compared to 6.0% in patients who did not receive any surgery ($p<0.001$) (see Table 3). Only two operated patients

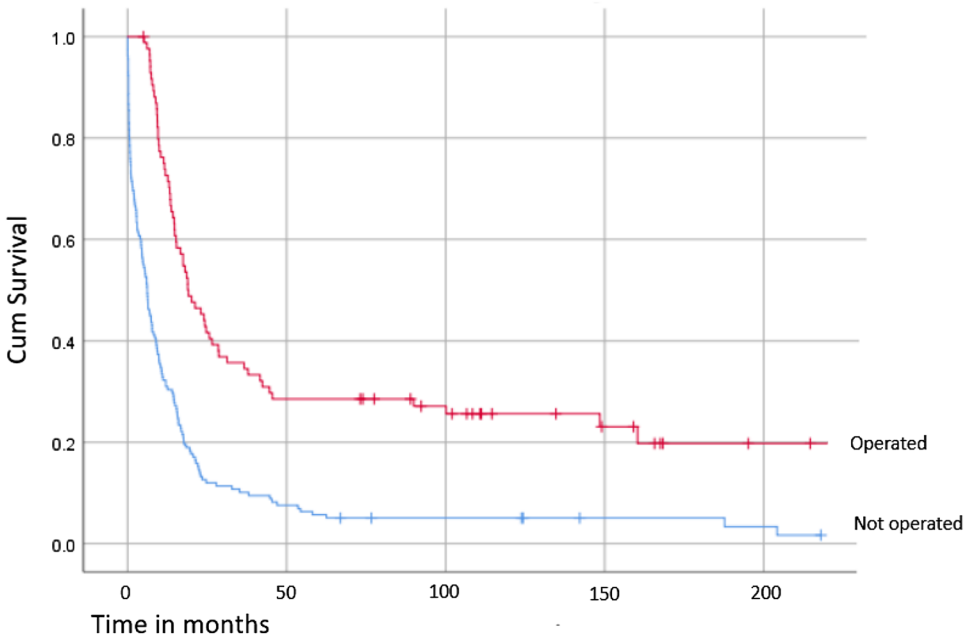


Fig. 1 Kaplan–Meier curve for OS of surgical status in HR-NB with abdominal primaries ($p<0.001$)

Table 3 Radiotherapy outcomes

Outcomes for radiotherapy according to site of primary tumour						
Primary site	5 years OS			5 years EFS		
	Irradiated	Not irradiated	*p* value	Irradiated	Not irradiated	*p* value
Abdominal	18.6%	12.5%	0.008	16.3%	11.5%	0.035
Thoracic	0.0%	40.0%	0.518	0.0%	40.0%	0.518
Neck	100.0%	0.0%	0.107	0.0%	12.5%	0.192
Paraspinal	16.7%	16.7%	NS	16.7%	16.7%	0.126
Other	100.0%	0.0%	0.110	100.0%	0.0%	NS
Total	21.3%	14.2%	<0.001	17.0%	13.8%	0.076
Outcomes for radiotherapy without prior surgery						
Primary site	5 years OS			5 years EFS		
	Radiotherapy only	No therapy	*p* value	Radiotherapy only	No therapy	*p* value
Abdominal	6.7%	5.8%	<0.001	6.7%	5.0%	<0.001
Thoracic	–	10.0%	0.007	–	10.0%	0.013
Neck	–	0.0%	0.271	–	0.0%	0.095
Paraspinal	–	–	NS	–	0.0%	0.001
Other	100.0%	0.0%	0.110	100.0%	0.0%	0.126
Total	12.5%	5.4%	<0.001	12.5%	4.8%	0.001

OS overall survival, *EFS* event free survival

Stellenbosch University https://scholar.sun.ac.za

(1.9%) died from surgical complications due to post-operative bleeding (Fig. 2).

Patients, who were irradiated, had a better five-year OS of 21.3% and EFS of 14.2% compared to patients that were not irradiated with a 5-year OS of 17.0% ($p < 0.001$) and 5-year EFS of 13.8% ($p = 0.076$) (see Table 3). The five-year OS for patients with irradiated abdominal primaries was 18.6% compared to 12.5% in patients that were not irradiated ($p = 0.008$) (see Fig. 3). The five-year EFS for patients with irradiated abdominal primaries was 16.3% compared to 11.5% in patients who were not irradiated ($p = 0.035$). Patients who received only radiotherapy in combination with chemotherapy had a better five-year OS of 12.5%, compared to 5.4% in those who received chemotherapy alone ($p < 0.001$), with similar findings for EFS (12.5% with radiotherapy versus 4.8% without radiotherapy) ($p < 0.001$). (see Table 3).

Operated patients had a longer mean OS of 60.9 months (80.6 months versus 19.7 months) (median 12.8 months) compared to those, who did not receive any surgery

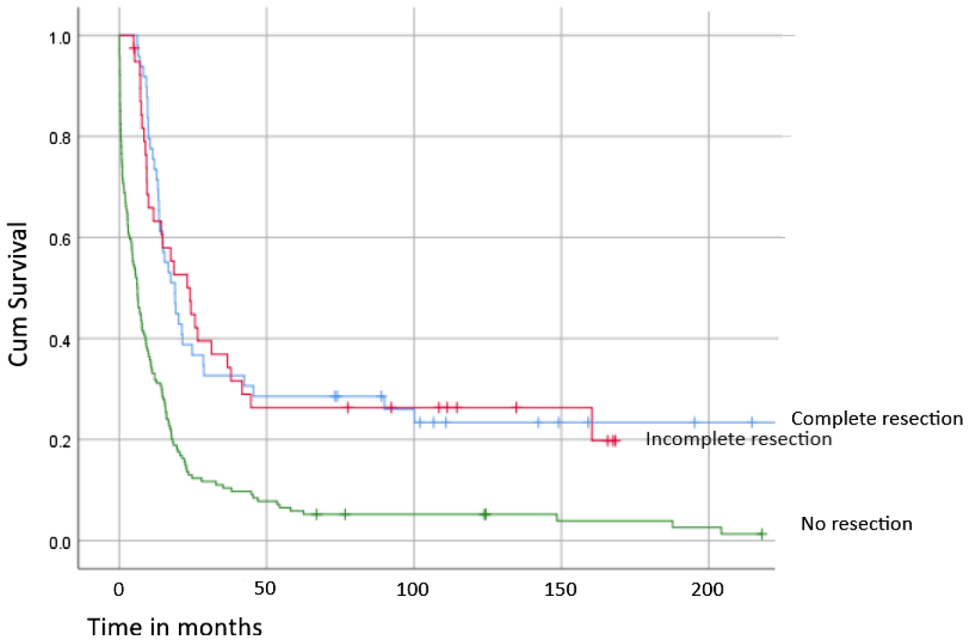


Fig. 2 The Kaplan–Meier curve for OS of the extent of resection in HR-NB with abdominal primaries ($p < 0.001$)

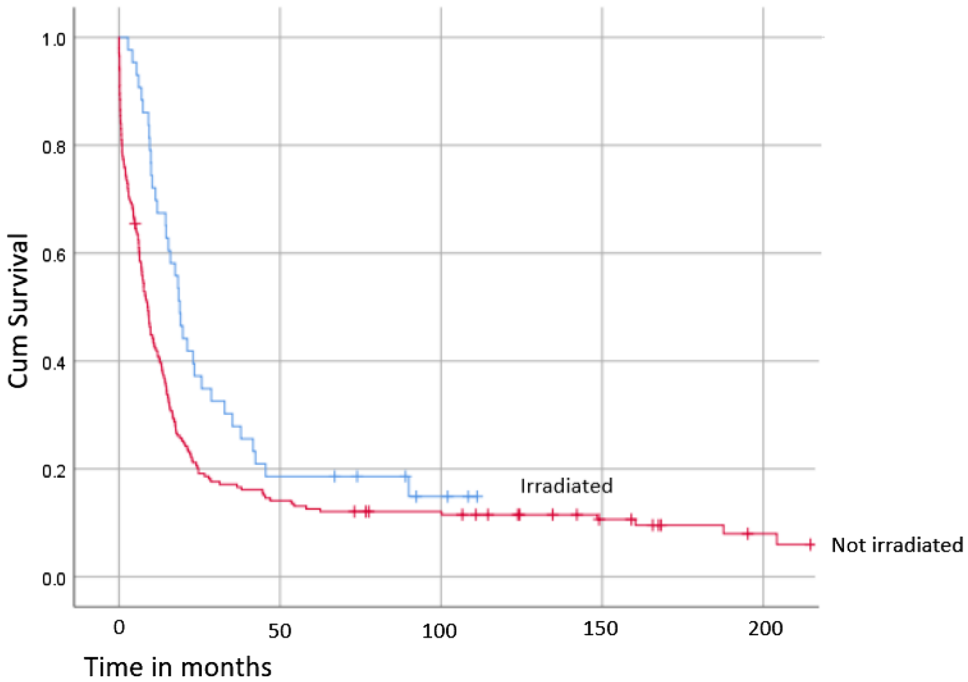


Fig. 3 Kaplan–Meier curve of OS of radiotherapy status for HR-NB with abdominal primaries ($p = 0.008$)

Stellenbosch University https://scholar.sun.ac.za

($p < 0.001$) (see Table 4). In patients who were irradiated, the mean OS was an average of 7.9 months longer (46.2 months versus 38.3 months)(median 9.7 months) compared to those who did not receive any radiotherapy ($p < 0.001$) (see Table 4).

On univariate analysis only thoracic primaries were of prognostic value compared to abdominal primaries ($p = 0.006$) in determining OS (see Table 5). The significant prognostic factors on multivariate analysis were age less than 18 months ($p < 0.001$), LDH < 750 U/L ($p < 0.001$) and

Table 4 Overall survival advantage for surgical, radiotherapy, and transplant interventions

Overall survival advantage HR abdominal NB according to surgery treatment					
Surgery total (mean)	Estimate (months)	Std. error	95% confidence interval		
			Lower limit	Upper limit	*p* value
Not operated	19.747	3.304	13.271	26.222	< 0.001
Operated	80.613	9.366	62.256	98.971	
Abdominal (mean)					
Not operated	19.455	3.530	12.536	26.374	< 0.001
Operated	69.664	9.751	50.552	88.776	
Thoracic (mean)					
Not operated	23.015	12.102	0.000	46.735	0.001
Operated	160.233	26.001	109.272	211.195	
Neck (mean)					
Not operated	10.981	3.696	3.736	18.226	0.225
Operated	49.567	25.244	0.089	99.044	
Paraspinal (mean)					
Not operated	3.958	1.741	0.547	7.370	< 0.001
Operated	62.896	24.042	15.773	110.019	
Overall survival advantage HR abdominal NB according to extent of resection					
Surgery (mean)	Estimate (months)	Std. error	95% confidence interval		
			Lower limit	Upper limit	*p* value
Complete	77.896	12.540	53.318	102.473	< 0.001
Incomplete	77.680	12.598	52.988	102.372	
None	19.466	3.250	13.096	25.836	
Overall survival advantage HR abdominal NB according to radiotherapy treatment					
Radiotherapy total (mean)	Estimate (months)	Std. error	95% confidence interval		
			Lower limit	Upper limit	*p* value
Irradiated	46.229	8.102	30.349	62.110	0.007
Not irradiated	38.350	4.578	29.377	47.324	
Abdominal (median)					
Irradiated	18.900	1.552	15.859	21.941	0.008
Not irradiated	8.967	0.859	7.283	10.651	
Thoracic (mean)					
Irradiated	29.350	18.383	0.000	65.381	0.518
Not irradiated	10.367	1.066	8.277	12.456	
Overall survival in high risk according to autologous hemopoietic stem cell transplant (HSCT)					
HSCT (mean)	5 years OS	Estimate (months)	Std. error	95% Confidence interval	
				Lower limit	Upper limit	*p* value
With	40%	37.867	10.115	18.042	57.691	0.268
Without	19%	17.433	2.741	12.062	22.805	

OS overall survival, *HSCT* hemopoietic stem cell transplant

Stellenbosch University https://scholar.sun.ac.za

Table 5 Univariate analysis of prognostic factors in HR-NB

Univariate analysis of the primary site as prognostic factor in HR-NB				
	Hazard ratio	95% CI for HR		*p* value
		Lower limit	Upper limit	
Primary site (abdominal comparator)				0.053
Thoracic vs abdominal primary	0.456	0.260	0.800	0.006
Neck vs abdominal primary	1.020	0.503	2.067	0.956
Paraspinal vs abdominal primary	0.765	0.406	1.443	0.408
Other sites vs abdominal primary	1.479	0.696	3.146	0.309
Univariate analysis of prognostic factors in HR abdominal NB				
Surgical status (operated comparator)	Hazard ratio	95% CI for HR		*p* value
		Lower limit	Upper limit	
Not operated vs operated	2.647	1.968	3.560	< 0.001
Resection status (complete surgery comparator)				< 0.001
Incomplete vs compete surgery	1.019	0.626	1.658	0.939
No surgery vs compete surgery	2.604	1.811	3.744	< 0.001
Radiotherapy status (irradiated comparator)				
Not irradiated vs radiated	1.615	1.128	2.314	0.009
Age group (< 18 months comparator)				< 0.001
18–60 months	2.626	1.732	3.980	< 0.001
> 60 months	1.975	1.226	3.181	0.005
Stage (Stage 1, 2 and 4s comparator)				0.001
Stage 3	1.852	0.630	5.440	0.263
Stage 4	3.609	1.329	9.800	0.012
LDH (< 750 U/L comparator)				< 0.001
> 750 U/L vs < 750 U/L	1.729	1.254	2.386	0.001
Unknown vs < 750 U/L	3.115	2.043	4.750	< 0.001
Ferritin (< 120 g/dl comparator)				0.006
> 120 g/dl vs < 120 g/dl	1.713	1.200	2.445	0.003
Unknown vs < 120 g/dl	1.699	1.180	2.447	0.004
Pathology (FH comparator)				0.019
UH vs FH	1.953	1.206	3.161	0.006
Unknown vs FH	1.880	1.177	3.002	0.008
MYCN status (NA comparator)				0.047
Amplified vs NA	0.871	0.537	1.412	0.575
Unknown vs NA	1.292	0.844	1.977	0.239
Post-induction metastatic remission status (in remission comparator)				< 0.001
Not in remission vs in remission	5.153	3.435	7.729	< 0.001
Unknown status vs in remission	8.903	3.619	21.901	< 0.001

favourable histology ($p = 0.042$). For abdominal primaries, metastatic remission status after induction chemotherapy ($p < 0.001$), surgical status ($p = 0.027$), and radiotherapy status ($p = 0.040$) were of prognostic importance for OS (see Table 6).

Operated patients relapsed with metastatic disease (24.5%; $n = 26/106$), which was similar for irradiated patients with metastatic relapse (10.6%; $n = 5$) and metastatic progression (42.6%; $n = 20$) (see Supplementary Table 2).

Stellenbosch University https://scholar.sun.ac.za

Table 6 Multivariate analysis of prognostic factors in HR abdominal NB

Multivariate analysis of prognostic factors in HR abdominal NB at diagnosis				
	Hazard ratio	95% CI for HR		*p* value
		Lower limit	Upper limit	
Age group (< 18 months comparator)				< 0.001
18–60 months	2.331	1.529	3.554	< 0.001
> 60 months	1.806	1.106	2.948	0.018
LDH (< 750 U/L comparator)				< 0.001
> 750 U/L vs < 750 U/L	1.698	1.215	2.372	0.002
Unknown vs < 750 U/L	2.922	1.910	4.470	< 0.001
Pathology (FH comparator)				0.042
UH vs FH	1.808	1.109	2.948	0.018
Unknown vs FH	1.796	1.116	2.889	0.016
Multivariate analysis of prognostic factors in HR abdominal NB during management				
	Hazard ratio	95% CI for HR		*p* value
		Lower limit	Upper limit	
Age group (< 18 months comparator)				0.017
18–60 months	1.842	1.172	2.895	0.008
> 60 months	1.425	0.848	2.396	0.182
LDH (< 750 U/L comparator)				0.004
> 750 U/L vs < 750 U/L	1.602	1.149	2.233	0.005
Unknown vs < 750 U/L	2.016	1.276	3.186	0.003
Post-induction metastatic remission status (in remission comparator)				< 0.001
Not in remission vs in remission	3.732	2.405	5.792	< 0.001
Unknown status vs in remission	4.130	1.543	11.057	0.005
Surgical status (operated comparator)				
Not operated vs operated	1.541	1.052	2.258	0.027
Radiotherapy status (irradiated comparator)				
Not irradiated vs radiated	1.416	1.016	1.973	0.040

CI confidence interval, *HR* high-risk, *NB* neuroblastoma, *FH* favorable histology, *UH* unfavorable histology

Discussion

Surgery is the most fundamental treatment modality in the multi-modality treatment of neuroblastoma with curative intent to ensure loco-regional control of the disease, regardless of metastasis [2, 14]. In stage III patients, complete resection affords better survival, especially in those with unfavorable tumor biology [15, 16]. In stage IV tumors, either complete resection or gross total resection (GTR) of 95% was recommended when possible. The COG A3973 study concluded that even a near GTR of greater than 90% obtained superior OS [17]. After surgery, age and tumor biology are the most powerful determinants of outcome in patients with neuroblastoma. In this study, only age below 18 months was significant as a prognostic factor. Surgical skill and experience may influence the subjective evaluation of resectability by the surgeon [18]. Improvements in EFS and OS in resected tumors may thus be a reflection of a more favorable biological profile and those who could not be resected had an adverse prognosis at diagnosis.

During the HR-NBL1/SIOPEN trial between 2002 and 2015, 98% of patients underwent surgery compared to only 35.9% of the patients with HR disease reported in this South African study, while only 15.9% of HR disease tumors received radiotherapy compared to 88% in the HR-NBL1/SIOPEN trial [19]. This may have been due to

Stellenbosch University https://scholar.sun.ac.za

a combination of factors including inequitable access to paediatric surgical expertise, shortage of operating slots, availability of radiotherapy facilities, and a chemotherapy-only strategy for metastatic patients [20].

The evidence of the prognostic significance and ideal extent of resection is conflicting. Multiple changes in systemic treatment, such as the quality and quantity of autologous transplants and molecular targeted therapies, as well as revisions of risk classification systems have complicated evaluations [21, 22]. Retrospective studies at Memorial Sloan Kettering (MSK) have proven superior survival outcomes for children with complete surgical resections (CSR) versus partial surgical resections (PSR), especially in metastatic disease [23, 24]. The MSK two-year survival rates of CSR compared to PSR were 80% versus 38%. On the basis of this, it is possible to conclude that CSR was superior to PSR, even for stage IV patients with bone marrow involvement [24–26]. This could not be validated in other reports [23]. The difference in survival outcomes for MYCN amplified tumours compared to non-amplified tumours were proportional to surgical outcomes for PSR compared to CSR [27]. This study demonstrated significant survival advantage if patients had tumor resection, regardless of extent if more than 50%. On multivariate analysis, the extent of resection did not reach significance, with metastatic remission being the most significant prognostic factor.

In the HR-NBL1/SIOPEN Trial, complete resection had a better five-year OS compared to incomplete resection (39% vs 30%) [19]. A meta-analysis of 19 studies in patients with HR-NB concluded that GTR did confer an OS advantage above subtotal resection (STR) at both three years and five years. However, this was only true for STR over biopsy only at three years [21]. Only in asymptomatic stage II patients with LR disease did less than 50% resection confer excellent outcomes with surgery alone [28]. Chemotherapy was restricted to patients with progression of symptomatic disease or less than 50% resection [28]. In South Africa, a survival advantage was demonstrated across all risk classifications at 2 years and 5 years with complete or incomplete resections.

The role of radiotherapy has been investigated in emergency presentations, palliation and local and metastatic disease in relation to chemotherapy and surgery, as has the role of in the transplant setting [4, 5]. Radiotherapy plays a significant role in high-risk disease, regardless of resection or nodal involvement [29, 30]. In Egypt, patients who received radiotherapy to primary sites had a superior three-year OS of 57.6% compared to 23.4% in patients who were not irradiated ($p = 0.007$) [31]. Similarly this was reflected in the three-year EFS rate, with 42.5% for irradiated patients compared to 19.5% in patients not receiving radiotherapy ($p = 0.032$) [31].

Reports estimate that 40% of HR patients will relapse [32]. Local relapses at the primary tumor and metastatic sites play a major role in mortality, especially in high-risk patients [30, 31]. The NB97 radiotherapy approach focused on residual tissue of the primary tumor site to compensate for the outcome disadvantage of incomplete response to induction therapy [32]. It was concluded that the administration of radiotherapy could compensate for the residual tumor [32]. Patients who were irradiated at metastatic sites had better OS than patients who did not receive radiation to metastatic sites [33]. Metastatic recurrence was less likely in previously irradiated sites than in unirradiated sites [34]. In this study, patients who could undergo surgery relapsed mainly at metastatic sites (24.5%) as compared to the primary tumor bed (6.6%). The reason could be that less than 16% of patients were irradiated (*see* Supplementary Figure 1). Metastatic progression was the main event in both patients that were not irradiated (57.7%) as well as irradiated patients (42.6%).

In keeping with expert opinion in the SIOP-PODC guidelines, in the absence of a surgical resection, radiotherapy to the primary tumor, with or without metastatic sites, had a short-term benefit in patients, with a superior two-year OS in unresected patients receiving radiotherapy versus patients not receiving radiotherapy [2].

It is not clear to what extent local therapies influence outcomes in HR-NB in therapies that include single or tandem autologous transplants, molecular targeted therapy and maturation therapy [20]. Yet, in variable resource settings where chemotherapy, radiotherapy and surgery are the only treatment options, the impact of surgery with or without radiotherapy on survival has been advantageous. The benefit is amplified with a combination of both. In settings where autologous transplants and targeted therapies are not available, other treatment modalities are paramount in metastatic sites, especially if there were isolated or limited number of metastatic sites [2].

Stellenbosch University https://scholar.sun.ac.za

Limitations

The study was retrospective with non-randomized cohorts and treatment protocols between POUs were not standardized. The determination of risk stratification was limited by the absence of genetic information on the tumors. Documentation of the extent of resection was not standardized. Certain cohort samples were too small to determine significant results.

Conclusion

Where possible, resections should be attempted, regardless if a resection is a complete total resection, near gross total resection or incomplete resection, provided that resections are not mutilating or increase the risk for mortality. Radiotherapy as standard of care should be part of standard management and metastatic control with autologous bone marrow transplants should be considered, if possible, in LMICs. A national treatment protocol should be introduced to standardize assessment and management of HR neuroblastoma with the aim of improving outcomes.

Acknowledgements Dr. van Heerden, as staff member of the Department of Paediatric Haematology and Oncology, Antwerp University Hospital, University of Antwerp, acknowledges the Department for research support. The authors acknowledge the SACCSG, for supporting the study, and the South African Children's Tumour Registry (SACTR), for providing statistical data.

Author contributions JvH conceptualized and designed the study, collected data, performed the data analysis and wrote the manuscript. MK assisted with concept development, as well as design of the study, supervised data analysis, critically reviewed and revised the manuscript. TE performed the statistical analysis. GPH, as expert pediatric surgeon, supervised the surgical content and contributed patient-related data. All other authors collected data in their respective pediatric oncology units and contributed significantly to the manuscript.

Compliance with ethical standards

Conflict of interest There is no conflict of interest to disclose.

Appendix

See Table 7.

Table 7 INRG classification

INRG stage	Age (months)	Histologic category	Grade differentiation	MYCN	11 q aberration	Ploidy	Pretreatment risk group
L1/L2	Any	GN maturing GNB intermittent	Any	Any	Any	Any	A—very low risk
L1	Any	Any, except GN maturing GNB intermittent	Any	nAmp	Any	Any	B—very low risk
				Amp	Any	Any	K—high risk
L2	< 18	Any, except GN maturing GNB intermittent	Any	nAmp	No	Any	D—low risk
					Yes	Any	G—intermediate risk
	≥ 18	GNB nodular, neuroblastoma	Differentiated	nAmp	No	Any	E—low risk
					Yes		H—intermediate risk
			Poorly differentiated or undifferentiated	nAmp	Any		
			Any	Amp	Any	Any	H—high risk
M	< 18	Any	Any	nAmp	Any	Hyperdiploid	F—low risk
	< 12	Any	Any	nAmp	Any	Diploid	I—intermediate risk
	12 to < 18	Any	Any	nAmp	Any	Diploid	J—intermediate risk
	< 18	Any	Any	Amp	Any	Any	O—high risk
	≥ 18	Any	Any	Any	Any	Any	P—high risk
MS	< 18	Any	Any	nAmp	No	Any	C—very low risk
					Yes	Any	O—high risk
				Amp	Any	Any	R—high risk

Stellenbosch University https://scholar.sun.ac.za

References

1. Park JR, Eggert A, Caron H (2008) Neuroblastoma: biology, prognosis, and treatment. Pediatr Clin North Am 55(1):97–120
2. Parikh N, Howard S, Chantada G et al (2015) SIOP-PODC adapted risk stratification and treatment guidelines: recommendations for neuroblastoma in low- and middle-income settings. Pediatr Blood Cancer 62(8):1305–1316
3. Avanzini S, Pio L, Erminio G, Granata C et al (2017) Image-defined risk factors in unresectable neuroblastoma: SIOPEN study on incidence, chemotherapy-induced variation, and impact on surgical outcomes. Pediatr Blood Cancer 64(11):e26605
4. Irtan S, Brisse HJ, Minard-Colin V, Schleiermacher G et al (2015) Image-defined risk factor assessment of neurogenic tumors after neoadjuvant chemotherapy is useful for predicting intra-operative risk factors and the completeness of resection. Pediatr Blood Cancer 62(9):1543–1549
5. Robbins JR, Krasin MJ, Pai Panandiker AS, Watkins A et al (2010) Radiation therapy as part of local control of metastatic neuroblastoma: the St Jude Children's Research Hospital experience. J Pediatr Surg 45(4):678–686
6. Inserra A, Narciso A, Paolantonio G, Messina R et al (2016) Palliative care and pediatric surgical oncology. Semin Pediatr Surg 25(5):323–332
7. Feudtner C, Blinman TA (2013) The pediatric surgeon and palliative care. Semin Pediatr Surg 22(3):154–160
8. Bowman L, Hancock M, Santana V et al (1991) Impact of intensified therapy on clinical outcome in infants and children with neuroblastoma: the St Jude Children's Research Hospital experience, 1962 to 1988. J Clin Onco 9(9):1599–1608
9. Stram D, Matthay K, O'Leary M et al (1996) Consolidation chemoradiotherapy and autologous bone marrow transplantation versus continued chemotherapy for metastatic neuroblastoma: a report of two concurrent Children's Cancer Group studies. J Clin Oncol 14(9):2417–2426
10. Peinemann F, Tushabe D, Berthold F (2015) Rapid COJEC versus standard induction therapies for high-risk neuroblastoma. Cochrane Database Syst Rev 19(5):CD010774. https://doi.org/10.1002/14651858.CD010774.pub2
11. Brodeur G, Pritchard J, Berthold F et al (1993) Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol 11(8):1466–1477
12. Park J, Bagatell R, Cohn S et al (2017) Revisions to the international neuroblastoma response criteria: a consensus statement from the national cancer institute clinical trials planning meeting. J Clin Oncol 35(22):2580–2587
13. Wagner III WE (2019) Using IBM® SPSS® statistics for research methods and social science statistics. Sage Publications, Incorporated, California
14. Bolognese A, Izzo L (eds) (2009) Surgery in multimodal management of solid tumours. Chapter 1: surgery and tumours. Springer-Verlag, Italia, pp 5–16
15. Modak S, Kushner B, LaQuaglia M, Kramer K, Cheung N (2009) Management and outcome of stage 3 neuroblastoma. Eur J Cancer 45:90–98
16. Powis M, Imeson J, Holmes S (1996) The effect of complete excision on stage III neuroblastoma: a report of the European Neuroblastoma Study Group. J Pediatr Surg 31:516–519
17. Von Allmen D, Davidoff M, London W, Van Ryn M et al (2017) Impact of extent of resection on local control and survival in patients from the COG A3973 study with high-risk neuroblastoma. JCO J Clin Oncol 35(2):208–216
18. von Allmen D, Davidoff A, London WB, Van Ryn C et al (2017) Impact of extent of resection on local control and survival in patients from the COG A3973 study with high-risk neuroblastoma. J Clin Oncol 35(2):208–216
19. Holmes K, Poëtschger U, Sarnacki S, Monclair T et al (2018) The influence of surgical excision on survival in high-risk neuroblastoma revisited after introduction of ch14.18/CHO immunotherapy in the HR-NBL1/SIOPEN trial. J Clin Oncol. https://doi.org/10.1200/JCO.2018.36.15_suppl.10521
20. Hadley G, Van Heerden J (2017) High-risk neuroblastoma in a sub-Saharan African country: telling it like it is. Trop Doct 47(4):370–374
21. Yang X, Chen J, Wang N, Liu Z et al (2019) Impact of extent of resection on survival in high-risk neuroblastoma: a systematic review and meta-analysis. J Pediatr Surg 54(7):1487–1494
22. Englum B, Railon K, Speicher P, Gulack B et al (2015) Value of surgical resection in children with high-risk neuroblastoma. Pediatr Blood Cancer 62(9):1529–1535
23. Mullassery D, Farrelly P, Losty P (2014) Does aggressive surgical resection improve survival in advanced stage 3 and 4 neuroblastoma? A systematic review and meta-analysis. Pediatr Hematol Oncol 31(8):703–716
24. La Quaglia M, Kushner B, Su W, Heller G et al (2004) The impact of gross total resection on local control and survival in high-risk neuroblastoma. J Pediatr Surg 39(3):412–417
25. Chamberlain R, Quinones R, Dinndorf P et al (1995) Complete surgical resection combined with aggressive adjuvant chemotherapy and bone marrow transplantation prolongs survival in children with advanced neuroblastoma. Ann Surg Oncol 2:93–100
26. Kiely E (1994) The surgical challenge of neuroblastoma. J Pediatr Surg 29:128–133
27. Nakagawara A, Ikeda K, Yokoyama T et al (1988) Surgical aspects of MYCN oncogene amplification of NB. Surgery 104:34–40
28. Strother DR, London WB, Schmidt ML, Brodeur GM et al (2012) Outcome after surgery alone or with restricted use of chemotherapy for patients with low-risk neuroblastoma: results of Children's Oncology Group study P9641. J Clin Oncol 30(15):1842–1848
29. Gatcombe HG, Marcus RB Jr, Katzenstein HM et al (2009) Excellent local control from radiation therapy for high-risk neuroblastoma. Int J Radiat Oncol Biol Phys 74(5):1549–1554
30. Ferris MJ, Danish H, Switchenko JM et al (2017) Favorable local control from consolidative radiation therapy in high-risk neuroblastoma despite gross residual disease, positive margins, or nodal involvement. Int J Radiat Oncol Biol Phys 97(4):806–812
31. El-Sayed MI, Ali AM, Sayed HA, Eman MZ (2010) Treatment results and prognostic factors of pediatric neuroblastoma: a retrospective study. Int Arch Med 3:37
32. Polishchuk AL, Li R, Hill-Kayser C et al (2014) Likelihood of bone recurrence in prior sites of metastasis in patients with high-risk neuroblastoma. Int J Radiat Oncol Biol Phys 89:839–845
33. Simon T, Hero B, Bongartz R, Schmidt M, Müller RP, Berthold F (2006) Intensified external-beam radiation therapy improves the outcome of stage 4 neuroblastoma in children %3e1 year with residual local disease. Strahlenther Onkol 182:389–394
34. Casey DL, Pitter KL, Kushner BH et al (2018) Radiation therapy to sites of metastatic disease as part of consolidation in high-risk neuroblastoma: can long-term control be achieved? Int J Radiat Oncol Biol Phys 100(5):1204–1209

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Stellenbosch University https://scholar.sun.ac.za

Affiliations

Jaques van Heerden[1] · Mariana Kruger[1] · Tonya Esterhuizen[2] · Marc Hendricks[3] · Jennifer Geel[4] · Ané Büchner[5] · Gita Naidu[6] · Jan du Plessis[7] · Barry Vanemmenes[8] · Ronelle Uys[1] · G. P. Hadley[9] · For the South African Children's Cancer Study Group

1 Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Tygerberg Hospital, Stellenbosch University, Cape Town, South Africa

2 Biostatistics Unit, Faculty of Medicine and Health Sciences, Stellenbosch University, Stellenbosch, South Africa

3 Department of Paediatrics and Child Health, Faculty of Health Sciences, Paediatric Haematology and Oncology Service, Red Cross War Memorial Children's Hospital, University of Cape Town, Cape Town, South Africa

4 Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Health Sciences, Charlotte Maxeke Johannesburg Academic Hospital, University of the Witwatersrand, Johannesburg, South Africa

5 Paediatric Haematology and Oncology, Department of Paediatrics, Steve Biko Academic Hospital, University of Pretoria, Pretoria, South Africa

6 Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Health Sciences, Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand, Johannesburg, South Africa

7 Division of Paediatric Haematology and Oncology, Department of Paediatrics, Faculty of Health Sciences, Universitas Hospital, University of the Free State, Bloemfontein, South Africa

8 Division of Paediatric Haematology and Oncology Hospital, Department of Paediatrics, Frere Hospital, East London, South Africa

9 Department of Paediatric Surgery, Faculty of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 9

The implementation of a national paediatric oncology protocol for neuroblastoma in South Africa (2020)

Ethics no: S18/07/138

Submitted to Cancer Causes and Control November 2020

Van Heerden, Jaques[1,2]; Kruger, Mariana [1].

Affiliations

[1] Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa

[2] Paediatric Haematology and Oncology, Department of Paediatrics Antwerp University Hospital, Antwerp, Belgium

Correspondence:

Dr Jaques van Heerden, Department of Paediatric Haematology and Oncology, University Hospital of Antwerp, Drie Eikenstraat 655, Edegem, 2650 Belgium. E-mail: Jaques.vanheerden@uza.be

Contributors' Statement:

Both authors conceptualised and designed the study. Jaques van Heerden collected data and wrote the manuscript. Mariana Kruger critically reviewed and revised the manuscript.

Running title: Implementation of a national paediatric oncology protocol

Abbreviations:

ASCT	Autologous stem cell transplantation
CFIR	Consolidated Framework for Implementation Research
HR	High risk
IR	Intermediate risk
LMIC	Low- and middle- income country
LR	Low risk
NB	Neuroblastoma
NHI	National Health Insurance
SACCSG	South African Children's Cancer Study Group
OS	Overall survival
PI	Principal investigator
VLR	Very low risk

Stellenbosch University https://scholar.sun.ac.za

ABSTRACT

Background:

The aim of the World Health Organization-International Paediatric Oncology Society is to improve childhood cancer survival in low- and middle-income countries to 60% by 2030. This can be achieved by using standardised evidence-based national treatment protocols for common childhood cancers.

Objectives:

The aim of the study was to describe the development and implementation of the SACCSG NB-2017 neuroblastoma (NB) treatment protocol as part of the treatment harmonisation process of the South African Children's Cancer Study Group.

Methods:

The Consolidated Framework for Implementation Research was used to identify factors that could influence the implementation of the national NB protocol as a health care intervention. The evaluation was done according to five interactive domains for implementation: intervention characteristics, inner setting, outer setting, individual or team characteristics and the implementation process.

Results:

The protocol was developed over 26 months by 26 physicians involved in childhood cancer management. The process included an organisational phase, a resource identification phase, a development phase and a research ethics approval phase. Challenges included nationalised inertia, variable research ethical approval procedures with unnecessary delays and uncoordinated clinical trial implementation.

Conclusion:

The implementation of the national NB protocol demonstrated the complexity of the implementation of a national childhood cancer treatment protocol. However, standardised paediatric cancer treatment protocols based on local expertise and resources in limited settings are feasible.

Keywords:

South Africa, neuroblastoma, national protocols, children, Consolidated Framework for Implementation Research

Stellenbosch University https://scholar.sun.ac.za

ARTICLE

Introduction

According to both the European Commission (ORPHA number 635: neuroblastoma) [1] and the United States Rare Diseases Act of 2002 [2], childhood malignancies such as neuroblastoma (NB) are classified as rare diseases. Although great clinical and biological advances have been made with regard to paediatric tumours worldwide, the multitude of approaches demand significant human and financial resources [2]. A disadvantage is the isolated development of management protocols that are not reproducible in other settings due to non-standardisation [2]. A good example regarding NB was the development of various classification systems and treatment approaches in North America by the Children's Oncology Group (COG), the International Society of Paediatric Oncology Europe Neuroblastoma Group (SIOPEN) and other paediatric oncology societies in Japan, Australia and New Zealand [3,4]. An international collaboration of various NB workgroups therefore led to the establishment of the International Neuroblastoma Risk Group (INRG) and the development of the INRG classification system, based on pooled data from multiple countries. This collaboration led to clinical trials with standardised protocols with a greater number of included participants, which improved statistical significance [5]. The initiative of the INRG and international NB clinical trials is evident in the improved outcomes for high-risk (HR) disease from five-year overall survival (OS) of 20% to 57% due to this cooperative research over the past 20 years [6]. Yet, due to the lack of advanced laboratory capacities in low- and middle-income countries (LMICs), the genetic information standardly used as part of this classification system in high-income countries (HICs) limits the use of the NB stratification system in LMICs.

The South African Children's Cancer Study Group (SACCSG) established a South African Children's Tumour Registry in 1987 [7,8]. Limited data on the treatment of NB beyond a single institutional report by Hesseling et al. in 1990 was available in South Africa prior to the start of the process to develop a national NB treatment protocol [9]. To date, treatment strategies for NB in South Africa have been diverse and based on the experience of individual paediatric oncologists [10]. The treatment of NB was managed according to the available resources and used multiple international protocols. The SACCSG supported the development of a national NB management protocol in 2016, which led to the SACCSG NB-2017 study. The protocol was developed in line with the World Health Organization (WHO)-International Paediatric Oncology Society (SIOP) aim to improve childhood cancer survival in LMICs to 60% by 2030 [11].

The aim of this article is to describe the development and implementation of the SACCSG NB-2017 clinical trial according to a validated implementation research framework. The Consolidated Framework for Implementation Research (CFIR) domains and associated constructs were used for evaluation purposes based on the data gathered during the various phases of the development of the protocol [12]. Based on the CFIR domains and constructs, tables were constructed from e-mails, meeting notes and workshop discussions during the development of the clinical trial [12,13]. The CFIR was chosen because it allowed for both system-level evaluation and linking of the influence of individual action and behaviour during the implementation process [13,14]. The CFIR allowed for the evaluation of the national governance structures, paediatric oncology units (POUs) and individuals involved in the implementation process [13,14]. A descriptive overview of the analysis or

Stellenbosch University https://scholar.sun.ac.za

interpretation was used to describe the thematic content of the clinical trial development and implementation process.

Setting

South Africa has a heterogeneous medical system [15]. Public health care is proportionally funded by the central government in each of the nine provinces. The health authorities within each province determine the financial expenditure for development of medical services in that province. The private health care system is funded on a pay-for-service business model, and private medical insurance plans are available to those who can afford the contribution tariffs [15]. Since the inception of democracy in 1994, the government has introduced several development strategies to improve health care and increase access to and affordability of cancer care services and research [16]. One example is the free primary health care for children under five years [16,17]. Access was addressed by expanding the health care network to decrease travel distances [17].

Several regulatory processes that support development of national treatment protocols for rare diseases have been developed since 1994 in South Africa. The National Cancer Registry is managed under the umbrella of the National Institute of Communicable Diseases [18]. The National Health Research Ethics Council, established under the National Health Act No 61 of 2003, provides guidance regarding health care research in line with international guidelines [19].

Development of the national neuroblastoma clinical trial

The SACCSG initiated the harmonisation of management for paediatric tumours in 2010. The aim was to standardise the management of childhood malignancies across all South African POUs. Individual tumour workgroups with a principal investigator were established to develop national treatment protocols. The workgroups consisted of collaborations among various health care teams in the management of childhood malignancies and included paediatric oncologists, surgeons, radio-oncologists as well as laboratory and imaging services from several hospitals. Each workgroup was responsible for evaluation of the resources of all the POUs across South Africa to manage malignancies, evaluation of contemporary clinical trials for the relevant tumours and development of a standard of care management protocol that could be implemented and executed in all POUs of the country and adapted for the local context [20,21,22].

An NB workgroup was established in 2016, and a treatment protocol was developed. To prepare for this national clinical trial, a retrospective study was undertaken to evaluate the management of NB between 2000 and 2014 as well as a survey to evaluate currently available resources for the management of NB in South Africa, which resulted in a publication in 2019 [10]. The protocol development was done via online discussions, document reviews, paediatric oncology meetings and tumour-related workshops. After finalisation of the management protocol through consensus, research ethics approval was obtained.

Study objectives

The study objectives were as follows:

1) To describe the development of the SACCSG NB-2017 clinical trial as a future resource for similar projects.

Stellenbosch University https://scholar.sun.ac.za

2) To assess the facilitators of and barriers to both the development and implementation of a national paediatric oncology clinical trial.

Methods and analysis

The evaluation was done according to five interactive domains for implementation: intervention characteristics, inner setting, outer setting, individual or team characteristics and the implementation process. The first author (JvH) allocated the themes and described the relevance for the evaluation of the implementation of the trial. The second author (MK) critically evaluated and edited the text, tables, themes and descriptions. The implementation evaluation was completed by consensus between the two authors.

Development of the SACCSG NB-2017 clinical trial

South Africa has 13 public POUs and six private health care POUs, situated in seven of the nine provinces (Table 1). These POUs are linked to seven universities where the research was conducted. The SACCSG NB-2017 clinical trial was developed mainly by paediatric oncologists, including the principal investigator (PI) and co-principal investigator, as well as 12 additional paediatric oncologists. Four additional clinical contributors (not the primary paediatric oncologists) also participated. The sub-discipline guidelines were developed by eight discipline-specific experts in conjunction with the PI. These experts included paediatric surgeons (n = 2), radio-oncologists (n = 2), anatomical pathologists (n = 2), a nuclear physician (n = 1) and a laboratory haematologist (n = 1).

The first SACCSG NB protocol meeting was held in 2016 whereafter the protocol development took 26 months (Table 2). The process of developing this protocol constituted four parts (Figure 1 and Appendix A), namely establishing need and consensus, identifying the local health care resources, including medical experts and project managers, facilitating and contributing to the development of the protocol and ethics approvals.

Establishing need and national paediatric oncology organisation approval

The SACCSG process for any national paediatric oncology study involved presentation and approval at an official SACCSG meeting with a minimum of one representative per South African POU present. The NB protocol was presented at the SACCSG protocol workshop in Durban, South Africa, in September 2016. The presentation included the scope, aims and academic studies associated with the protocol development. The consensus for development served as an invitation for interested physicians to involve themselves in the protocol development.

Establishing resources for protocol development

Local medical expertise: Each POU identified a physician responsible for local data management, interdisciplinary management and protocol oversight. These physicians became members of the SACCSG NB working group who evaluated the literature and protocol drafts and contributed to ensure feasibility in their local setting. The group also included physicians with an interest in palliative care to develop guidelines for non-curative management.

Stellenbosch University https://scholar.sun.ac.za

Sub-disciplinary expertise: Physicians with NB experience in the fields of paediatric surgery, radio-oncology, pathology, laboratory haematology and nuclear medicine were invited and participated in creating guidelines for the management of NB by each sub-discipline. These physicians developed discipline-specific management guidelines and amended protocol drafts and adapted the international standards for the local setting.

Local logistics: During November 2016, a survey was completed by each local hospital investigator. The survey evaluated the resources available in the respective hospitals for treating children with NB (Appendix B).

National NB experience: A retrospective study to evaluate the management and outcomes of NB in South Africa between 2000 and 2014 was approved by the hospital investigators. In January 2017, the protocol was approved by the University of Kwa-Zulu Natal Biomedics and Research Ethics Council (BREC Ref no: BE572/16) and the data collection was started, which resulted in a publication in 2019 [10].

International experience: The members of the SACCSG NB working group reviewed the literature for evidence-based NB management from international NB working groups to guide decisions during the protocol development. International experts in NB management were consulted.

Financial resources: The vzw Kinderkankerfonds, Belgium, provided developmental funds [23].

Clinical trial initiation and development

The SACCSG NB-2017 clinical trial aimed to standardise an NB management protocol as a single-arm clinical trial in South Africa to improve outcome. As the protocol served as an exercise for implementation, the decision by the NB working group was to include only OS (two years and five years) and event-free survival (two years and five years) in the protocol as primary endpoints.

The development was done according to two parallel action plans:

Consensus decisions were based on four key criteria: established international research evidence, local expertise, availability of resources for the clinical trial in all POUs in South Africa, and financial costs and sustainability. Protocol-specific consensus recommendations can be seen in Table 1.

Firstly, regarding tumour-related diagnostic and chemotherapy protocols, the diagnostic and evaluation requirements, risk stratification and protocols relating to chemotherapy were collaboratively developed by die paediatric oncology physicians (hospital investigators). The treatment approaches were adopted by all POUs of the NB working group. The development was done in three stages: Firstly, the management principles were established during the SACCSG protocol development meeting at the Stellenbosch Institute for Advanced Study in May 2017 (Appendix C). Summaries of the literature review were presented, and recommendations were proposed for the protocol section. It was concluded that the protocol would primarily be a standard of care, curative treatment protocol and would secondarily be supported by a palliative section. There would be a single treatment arm with no randomisation. Due to the complexity of the pathology, the operational research data and outcome-based indicators should be collected prospectively. The second meeting was an online SACCSG NB working group meeting hosted on the web-based Cure4Kids platform, Africa

Stellenbosch University https://scholar.sun.ac.za

Room, St. Jude Children's Research Hospital, in January 2018 (Appendix C). The sections on which consensus had been reached during the first and second draft reviews were discussed, and the concluded sub-discipline guidelines were presented to the NB working group. Consensus was not reached on the induction chemotherapy for HR NB nor on the scope of the autologous stem cell transplant (ASCT) section. The third meeting was an NB working group meeting at the Radisson Hotel, Sandton, in April 2018 (Appendix C). The remaining sub-discipline guidelines were presented. Final consensus was reached on all sections after pre-prepared two-member debates on the positive and negative aspect for implementation in the South African setting.

Secondly, associated sub-discipline guidelines were developed by the principal investigator and sub-discipline experts based on literature reviews, expert opinions and practical considerations for the South African setting. The guidelines were incorporated as guidelines that allowed for the adaptation during management, subject to available local health care resources.

The protocol was developed for all NB-related management aspects independent of risk stratification or funding options. Therefore, private funding would benefit those who wanted greater access to advanced treatment options such as ASCT or treatment options such as targeted therapy that are not available in the country.

Ethics reviews and implementation

During this period, all the necessary documentation was prepared for academic evaluation and ethics clearance by universities and for ethics clearance by governmental (provincial and national) and hospital authorities. Approval of the protocol constituted 42 applications to different authorities. The duration of the ethics review committee evaluations varied from 1 month to 20 months (1 still pending) (range 1-20 months, mean = 5 months, median = 2 months). The total duration for an application (academic and research ethics review) for the POUs varied from 2 months to 20 months (still pending) (range 2-20 months, mean = 10 months, median = 12 months) (Table 2).

Facilitators of and barriers to development and implementation of a national paediatric oncology clinical trial

The implementation of the SACCSG NB-2017 clinical trial was important in establishing a multidisciplinary, national standardisation of the treatment of this rare tumour in children. The management of NB necessitates multiple disciplines in the diagnosis, treatment and continued evaluation of the pathology. These interactions in a resource-strained setting are often challenged by varied experience in management and perceived treatment goals [24]. The CFIR provided an organising framework to identify implementation factors and essential lessons during the development and implementation of the protocol (Table 3).

Development and sustainability

A collaborative clinical trial is only possible when each collaborator remains continually responsible for his/her delegated functions through ownership and contribution of local knowledge [24,25]; therefore, hospital investigators functioned as liaison between the protocol and other disciplines, which prevented unilateral implementation. The NB workgroup was established to ensure that each POU had resources in place to execute the SACCSG NB-2017 clinical trial. The prescribed benefits by

Stellenbosch University https://scholar.sun.ac.za

private medical insurance for treatments and investigations dictate and to a large degree guide the management of treatment in private hospitals. Health care systems managing fixed budgets are attentive to problems of implementation in order to maximise the health care funds [24,25].

Individual factors

Historically, POUs in South Africa were self-determining departments. The NB management protocols were based on strong evidence from international protocols, systematic reviews and clinical practice guidelines [26]. In some instances, a deviation from known local practices was required to achieve a single standard of care that would be feasible in all POUs regardless of unequal access to resources. In general, established clinical practice is slow to change, referred to as 'clinical inertia' [27], which was present especially in determining the standard induction chemotherapy for HR NB. The retrospective review showed numerous induction regimes that were used from 2000 to 2014 [10,26]. Yet, in the South African context, none proved superior when considering post-induction remission and outcomes although the toxicity profile of OPEC/OJEC proved more beneficial than the RAPID COJEC protocol and doxorubicin-containing protocols [26].

The interests and priorities of each POU and team determined their culture or attitude towards the clinical trial and its implementation. A mayor contributing factor was the lack of time and resources to facilitate implementation and complete administration. The protracted process for state research ethics applications increased the lapse of time between training on the study procedures and initiation of the study after approval had been granted. Two sites delayed ethics application by requiring new academic evaluations after an academic evaluation had been done at the principal investigator's site.

External regulatory environment

The National Cancer Strategic Framework for South Africa 2017-2022 does not address the needs of paediatric oncology frameworks [27], nor does it acknowledge paediatric oncology as a discipline independent from adult oncology services. Therefore, development of the SACCSG NB-2017 clinical trial was initiated by the SACCSG and the NB workgroup on the basis that management of NB would be based on international evidence with the available national resources.

Systems and technologies

Each POU had its own system of data documentation, retrieval and storage. These included files (paper-based) or basic computerised data capture systems, each with its own advantages and disadvantages. Understanding the challenges of transitioning from traditional data systems to an online system such as REDCap [28], the study was started with paper-based and Excel-based databases with transition to REDCap. With the PI hosting the database at a single university, access restrictions had to be navigated for the workgroup with university approval needed for each collaborating researcher.

Implementation process

No standardised implementation process existed for national paediatric oncology clinical trials in South Africa. After the national retinoblastoma clinical trial initiated in 2012 [21], the SACCSG NB-2017 clinical trial is one of three newly developed national clinical trials for implementation. Due to varying

Stellenbosch University https://scholar.sun.ac.za

duration of research ethics approvals, a coordinated implementation was not possible. During this period, two POUs were established for treating children with malignancies, from which research ethics approval had to be obtained. The movement of evidence-based practices into routine clinical practice demands focused efforts; therefore, the protocol was based on current POU practices [24,25]. Yet, linked to resources, a greater number of training sessions in the utilisation of datasets and documentation were needed in order to activate the team.

Resources

The lacking resources in NB management identified were human resources, provision of supportive care during chemotherapy toxicity and advanced treatment options such as ASCT [24,25]. A multidisciplinary team representing multiple departments is needed to manage patients with NB. The outcome of a patient is therefore linked to treatment modalities that collectively determine a treatment result. If treatment response is inadequate, surgery is not possible and the administration of radiotherapy becomes more important. The inconsistent availability of isotopes for mIBG scans limited the important treatment evaluation modality.

Another constraint was time as the implementation of a clinical trial requires a great amount of time from each investigator. In the South African context, the paediatric oncologist has a dual role as clinician and researcher, added to numerous other duties, which include both undergraduate and postgraduate education. Some POUs have one clinician who is the proxy hospital researcher for all tumour-specific clinical trials. Research assistants could strengthen the research capacity in POUs.

Challenges

Evidence-based, practice-changing clinical trials in POUs to improve the health systems that currently govern the institutions should support research initiatives, ethics committees should contribute to the ease of implementation of quality research and the vision of each POU should promote the implementation of clinical trials. This includes a balance between clinical duties and research.

Introducing new standardised protocols and new technologies such as REDCap for data capturing into an established administrative system necessitates training and increasing the skills of the staff of a POU to initiate and maintain databases [12,28]. The continued functionality of the data system is reliant on more than a single person to ensure sustained function of the system.

The reliability of the initial data whilst implementing the clinical data system could be limited since only a small number of participants were enrolled in this study [24,29]. This was the knock-on effect of the delayed ethics approvals, staggered guidance with initiating enrolment of patients and development of various paper, electronic and online data tools.

As part of the health care system in South Africa, the development of paediatric oncology services faces obstacles that include unequal distribution of resources, increased disease burden of both communicable and non-communicable diseases, limited management and leadership experience to transform the health care system, and limited research support and development to optimise the implementation of national clinical trials [16].

Stellenbosch University https://scholar.sun.ac.za

International implementation of paediatric health care initiatives

The components of national childhood malignancy strategies in LMICs include accredited POUs, financial coverage of paediatric oncology treatment, paediatric cancer registries and a national paediatric oncology governing body [30]. Of great importance is the development of national standards. In South Africa, the same challenges of non-standardisation and limited resources were cited in the treatment of Hodgkin lymphoma and retinoblastoma [20,21]. In contrast to HIV/AIDS and tuberculosis care in South Africa in which improvement of outcomes has been achieved by making treatment available over a great network, standardised care in childhood cancer relies on early detection and referral to centralised POUs [31,32,33]. Yet, common denominators for childhood cancer, HIV/AIDS and tuberculosis programmes were the variable needs of patients and medical staff and an increased need for resources and support during implementation of programmes and doing research [31,32]. Increased resources proved beneficial for improved outcomes during the implementation of acute lymphoblastic leukaemia protocols in South America [33]. A national standardised protocol based on resources and availability of supportive care in the Dominican Republic improved the two-year OS for children diagnosed with acute lymphoblastic leukaemia from 40% to 70% by reducing the intensity of treatment and toxicity [34]. Morocco introduced the risk-adapted stratification and treatment guidelines for NB, which has decreased the challenges for the accurate diagnosis and optimal treatment of NB [35].

Recommendations

The development and approval of a national clinical trial would be facilitated by reciprocal or centralised ethics and academic approvals [36,37]. The same applies to external regulation of the government as well as hospital and provincial approvals. A homogenous approach to the application systems at universities would provide the first step in simplifying the process.

Acknowledging the need for funding and research support by both governmental and non-governmental organisations for national projects should gain greater priority. This would improve establishing national data collection platforms and contribute to the financial sustainability of health care systems.

The National Department of Health has to implement a strategic policy relating to the care of children with oncological diseases in South Africa. Greater government support and endorsement would highlight the care of children with cancer in South Africa.

Worldwide, health care settings are becoming more dynamic and more resource constrained yet interconnected due to electronic resources and are driven by equally complex political and economic factors [38]. Accordingly, maximising health care results has become a policy requirement internationally [38]. Therefore, even in LMICs, health care systems and health sciences should develop in parallel to meet the services need [29,38].

Conclusion

LMICs, such as South Africa, have the capacity to establish a framework for improved clinical care, greater research capacity and continued sustainable evaluation of management for better outcomes in NB management. The SACCSG NB-2017 collaborative national clinical trial constitutes the

Stellenbosch University https://scholar.sun.ac.za

confluence of local experience and multiple incorporated international guidelines. This implementation evaluation can serve as the stimulus for other LMICs to establish NB programmes according to their individual resources.

Conflict of interest

There is no conflict of interest to disclose.

Acknowledgements

The SACCSG Neuroblastoma Workgroup: Dr Jaques van Heerden, Prof Mariana Kruger, Dr Marc Hendricks, Dr Ronelle Uys, Dr Jan du Plessis, Dr Ané Büchner, Prof Janet Poole, Dr Ele Madzhia, Prof Gita Naidu, Dr Barry van Emmenes, Dr Johani Vermeulen and Dr Liezl du Plessis.

Contributors to the NB protocol and guidelines: Prof GP Hadley and Dr Derek Harrison (Surgery), Prof Jeanette Parks and Dr Thanushree Naidoo (Radiotherapy), Prof Pawel Schubert and Prof Komala Pillay (Anatomical Pathology), Dr Anita Brink (Nuclear Medicine), Prof Leriska Haupt (Haematology), Dr Anel van Zyl and Prof Jennifer Geel (Paediatric Oncology); Dr Nicolene Moonsumy (Paediatric Haematology), Ms Judy Schoeman (Dietetics and system design).

Prof Kate Matthey and Prof Nicolas André are acknowledged for their contributions during personal or e-mail consultations during the development of the prospective protocol.

Dr van Heerden, as staff member of the department of Paediatric Haematology and Oncology, Antwerp University Hospital, University of Antwerp, acknowledges the department for research support as well as the vzw Kinderkanderfonds, Belgium for the initial research grants.

The authors acknowledge the SACCSG for supporting the study and the South African Children's Tumour Registry (SACTR) for providing statistical data.

References:

1. Rath, A; Ali, H. Orphanet Report Series - List of rare diseases and synonyms listed in alphabetical order - January 2020 http://www.orpha.net/orphacom/cahiers/docs/GB/List_of_rare_diseases_in_alphabetical_order.pdf (Accessed 15 June 2020)
2. Kotecha RS, Kees UR, Cole CH, Gottardo NG. Rare childhood cancers - an increasing entity requiring the need for global consensus and collaboration. Cancer Med. 2015;4(6):819-824.
3. Monclair T, Brodeur GM, Ambros PF, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. J Clin Oncol. 2009; 27(2): 298-303.
4. Pinto NR, Applebaum MA, Volchenboum SL, et al. Advances in Risk Classification and Treatment Strategies for Neuroblastoma. J Clin Oncol. 2015; 33(27): 3008-3017.
5. Arora, R; Bakhshi, S. Indian Pediatric Oncology Group (InPOG) –Collaborative Research in India Comes of Age, Pediatric Hematology Oncology Journal (2016), doi: 10.1016/j.phoj.2016.04.005.
6. Fletcher JI, Ziegler DS, Trahair TN, Glen MM, et al. Too many targets, not enough patients: rethinking neuroblastoma clinical trials. Nat Rev Cancer. 2018; 18(6): 389–400.

Stellenbosch University https://scholar.sun.ac.za

7. The South African Children's Cancer Study Group. About SACCSG. www.saccsg.co.za (Accessed 15 June 2020)
8. Stefan DC, Stones DK, Wainwright RD, Kruger M, et al. Childhood cancer incidence in South Africa, 1987 - 2007. SAMJ: South African Medical Journal. 2015; 105(11): 939-947.
9. Hesseling PB, Ankone K, Wessels G; et al. Neuroblastoma in southern Africa: epidemiological features, prognostic factors and outcome. Ann Trop Paediatr. 1999;19(4):357-363.
10. Van Heerden, J, Hendricks, M, Geel, J, et al. Overall survival for neuroblastoma in South Africa between 2000 and 2014. Pediatr Blood Cancer. 2019; 66: e27944 https://doi.org/10.1002/pbc.27944.
11. International Society of Paediatric Oncology. WHO Global Initiative for Childhood Cancer. https://siop-online.org/who-global-initiative-for-childhood-cancer/ Accessed October 2020.
12. Damschroder LJ, Aron DC, Keith RE, Kirsh SR, et al. Fostering implementation of health services research findings into practice: a consolidated framework for advancing implementation science. Implementation Science. 2009; 4(1): 1035–1039.
13. Means AR, Kemp CG, Gwayi-Chore MC, et al. Evaluating and optimizing the consolidated framework for implementation research (CFIR) for use in low- and middle-income countries: a systematic review. Implement Sci. 2020;15(1):17.
14. Rapport F, Smith J, O'Brien TA, et al. Development of an implementation and evaluation strategy for the Australian 'Zero Childhood Cancer' (Zero) Program: a study protocol. BMJ Open 2020;10: e034522. doi:10.1136/bmjopen-2019-034522
15. South African Government. Health. www.gov.za (Accessed 15 June 2020).
16. Maphumulo WT, Bhengu BR. Challenges of quality improvement in the healthcare of South Africa post-apartheid: A critical review. Curationis. 2019;42(1):e1-e9.
17. Burger R, Christian C. Access to health care in post-apartheid South Africa: availability, affordability, acceptability. Health Economics, Policy and Law. 2018; 1–13: doi:10.1017/S1744133118000300.
18. National Cancer Registry, National Institute for Communicable diseases. https://www.nicd.ac.za/centres/national-cancer-registry Accessed 13 August 2020.
19. National Health Research Ethics Council. Department of Health. http://nhrec.health.gov.za Accessed 13 August 2020.
20. Geel J, Hendricks M, Eyal K; et al. O-045 Harmonisation of Hodgkin Lymphoma Treatment for Children, Adolescents and Young Adults in South Africa. Action Research. Paper presented at: 50th Congress of the International Society of Paediatric Oncology (SIOP); 2018 November 16-19; Kyoto, Japan.
21. Kruger M, Wainwright L, Davidson A; et al. O-093. Report of First National Collaborative Treatment Protocol of Retinoblastoma in South Africa: An Interim Analysis, Action Research. Paper presented at: 50th Congress of the International Society of Paediatric Oncology (SIOP); 2018 November 16-19; Kyoto, Japan.
22. Hendricks M, Cois A, du Plessis J ; et al. PO112 SIOP19-0819 Favourable Outcomes for South African Children and Adolescents with Mature and Immature Teratomas (1990-2015): First Report by the South African Children Cancer Study Group. Action Research. Paper presented at: 51th Congress of the International Society of Paediatric Oncology (SIOP); 2019 October 23-26; Lyon, France.
23. Vzw Kinderkankerfonds. https://www.kinderkankerfonds.be/home/ Accessed 8 August 2020.
24. Hosey GM, Rengiil A, Maddison R, Agapito AU, Lippwe K, Wally OD, et al. US associated Pacific islands health care teams chart a course for improved health systems: implementation and

Stellenbosch University https://scholar.sun.ac.za

evaluation of a non-communicable disease collaborative model. J Health Care Poor Underserved. 2016;27(4A):19.

25. Shi J; Jiang C; Tan D; Yu D; et al.: Advancing Implementation of Evidence-Based Public Health in China: An Assessment of the Current Situation and Suggestions for Developing Regions. BioMed Research International. 2016; 1–8. doi:10.1155/2016/2694030
26. Van Heerden J, Geel J, Hendricks M, et al. The evaluation of induction chemotherapy regimens for high-risk neuroblastoma in South African children, Pediatric Hematology and Oncology. 2020; 37:4, 300-313.
27. The National Department of Health. The National Cancer Strategic Framework for South Africa 2017-2022. www.health.gov.za (Accessed 25 May 2020).
28. PA Harris, R Taylor, R Thielke, J Payne, N Gonzalez, JG. Conde, Research electronic data capture (REDCap) – A metadata-driven methodology and workflow process for providing translational research informatics support*, J Biomed Inform. 2009 Apr;42(2):377-81.*
29. Roundtable on Translating Genomic-Based Research for Health; Board on Health Sciences Policy; Health and Medicine Division; National Academies of Sciences, Engineering, and Medicine. Applying an Implementation Science Approach to Genomic Medicine: Workshop Summary. Washington (DC): National Academies Press (US); 2016 Jul 6. 2, Implementation Science: Methods and Approaches. Available from: https://www.ncbi.nlm.nih.gov/books/NBK373700/ (Accessed 15 April 2020).
30. Gupta S, Rivera-Luna R, Ribeiro RC, Howard SC (2014) Pediatric Oncology as the Next Global Child Health Priority: The Need for National Childhood Cancer Strategies in Low- and Middle-Income Countries. PLoS Med 11(6): e1001656.
31. Harries AD, Lawn S, Getahun H, Zachariah R, Havlir D. Journal of the International AIDS Society 2012, 15:17396.
32. Naidoo N, Zuma N, Khosa NS, Marincowitz G, Railton J, Matlakala N, Marinocowitz G, Railton J, et al. Qualitative assessment of facilitators and barriers to HIV programme implementation by community health workers in Mopani district, South Africa. PLoS ONE. 2018; 13(8): e0203081.
33. Howard S, Pedrosa M, Lins M, Pedrosa A, Pui C, et al. Establishment of a Pediatric Oncology Program and Outcomes of Childhood Acute Lymphoblastic Leukemia in a Resource-Poor area. JAMA. 2004; 291 (20): 2471-2475.
34. Hunger SP, Reyes D, Negrin O, Montero M, De la Rosa L, et al Decreased early mortality and increased survival with less intensive therapy for acute lymphoblastic leukemia (ALL) in the Dominican Republic. Pediatr Blood Cancer. 2011; 57: 761.
35. Salman Z, El Kababri M, Hessissen L, Khattab M, Matthay K. An Intensive Induction Protocol for High Risk Neuroblastoma in Morocco. Journal of Global Oncology 2016 2:3_suppl, 80s-81s.
36. Government of Canada, Panel on Research Ethics. Module 10: Multi-Jurisdictional Research Ethics Review. 2019. https://ethics.gc.ca/ Accessed September 2020.
37. Dove ES, Garattini C. Expert perspectives on ethics review of international data-intensive research: Working towards mutual recognition. Research Ethics. 2018;14(1):1-25.
38. Bauer MS, Damschroder L, Hagedorn H, Smith J, Kilbourne AM. An introduction to implementation science for the non-specialist. BMC Psychol. 2015;3(1):32.

Stellenbosch University https://scholar.sun.ac.za

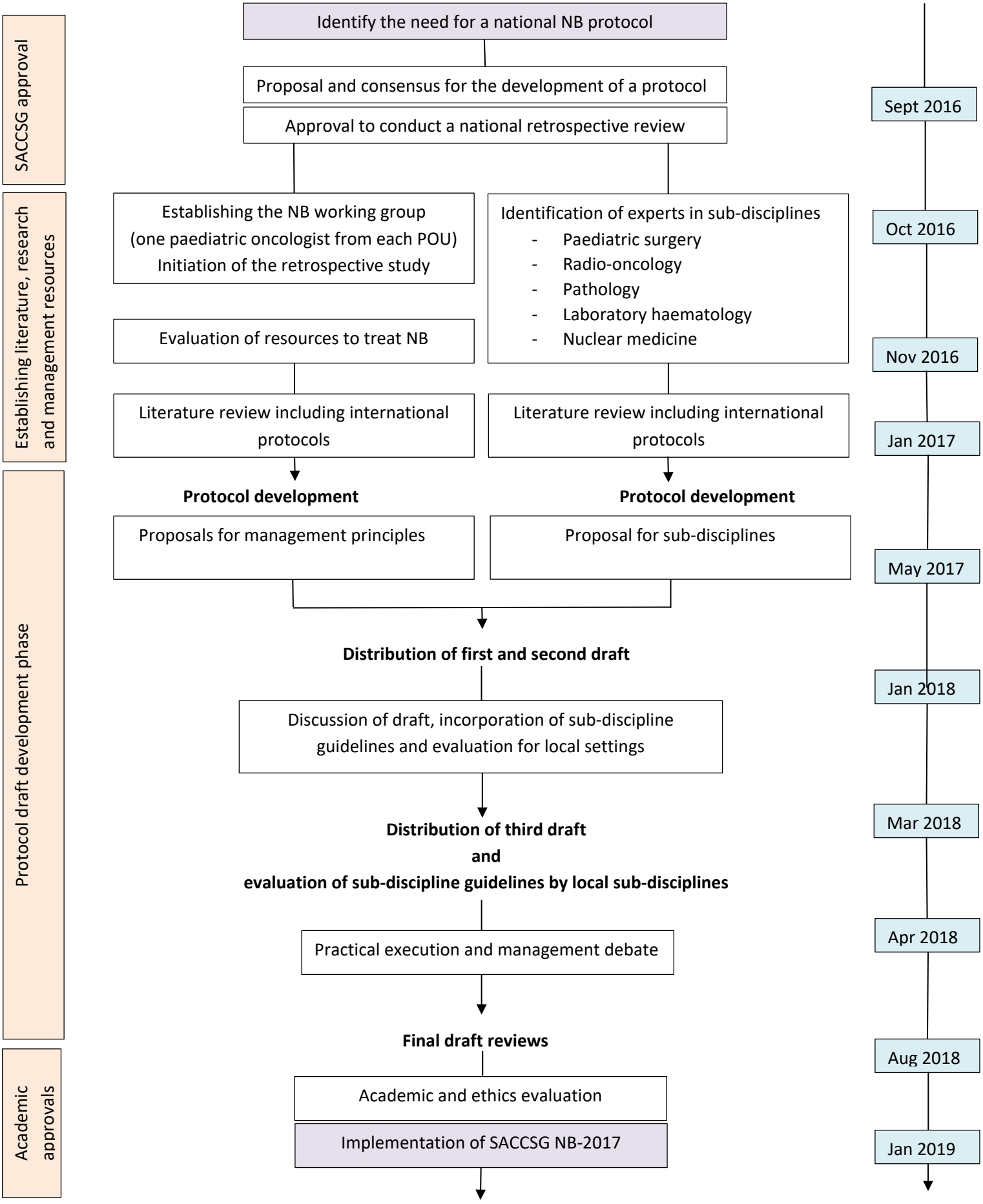
Figures:

Tables:

Appendices:

Stellenbosch University https://scholar.sun.ac.za

Figure 1: The protocol and guidelines development process



Stellenbosch University https://scholar.sun.ac.za

Tables

Table 1: Consensus protocol recommendations based on the four key criteria

Recommendations for SACCSG NB-2017	International evidence	Local expertise	Resource availability and implementation	Cost and sustainability
Diagnosis - Clinical evaluation plus NB-defining imaging plus raised u-catecholamine levels - Tissue biopsy - NB-defining cells on bone marrow aspiration and trephine	SIOP-PODC recommendations for NB in LMICs	Variable availability of diagnostic investigations Protocol should be flexible	Resources that are available to each POU	Standard of care benefits public medical service costs and adheres to private health care benefits
Staging Investigations as part of staging: - Skeletal survey - Ultrasound of the abdomen - CT/MRI - BMAT - Bone scan - mIBG scan	SIOP-PODC recommendations for NB in LMICs	Variable availability of diagnostic investigations Protocol should be flexible for each POU to complete staging Must include BMAT	Resources that are available to each POU	Standard of care benefits public medical service costs and adheres to private health care benefits
Laboratory investigations Investigations as part of diagnostic phase: - FBC/UCE/LFT - LDH/ferritin - u-catecholamine levels	SIOP-PODC recommendations for NB in LMICs	SIOP-PODC recommendations for NB in LMICs	Resources that are available to each POU	Standard of care benefits public medical service costs and adheres to private health care benefits
Nuclear investigations Where available, the following investigations should be done: - Nuclear GFR - Bone scan (only when mIBG scan is not available) - mIBG scan	SIOP-PODC recommendations for NB in LMICs	- mIBG scan not sooner than two weeks post chemotherapy - Just before following course of chemotherapy	- Not all units have access to nuclear investigations - Isotopes for mIBG scans not always available or available on time	Standard of care benefits public medical service costs and adheres to private health care benefits

Stellenbosch University https://scholar.sun.ac.za

- PET scan (in mIBG-non-avid tumours)		- No benefit for an intermediary evaluation when done during chemotherapy - Postinduction mIBG scan should be done - PET scan in +/- 10% mIBG-non-avid patients - Bone scan can be used at diagnosis but not for disease response evaluations		Sustainability dependent on isotope production
Platinum alkylators	Nuclear GFRs are the most sensitive	- Renal function screening with Schwartz formula and nuclear GFRs - Use of the Boston ototoxicity scale		
Tumour sampling According to available expertise: - Biopsy - Fine-needle aspiration	SIOP-PODC recommendations for NB in LMICs	- Fine-needle aspiration technique according to pathology departments with expertise - Core biopsy according to guidelines of COG and SIOPEN protocols	Resources that are available to each POU	Consider not sampling patients with a possibly poor outcome with advanced disease Standard of care benefits public medical service costs and adheres to private health care benefits
Bone marrow aspiration Indications: - Diagnosis - Staging	SIOP-PODC recommendations for NB in LMICs	SIOP-PODC recommendations for NB in LMICs	Resources that are available to each POU	Standard of care benefits public medical service costs and

Stellenbosch University https://scholar.sun.ac.za

- MYCN and genetics				adheres to private health care benefits
Pathology Indications: - Diagnosis - MYCN and staging	SIOP-PODC recommendations for NB in LMICs	Indications: - Diagnosis - MYCN and staging	Resources that are available to each POU	Standard of care benefits public medical service costs and adheres to private health care benefits
Genetic markers - MYCN	SIOP-PODC recommendations for NB in LMICs	- MYCN - Where not available on site to be determined off site	Resources that are available to each POU	Standard of care benefits public medical service costs and adheres to private health care benefits
Risk stratification - Determine which risk classification system is preferred - Discuss which risk stratifications to incorporate	SIOP-PODC recommendations for NB in LMICs	- LDH and ferritin should be utilised more - MYCN should be used especially in patients when it determines whether a patient will be upstaged - Choice of a radiological-based risk stratification - Upstage the following patients with MYCN-amplification: Stage 4 and Stage 4s tumour < 18 months, all Stage 2 tumours and Stage 3 in the absence of known LDH and ferritin values	Few VLR and LR patients documented. The approaches are comparable; therefore, only an LR stratification will be used, which includes the VLR stratification.	Sustainable
Curative treatment				

Stellenbosch University https://scholar.sun.ac.za

- Protocol to be a curative protocol with a palliative section - One protocol rather than separate protocols for each risk stratification				Standard of care benefits public medical service costs and adheres to private health care benefits Sustainability dependent on access to chemotherapy
LR and VLR Approach to patients with VLR and LR tumours	SIOP-PODC recommendations for NB in LMICs	- Local expertise comparable to international recommendations	Request to define 'symptomatic tumour pathology' – the Philadelphia score was included	
IR Approach to patients with IR tumours	SIOP-PODC recommendations for NB in LMICs	- The greatest challenge is to define true IR compared to false IR due to limitations in genetic testing - Utilise LDH and ferritin to decide on upstaging from IR to HR - Limited options for relapse patients; therefore, rather upstage a patient than trying to salvage a false-IR patient	- Developing a risk stratification for South African purposes - Based on LDH and ferritin as surrogates for MYCN and genetic aberrations - Compare the South African stratification with international stratifications	
HR Approach to patients with HR tumours	SIOP-PODC recommendations for NB in LMICs	*Induction chemotherapy* - Preliminary data indicated neither doxorubicin-based induction chemotherapy, OPEC/OJEC nor rapid COJEC were superior protocols	*Induction chemotherapy* - OPEC/OJEC was chosen to be the induction chemotherapy - Rapid COJEC could be used where private funding was available, where a POU could	ASCT dependent on ability of referral transplant centres to treat patients ASCT dependent on access to conditioning chemotherapy and supportive care options

Stellenbosch University https://scholar.sun.ac.za

		- Rapid COJEC most toxic - Consider increased toxicity with malnourished children *Surgery* - Surgery is important - Guidelines and inclusion of surgeons in development for surgical indications are important *Radiotherapy* - Greater utilisation of radiotherapy is needed - Radiotherapy as single local therapy should be administrated even in the absence of surgery - Radiotherapy should be done if up to five localised metastatic lesions are present - Greater application in palliative care *Consolidation* - More consultations for ASCT should be done	provide supportive care and where an ASCT option was available - A single-day administration was favoured above over three days to manage bed availability and reduce costs *Surgery* - Active participation of paediatric surgeons - Resection of 90-100% should be aim (not 100% only) *Consolidation* - All four transplant units are willing to receive consultations - Request for standardised indications for ASCT and referral	Access to CRA is limited

Stellenbosch University https://scholar.sun.ac.za

		- Inconsistent availability of busulfan and melphalan *MRD treatment* - CRA is advised in conjunction with ASCT - Outside ASCT limited data		
Stage 4s Approach to patients with Stage 4s tumours	SIOP-PODC recommendations for NB in LMICs		Request to define 'symptomatic tumour pathology' – the Philadelphia score was included	Standard of care benefits public medical service costs and adheres to private health care benefits
Perinatal NB Approach to patients with perinatal NB tumours	- A small adrenal mass detected before birth until six months postnatal age has a favourable outcome (> 95% four-year OS) without surgical or medical treatment - Monitoring: Physical exam and ultrasonography every 6-8 weeks until the mass resolves or until surgical resection is indicated for growth or mass causes symptoms		Request to define 'symptomatic tumour pathology' – the Philadelphia score was included	
Palliative treatment Palliative options that included chemotherapy and preservation of QoL	Standardised symptomatic palliative care	- Experience limited to standardised symptomatic palliative care, dexamethasone and radiotherapy - Protocol for chemotherapy-based guidelines needed		Standard of care benefits public medical service costs and adheres to private health care benefits

Stellenbosch University https://scholar.sun.ac.za

	Metronomic therapy involving combination with cyclophosphamide, etoposide and valproic acid for refractory and relapsing NB Propranolol- and doxorubicin-based metronomic protocols Dexamethasone and radiotherapy for bone pain			
Surgery - Indications for surgery - Objectives of surgery	LR – only symptomatic or progressive tumours IR – non-mutilating > 50% resection HR – non-mutilating > 90% resection Lymph node biopsies	Preliminary data suggested LR and IR tumours should be resected because of high absconding and lost to follow-up rate, thus long-term follow-up not always viable HR - Resection of 90-100% should be aim (not 100% only) - 50-90% resection still more advantages than no resection	Guidelines for paediatric surgery evaluation	Standard of care benefits public medical service costs and adheres to private health care benefits

Stellenbosch University https://scholar.sun.ac.za

		Second-look surgery viable option to gain complete resection		
Radiotherapy - Indications for radiotherapy - Objective of radiotherapy	SIOP-PODC recommendations for NB in LMICs	*Guidelines to be developed by radiotherapy services* LR no indication for radiotherapy Enlarging hepatic mass, no radiotherapy should be used as liver fibrosis is a common complication. Liver transplant options are not possible. Therefore, chemotherapy should be used for enlarging liver tumours. IR – With residual tumour, radiotherapy is indicated to prevent progression or relapse HR – Radiotherapy as single local therapy should be administrated even in the absence of surgery. Radiotherapy should be done if up to five localised metastatic lesions are present.		Standard of care benefits public medical service costs and adheres to private health care benefits Sustainable but limited to competing indications of adult oncology services and other paediatric tumour types with a better prognosis
ASCT - Indications for ASCT - Objective of ASCT	SIOP-PODC recommendations for NB in LMICs	- Transplant specialists most experienced with Bu/Mel - Request for standardised indications for ASCT and referral		Standard of care benefits public medical service costs and adheres to private health care benefits Sustainability dependent on access to conditioning chemotherapy, aphesis

Stellenbosch University https://scholar.sun.ac.za

					services and capacity of transplant services
National standardisation - Germ cell tumours prospective protocol - Hodgkin lymphoma prospective protocol			Guidelines for standardisation: - Hyperhydration with HCTZ as diuretic - Renal function screening with Schwartz formula and nuclear GFRs - Use of the Boston ototoxicity scale	Prevention of morbidity and mortality is cost-effective	Feasible and sustainable
Public versus private funding	Equal access and best possible care			Best care based on international evidence, toxicity, provision of supportive care and POU capacity	Based on ethical principles
National resources - Diagnostics - Transplant services - Database			- Starting with paper database - Eventually REDCap as national database		University based (Stellenbosch University): Sustainable
Study inclusion				- All patients with NB should be registered with the study including patients 'off protocol' as part of implementation of research	

Abbreviations: ASCT – autologous stem cell transplant; CRA – cis-retinoic acid; CT – computerised tomography; FDG-PET – fluorodeoxyglucose positron emission tomography; FISH – fluorescent in situ hybridisation; GFRs – glomerular filtration rate; GPR – good partial response; HCTZ – hydrochlotiazide (diuretic); IHC – immunohistochemistry; ^{123}I- MIBG – metaiodobenzylguanidine; INPC – International Neuroblastoma Pathology Classification; H&E – hematoxylin and eosin stain; LDH – lactate dehydrogenase; mCR – metastatic complete response; MRI – magnetic resonance imaging; NB – neuroblastoma; POU – paediatric oncology unit; PR – partial response; RT-PCR – reverse transcription polymerase chain reaction

Stellenbosch University https://scholar.sun.ac.za

Table 2: Protocol approvals

Site	Academic approval	Ethics approval	Reciprocal approval	Ethics approval duration	Applicant PI/HI	Duration of process (months)	Hospital approval	Provincial approval	National approval
Governmental institutions									
Site A	Yes	Yes	PI site	1	PI	2	Yes	Yes	Yes
Site B	Yes	Yes	No (initiated after application)	4	Both	9	Yes		
Site C	No	N/A	No	2	PI	5	Yes	Yes	
Site D	No	N/A	No	2	Both	9	Yes		
Site E	No	Yes	Yes	1	PI	1	Yes	Yes	
Site F	No						Yes		
Site G	No	Yes	No	4	PI	12	Yes	Yes	
Site H	No	Yes	No		PI	12	Yes	Yes	
Site I	No	Yes (separate applications)	No	9	HI	14	Yes	Yes	
Site J	No	Yes (separate applications)		8	HI	14	Yes		
Site K	No	Yes	No	2	PI or HI	12	Yes		
Site L	No	Yes	No	2	HI	13	Yes		
Site M	No	Yes	No	20 (pending)	HI	20 (pending)	Yes	Yes	
Subtotal	2	9	1	Mean 5 (1-20) Median 2		Mean 10 (1-20) Median 12	13	7	1
Total	33								
Private institutions									
Site P1	Done in two academic centres	N/A	No	Linked to academic approvals or individual hospital approvals	HI	Linked to academic approval	Yes	Yes	Yes
Site P2		With Site C	No		HI		Yes	Yes	
Site P3		N/A	No		HI		Yes	Yes	
Site P4		N/A	No		HI		Yes	Yes	
Site P5		With Site J	No		HI		Yes	Yes	
Site P6		N/A	No		HI		Yes	Yes	

Abbreviations: N/A – not applicable; HI – hospital investigator; PI – principal investigator

Stellenbosch University https://scholar.sun.ac.za

Table 3: Mapping of the SACCSG NB-2017 management protocol according to the Consolidated Framework for Implementation Research

Domains	Constructs	
Intervention characteristics	**Facilitator**	**Challenge**
Source	- SACCSG harmonisation initiative - PI interest - Higher degree purposes	Developing interest in all POUs
Evidence and strength	- International studies (mainly HICs) - Guidelines for LMICs (SIOP-PODC)	Generating South African-based evidence
Relative advantage	- Standardisation of national management - Multidisciplinary approach - Optimising national resources - Collective management opinion	Determining standards in South Africa
Adaptability	- Based on the SIOPEN protocols - Integration of the SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for NB in LMIC settings - Integration of evidence-based studies	Availability of protocol modalities in HICs differ from South Africa
Complexity	- NB has distinct heterogenous entities - Historical evidence delineated clear starting points - Both senior health care workers and health care workers with local knowledge in several disciplines were available as resources	- Standardised protocol for each heterogenous clinical group - Evidence is based on the HIC experience whilst published guidelines could be implemented in various ways - Standardisation required compromise from POUs
Design quality and packaging	- Standardised SACCSG formats were in place - Visual flow diagrams were included to supplement text - An abbreviated guidelines document was produced - Electronic backup was provided as USB-sticks - Electronic ‘fill-in’ documents and Excel database were provided - Consent and assent forms were translated into eight official languages of South Africa	- Development of an online database via REDCap was secondary to other databases

Stellenbosch University https://scholar.sun.ac.za

Costs	- Standard of care without additional costs	- Private health care management limitations - Private health care is not always aligned with international consensus - Public care directives have not been updated or never been evaluated for inclusion in standard of care
Inner setting		
Networks and communication	- Various meeting platforms available - Electronic communication facilitated discussion - Increased interdepartmental collaboration - Pooling of national resources	- Limited availability of time and managing it as resource - Need for consistent workgroup meetings - Clarity around the responsibility of HIs
Culture	- Historically independent development of POUs and protocols - Work structure limits research time	- Networking and gaining second opinions - REDCap and other databases are not readily used by everyone - Changing staff in POUs prevents continuity of oversight - Obtaining consent or assent in a palliative setting is challenging
Structural characteristics	- Need to standardise national procedures and reporting	- Staffing changes and shortages - Limited capacity for research administration - Continual need for orientation - Need for electronic database development
Implementation climate	- Varied availability to facilitate implementation - Electronic protocols available to various team members	- Varied response to changes - Limited orientation towards the protocols - Reservations from SACCSG that national protocols were used as degree opportunities
Readiness for implementation	- Clinically, the protocol was designed to be standard of care - Paper-based data collection at start	- Obtaining consent and assent is time consuming - Development of REDCap database to be completed - Time for data capturing is a scarce resource
Outer setting		
Patient needs and resources	Consent and assent forms translated into eight official South African languages	
External policy and incentives	- Inter-POU communication and exchange of expertise - Governmental resource management	Added administration
Peer pressure	- Level of quality assurance on national and international level	Other LMICs have superior management programmes with better outcomes than South Africa

Stellenbosch University https://scholar.sun.ac.za

	- Personal development in research and tumour-based knowledge - Develops sustainable systems	
Individual or team characteristics		
Knowledge and beliefs about intervention	- Limit unequal management approaches - Management is not oncologist dependent	Individual POUs have historical preferences
Common goal	Harmonisation of management and optimising resources	- Establishing a common goal is challenging - Remaining motivated to achieve the goals is challenging
Self-efficacy	Individual researchers	Individual motivation towards the project differs
Combined efforts	SACCSG has an agreed mandate to develop national protocols	- Support from senior leadership in the SACCSG - Support for others to pursue interests that are not common to all POUs
Implementation process		
Planning	- National: PI - Local: Hospital investigators with local sub-discipline departments	- Time needed for implementation support - Various needs from different POUs for implementation
Engaging	- Learning skills regarding research and cooperative studies - Development of interdepartmental relations in each hospital	- Need for engagement of PI - Need for constant follow-up of PI
Executing	- Learning the protocol is important - Continuous administration is needed	Each hospital investigator to self-regulate her/his units
Reflecting and evaluating	- Operational research is part of the NB protocol - Interim evaluations have been planned	Implementation evaluations should be more frequent

Abbreviations: SACCSG – South African Children's Cancer Study Group; PI – principal investigator; POUs – paediatric oncology units; HICs – high-income country; NB – neuroblastoma; SIOP – International Society for Paediatric Oncology; PODC – paediatric oncology for developing countries; LMIC – low- and middle-income country

Stellenbosch University https://scholar.sun.ac.za

Appendices

Appendix A: Timeline of the protocol development

Date	Action
September 2016	SACCSG Durban protocol meeting National consensus to develop an NB management protocol
October 2016	Initiation of the NB working group
October 2016	Initiation of the retrospective NB study
November 2016	Survey of hospital resources to treat children with NB
January 2017	Initiation of development of protocol
April 2017	Initiation of pathology guidelines
May 2017	SACCSG protocol development meeting in Stellenbosch
June 2017	Initiation of radiotherapy guidelines
July 2017	Initiation of autologous bone marrow transplant guidelines
July 2017	Initiation of nuclear medicine guidelines
September 2017	First draft evaluation (supervisor)
November 2017	Second draft evaluation (NB working group)
January 2018	Online NB working group meeting on the Cure4Kids platform
February 2018	Initiation of consent and assent forms
March 2018	Third draft evaluation (NB working group)
April 2018	SACCSG NB-2017 management protocol workshop
April 2018	Preliminary retrospective study findings presented
May 2018	Initiation of palliative care guidelines
June 2018	Translation of consent and assent forms into eight official languages
August 2018	Ethical approval SU
September 2018	Ethics application UKZN
October 2018	Academic evaluation of protocol UCT
December 2018	Ethics application UCT
January 2019	First and second site initiated for protocol
February 2019	Ethics application Wits
March 2019	Ethics application UFS
March 2019	Ethics application Frere Hospital East London
March 2019	Ethics application University of Limpopo
April 2019	Third site opened
March 2019	Ethics application Eastern Cape Provincial Department of Health
May 2019	UCT ethics clearance
May 2019	Eastern Cape Provincial Department of Health ethical clearance
June 2019	Fourth site opened
August 2019	Ethics applications UP and SMU
September 2019	UFS ethics clearance
December 2019	Wits ethical clearance
December 2019	UP ethics clearance
February 2020	SMU ethics clearance

Abbreviations: SACCSG – South African Children's Cancer Study Group; NB – neuroblastoma; SU – Stellenbosch University; UKZN – University of Kwa-Zulu Natal; UCT – University of Cape Town; Wits – University of the Witwatersrand; UFS – University of the Free State; UP – University of Pretoria, SMU – Sefako Makgatho Health Sciences University

Stellenbosch University https://scholar.sun.ac.za

Appendix B: Survey establishing the resources for the treatment of neuroblastoma in South Africa

	Yes	No	Maybe/ sometimes
Imaging			
Chest X-ray			
Skeletal survey			
Abdominal ultrasound			
Neck ultrasound			
Ultrasound doppler			
CT scan			
MRI scan			
Nuclear medicine studies			
- Bone scan			
- mIBG scan			
- PET scan			
Blood tests			
Full blood count			
Reticulocytes			
Liver enzymes			
LDH			
Ferritin			
Kidney functions			
Urine analysis			
Urine catecholamine levels			
Diagnostics			
Bone marrow aspiration			
Trephine			
Tissue biopsy			
Pathology			
Direct microscopy according to INPC classification			
H&E stain			
Immuno-histochemistry			
MYCN FISH			
Other genetic and molecular studies			
Chemotherapy			
Vincristine			
Vinblastine			
Cyclophosphamide IV			
Cyclophosphamide PO			
Carboplatin			
Cisplatin			
Etoposide IV			
Etoposide PO			
Adriamycin			
Ifosfamide			
Topotecan			
Surgery			
General surgeons			
Paediatric surgeons			

Stellenbosch University https://scholar.sun.ac.za

Cardio-thoracic surgeons			
Neurosurgery			
Radiotherapy			
Access			
ASCT service			
Access			
Able to refer			
mIBG-therapy			
Access			
Supportive care			
Antibiotics			
Blood products			
Paediatric Intensive Care Unit			
Other			
cis-retinoic acid			
G-CSF			
Propranolol			
Celecoxib			
Methotrexate oral			

Abbreviations: CT – computerised tomography; MRI – magnetic resonance imaging; mIBG – meta-Iodobenzylguanidine; PET – positron emission tomography; INPC – International Neuroblastoma Pathology Classification; H&E – hyosine and eosine; FISH – in situ fluorescent hydridasation; IV – intravenous; PO – per os (orally); G-CSF – granulocyte colony-stimulating factor

Stellenbosch University https://scholar.sun.ac.za

Appendix C: Outcomes of protocol development meetings

Stellenbosch Institute for Advanced Study, Stellenbosch, May 2017
The protocol would be a standard of care, curative treatment protocol. - LR disease (no VLR disease). - IR disease. - HR disease. - Perinatal disease. - Stage 4s disease. - Emergency presentations. The protocol would be supported by a palliative section. There would be no randomisations, but a singular treatment arm was proposed to be implemented nationally. Operational research data and outcomes-based indicators should be collected. The primary study question should be based on the outcomes of a national protocol. There would be no secondary study questions.
Web-based Cure4Kids platform, Africa Room, St. Jude Children's Research Hospital, January 2018
The sections on which consensus had been reached during the first and second draft reviews were discussed. Sub-discipline guidelines were presented to the NB working group. - Nuclear medicine guidelines were discussed and accepted. - Radiotherapy guidelines were discussed and accepted. - Radiotherapy without surgery would be a strong recommendation. - Chemotherapy recommendations were discussed. - De-escalation of treatment for LR disease (from OPEC/OJEC) was recommended. - French-based Adriamycin-containing protocol for IR disease was accepted. - Consensus was not reached on induction chemotherapy for HR NB. - Doxorubicin-containing protocols were excluded, but the discussion of the choice between rapid COJEC and OPEC/OJEC was not concluded. The scope of the ASCT section was not determined. A multi-arm approach to the palliative care guidelines should be adopted. - Symptomatic palliation. - Chemotherapy based (OPEC/OJEC). - Metronomic therapy based. - Role of mIBG-therapy.
NB working group at the Radisson Hotel, Sandton, April 2018
Overview, aims and objectives of the protocol. - Signed off with unanimous consensus. Diagnosis, inclusion criteria and staging systems. - Presented and discussed – signed off with unanimous consensus. Treatment approaches and chemotherapy and discussion. - Treatment in emergency situations: Preference for radiotherapy in paraspinal emergency but chemotherapy in hepatic emergencies. - LR and IR disease – signed off with unanimous consensus. - Stage 4s and perinatal NB – need a better definition of 'symptomatic' disease: Philadelphia score suggested. - HR disease – discussion between rapid COJEC and OPEC/OJEC. OPEC/OJEC approved for national protocol with an ethical caveat. Ethics recommendations.

Stellenbosch University https://scholar.sun.ac.za

- Rapid COJEC is still recommended for induction chemotherapy with HR NB but only has survival advantage if a patient is transplanted. Remain cognisant when patients have further treatment options.

ASCT services

- Recommendations presented.
- ASCT transfer documentation accepted.
- Concern regarding access to chemotherapy for ASCT.
- Aim of operative research to evaluate ASCT services for future improvements on recommendations.

The remaining sub-discipline guidelines were presented.

- Pathology and cytology were discussed and accepted.
- Surgery recommendations were debated and accepted.

Palliation.

- Recommendations were evaluated and accepted.
- The aim of operative research is to evaluate palliation services for future improvements on recommendations.

Documentation and reporting.

- Electronic formats of paper-based documents will be provided.
- Excel spreadsheet will be provided for local records.
- Online database (REDCap) will be developed in the future.

Final consensus was reached on all sections.

Final approval will be done electronically once suggestions have been included.

Abbreviations: HR – high risk; IR – intermediate risk; LR – low risk; VLR – very low-risk; NB – neuroblastoma; ASCT – autologous stem cell transplant

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 10

Inequity in paediatric oncology in South Africa – the neuroblastoma case study (2020)

Ethics no: S18/07/138

Accepted for publication in the South African Journal of Oncology, 21 January 2021

van Heerden, Jaques[1,2]; Esterhuizen, Tonya Marianne[3]; Kruger, Mariana[1].

Affiliations

[1] Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa

[2] Paediatric Haematology and Oncology, Department of Paediatrics Antwerp University Hospital, Antwerp, Belgium

[3] Division of Epidemiology and Biostatistics, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

Corresponding author

E-mail address: Jaques.vanheerden@uza.be

Department of Paediatric Haematology and Oncology, University Hospital of Antwerp, Drie Eikenstraat 655, Edegem, 2650, Belgium.

Contributors' Statement

MK conceptualized the study. JvH assisted with concept development, designed the study, collected the data, performed the data analysis and wrote the manuscript. MK critically reviewed and revised the manuscript. TME was the statistician for data analysis and reviewed the manuscript.

Abbreviations

ASCT	Autologous stem cell transplant
CI	Confidence interval
DoH	Department of Health
EC	Eastern Cape
FS	Freestate
GP	Gauteng Province
HIC	High-income countries
HR	High-risk

Stellenbosch University https://scholar.sun.ac.za

IR	Intermediate-risk
LMIC	Low- and middle-income countries
LIC	Low- income country
LP	Limpopo
LR	Low-risk
MIC	Middle- income country
MP	Mpumalanga
NB	Neuroblastoma
NC	Northern Cape
NW	North West
OS	Overall survival
POU	Paediatic Oncology Unit
SA	South Africa
SIOP	International Society for Paediatric Oncology
UN	United Nations
WC	Western Cape

Stellenbosch University https://scholar.sun.ac.za

ABSTRACT

Background

According to the South African Constitution, adopted in 1996, everyone has the right to access health care services, but children also have the right to health care that will ensure their survival. The aim with this study was to determine whether there was access to equitable paediatric oncology services for the management of neuroblastoma in South Africa.

Methods

A literature review was carried out, focusing on access to health care in South Africa for children with neuroblastoma who had been diagnosed between 2000 and 2014. The paediatric oncology units (POUs) were classified from Setting 1 to 4 in accordance with the International Society of Paediatric Oncology resource settings for neuroblastoma diagnosis. In addition, supplementary data from a retrospective study of the management of neuroblastoma in South Africa during the study period was evaluated.

Results

The neuroblastoma care services in South Africa were not uniformly resourced and accessible across the provinces in the period 2000 to 2014. The Gauteng and Western Cape provinces (2/9 provinces) had excellent health care services that included access to transplant facilities, while Mpumalanga, Northern Cape and North-West (3/9 provinces) had no paediatric oncology services. Traveling distances to health care services pose major challenges due to distances from health care centers, while number of medical staff to provide oncology care for children were unequally distributed. The South African Constitution did not define basic health care for children, nor did the National Cancer Control plan acknowledged childhood cancer as a defined entity without provision until 2022.

Conclusion

Children diagnosed with neuroblastoma do not have equitable access to health care as stated in the South African Constitution. The case of neuroblastoma highlights the inequitable access to childhood care as a whole in South Africa. As the health of children is a national priority, it is therefore necessary to sensitize policy makers to the needs of children with cancer.

ARTICLE

Introduction

When the Republic of South Africa ratified the United Nations (UN) Convention on the Rights of the Child in 1995 and subsequently enshrined children's rights to health care in 1996 in its Constitution, the country committed to provide children with equitable health care.[1] Section 27 of the South African Constitution affords children access to health care as citizens of South Africa, and they have the right to basic health care services under section 28.[2] These two rights in the Bill of Rights *facilitate* the access to health care.[1] Children may lodge a claim against the state for the provision of health care services when their parents are unable to afford health care services.[1]

Stellenbosch University https://scholar.sun.ac.za

The South African Constitution states that the state should also take *reasonable* action to comply with the provision of health care.[2,4] It does not fully define the nature of the health services beyond emergency medical care and basic health services, which may be interpreted as primary health care or preventative health care.[3] Section 28 of the Bill of Rights stipulates that children have a right to basic nutrition, shelter, basic health care services and social services.[2] The Constitution protects the right to life, and as oncological diseases are life-threatening, oncological health care should be defined as an essential health care service.[2]. The government should provide health care in accordance with its available resources but may not allocate a disproportionate share of the budget to one sector of health care, and thereby create shortages for other health care services.[3] To be able to prioritise health care services, major public health needs should be identified for state funding [4].

Childhood cancer is one of the leading causes of mortality in high-income countries.[5] Yet, 90% of the world's paediatric population lives in low- and middle-income countries where 84% of the global childhood cancer burden occurs.[6] This is the estimate, taking into account that there may be a 10% to 45% underestimation of childhood cancer incidences, partially due to the lack of cancer registries and poor access to oncological health care.[6] In South Africa, the number of underdiagnosed patients is estimated to be in the same region as in other low- and middle-income countries.[7] As section 37 of the Constitution states that emergency health care is a right, children with cancer should have the right to life-saving treatment regardless of where in South Africa they live.

According to the World Health Organization (WHO), the definition of access to medical care pertains to physical access, economic access to information about health care.[8] Physical access is defined as that 'health facilities, goods and services must be within safe physical reach for all sections of the population, especially vulnerable or marginalized groups'. Economic accessibility is defined as 'a measure of people's ability to pay for services without financial hardship. It takes into account not only the price of the health services but also indirect and opportunity costs (e.g. the costs of transportation to and from facilities and of taking time away from work)'.[8] Access consists of *services* that can provide the needed care, *timeliness* of receiving the care when it is recognised, a *workforce* that can provide the care, and *coverage* or the means to access health care.[9,10]

We aimed to evaluate access to equitable paediatric oncology services for the treatment of neuroblastoma (NB), a childhood malignancy, in line with the stipulations of the South African Constitution. The three issues for evaluation were equal access to NB care, equal paediatric oncology services, and other equal resources needed for childhood cancer diagnosis and treatment. Furthermore, we wished to determine whether the state had taken reasonable action for NB health care towards achieving the aim of the WHO International society for Paediatric Oncology (SIOP) to improve childhood cancer survival in low- and middle-income countries (LMICs) to 60% by 2030.[11]

Materials and methods

Electronic literature reviews were conducted on the constitutional, legal, and ethical issues pertaining to equality of medical care and access to medical care in the South African setting. Searches were conducted on PubMed, Google Scholar, WorldCat and JSTOR with search terms 'access to medical care', 'rights to medical care', 'equal medical care', 'cancer', 'children' and 'South Africa'. The reference lists of publications were screened to supplement the search results.

Stellenbosch University https://scholar.sun.ac.za

Setting

South Africa consists of nine provinces, subdivided into nearly 300 districts. [12,13] The health care in the country is administrated by three systems: The national, provincial and the district health systems. [12,13] The national Department of Health (DoH) coordinates the public and private health care services at national, provincial and district levels, while administrative, financial, and supportive services are regulated at the provincial and district levels.[14] In 2012, South Africa's DoH initiated a National Health Insurance (NHI) plan as an efficient, equitable and sustainable health system.[15] This social health insurance plan was developed to make health care more accessible and affordable for citizens who have no other way of funding such care individually, but it has not yet been implemented due to funding still being sourced.[15]

Data

Based on the South African Children's Cancer study group's retrospective study of the management and outcomes of NB between 2000 and 2014, we evaluated the burden of three prognostic factors, age at diagnosis, stage, and risk stratification, associated with NB in each province of South Africa. Furthermore, we evaluated the human resources and paediatric oncology services during this period by comparing the provincial paediatric oncology services that manage children diagnosed with NB. Paediatric oncology services were evaluated according to the International Society of Paediatric Oncology (*SIOP*) resource settings for NB diagnosis, staging, and risk stratification (*Appendix A*).[16] A multidisciplinary team including subspecialist doctors, nurses and laboratory staff was involved in managing childhood malignancies.[16] We surveyed only former and current paediatric oncologists and paediatric surgeons attached to paediatric oncology services to establish the number of physicians working in paediatric oncology associated with individual POUs. Where possible, annual departmental hospital reports were cross-referenced for confirmation. To evaluate access to POUs, three random furthest points with a named settlement in each province were chosen. The distance and travel duration between the settlement and the nearest paediatric POU were determined with Google Maps®.[17]

Statistical analysis

Data from a retrospective study on the management and outcomes of South African children diagnosed with NB between 2000 and 2014 were used to determine the overall survival (OS) and associated 95% confidence intervals (CI) for each province. These data were described using Kaplan Meier curves with differences evaluated using log rank tests. The Kaplan Meier curves were assessed using IBM SPSS Version 25 (IBM Corporation, USA) statistical software.[18] For all calculations a *p*-value less than 0.05 was considered significant.

Results

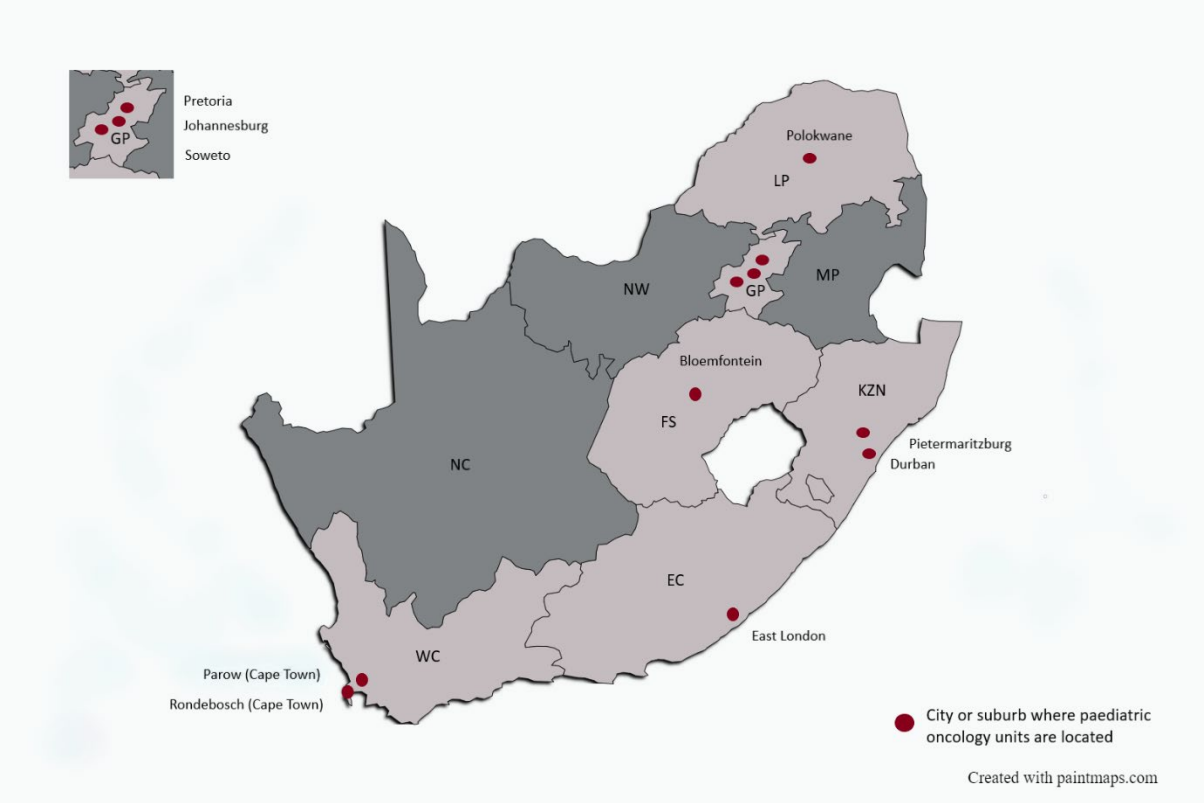
Geographic characteristics

Nearly a third (n=124, 32.2%) of the 385 children diagnosed with NB in South Africa between 2000 and 2014 (*Figure 1*) were treated in the Western Cape (WC), (n=114, 29.6%) were treated in Gauteng (GP), and (n=62, 16.1%) in the Free State (FS), followed by KwaZulu-Natal (KZN) (n=55, 14.3%) and the

Stellenbosch University https://scholar.sun.ac.za

Eastern Cape (EC) (n=30, 7.8%). Data from Limpopo (LP) was not included in this study because permission to access data could not be obtained.

Figure 1: The provinces and cities of South Africa with paediatric oncology units from 2000 to 2014



Abbreviations: EC – Eastern Cape; FS – Free State; GP – Gauteng; KZN – KwaZulu-Natal; LP – Limpopo; MP – Mpumalanga; NC – Northern Cape; NW – North West; WC – Western Cape

Age at diagnosis

The median age at diagnosis for the total cohort was 39.9 months (interquartile range (IQR), 15.4-49.6 months) (*Table 1*). The median age at diagnosis of patients from EC was 34.3 months (IQR, 19.1-48.2 months), 36.6 months (IQR, 12.2-81.9 months) for patients from FS, 36.8 months (IQR, 16.6-51.4 months) for patients from GP, 26.5 months (IQR, 13.5-41.4 months) for patients from WC, and 21.3 months (IQR, 13.5-48.0 months) for patients from KZN. In all the provinces the largest age group was the 19- to 60-month-old children. FS had the highest percentage (32.3%) of children older than five years. In all POUs the predominant age group was the 19- to 60-month-old children (*Table 2*). Johannesburg was the POU with the highest percentage (32.3%) of children over five years.

Tumour staging at diagnosis

Stage 4 or metastatic disease was the most prevalent (n=273, 70.9%) (*Table 3*). All provinces predominantly had Stage 4 disease, but EC (83.3%) and KZN (80.0%) had the highest percentages compared to 68.5%, 68.4% and 66.1% in WC, GP and FS respectively. WC had the highest percentage Stage 1 or localised disease (n=10, 8.1%).

Stellenbosch University https://scholar.sun.ac.za

The POUs (*Table 4*) with the highest percentages of patients with Stage 4 or metastatic disease were Pietermaritzburg (KZN), East London (EP), and Pretoria (GP), with 100%, 83.3% and 82.1% respectively. Rondebosch in Cape Town (WP) (n=9, 9.9%) and Johannesburg (GP) (n=3, 5.9%) had the highest percentage of Stage 1 or localised disease.

Risk stratification at diagnosis

High-risk (HR) disease was the most prevalent (n=294, 76.4%) (*Table 5*). All provinces predominantly had HR disease, but EC (90.0%) and FS (95.2%) had the highest percentages compared 81.6%, 65.3% and 61.8% in GP, WC and KZN respectively. The percentage of HR disease must be seen in the context of 29.1% of KZN patients not being able to be risk stratified. WC had the highest percentage of low-risk (LR) disease (n=26, 21.0%). The four POUs (*Table 6*) with the highest percentages of patients diagnosed with HR disease were Pietermaritzburg (KZN), Bloemfontein (FS), Pretoria (GP), and East London (EP), with 100%, 95.2%, 92.3% and 90% respectively followed by Johannesburg (GP) (82.4%), Parow in Cape Town (WP) (81.8%), Soweto (GP) (62.5%), Rondebosch in Cape Town (WP) (59.3%) and Durban (KZN) (57.1%). Rondebosch in Cape Town (WP) (n=22, 24.4%), Johannesburg (GP) (n=7, 13.7%) and Parow in Cape Town (WP) (n=4, 12.1%) had the highest percentage of LR disease followed by Pretoria (GP), Soweto (GP), Durban (KZN), East London (EL) and Bloemfontein (FS) with 7.7%, 4.2%, 4.1%, 3.3% and 3.2% respectively. Pietermaritzburg (KZN) had no patients with LR disease.

Evaluation of access to paediatric oncology services in South Africa *(Table 7)*

GP was the province with the smallest surface area (18 176 km^2), with the shortest travelling distances to services (83.5 km – 119.0 km), and with the shortest travel duration (59 min – 1 h 34 min). NC was the province with the largest surface area (372 889 km^2), with the furthest travelling distances to services (283.7 km – 1 105.5 km) and the longest travel duration (2 h 55 min – 16 h 32 min). WC, with established paediatric oncology services, had comparable distances (427.4 km – 595.8 km) and travel durations (4 h 22 min – 6 h 45 min), with MP (141.1 km – 435.1 km; 1 h 48 min – 4 h 56 min) and NW (336.1 km – 641.8 km; 3 h 40 min – 6 h 46 min), who had no paediatric oncology services.

National access to neuroblastoma care

Based on geographical distances in South Africa (*Figure 1*), road access and travelling time to cover the distances as well as transport options for patients – *timeliness* (*Table 7*) of access to care were not equal. The Constitution guarantees the *facilitation of gaining* access to health care.[2] Both the 2009 public inquiry into access to health care services and the 2017 Foundation for Human Rights paper on monitoring the right of access to health care in South Africa documented ongoing limited resources and access to both patient transport services and emergency transport.[19,20] The greatest burden fell on children and patients from rural areas who needed interprovincial transfers.[21, 22] Not only did patients in MP, NC and NW not have NB medical services in their own provinces, but there was also limited transport for them to access NB care in other provinces.

Stellenbosch University https://scholar.sun.ac.za

Anti-neoplastic agents are important for the treatment of NB.[21] Until 2016, approximately 20 basic and essential anti-neoplastic agents listed in the WHO essential anti-cancer medications had not been listed on the South African Essential Drugs list.[23] Subsequent Essential Medicines Formularies for Tertiary and Quaternary Care did also not include anti-neoplastic agents as needed for childhood malignancies.[24, 25]

A multidisciplinary team is crucial for the management of NB. The disciplines should include paediatric oncologists, paediatric surgeons, radio-oncologists, radiologists, pathologists, nuclear physicians, bone marrow transplant specialists, and supportive care services (blood transfusion services, pharmacy services and dieticians), but of special importance is the nursing staff.[16] If the provinces without paediatric oncology services are not taken into account, GP and WC, who had the smallest percentage of children under the age of 15 years (respectively 24.5% and 26.7%),[26] had the best access to health care between 2000 and 2014, with more paediatric oncologists and paediatric surgeons than the other provinces (*Table 8 and 9*). Even in the context of this disproportionate distribution of human resources, both the 2009 public inquiry and the 2017 Foundation for Human Rights working paper concluded that there was a shortage of skilled health care workers, especially nursing staff, in the public sector, and that their numbers were still decreasing.[20,27]

Equality of the paediatric oncology services delivering neuroblastoma care

The management of NB includes chemotherapy, surgery, and radiotherapy.[28, 29] In localised NB trimodal therapy is curative, but in metastatic NB or NB with adverse biology, trimodal therapy leads to a survival of only 20%.[16, 28, 29] Autologous bone marrow transplant preceded by ablative bone marrow therapy, immunotherapy and maturation therapy with *cis*-retinoic acid are vital, but were not available in South Africa during this time.[28, 29].

Between 2000 and 2014 the POUs in South Africa delivered different levels of neuroblastoma management based on the available health care resources in each hospital *(Table 10)*. Important in the management of high-risk NB was autologous bone marrow transplant. Pietermaritzburg (KZN) and Polokwane (LP) were Setting 1 POUs with access only to basic levels of health care (*Appendix A*). Bloemfontein (FS), Durban (KZN), and East London (EC) were Setting 2 POUs with access to the full range of health care management needed, excluding access to bone marrow transplant facilities for children. Rondebosch and Parow (Cape Town, WC), Johannesburg, Pretoria and Soweto (GP), with autologous transplant capabilities, were Setting 3 POUs with access the full range of health care management facilities, including bone marrow transplant. None of the POUs were classified as Setting 4 POUs, since South Africa does not provide immunotherapy for the treatment of NB.

The existence of a facility does however not guarantee access to it, or that access to it would be gained.[30] In South Africa, when a paediatric surgeon was not available, a general surgeon performed surgical interventions when diagnosing NB with the aid of a biopsy or operated on the primary tumour. Radiotherapy services did not routinely reserve time for paediatric NB patients who needed irradiation. Moreover, in both these situations children had to compete not only for resources, but also with the adult population to gain access to life-saving services.[31] High-risk NB had poor outcomes, high relapse rates and a high need for resources.[16] When the justice principle is applied for access to limited surgical, radiotherapy and transplantation services, these characteristics might work against

Stellenbosch University https://scholar.sun.ac.za

patients with NB due to competition for resources rather allocated to burden of disease of adult non-communicable diseases. This situation was compounded when paediatric oncology services competed with adult services.[31]

The right to life – a right to be treated for neuroblastoma and treatment-related complications

Worldwide localised NB without adverse biology (low- and intermediate-risk disease) had five-year overall survival (OS) rates of upwards from 80%.[16,28,29] In metastatic NB or NB with adverse biology (high-risk disease) with multimodal therapy, including autologous stem cell transplant (ASCT) and immunotherapy, the five-year OS rates were 60%.[15,27,28] In South Africa, with limited access to ASCT and no immunotherapy, the five-year OS was approximately 20%.[32] The inequitable distribution of NB management-related resources had an impact on the survival, as the two provinces GP and WC, with a full range of health care services, had survival rates above the national average of 22.6% (*Table 11*). KZN did not have a paediatric oncologist to complement the multidisciplinary team until 2013 (the end of the study period) and had the lowest survival rate of 5.5%. The effect of inequitable access to NB care could be demonstrated by comparing Pretoria and Soweto with Johannesburg. All are Setting 3 paediatric oncology services, but during the study period Johannesburg received referrals from Southern GP (roughly 9 088 km^2) and Soweto received referrals from Southern GP and NW (113 970 km^2), while Pretoria received referrals from Northern GP, MP and LP (until a POU was opened in Ga-Rankuwa) (roughly 211 337 km^2), which were significantly further away from the child's residence. This potentially may have led to late diagnosis and delays in referrals to central hospitals, contributing to a poorer 10-year OS for Pretoria (5.1%) and Soweto (16.7%), compared to 39.2% in Johannesburg.[17]

Chronic care or long-term life-saving health care

Since 2009, non-communicable diseases in South Africa, including cancer, have contributed the greatest percentage to the burden of disease in the country.[20] It is estimated that one in every five children up to late adolescence in South Africa were in need of long-term lifesaving health care or chronic health care due to a previously life-limiting condition, such as with cancer and palliative care [33]. Yet, when the right to life-saving health care on the basis of a chronic condition was challenged in the Constitutional Court in the Soobramoney case: Soobramoney v Minister of Health (KwaZulu-Natal) 1998 (1) SA 765 (CC), the Court decided that emergency medical treatment did not include chronic treatment.[34] Therefore, autologous stem cell transplant (ASCT), which contributes a 15% to 20% increase in survival in high-risk NB,[16] is not guaranteed as a right to life under the determination of the Constitutional Court and neither is any part of paediatric oncology care apart from *acute* life-threatening emergencies such as acute emergencies at diagnosis such as spinal cord compression symptoms and respiratory distress or neutropenic fever, heart failure caused by chemotherapy-induced anaemia, bleeding due to thrombocytopaenia.[16]

Discussion

Neuroblastoma is a childhood malignancy of the neuro-endocrine system, contributing 15% of the total deaths in the paediatric oncology population and only 20% of cases survive for longer than five

Stellenbosch University https://scholar.sun.ac.za

years due to late diagnosis and advanced disease in low- and middle-income countries.[35] .[16] The five-year OS rate in South Africa is 27% in at country with a youthful population of 34.3% of the population under the age of 15 years.[21].[36] Since the start of democracy in 1994, the DoH has developed beneficial programmes for children, which include the national integrated nutrition programme, the programme for the prevention of maternal HIV to child transmission, and the early childhood development and basic education programmes.[36] Free basic child health care services for children under the age of five years are included in these programmes. A bias in favour of younger children and preventative medicine is evident in all these programmes, with the health needs of children with chronic diseases, older children and adolescents being neglected.[37] Provincial health departments have directed resources towards paediatric oncology care with initiatives such as the Essentials for Palliative Care and the KwaZulu-Natal paediatric outreach programmes,[33] but paediatric oncology resources through South Africa remain unequal. Gauteng had the highest number of children under the age of 15 years, and the Western Cape only the fifth highest with respectively 17.7% and 11.5%.[26] Yet both provinces had the most resources to manage NB. Mpumalanga, with 36.1% of the children under 15 years, and Limpopo with 39.1%, were the two provinces with the least resources and the highest percentage of children in the country [26]. In 2020 Mpumalanga still had no paediatric oncology services and referred children with NB to Pretoria.

The WHO-SIOP joint goal is to achieve a 60% childhood cancer cure rate worldwide.[37,38] The South African government lacked the stewardship to implement National Core Standards, including programmes related to cancer care, in the country.[39] Most cancer-related programmes were adult-centred.[40] The Ministerial Advisory Committee on the Prevention and Control of Cancer (MACC) was established in 2013 and the Strategic Plan for the Prevention and Control of Non-Communicable Diseases ran from 2013 to 2017, but childhood cancer was not a priority, as the focus was again on prevention rather than cure, not applicable in childhood cancer.[40] In the 2017 to 2022 National Cancer Strategic Framework for South Africa the commitment to paediatric cancers was not stated beyond a paragraph on childhood cancer epidemiology.[41] Therefore, although NB has a peak incidence in children between the ages of two and five years,[16] the paediatric programmes' bias in favour of younger children does not include non-communicable disease management. Access to treatment in the private health care setting for childhood cancers, thus for NB, is better than in the public setting. The two-tiered health system benefits the financially independent of the population or those who can afford private health insurance.[42]

Although NB is classified as a rare disease, [43,44] optimising its management in South Africa is important from the principle of justice as part of setting a basic standard of health care for rare diseases.[45] The international age-standardised rate of NB in countries with standardised cancer registries is 10.6 cases per million.[46] In South Africa, with at least a 50% under-diagnosis of childhood malignancies,[7] the incidence is far less, at 2.7 cases per million.[7] Therefore, improving the quality of awareness of neuroblastoma, as with all childhood cancers, improving diagnostic capabilities and bringing about increased access to paediatric oncology care are basic, life-saving health care services to which children have a right under the Constitution.

The absence of a definition in the Bill of Rights for 'basic health care services' as they pertain to children may be due to the relatively young Constitution or a means for the government not to commit to defined services. As a signatory of the UN Convention on the Rights of the Child, the South African government must prioritise the needs of children as the most vulnerable members of the South

Stellenbosch University https://scholar.sun.ac.za

African society.[31] Section 7(2) of the Constitution requires the state to 'respect, protect, promote and fulfil the rights in the Bill of Rights'. Concerning the right of access to health care services, *respect* determines that the state not unreasonably limit people's access to health care services, whether in the public or private sector.[34] Thus, a *reasonable* measure to ensure that children, including children with malignancies, not only survive, but also thrive and reach their full capabilities according to the UN Convention, is to address discriminatory policies and practices:[34] defining basic health care for children and acknowledge the need for chronic health care services for children in South Africa. After 25 years of democracy, the scope of paediatric oncology should be acknowledged and a separate national cancer control plan for children formulated to address the paediatric epidemiology, pathophysiology and management needs of children with cancer.

Conclusion

In the case study of children diagnosed with neuroblastoma, it was determined that the patients were not afforded equitable access to care, were not afforded the same level of care based on resources, and were not afforded the right to life by means of access to medical services as laid down by the South African Constitution. This case of neuroblastoma illustrates the measure of access to care for all paediatric malignancies in South Africa, which is currently a low priority in national cancer control because of the paucity of initiatives by policymakers for children with oncological diseases.

Conflict of interest

There is no conflict of interest to disclose.

Acknowledgements

Permission was obtained via the Wiley Library to reproduce a table for academic purposes from Parikh, et al. Clinical Practice Guidelines: SIOP-PODC Adapted Risk Stratification and Treatment Guidelines: Recommendations for Neuroblastoma in Low- and Middle-Income Settings, during September 2020.

We acknowledge the members of the South African Children Cancer Study Group and *South African Association* of *Paediatric Surgeons* for providing data for survival analysis, historical information that benefitted the analyses. We wish to express our gratitude to the administrative staff from the hospitals that were able to provide annual hospital reports for cross-referencing statistics.

The SACCSG Neuroblastoma Workgroup: Dr Jaques van Heerden, Prof Mariana Kruger, Dr Marc Hendricks, Dr Ronelle Uys, Dr Jan du Plessis, Dr Ané Büchner, Prof Janet Poole, Dr Ele Madzhia, Prof Gita Naidu, Dr Barry van Emmenes, Dr Johani Vermeulen and Dr Liezl du Plessis.

[3769 words]

References:

1) Buchner-Eveleigh M. Children's rights of access to health care services and to basic health care services: a critical analysis of case law, legislation and policy. De Jure Law Journal. 2016; 49(2): 307-325.
2) The South African Government. Constitution of the Republic of South Africa, 1996. https://www.gov.za/documents/constitution-republic-south-africa-1996. Accessed September 2020.

Stellenbosch University https://scholar.sun.ac.za

3) Pillay K. Tracking South Africa's progress on health care rights: Are we any closer to achieving the goal? Law, democracy and development. 2003; 7(1): 55-81.
4) Hassim A, Heywood M, Berger J. Health and Democracy. Chapter 2: The Constitution and public health policy. p 30-45. http://section27.org.za/2007/06/health-and-democracy/# Accessed September 2020.
5) Johnston WT, Erdmann F, Newton R, Steliarova-Foucher E, Schüz J, Roman E, et al:. Childhood cancer: Estimating regional and global incidence. Cancer Epidemiol. 2020; 101662. doi: 10.1016/j.canep.2019.101662.
6) Magrath I, Steliarova-Foucher E, Epelman S, Ribeiro RC, Harif M, Li CK; et al.: Paediatric cancer in low-income and middle-income countries. The Lancet Oncology. 2013; 14(3), e104–e116. doi:10.1016/s1470-2045(13)70008-1.
7) Stefan DC, Stones DK, Wainwright D, Kruger M, Davidson A, Poole J; et al.: Childhood cancer incidence in South Africa, 1987 - 2007. S Afr Med Journal. 2015; 105(11): 939-949.
8) Universal Health Coverage and Universal Access, Bulletin of the World Health Organization 2013; 91:546-546A.
9) Agency for Healthcare Research and Quality. Elements of Access to Health Care. Chartbook on Access to Health Care. https://www.ahrq.gov/research/findings/nhqrdr/chartbooks/access/elements.html (Accessed Sept 2020)
10) Institute of Medicine, Committee on Monitoring Access to Personal Health Care Services. Access to health care in America. Washington, DC: National Academy Press; 1993. https://www.ncbi.nlm.nih.gov/books/NBK235882/
11) International Society of Paediatric Oncology. WHO Global Initiative for Childhood Cancer. https://siop-online.org/who-global-initiative-for-childhood-cancer/ Accessed October 2020.
12) Department of Government Communication and Information System (South Africa) (2013), "Categories of municipalities", available at: www.gov.za/aboutgovt/localgovernment.htm (accessed September 2020).
13) Department of Health (South Africa) (2010), "National service delivery agreement: a long and healthy life for all South Africans", available at: www.health-e.org.za/wp-content/uploads/2013/05/3771ccea0610904ff0c3de0f09f210391.pdf (September 2020).
14) Katuu S. Healthcare systems: typologies, framework models, and South Africa's health sector International Journal of Health Governance. 2018; 23(2):134-148.
15) Comny A. South African health care system analysis. Public Health Review. 2018; 1(1): 1-8.
16) Parikh N, Howard S, Chantada G, Israels T, Khattab M, Alcasabas P, et al. SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for neuroblastoma in low- and middle-income settings. Pediatr Blood Cancer. 2015; 62(8): 1305-1316.
17) Google (n.d.). [Multiple destinations in between cities in South Africa]. Retrieved September 13, 2020. https://www.google.com/maps
18) IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.
19) South African Human Rights Commission. Public inquiry into the right to access to health care services. 2009. Chapter 4: Access to Health Care. p41-42.
20) Weyss B, Webster D, Selebalo H. Studies in poverty and inequality institute. Monitoring the right of access to health care in South Africa. Working paper 17. September 2017. Chapter 3: Quality indicators p69.

Stellenbosch University https://scholar.sun.ac.za

21) Harris, B., Goudge, J., Ataguba, J., McIntyre, D., Nxumalo, N., Jikwana, S., & Chersich, M. (2011). Inequities in access to health care in South Africa. Journal of Public Health Policy, 32, S102-S123. Retrieved September 25, 2020, from http://www.jstor.org/stable/20868835
22) South African Human Rights Commission. Public inquiry into the right to access to health care services. 2009. Chapter 6: Access to Health Care. p50.
23) Perumal-Pillay VA, Suleman F. Quantitative evaluation of essential medicines lists: the South African case study. BMC Health Serv Res. 2016; 16: 687 https://doi.org/10.1186/s12913-016-1937-x.
24) Department of Health, South Africa. Tertiary and Quaternary Level Essential Medicines Recommendations. Reviewed items June 2017. Pretoria, Cape Town, South Africa 2017.
25) Department of Health, South Africa. Tertiary and Quaternary Level Essential Medicines Recommendations. Reviewed items June 2020. Pretoria, Cape Town, South Africa 2020.
26) Statistics South Africa (2020) Mid-year population estimates by population group, age and sex, 2020. Mid-year population estimates report, 2020. Pretoria, Cape Town: Statistics South Africa. www.statSA.gov.za Accessed September 2020.
27) South African Human Rights Commission. Public inquiry into the right to access to health care services. 2009. Chapter 3.4: Human Resources. p34-38.
28) Pinto, N; Applebaum, M; Volchenboum, S; Matthay, K; et al. Advances in Risk Classification and Treatment Strategies for Neuroblastoma. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology 2015; 33(27):* 3008-17.
29) Whittle, S; Smith, V; Doherty, E; Zhao, S; McCarty, S; Zage, P. Overview and recent advances in the treatment of neuroblastoma. Expert review of anticancer therapy. 2017;17(4):369-86.
30) Gulliford M, Figueroa-Munoz J, Morgan M, Hughes D, Gibson B, Beech R, et al. What does "access to health care" mean? Journal of Health Services Research & Policy, 2002; 7(3): 186–188.
31) Shung-King M, Lake L, Sanders D & Hendricks M (eds) (2019) South African Child Gauge 2019. Cape Town: Children's Institute, University of Cape Town.
32) Van Heerden, J, Hendricks, M, Geel, J, et al. Overall survival for neuroblastoma in South Africa between 2000 and 2014. Pediatr Blood Cancer. 2019; 66:e27944 https://doi.org/10.1002/pbc.27944.
33) Westwood A, Slemming W. Long term health conditions in children: Towards comprehensive care. Shung-King M, Lake L, Sanders D & Hendricks M (eds) (2019) South African Child Gauge 2019. Cape Town: Children's Institute, University of Cape Town. p95-114.
34) Berger J. Health and democracy: a guide to human rights, health law and policy in post-apartheid South Africa. Editors: Hassim A, Heywood M, Berger J. Cape Town, South Africa: SiberInk, 2007. Chapter 2: The Constitution and public health policy. 2010 (4): p30-69. www.section27.org (Accessed September 2020).
35) International Agency for Research on Cancer. World Cancer Report 2014. Lyon 2014. Editors Stewart BW, Wild CP. World Health Organization. Chapter 5.16. Tumours of the nervous system, p511.
36) Van Heerden, J, Hendricks, M, Geel, J, et al. Overall survival for neuroblastoma in South Africa between 2000 and 2014. Pediatr Blood Cancer. 2019; 66:e27944
37) World Health Organization. Improving Cancer Cure rates. https://www.who.int/activities/improving-childhood-cancer-cure-rate Accessed October 2020.

Stellenbosch University https://scholar.sun.ac.za

38) International Society of Paediatric Oncology. WHO Global Initiative for Childhood Cancer. https://siop-online.org/who-global-initiative-for-childhood-cancer/ Accessed October 2020.
39) Maphumulo WT, Bhengu BR. Challenges of quality improvement in the healthcare of South Africa post-apartheid: A critical review. Curationis 2019; 42(1): a1901. https://doi.org/10.4102/curationis. v42i1.1901.
40) Department of Health, South Africa. Strategic Plan for the Prevention and Control of Non-Communicable Diseases 2013-2017. Pretoria, Cape Town, South Africa (2013).
41) Department of Health, South Africa. National Cancer Strategic Framework for South Africa 2017 – 2022. Pretoria, Cape Town, South Africa (2017).
42) Conmy A. South African health care system analysis. Public Health Review. 2018; 1(1): 1-8.
43) EURORDIS - What is a rare disease? http://www.eurordis.org/about-rare-diseases.(Accessed September 2020).
44) Orphanet website: about rare diseases. http://www.orpha.net/consor/cgibin/Education_AboutRareDiseases. (Accessed September 2020).
45) O'Connor DJ, Buckland J, Almond N, Boyle J, Coxon C, Gaki E, et al. Commonly setting biological standards in rare diseases, Expert Opinion on Orphan Drugs. 2019; 7:7-8, 305-314.
46) Steliarova-Foucher E, Stiller CA, Hesselinge P, Moreno F, Ries L, Shin H. International Incidence of Childhood Cancer Volume III Chapter IVa. Neuroblastoma and ganglioneuroblastoma. http://iicc.iarc.fr/includes/results/comparative/0401_Neuroblastoma.pdf (Accessed September 2020).

Stellenbosch University https://scholar.sun.ac.za

Tables

Figures

Appendices

Stellenbosch University https://scholar.sun.ac.za

Table 1: Neuroblastoma age groups at diagnosis from 2000 to 2014 per province in South Africa

Province			EC	FS	GP	KZN	WC	Total
Median in months (IQR)			34.3 (19.1-48.2)	36.6 (12.2-81.9)	36.8 (16.6-51.4)	21.3 (13.5-48.0)	26.5 (13.5-41.4)	39.9 (15.4-49.6)
Age	0-18 mo	N(%)	5 (16.7%)	16 (25.8%)	32 (28.1%)	17 (30.9%)	39 (31.5%)	109 (28.3%)
	19-60 mo		22 (73.3%)	26 (41.9%)	60 (52.6%)	27 (49.1%)	67 (54.0%)	202 (52.5%)
	>60 mo		3 (10.0%)	20 (32.3%)	22 (19.3%)	11 (20.0%)	18 (14.5%)	74 (19.2%)
Total		N	30	62	114	55	124	385

Abbreviations: EC – Eastern Cape; FS – Free State; GP – Gauteng; KZN – Kwa-Zulu Natal; WC – Western Cape; mo - months

Table 2: Neuroblastoma age groups at diagnosis from 2000 to 2014 per city in South Africa

Province			EC	FS	GP			KZN		WC		
City			EL	BLN	JHB	PTA	SWT	DBN	PMB	PRW	RBH	Total
Age	0-18 mo	N(%)	5 (16.7%)	7 (17.9%)	16 (25.8%)	18 (35.3%)	7 (29.2%)	16 (32.7%)	1 (16.7%)	10 (30.3%)	29 (31.9%)	109 (28.3%)
	19-60 mo		22 (73.3%)	22 (56.4%)	26 (41.9%)	23 (45.1%)	15 (62.5%)	23 (46.9%)	4 (66.7%)	16 (48.5%)	51 (56.0%)	202 (52.5%)
	>60 mo		3 (10.0%)	10 (25.6%)	20 (32.3%)	10 (19.6%)	2 (8.3%)	10 (20.4%)	1 (16.7%)	7 (21.2%)	11 (12.1%)	74 (19.2%)
Total		N	30	62	39	51	24	49	6	33	91	385

Abbreviations: EC – Eastern Cape; EL – East London; FS – Free State; BLN – Bloemfontein; GP – Gauteng; JHB – Johannesburg; PTA – Pretoria; SWT – Soweto; KZN – Kwa-Zulu Natal; DBN – Durban; PMB – Pietermaritzburg; WC – Western Cape; PRW – Parow (Cape Town); RBH – Rondebosch (Cape Town); mo - months

Table 3: Neuroblastoma staging at diagnosis from 2000 to 2014 per province in South Africa

Province			EC	FS	GP	KZN	WC	Total
INSS	Stage 1	N(%)	0 (0.0%)	2 (3.2%)	3 (2.6%)	1 (1.8%)	10 (8.1%)	16 (4.2%)
	Stage 2		1 (3.3%)	1 (1.6%)	9 (7.9%)	0 (0.0%)	5 (4.0%)	16 (4.2%)
	Stage 3		3 (10.0%)	16 (25.8%)	20 (17.5%)	5 (9.1%)	22 (17.7%)	66 (17.1%)
	Stage 4		25 (83.3%)	41 (66.1%)	78 (68.4%)	44 (80.0%)	85 (68.5%)	273 (70.9%)
	Stage 4s		1 (3.3%)	2 (3.2%)	4 (3.5%)	5 (9.1%)	2 (1.6%)	14 (3.6%)
Total		N	30	62	114	55	124	385

Abbreviations: INSS – International Neuroblastoma Staging System; EC – Eastern Cape; FS – Free State; GP – Gauteng; KZN – Kwa-Zulu Natal; WC – Western Cape

Stellenbosch University https://scholar.sun.ac.za

Table 4: Neuroblastoma staging at diagnosis from 2000 to 2014 per city in South Africa

Province			EC	FS	GP			KZN		WC		
City			EL	BLN	JHB	PTA	SWT	DBN	PMB	PRW	RBH	Total
INSS	Stage 1	N(%)	0 (0.0%)	2 (3.2%)	3 (5.9%)	0 (0.0%)	0 (0.0%)	1 (2.0%)	0 (0.0%)	1 (3.0%)	9 (9.9%)	16 (4.2%)
	Stage 2		1 (3.3%)	1 (1.6%)	5 (9.8%)	3 (7.7%)	1 (4.2%)	0 (0.0%)	0 (0.0%)	1 (3.0%)	4 (4.4%)	16 (4.2%)
	Stage 3		3 (10.0%)	16 (25.8%)	9 (17.6%)	4 (10.3%)	7 (29.2%)	5 (10.4%)	0 (0.0%)	6 (18.2%)	16 (17.6%)	66 (17.1%)
	Stage 4		25 (83.3%)	41 (66.1%)	31 (60.8%)	32 (82.1%)	15 (62.5%)	38 (77.6%)	6 (100%)	24 (72.7%)	61 (67.0%)	273 (70.9%)
	Stage 4s		1 (3.3%)	2 (3.2%)	3 (5.9%)	0 (0.0%)	1 (4.2%)	5 (10.2%)	0 (0.0%)	1 (3.0%)	1 (1.1%)	14 (3.6%)
Total		N	30	62	39	51	24	49	6	33	91	385

Abbreviations: EC – Eastern Cape; EL – East London; FS – Free State; BLN – Bloemfontein; GP – Gauteng; JHB – Johannesburg; PTA – Pretoria; SWT – Soweto; KZN – Kwa-Zulu Natal; DBN – Durban; PMB – Pietermaritzburg; WC – Western Cape; PRW – Parow (Cape Town); RBH – Rondebosch (Cape Town)

Table 5: Neuroblastoma risk stratification at diagnosis from 2000 to 2014 per province in South Africa

Province			EC	FS	GP	KZN	WC	Total
Risk	LR	N(%)	1 (3.3%)	2 (3.2%)	11 (9.7%)	2 (3.6%)	26 (21.0%)	42 (10.9%)
	IR		1 (3.3%)	1 (1.6%)	10 (8.8%)	3 (5.5%)	14 (11.3%)	29 (7.5%)
	HR		27 (90.0%)	59 (95.2%)	93 (81.6%)	34 (61.8%)	81 (65.3%)	294 (76.4%)
	Unknown		1 (3.3%)	0 (0.0%)	0 (0.0%)	16 (29.1%)	3 (2.4%)	20 (5.2%)
Total		N	30	62	114	55	124	385

Abbreviations: INSS – International Neuroblastoma Staging System; EC – Eastern Cape; FS – Free State; GP – Gauteng; KZN – Kwa-Zulu Natal; WC – Western Cape; LR- low-risk; IR – intermediate risk; HR – high-risk

Stellenbosch University https://scholar.sun.ac.za

Table 6: Neuroblastoma risk stratification at diagnosis from 2000 to 2014 per city in South Africa

Province			EC	FS	GP			KZN		WC		
City			EL	BLN	JHB	PTA	SWT	DBN	PMB	PRW	RBH	Total
Age	LR	N(%)	1 (3.3%)	2 (3.2%)	7 (13.7%)	3 (7.7%)	1 (4.2%)	2 (4.1%)	0 (0.0%)	4 (12.1%)	22 (24.4%)	42 (10.9%)
	IR		1 (3.3%)	1 (1.6%)	2 (3.9%)	0 (0.0%)	8 (33.3%)	3 (6.1%)	0 (0.0%)	2 (6.1%)	12 (13.2%)	29 (7.5%)
	HR		27 (90.0%)	59 (95.2%)	42 (82.4%)	36 (92.3%)	15 (62.5%)	28 (57.1%)	6 (100%)	27 (81.8%)	54 (59.3%)	294 (76.4%)
	Unknown		1 (3.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	16 (32.7%)	0 (0.0%)	0 (0.0%)	3 (3.3%)	20 (5.2%)
Total		N	30	62	39	51	24	49	6	33	91	385

Abbreviations: EC – Eastern Cape; EL – East London; FS – Free State; BLN – Bloemfontein; GP – Gauteng; JHB – Johannesburg; PTA – Pretoria; SWT – Soweto; KZN – Kwa-Zulu Natal; DBN – Durban; PMB – Pietermaritzburg; WC – Western Cape; PRW – Parow (Cape Town); RBH – Rondebosch (Cape Town); LR- low-risk; IR – intermediate risk; HR – high-risk

Stellenbosch University https://scholar.sun.ac.za

Table 7: Provincial distances and traveling times to the nearest paediatric oncology unit during 2000 to 2014

Destination to nearest POU	Distance	Travel time
Eastern Cape (168 966 km2)		
Elyolo to Port Elizabeth	338.8 km	3 h 45 min
Aliwal North to East London	357.8 km	3 h 53 min
Pamlaville to East London	490.3 km	6 h 29 min
Free State (129 825km2)		
Memel to Bloemfontein	453.9 km	4 h 37 min
Maseru (border with Lesotho) to Bloemfontein	143.9 km	1 h 47 min
Orania to Bloemfontein	222.9 km	2 h 23 min
Gauteng (18 176 km^2)		
Klipdrif to Soweto	90.8 km	1 h 8 min
Loding to Pretoria	119.0 km	1 h 34 min
Devon to Johannesburg	83.5 km	59 min
Kwa-Zulu Natal (94 361 km2)		
Manguzi to Durban	422.0 km	5 h 3 min
Port Edward to Durban	163.7 km	1 h 35 min
Bonjanjeni to Pietermaritzburg	202.1 km	2 h 22 min
Limpopo (125 754 km2)		
Musina to Polokwane	196.4 km	2 h 19 min
Dwaalboom to Polokwane	362.0 km	3 h 54 min
Hoedspruit to Polokwane	216.8 km	2 h 32 min
Mpumalanga (76 495 km2)		
Mbuzini to Pretoria	453.1 km	4 h 56 min
Delfkom to Pretoria	391.4 km	4 h 21 min
Lefiso to Pretoria	141.1 km	1 h 48 min
North West (104 882 km2)		
Vorstershoop to Bloemfontein	544.6 km	5 h 46 min
Vorstershoop to Pretoria	641.8 km	6 h 46 min
Supingstad to Pretoria	336.1 km	3 h 40 min
Northen Cape (372 889 km2)		
Mier to Cape Town	1105.5km	8 h 43 min
Mier to Bloemfontein	874.9 km	16 h 32 min
Alexander Bay to Cape Town	786.7 km	7 h 36 min
Noupoort to Bloemfontein	283.7 km	2 h 55 min
Western Cape (129 462 km2)		
Tsitsikama to Cape Town	585.6 km	6 h 45 min
Kliprand to Cape Town	595.8 km	4 h 22 min
Murraysburg to Cape Town	427.4 km	6 h 43 min

Stellenbosch University https://scholar.sun.ac.za

Table 8: The number of paediatric oncologists in each province between 2000 and 2014

2000			**2014**		
	Paediatric oncologist	Paediatric haematologist		Paediatric oncologist	Paediatric haematologist
Eastern Cape					
EL	1	0	EL	0	0
PE	0	0	PE	0	0
Sub-total	1	0	**Sub-total**	0	0
Provincial totals	**Doctors**		**Provincial totals**	**Doctors**	
2 928 000	1 to 2.9 mil	0	2 570 000	0	0
Free State					
Bloemfontein	1	0	Bloemfontein	3	0
Provincial totals	**Doctors**		**Provincial totals**	**Doctors**	
1 000 000	1 to 1 mil	0	980 000	1 to 326 666	0
Gauteng					
Pretoria	2	0	Pretoria	2	0
Soweto	1	0	Soweto	3	0
Jhb	1	0	Jhb	3	0
Sub-total	4	0	**Sub-total**	8	0
Provincial totals	**Doctors**		**Provincial totals**	**Doctors**	
2 939 000	1 to 734 750	0	3 743 000	1 to 467 875	0
Kwa-Zulu Natal					
Durban	0	2	Durban	0	3
PMB	0	0	PMB	1	1
Sub-total	0	2	**Sub-total**	1	4
Provincial totals	**Doctors**		**Provincial totals**	**Doctors**	
4 149 000	0	1 to 2 074 500	4 062 000	1 to 4 062 000	1 to 1 037 250
Limpopo					
Polokwane	0	0	Polokwane	0	0
Provincial totals	**Doctors**		**Provincial totals**	**Doctors**	
2 421 000	0	0	2 310 000	0	0
Mpumalanga					
Mbombela (Nelspruit)	0	0	Mbombela (Nelspruit)	0	0
Provincial totals	**Doctors**		**Provincial totals**	**Doctors**	
1 520 000	0	0	1 564 000	0	0
Northern Cape					
Kimberley	0	0	Kimberley	0	0
Provincial totals	**Doctors**		**Provincial totals**	**Doctors**	
398 000	0	0	408 000	0	0
North West					
Ga Rankuwa	0	0	Ga Rankuwa	0	0
Provincial totals	**Doctors**		**Provincial totals**	**Doctors**	
1 165 000	0	0	1 293 000	0	0

Stellenbosch University https://scholar.sun.ac.za

Western Cape					
Rondebosch	3	1	Rondebosch	3	0
Parow	2	0	Parow	3	0
Sub-total	5	1	**Sub-total**	6	0
Provincial totals	**Doctors**		**Provincial totals**	**Doctors**	
1 609 000	1 to 321 800	1 to 1 609 000	1 866 000	1 to 311 000	0
South Africa					
Total	12	3	**Total**	17	4
18 129 000	1 to 1 510 750	1 to 6 043 000	18 795 000	1 to 1 105 588	1 to 4 698 750

Stellenbosch University https://scholar.sun.ac.za

Table 9: The number of paediatric surgeons in each province between 2000 and 2014

	2000		2014
	Paediatric surgeons		Paediatric surgeons
Eastern Cape			
East London	3	EL	3
PE	1	PE	1
Sub-total	4	**Sub-total**	4
Provincial total		**Provincial total**	
2 928 000	1 to 732 000	2 570 000	1 to 642 500
Free State			
Bloemfontein	1	Bloemfontein	1
Provincial total		**Provincial total**	
1 000 000	1 to 1 000 000	980 000	1 to 980 000
Gauteng			
Soweto		Soweto	3
Jhb	4	Jhb	4
Jhb (Pvt)		Jhb (Pvt)	3
Pretoria	2	Pretoria	2
Pretoria (Pvt)	2	Pretoria (Pvt)	4
Sub-total	8	**Sub-total**	16
Provincial total	**Doctors**	**Provincial total**	**Doctors**
2 939 000	1 to 367 375	3 743 000	1 to 233 937
Kwa-Zulu Natal			
Durban	3	Durban	3
PMB	1	PMB	2
Sub-total	4	**Sub-total**	5
Provincial total	**Doctors**	**Provincial total**	**Doctors**
4 149 000	1 to 1 037 250	4 062 000	1 to 812 400
Limpopo			
Polokwane	0	Polokwane	1
Provincial totals	**Doctors**	**Provincial totals**	**Doctors**
2 421 000	0	2 310 000	1 to 2 310 000
Mpumalanga			
Mbombela (Nelspruit)	0	Mbombela (Nelspruit)	0
Provincial totals	**Doctors**	**Provincial totals**	**Doctors**
1 520 000	0	1 564 000	0
Northern Cape			
Kimberley	0	Kimberley	0
Provincial totals	**Doctors**	**Provincial totals**	**Doctors**

Stellenbosch University https://scholar.sun.ac.za

398 000	0	408 000	0
North West			
Ga Rankuwa	1	Ga Rankuwa	2
Provincial totals	**Doctors**	**Provincial totals**	**Doctors**
1 165 000	1 to 1 165 000	1 293 000	1 to 646 500
Western Cape			
Rondebosch Parow **Subtotals**	3 2 5	Rondebosch Parow **Sub-total**	4 2 6
Provincial totals	**Doctors**	**Provincial totals**	**Doctors**
1 609 000	1 to 321 800	1 866 000	1 to 311 000
South Africa			
Total	23	**Total**	35
18 129 000	1 to 788 217	18 795 000	1 to 537 000

Stellenbosch University https://scholar.sun.ac.za

Table 10: Evaluation of Paediatric oncology units according to the SIOP-PODC Resource Settings for Neuroblastoma Diagnosis, Staging, and Risk Stratification

	Basic bloods	LDH and ferritin	X-ray	U/S	CT/MRI	BMT	mIBG	MYCN	Chemotherapy	Surgery	Radiotherapy	ASCT	Level Setting
	Eastern Cape												
East London	On site	On site	On site	On site	On site	On site	Off-site	Off-site	On site	On site	On site	No	2
	Free State												
Bloemfontein	On site	On site	On site	On site	On site	On site	On site	Off-site	On site	On site	On site	No	2
	Gauteng												
Johannesburg	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	Yes	3
Pretoria	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	Yes	3
Soweto	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	Yes	3
	Kwa-Zulu Natal												
Durban	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	No	2
Pietermaritzburg	On site	On site	On site	On site	On site	On site	Off-site	Off-site	On site	On site	On site	No	1
	Limpopo												
Polokwane	On site	On site	On site	On site	On site	On site	Off-site	Off-site	On site	On site	Off-site	No	1
	Mpumalanga, Northern Cape and North West												
No POUs	None	None	None	None	None	None	None	None	None	None	None	None	None
	Western Cape												
Rondebosch	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	Yes	3
Parow	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	On site	Yes	3

Table key: "On site" refers to services in the same hospital or same hospital complex in the same city. "Off-site" refers to services in another hospital complex, another city or province.
Abbreviations: U/S – ultrasonography, CT – computed tomography, MRI – magnetic resonance imaging, BMT – bone marrow aspirate and trephine, mIBG - ASCT – autologous stem cell transplant, POU –paediatric oncology unit

Stellenbosch University https://scholar.sun.ac.za

Table 11: Provincial and paediatric Oncology Unit (POU) overall survival outcomes

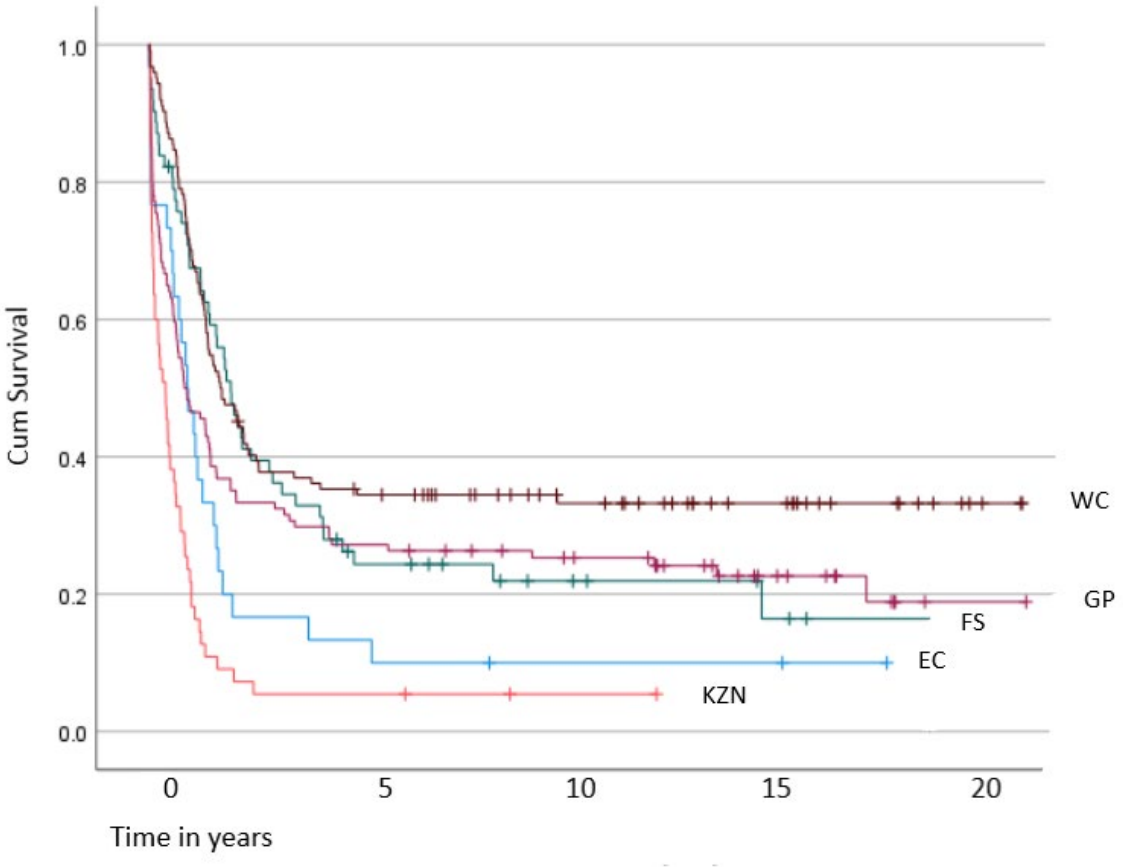
	N (%)	10-year OS	Std. Error	Median 95% CI		p-value
				Lower Bound	Upper Bound	
Provinces						
Eastern Cape	30 (7.8%)	10.0%	2.214	5.528	14.206	<0.001
Free State	62 (16.1%)	21.0%	3.001	15.419	27.181	
Gauteng	75 (19.5%)	22.8%	3.129	3.001	15.266	
Kwa-Zulu Natal	55 (14.3%)	5.5%	1.271	1.709	6.691	
Western Cape	124 (32.2%)	33.9%	3.191	12.313	24.820	
Total	385	22.6%	1.238	10.807	15.660	
Paediatric Oncology Units						
Bloemfontein	62 (16.1%)	21.0%	3.001	15.419	27.181	<0.001
Durban	49 (12.7%)	4.1%	1.960	0.000	6.441	
East London	30 (7.8%)	10.0%	2.214	5.528	14.206	
Johannesburg	51 (13.2%)	39.2%	42.383	0.000	130.070	
Pietermaritzburg	6 (1.6%)	16.7%*	1.225	3.000	7.800	
Pretoria	39 (10.1%)	5.1%	2.809	0.000	11.006	
Rondebosch	91 (23.6%)	39.6%	5.357	12.368	33.366	
Soweto	24 (6.2%)	16.7%	3.715	0.000	14.182	
Parow	33 (8.6%)	18.2%	1.589	11.253	17.480	
Total	385	22.6%	1.238	10.807	15.660	

* Although the survival curve for Pietermaritzburg had already reached its plato, the value reflects a 5-year OS (as opposed to a 10-year OS for the other cities).

Stellenbosch University https://scholar.sun.ac.za

Figures

Figure 2: Kaplan Meier curves overall survival outcomes for patients diagnosed with neuroblastoma between 2000 and 2014 in each province (p<0.001)



Abbreviations: EC -Eastern Cape, FS – Free State, GP – Gauteng Province, KZN – Kwa-Zulu Natal, WC – Western Cape

Stellenbosch University https://scholar.sun.ac.za

Appendices:

Appendix A: International Society of Paediatric Oncology (*SIOP*) Resource Settings for Neuroblastoma diagnosis, staging, and risk stratification

	Setting 1	**Setting 2**	**Setting 3**	**Setting 4**
Diagnosis	History, Physical examination, Histology of small round blue cell tumour or bone marrow metastases Urinary catecholamines (if available)			
Staging	CXR and skeletal survey, Abdominal ultrasound, Bilateral BM aspirate & biopsy	CT neck/ chest/ abdomen/ pelvis 99mTc-bone Scan Bilateral BM aspirate & biopsy	CT neck/ chest/ abdomen/ pelvis 123I- MIBG or 18FDG-PET MRI head or spine if involved Bilateral BM aspirate & biopsy	CT scan neck/ chest/ abdomen/ pelvis 123I- MIBG or 18FDG-PET MRI head or spine if involved Bilateral BM & biopsy
Laboratory	CBC, liver enzymes, LDH, ferritin, creatinine, urinalysis	CBC, liver enzymes, LDH, ferritin, creatinine, urinalysis Urine HVA/ VMA	CBC, liver enzymes, LDH, ferritin, creatinine, urinalysis Urine HVA/ VMA Tumour lysis labs if INSS 4 (electrolytes, Ca Mg PO4, uric acid)	CBC, liver enzymes, LDH, ferritin, creatinine, urinalysis Urine HVA/VMA Tumour lysis labs if INSS 4 (electrolytes, Ca Mg PO4, uric acid)
Pathology	H&E stain	H&E stain IHC	H&E stain, IHC INPC classification (if available) (differentiation grade, MKI) MYCN	H&E stain, IHC INPC classification MYCN, DNA Ploidy segmental chromosome abnormalities
Infrastructure	Nursing, Inpatient Hospital	Nursing, Inpatient hospital	Nursing, Inpatient Hospital	Nursing, Inpatient Hospital

Stellenbosch University https://scholar.sun.ac.za

	Access to RBC or whole blood	Access to RBC & Platelets Paediatric Surgeon Family Housing Intensive Monitoring Capabilities	Rapid Access to all Blood Products Paediatric Surgeon Family Housing Pediatric ICU Isolation and Transplant Facility	Rapid Access to all Blood Products Paediatric Surgeon Family Housing Pediatric ICU Isolation and Transplant Facility
Therapeutics	Antibiotics Standard Chemotherapy	Antibiotics Standard Chemotherapy Radiation Therapy	Antibiotics Standard Chemotherapy Radiation Therapy Transplant Conditioning Agents Isotretinoin	Antibiotics Standard Chemotherapy Radiation Therapy Transplant Conditioning Agents Isotretinoin Anti-GD2 antibody

Abbreviations: CT, computerized tomography; CBC, complete blood count; LDH, lactic dehydrogenase; H&E, hematoxylin and eosin stain; IHC, immunohistochemistry; RBC, red blood cell; HVA, homovanillic acid; VMA, vannilylmandelic acid; 123I- MIBG, *meta*-iodobenzylguanidine; FDG-PET, fluorodeoxyglucose positron emission tomography; MRI, magnetic resonance imaging

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 11

Interim analysis of the prospective national neuroblastoma protocol (SACCSG NB-2017) in South Africa

The prospective SACCSG NB-2017 study has started recruitment in South Africa in January 2019 with two paediatric oncology units (POUs), respectively at Tygerberg Hospital (Cape Town) and Inkosi Albert Luthuli Central Hospital-Grey's Hospital (Durban-Pietermaritzburg in Kwa-Zulu Natal). Currently 12 paediatric oncology units (POUs) in the public sector, four POUs in the private sector and one POU in Namibia are participating in the study.

The original estimated inclusion of patients has been estimated at 30-40 patients per year, based on the retrospective study data. There are currently only 14 patients included, which is less than those expected (*Table 1*). When evaluating the provincial study inclusions (*Table 2*) less than 50% of the potential patients have been included into the study.

Table 1: Patients included in the SACCSG NB-2017 study since January 2019

SACCSG NB-2017										
ID nr	**Hospital**	**DOD**	**Sex**	**Age (mo)**	**Primary**	**Stage**	**MYCN**	**INRG**	**Current phase of treatment**	**Outcome**
NB-0001	EL	15/03/2019	F	67	Neck [R]	4	NA	HR	Completed*	Alive
NB-0002	EL	03/05/2019	F	7	Abd	4	NA	HR	Induction	Died
NB-0003	TBH	25/04/2019	M	19	PS	4	NA	HR	Maintenance	Alive
NB-0004	Bloem	24/06/2019	M	8	Abd	4	NA	HR	ASCT	Alive
NB-0005	KZN	06/04/2019	M	53	Abd	3	NA	IR	Post treatment	Alive
NB-0006	KZN	03/07/2019	M	86	Abd	4	Amp	HR	Palliation	Died
NB-0007	KZN	09/07/2019	M	23	Abd	4	Amp	HR	Palliation	Died
NB-0008	Pta	15/05/2020	F	58	Thx	4	NA	HR	Induction	Alive
NB-0009	Bloem	25/06/2020	F	8	Abd	4	NA	IR	Induction	Alive
NB-0010	Bloem	27/08/2020	M	76	Abd	4	Amp	HR	Induction	Alive
NB-0011	Bloem	04/07/2020	M	26	Abd	4	NA	HR	Induction	Alive
NB-0012	Bloem	06/02/2020	M	50	Abd	4	Amp	HR	Induction	Alive
NB-0013	RBH	3/1/2020	F	96	Abd	4	NA	HR	ASCT	Alive
NB-0014	PE	1/10/2020	F	26	Abd	4	T/F	HR	Induction	Alive
Patients on prospective protocol not yet registered										
Patients 9										

***** patient has since relapsed and started relapse treatment

Abbreviations: DOD – Date of diagnosis; mo – months; INRG – International neuroblastoma risk group; EL – East London Frere Hospital; TBH – Tygerberg Hospital; Bloem – Bloemfontein; Pta – Pretoria Steve Biko Academic Hospital; RBH – Rondebosch Private Hospital, PE – Port Elizabeth Provincial Hospital, F – female; M – male; Abd – Abdominal; PS – paraspinal; Thx – thorax; NA – not amplified, Amp – amplified, HR – high-risk, IR – intermediate risk, ASCT – autologous stem cell transplant.

There was a male predominance with a male to female ratio of 1.3:1, and patients had been most frequently diagnosed in the 18-60-months category (n=7; 50.0%). The median age was 26 months (range 7 months - 8 years, mean 43.1 months).

Stellenbosch University https://scholar.sun.ac.za

The most common site of the primary was an abdominal tumour (n = 11; 78.6%) with 92.8% (n=13) of patients were diagnosed with stage 4 disease. MYCN was amplified in 69.2% (n=9/13) of tumours. The cohort was dominated by patients with HR disease (n=13, 92.8%). Three (21.4%) patients have died and one (7.1%) relapsed with a parietal bone lesion.

Table 2: Patients included in the SACCSG NB-2017 study since January 2019 according to provinces

		Expected number per year		
Province	**Children under 15 years N (%)**	**International 10.5/ mil per year**	**SA* 2.7/ mil per year**	**Study per year (Total) ****
EC	2 218 998 (14.9)	23.3	6.0	2 (4)
FS	833 571 (5.6)	8.8	2.3	2.5 (5)
GP	2 647 499 (17.7)	27.8	7.1	4 (8)
KZN	2 593 861 (17.4)	27.2	7.0	1.5 (3)
LP	1 971 421 (13.2)	20.7	5.3	0 (0)
MP	1 381 452 (9.2)	14.8	3.7	No POUs
NC	374 996 (2.5)	3.9	1.0	0 (0)
NW	1 203 386 (8.0)	12.6	3.2	0(0)
WC	1 710 772 (11.5)	18.0	4.6	1.5 (3)
Total SA	14 935 956 (100)	157.1	40.2	11.5 (23)

* incidence based on reports by Stefan. et al. 2015

** Includes patients identified for the study without a study number

Abbreviations: EC – Eastern Cape; FS – Freestate; GP – Gauteng; KZN – Kwa-Zulu Natal; LP – Limpopo; MP – Mpumalanga; NC- Northern Cape; NW – North West; WC – Western Cape, SA – South Africa, POUs – Paediatric oncology units

Challenges since the opening of the study

a) Ethics applications *(see Chapter 9 for an extended discussion)*

South African Universities have no common or standardised ethics application procedure. Neither is reciprocal ethics approval between universities established. The differences in ethical applications included: a) whether the principal investigator or hospital investigator had to complete the application differed between committees, b) whether a new academic evaluation had to be done, even after a full academic and ethical evaluation was done at the primary site, differed between committees, c) reciprocal agreement was not standard practice, d) the application forms and requirements differed between committees. Where the same documentation was needed, the format requirements differed. The implication was that a single document had to be reworked multiple times, e) the hospital approval differed between sites: either hospital superintendents, departmental heads or academic heads were responsible, but was not clear to applicant whom to direct requests to.

b) Personal experience

Some patients have not been included, because participating POUs have felt the psycho-emotional situation such as pre-terminal presentations, not conducive for including patients on the palliative aspect of the study. One hospital investigator felt that including a patient for trial purposes in proximity to a poor prognostic or palliative intent conversation with a parent was difficult to do and might be insensitive to the family.

c) Reluctance of medical staff in research participation

Stellenbosch University https://scholar.sun.ac.za

The SACCSG decided that during all national prospective studies, that at least one paediatric oncologist from each POU should be part of the working group to lead the study in each hospital. This person would make sure that the multidisciplinary team in their hospital could manage the patients according to the protocols. The degree of participation from working group members have varied for a number of reasons: 1) NB was not a particular interest for some of the member. 2) Closely linked to the subject matter is the interest to do research. A number of physicians chose to be clinicians and do not want to undertake research but find themselves in tertiary or academic centres where research is expected as part of their employment requirements. 3) With a limited number of physicians in each POUs, some have felt they had to take on responsibilities outside of their interest. 4) One site had difficulty in securing a dedicated hospital investigator due to rotating staff. 5) Some investigators found it difficult to communicate about the recruitment or avoided the subject. 6) Historically POUs developed in autonomous settings with limited co-operative research done on national level. POUs were accustomed to developing local protocols for the management of malignancies and may find it challenging to adapt to the new trial. 7) Obtaining consent for treatment in South Africa with 11 official languages can be challenging. To add the complexity of a trial, even as basic as a single arm, standard care protocol, adds a degree of complexity.

d) Low level of compliance monitoring

The responsibility of familiarising oneself with a new national treatment trial paired with the infrequent diagnosis of a patient with NB per POU contributes to low retention of the study protocol. Hospital investigators reported that clinical burdens limited the ability to refamiliarize themselves to the protocol. This frequency of support by the principal investigator should be increased.

e) The corona-pandemic

The daily clinical burdens have also been cited not to include patients. Since the outbreak of the COVID-19 pandemic an already overburdened South African medical system, increased pressures arose. This had the effect that academic and administrative responsibilities were secondary to pandemic prevention responsibilities. South Africa measures were very strict and protracted [1]. The lockdown commenced on 25 March 2020 and the first de-escalation from level 5 to level 4 on 1 May 2020 [1]. During lockdown interprovincial travel was not permitted except for personal emergencies which had to be approved by governmental institutions [1]. This excluded medical emergencies. Interprovincial travel was resumed from the initiation of level 2 lockdown on 18 August 2020 [1]. The effect on patients needing trans-provincial services is not clear, but the expectation that delayed diagnoses and relapses will increase. The Kingdom of Lesotho, an example of a country landlocked by a single country, mostly refers patients to Bloemfontein, South Africa. NB patients were still permitted to receive their treatment in South Africa with hospital documentation stating the need for cross-border travel [1]. New cases of NB could still be transferred to South Africa, but the administrative conditions were stricter and new patients had to be isolated until proven negative for COVID-19 since Lesotho is graded as a high-risk (red) country according to South African legislation [1].

Discussion

Research trough trials is important for the translation of knowledge to clinical practice [2]. Recruiting patients into trials demands a number of resources which includes obtaining consent during inclusion [3]. Consent is an ethical requirement for trial inclusion but is time-consuming [4]. It requires a motivated research to ensure a high level of inclusion and to maintain that motivation [3]. Research

Stellenbosch University https://scholar.sun.ac.za

in diverse ethnic settings and minority groups is less than westernised settings [2]. One factor, relevant to the South African setting, is the language barriers between the investigator and patient and family [2]. A translator may not be available and even if available could be challenging [2].

The judgement regarding inclusion of patients with advanced disease in research and the feelings of medical staff regarding the appropriateness to offer inclusion in research during pre-terminal stages, have been documented [5]. Lower than expected number of included patients have been reported in palliative research [5]. The unpredictable course and stage of the disease was also identified as a barrier [5]. A study by Steele; et al. reported that ethics boards and physicians were reluctant to approve and participate in paediatric palliative care research siting the burden on families as one reason [4]. The study concluded that families with children who have a life-threatening condition participating in research is both acceptable to parents and have a positive effect on the family [4].

Low investigator enthusiasm is well described to contribute to lower patient recruitment [5]. This factor is closely associated with trial experience and confidence in running a trial [6]. Consistantly having the same investigator improves both the recruitment rate and the consistency of data [6]. A consistent research team aids communication, especially for problem solving and for the continuity of research [7]. To ensure investigator compliance is maintained and/or improves, an initial orientation towards the protocol with continuing education on the study tools should be a standard to increase the level of compliance increases and maintain the intrest for participation [8].

The corona virus pandemic impacted the care for patients diagnosed with NB, and associated research, threefold: 1) Patients not yet diagnosed had to rely on accessible medical care, often primary care, in their local communities to distinguish symptoms from other common childhood diseases. 2) Patients already diagnosed with NB experienced added challenges to ensure that therapy was continued. 3) POUs had to adapt service delivery according to guidelines for the prevention of further infections.

Making the diagnosis

Solid tumours such as NB, often have an indolent onset [9] Therefore symptoms are not directly attributed to malignancies [9]. It is only when acute symptoms are present, for example symptoms associated with intestinal obstruction, respiratory distress or spinal compression, that medical services are sought [10]. Offenbacher et al. concluded that haematological malignancies often present with acute symptoms compared to solid tumours [10]. For this reason, they observed a decrease in solid tumour diagnosis, but not in haematological malignancies [10]. In the pandemic seeking help for minor symptoms were often delayed [11].

Access to medical care

At the peak of the epidemic in Italy and USA, POUs noted a reduction in the diagnoses of solid tumours [10,12]. In Milan, a national referral centre for Italy, a 45.7% reduction of solid tumour cases, which included NB, were observed during lockdown compared to the same months in previous years (Fisher exact test *P*-value 0.0416) [12]. The decrease was due to a decrease in interregional transfers [12]. In the South African retrospective study 70% of NB diagnosis were stage 4 disease [13]. The interim analysis showed 92.8% of patients presented with stage 4 disease, before and during lockdown. We postulate that with restricted movement during lockdown, the challenges that already existed in an

Stellenbosch University https://scholar.sun.ac.za

inequitable system, will be increased. The question might not be in the increase advanced staged disease, but the increase of deaths due to advanced disease, patients that died from complications of pancytopaenia post chemotherapy and abandonment of treatment. The decreased access to healthcare could have made access to life saving health care more difficult.

POUs adapting services

The main reasons POUs had to adapt their services in South Africa, as in many LMICs, were due to 1) personnel shortages, 2) insufficient equipment and 3) continued patient burden [14]. The South African health system had to contend with a high number of personnel having to isolated due to sickness, quarantine due to exposure or personnel that died due to coronavirus infections [14]. Like other LMICs, personnel had to be deployed to other overburdened services as well [14,15]. With a severe shortage of personal protective equipment (PPE's) and still having to maintain Occupational Health and Safety policies, service delivery had to be reorganized to maintain social distancing and hygiene precautions [14,15].

The pandemic affected all aspects of management including the diagnostic process. A study evaluating services in 194 retinoblastoma centres concluded that services that involved anaesthesia were partly or completely unavailable in 44.8% of POUs [16]. NB is in large dependent on tumour biopsies for diagnosis and genetic evaluation for risk stratification [17]. This further impacts the access to resection of primary tumours [18]. In India the care of patients with solid tumours were modified according to their ability to access health care [19]. For NB patients that could access health care the standard protocol was administered to patients that lived in the capital city of a POU were given chemotherapy at reduced dosages while patients that traveled from other states were given oral Cyclophosphamide and Etoposide [19].

To prevent the spread of the disease increased patient screening for COVID-19 was implemented and isolated COVID-19 units were established with separate management by dedicated staff [15]. Unnecessary displacement of patients or where travel was not possible, the role of telemedicine was increased during the lockdown. Patient contact was continued by telephone and internet-based services [20]. Patients that did not have access to internet were therefore further disadvantaged by mobility restrictions [20].

The impact on supportive care and co-morbidities

It has been estimated that overall survival in adult cancer patients will decrease by up to 10% due to the effects of the coronavirus pandemic [21]. In regions where food shortages, nutritional diseases and poverty are high, will see a decrease of income and a deepening of these health emergencies including in patients with neuroblastoma [22].

With travel restrictions the delivery of chemotherapy, antibiotics, anti-fungals and anti-viral supplies will most probably be disrupted [22]. In a region where HIV has a high incidence and patients might default treatment, HIV-related and associated malignancies might increase. Blood donation will decrease due to mobility restrictions with an eminent or increased shortage of blood products [22]. Therefore, patients on treatment intensive HR-NB protocols could face increased morbidity and mortality due to these shortages.

Stellenbosch University https://scholar.sun.ac.za

Not least of all these challenges, there will be an increased psycho-emotional burden on families due to decreasing resources, deaths due to the coronavirus and still having to cope with a neuroblastoma diagnosis [23].

Conclusion:

Many factors contribute to running a successful trial and recruitment patients. This is evident from the interim analysis of the SACCSG NB-2017 clinical trials and the sub-optimal inclusion of patients. The factors that contribute to investigator trial participation are systemic, institutional and individual to investigators. Although many factors can be mitigated, other factors are not and flexibility towards the research should be adopted with a greater emphasis on co-operative participation.

References:

1. The South African Government. Regulationas and Guidelines – Coronavirus COVID-19. https://www.gov.za/covid-19/resources/regulations-and-guidelines-coronavirus-covid-19 Accessed October 2020.
2. Hamel LM, Penner LA, Albrecht TL, Heath E, Gwede CK, Eggly S. Barriers to Clinical Trial Enrollment in Racial and Ethnic Minority Patients with Cancer. Cancer Control. 2016;23(4):327-337.
3. Fogel DB. Factors associated with clinical trials that fail and opportunities for improving the likelihood of success: A review. Contemporary Clinical Trials Communications. 2018; 11(9): 156-164.
4. Steele R, Cadell S, Siden H, et al. Impact of research participation on parents of seriously ill children. Journal of Palliative Medicine. 2014;17(7):788-796.
5. Hudson BF, Oostendorp LJ, Candy B, et al. The under reporting of recruitment strategies in research with children with life-threatening illnesses: A systematic review. Palliative Medicine. 2017;31(5):419-436.
6. Levett KM, Roberts CL, Simpson JM, Morris JM. Site-specific predictors of successful recruitment to a perinatal clinical trial. Clin. Trials. 2014; 11 (): 584-589.
7. Skjeggestad E, Norvoll R, Sandal GM, Gulbrandsen P. How do international medical graduates and colleagues perceive and deal with difficulties in everyday collaboration? A qualitative study. Scandinavian Journal of Public Health. 2017;45(4):428-435.
8. Snider SH, Flume PA, Gentilin SL, Lesch WA, Sampson RR, Sonne SC. Overcoming non-compliance with clinical trial registration and results reporting: One Institution's approach. Contemp Clin Trials Commun. 2020; 18:100557. Published 2020 Mar 17. doi: 10.1016/j.conctc.2020.100557
9. Tan TD, Franco EL. Diagnosis delays in childhood cancer. Cancer. 2007;110(4):703-713.
10. Offenbacher R, Knoll MA, Loeb DM. Delayed presentations of pediatric solid tumors at a tertiary care hospital in the Bronx due to COVID-19. Pediatr Blood Cancer. 2020. doi:10.1002/pbc.28615
11. Montoya J, Ugaz C, Alarcon S, Maradiegue E, García J, et al. COVID-19 in pediatric cancer patients in a resource-limited setting: National data from Peru. Pediatr Blood Cancer. 2020. doi:10.1002/pbc.28610
12. Chiaravalli S, Ferrari A, Sironi G, Gattuso G, Bergamaschi L, Puma N, et al. A collateral effect of the COVID-19 pandemic: Delayed diagnosis in pediatric solid tumors. Pediatr Blood Cancer. 2020; 67: e28640.

Stellenbosch University https://scholar.sun.ac.za

13. Van Heerden, J, Hendricks, M, Geel, J, et al. Overall survival for neuroblastoma in South Africa between 2000 and 2014. Pediatr Blood Cancer. 2019; 66: e27944 https://doi.org/10.1002/pbc.27944
14. Abratt RP. Patient care and staff well-being in oncology during the coronavirus pandemic – Ethical considerations. S. Afr. J. oncol. 2020;4(0): a129.
15. Schultz L, Link MP, Rheingold S, Hawkins DS, Dome JS, et al. Summary of COVID-19 clinical practice adjustments across select institutions. Pediatr Blood Cancer. 2020; 67: e28411. doi:10.1002/pbc.28411
16. Fabian ID, Stacey AW, Bowman R, Khetan V, et al. Retinoblastoma management during the COVID-19 pandemic: A report by the Global Retinoblastoma Study Group including 194 centers from 94 countries. Pediatr Blood Cancer. 2020. doi:10.1002/pbc.28584
17. Parikh N, Howard S, Chantada G, Israels T, Khattab M, Alcasabas P, et al. SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for neuroblastoma in low- and middle-income settings. Pediatr Blood Cancer. 2015; 62(8): 1305-1316.
18. Nerli RB, Sanikop AC, Sharma M, Ghagane SC. COVID-19 pandemic: A challenge to a child with cancer. Pediatr Blood Cancer. 2020; 67: e28512. doi:10.1002/pbc.28512
19. Pushpam D, Bakhshi S, Agarwala S. Chemotherapy adaptations in a referral tertiary care center in India for ongoing therapy of pediatric patients with solid tumors during COVID19 pandemic and lockdown. Pediatr Blood Cancer. 2020. doi:10.1002/pbc.28428.
20. Sajid MI, Altaf S, Mushtaq N, Belgaumi A, Fadoo Z. Pediatric hematology oncology during SARS-CoV-2: A brief communication of 28 patients and changes in clinical practice from a single institute in Pakistan. Pediatr Blood Cancer. 2020. doi:10.1002/pbc.28527.
21. The Lancet Oncology. Safeguarding cancer care in a post-COVID-19 world. Lancet Oncol. 2020;21(5):603. doi:10.1016/S1470-2045(20)30243-6.
22. Slone JS, Ozuah N, Wasswa P. Caring for Children with Cancer in Africa during the COVID-19 Crisis: Implications and Opportunities. Pediatric Hematology and Oncology. 2020; 1-5. doi:10.1080/08880018.2020.1772913.
23. Kotecha, R. S. (2020). Challenges posed by COVID-19 to children with cancer. The Lancet Oncology. doi:10.1016/s1470-2045(20)30205-9.

Stellenbosch University https://scholar.sun.ac.za

CHAPTER 12

Conclusions and recommendations

As there was little published data regarding neuroblastoma in Africa and specifically South Africa, a systematic investigation into the incidence and management of neuroblastoma in South Africa has been conducted towards this PhD dissertation. When collectively viewing the results of the various studies, the results indicate that South Africa faces similar challgenges as most LMIC with the management of neuroblastoma. The main elements are the underdiagnoses of cases, proportionately higher number of high-risk disease and poor outcomes.

This dissertation further has been able to reproduce the following findings similar to international studies: 1) the cut-point values for LDH with values of more than 750U/l and ferritin with values of more than 120ng/dl which indicate poor prognosis[1]; 2) the importance of local control measures in the absence of autologous stem cell transplantation; and 3) the predictive values of prognostic factors age, stage, biology and metastatic remission status to predict overall survival. Our research demonstrates the disparity in NB management in South Africa between the different POUs, between risk groups and especially in patients with high-risk disease. Furthermore, the approach to advanced disease has not been uniform in South Africa with variable outcomes between POU. The knowledge generated has assisted in the development of a national neuroblastoma treatment protocol as a single arm clinical trial. In the process the process exposesthe the resource challenges faced by a disproportionately funded system, but positively lays the basis for standardizing the approach to neuroblastoma in the country.

The strength of the research is present in the explorative approach to subject matter with lack of data from LMIC for a large cohort with neuroblastoma. The data collected to evaluate the various management approaches in South African POUs has assisted in comparing different treatment protocols and assisted with the planning of the national NB protocol by providing evidence for potentially the best treatment protocol for the local context. The most crucial finding is the role of induction chemotherapy for high-risk disease and use of additiveradiotherapy if surgery is not possible. The retrospective quality of the research has introduced limitations in certain aspects of the research, which include non-standardized risk stratifications and management definitions.

Although South Africa is a middle-income country, not all recommendations can directly be extrapolated to all LMICs. However, the approach applied to improve management of NB in the South African context may be replicated in other LMICs through evaluation of the local settingand the utilisation of local resources for clinical and research purposes.

A major recommendation is to implement a single standard of care protocol or clinical trial, which is sustainable in all the POUs in South Africa, even with resource differences, to improve the outcome of children with neuroblastoma. Previous studies indicate that to be truly successful in the improvement of outcomes, it is be necessary to participate in collaborative clinical trials in childhood cancer[39]. This study has proven that the risk stratification as per PODC recommendations is appropriate to use for prognostic purposes with the use of LDH and ferritin as surrogate tumour markers for MYCN and genetic mutations, in settings where it cannot be done. The LR and IR treatment compares to the PODC recommendations, but the South African guidelines for HR disease are platinum based without doxorubicin as platinum-based treatment protocol has been found to be less

Stellenbosch University https://scholar.sun.ac.za

toxic in the local context. Future research should also include the meticulous documentation of outcomes and optimization of national resources including stem cell transplant and mIBG-therapy with utilisation of the diagnostic and post induction IDRF[4], international neuroblastoma response criteria (INRC)[28] and the fastidious application of ASCT indications in HR disease as recommended in the SIOP-PODC protocols.

Recommendations for South Africa with relevance to LMICs

South Africa has great potential for national research related to NB. The focus should be on developing platforms that include various resource settings and strengthening the capacities of individual POUs to perform clinical trials related to NB. All disciplines involved in NB care should be part of the protocol development. The collaboration should include surgeons, radio-oncologists, laboratory and imaging-based physicians, thereby increasing the experience and capacity of treatmenting teams[40,41], with the interdependency of medical, surgical and radiology clinicians' interpretation of image defined risk factors for risk stratification and surgical planning are crucial[4,42].

Achieving early diagnosis remains a challenge in LMICs and especially in South Africa. It is therefore crucial to improve public awareness and include the warning signs of childhood cancer in the integrated management of childhood illnesses (IMCI) to assist primary health care workers to recognize childhood cancer early with speedy referral[43].

Current and future data-based systems should adopt a more research-orientated management of resources to ensure the provision of evidence for interventions implemented in the management of neuroblastoma. To improve NB cure requires the inclusion of advanced, expensive therapies such as ASCT, with clear indication for HR disease with the potential of cure. The handling and access of data inherently has ethical restrictions; yet the process of accessing this information should be made efficient to aid research rather than impeding the development or overburdening settings with high clinical responsibilities[44].

The surgery for NB is complex due to the encasing nature of the tumour[45]. If an experienced surgeon is not available, patients should be referred to a center with the necessary expertise in NB in the best interest of the child to ensure the most beneficial resection between 90% - 100% resection[46]. LMICs can rely on the subjective evaluation of surgeons, as was concluded in the HR-NBL1/SIOPEN trial[47], rather than through imaging. Standardized reporting should be part of the holistic surgical care[48]. By optimizing the utilization of national treatment modalities for chemotherapy, surgery and radiotherapy with considered indications for all treatment modalities, but especially autologous bone marrow transplantation, national resources could potentially be utilized responsibly and sustainably[49,50].

Of importance is the access to medicines for maintenance and relapse treatment. Cis-retinoic acid, in conjunction with an autologous transplant, can improve survival of up to 15 -20% in HR-NB through maturation therapy and is available in South Africa[4]. Topo-isomerase inhibitors, such as irinotecan and topotecan, and temozolomide have shown promise in the refractory, residual and relapse setting[51]. None of these medicins are currently on the essential medicine list for the treatment of neuroblastoma in South Africa but is potentially available with special motivation.

Stellenbosch University https://scholar.sun.ac.za

Palliative care options should be explored in South Africa for advanced disease and access to mIBG-therapy as a palliative treatment option may be a valuable treatment option[52]. With international collaborative groups exploring mIBG as part of upfront treatment and in the context of resistant disease[53], mIBG therapy in South Africa may be underutilized and could be part of innovative applications[52].

This study has proven the value of LDH and ferritin as tumour markers in the absence of access to determination of MYCN and can be implementd in LMICs.

Crucial long-term follow-up is necessary for all children treates with platinum-based treatment protocols, especially hearing and kidney function [54,55].

Greater initiatives towards bio-banking should be started. With the diversity of the genetic landscape in South Africa and the ever-increasing research into genetic and epigenetic targets in NB, biological knowledge could contribute to a greater understanding in the outcome variations as well as research into the pharmacokinetics and dynamics in the treatment of NB[53].

In conclusion, the approach to NB in resource restrained settings should focus on establishing a basis for management that will optimally use available resources in local settings. Recording data on the management and focus on the stepwise improvement of NB services in conjunction with other childhood malignancies should be part of the national health care system's overall management strategy. Such approaches will address the need to improve survival rates of children with cancer in alignment with the global World Health Organization 2030 oncology objectives.

References:

1. National Cancer Institute: Neuroblastoma http://www.cancer.gov/types/neuroblastoma (Accessed 6 September 2020).
2. National Cancer Institute: Surveillance, Epidemiology and End Results Database http://seer.cancer.gov (Accessed 6 September 2020).
3. Bhatnagar S, Sarin Y. Neuroblastoma: A Review of Management and Outcome. Indian J Pediatr. 2012; 79(6): 787-792.
4. Parikh N, Howard S, Chantada G, Israels T, Khattab M, Alcasabas P, et al. SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for neuroblastoma in low- and middle-income settings. Pediatr Blood Cancer. 2015; 62(8): 1305-1316.
5. Steliarova-Foucher E, Colombet M, Ries LAG, Moreno F, Dolya A, Bray F. International incidence of childhood cancer, 2001–10: a population-based registry study. Lancet Oncol 2017; 18: 719–31.
6. Kruger M, Hendricks M, Davidson A, Stefan CD, van Eyssen AL, Uys R, et al. Childhood cancer in Africa. Pediatr Blood Cancer. 2014; 61(4): 587-92.
7. Kattab M, Elkababri M, Cherkaoui S, Benmiloud S, Elhoudzi J, Hessissen L; et al. O-001. Results of an intensive induction protocol for high-risk neuroblastoma in Morocco (HR-NBL-MA-10). Paper presented at: 44th Congress of the International Society of Paediatric Oncology (SIOP); 2012 October 5-8; London, United Kingdom.
8. Moussa, E; et al. Combined Treatment Strategy and Outcome of High-Risk Neuroblastoma: Experience of the Children's Cancer Hospital-Egypt. Journal of Cancer Therapy, 2013, 4, 1435-1442.

Stellenbosch University https://scholar.sun.ac.za

9. Stefan D, Stones D, Wainright R, Kruger M, Davidson A, Poole J; et al. Childhood cancer incidence in South Africa, 1987–2007. S Afr Med J 2015; 105(11): 939-947.
10. Smith A, Altekruse S, Adamson P, Reaman GH, Seibel NL. Declining childhood and adolescent cancer mortality. Cancer 2014; 120(16): 2497-506.
11. Heck J, Ritz B, Hung RJ, Hashibe M, Bofetta P. The epidemiology of Neuroblastoma: a review. Paediatric and Perinatal Epidemiology 2009; 23(2): 125–143.
12. Bourdeaut F, de Carli E, Timsit S, Coze C, Chastager P, Sarnacki S; et al.: VIP hypersecretion as primary or secondary syndrome in neuroblastoma: A retrospective study by the Société Française des Cancers de l'Enfant (SFCE). Pediatr Blood Cancer 52 (5): 585-90, 2009.
13. Matthay K, Blaes F, Hero B; Plantz D, De Alarcon P, Mitchell WG; et al.: Opsoclonus myoclonus syndrome in neuroblastoma a report from a workshop on the dancing eyes syndrome at the advances in neuroblastoma meeting in Genoa, Italy, 2004. Cancer Lett 228 (1-2): 275-82, 2005.
14. Mahoney N, Liu G, Menacker S, Hogarthy MD, Maris JM. Pediatric horner syndrome: etiologies and roles of imaging and urine studies to detect neuroblastoma and other responsible mass lesions. Am J Ophthalmol 142 (4): 651-9, 2006.
15. Brodeur, G. Neuroblastoma: biological insights into a clinical enigma. Nature Reviews Cancer 2003; 3: 203-216.
16. Sanai, N; Alvarez-Buylla, A; Berger, M. Mechanisms of disease: neural stem cells and the origin of gliomas. N Eng Journal of Medicine. 2005; 353(8): 811–822.
17. De Weille, J. On the genesis of Neuroblastoma and Glioma. International Journal of Brain Science. Volume 2014. 1-4. De Weille, J. (2014). On the Genesis of Neuroblastoma and Glioma. International Journal of Brain Science, 2014, 1–14. doi:10.1155/2014/217503
18. Pizzo PA, Poplack DG, editors. Neuroblastoma, Chapter 30 p773-774. Principles and practice of pediatric oncology, 7th edition. Philadelphia: Lippincott-Williams and Wilkens, 2015.
19. Kogner P, Barbany G, Dominici C, Castello MA, Raschella G, Person H. Coexpression of Messenger RNA for TRK Protooncogene and Low Affinity Nerve Growth Factor Receptor in Neuroblastoma with Favorable Prognosis. Cancer Res 1993; 53(9): 2044-2050.
20. Nakagawara A, Azar C, Scavarda N, Brodeur G. Expression and function of TRK-B and BDNF in human neuroblastomas. Molecular and Cellular Biology. 1994;14(1):759-767.
21. Pinto, N; Gamazon, E; Antao, N; Myers, J; et al. Integrating Cell-Based and Clinical Genome-Wide Studies to Identify Genetic Variants Contributing to Treatment Failure in Neuroblastoma Patients. Clinical Pharmacology & Therapeutics 2014; 95: 644–652.
22. Yáñez et al. Two independent epigenetic biomarkers predict survival in Neuroblastoma. Clinical Epigenetics (2015) 7:16.
23. Decock, A; Ongenaert, M; Cannoodt, R; Verniers, K; De Wilde, B; Laureys, G; et al. Methyl-CpG-binding domain sequencing reveals a prognostic methylation signature in neuroblastoma. Oncotarget 2016; 7(2): 1960–1972.
24. Cohn, S; Diagnosis and classification of Small round-cell tumors of childhood. American Journal of Pathology, Vol. 155, No. 1, July 1999.
25. Cotterill, S; Pearson, A; Pritchard, J; et al.: Clinical prognostic factors in 1277 patients with neuroblastoma: results of The European Neuroblastoma Study Group 'Survey' 1982-1992. Eur J Cancer 36 (7): 901-8, 2000.

Stellenbosch University https://scholar.sun.ac.za

26. Hesseling PB, Ankone K, Wessels G, Schneider JW, Du Plessis, Moore L. Neuroblastoma in Southern Africa: Epidemiological features, prognostic factors and outcome. Ann Trop Paediatr 1999;19: 357–363.
27. Bleeker, G; van Eck-Smit, B; Zwindermann, K; et al. MIBG scans in patients with stage 4 neuroblastoma reveal two metastatic patterns, one is associated with MYCN amplification and in MYCN-amplified tumours correlates with a better prognosis. Eur J Nucl Med Mol Imaging (2015) 42:222–230.
28. Brodeur G, Seeger R, Barrett A; et al.: International criteria for diagnosis, staging, and response to treatment in patients with neuroblastoma. J Clin Oncol 6 (12): 1874-81, 1988.
29. Von Allmen, D; Davidoff, M; London, W; Van Ryn, M; Haas-Kogan, D; Kreissman, S; Khanna, G; Rosen, N; Park, J; La Quaglia, M. Impact of Extent of Resection on Local Control and Survival in Patients from the COG A3973 Study with High-Risk Neuroblastoma. JCO 2017; 35(2): 208-216.
30. Kushner, B; Wolden, S; LaQuaglia, M; et al. Hyperfractionated Low-Dose Radiotherapy for High-Risk Neuroblastoma After Intensive Chemotherapy and Surgery. JCO Jun 2001; 19(11): 2821-2828.
31. George, S; Tucker, E; Chesler, L. High-Risk Neuroblastoma: Current and Future Therapeutic Strategies. Ann Pediatr Child Health 2015; 3(8):1086-1091.
32. Basu, S; et al. Comparative Performance of Private and Public Healthcare Systems in Low- and Middle-Income Countries: A Systematic Review. Ed. Rachel Jenkins. *PLoS Medicine* 9.6 (2012): e1001244. *PMC*. Web. 8 Feb. 2018
33. Saarinen-Pihkala, U; Jahnukainen, K; Wikström, S; Koivusalo, A; Karikoski, R; Sariola, H; Hovi, L. Ultrahigh-risk group within the high-risk neuroblastoma category. J Pediatr Hematol Oncol 2013; 35(6):e254-9.
34. Monclair, T; Brodeur, G; Ambros, P; et al.: The International Neuroblastoma Risk Group (INRG) Staging System: An INRG Task Force Report. Journal of Clinical Oncology 2009; 27:2, 298-303.
35. Cohn, S; Pearson, A; et al. The International Neuroblastoma Risk Group (INRG) Classification System: An INRG Task Force Report J Clin Oncol 2009; 27(2):289-297.
36. Ferrari, A; Schneider, D; Bisogno. The founding of the European Cooperative Study Group on Pediatric Rare Tumors – EXPeRT, Expert Review of Anticancer Therapy 2013;13(1):1-3.
37. Arora, R; Bakhshi, S. Indian Pediatric Oncology Group (InPOG) –Collaborative Research in India Comes of Age, Pediatric Hematology Oncology Journal (2016), doi: 10.1016/j.phoj.2016.04.005.
38. Greengard, E; et al. Treatment of high-risk neuroblastoma in children: recent clinic trial results. Clin. Invest. 2013; 3(11), 1071–1081.
39. Van Heerden J, Zaghloul M, Neven A, de Rojas T, Geel J, et al. Pediatric Oncology Clinical Trials and Collaborative Research in Africa: Current Landscape and Future Perspectives. JCO Global Oncology. 2020; (6), 1264–1275.
40. Day S, Challinor J, Hollis R, Abramovitz L, et al: Peadiatric oncology nursing care in low- and middle-income countries: A need for baseline standards, in Magrath I (ed): Cancer Control 2015. Cancer Care in Emerging Health Care Systems. Woodbridge, United Kingdom, Peadiatric cancer, 2015, pp 111-116.
41. Bey P, Moreira C, Couitchere L, et al: Implementation of multidisciplinarity in French-African Group of Pediatric Oncology (GFAOP) sub-Saharan teams: A process build by the teams. Pediatr Blood Cancer 66(Suppl.4): e27989, 2019.

Stellenbosch University https://scholar.sun.ac.za

42. Parhar D, Joharifard S, Lo AC, Schlosser MP, Doadu OO. How well do image-defined risk factors (IDRFs) predict surgical outcomes and survival in patients with neuroblastoma? A systematic review and meta-analysis. Pediatr Surg Int. 2020; 36: 897–907.
43. Stones DK. Childhood Cancer: Early warning signs. CME. 2010; 8(7): 314-316.
44. Van Heerden J, Zaghloul M, Neven A, de Rojas T, Geel J, Patte C; et al.: Pediatric Oncology Clinical Trials and Collaborative Research in Africa: Current Landscape and Future Perspectives. JCO Global Oncology. 2020; 6:1264-1275.
45. Pohl A, Erichsen M, Stehr M, Hubertus J, Bergmann F, Kammer B, et. al. Image-defined Risk Factors Correlate with Surgical Radicality and Local Recurrence in Patients with Neuroblastoma. Klinische Pädiatrie. 2016, 228(03): 118–123.
46. Ryan AL, Akinkuotu A, Pierro A, Morgenstern DA, Irwin MS. The Role of Surgery in High-risk Neuroblastoma. Journal of Pediatric Hematology/Oncology. 2019; 1. doi:10.1097/mph.0000000000001607
47. Holmes K, Poëtschger U, Sarnacki S, Monclair T, Checchetto G, Chacon JG; et al.: The influence of surgical excision on survival in high-risk neuroblastoma revisited after introduction of ch14.18/CHO immunotherapy in the HR-NBL1/SIOPEN trial. Journal of Clinical Oncology. 2018; 36:15_suppl, 10521-10521.
48. Matthyssens LE, Nuchtern JG, Van De Ven CP, Gabra HOS, Bjornland K, Irtan S; et al.: A Novel Standard for Systematic Reporting of Neuroblastoma Surgery: The International Neuroblastoma Surgical Report Form (INSRF): A Joint Initiative by the Pediatric Oncological Cooperative Groups SIOPEN, COG, and GPOH [published online ahead of print, 2020 Jul 7]. Ann Surg. 2020;10.1097/SLA.0000000000003947.
49. Lau SCD, Unni MNM, Teh KH, Aziz MA, Muda Z, Thomas SA; et al.: Autologous stem cell transplantation following high-dose chemotherapy in children with high-risk neuroblastoma: Practicality in resource-limited countries. Pediatr Blood Cancer. 2020; 67: e28176. doi:10.1002/pbc.28176.
50. Jain R, Hans R, Totadri S, Trehan A, Sharma RR, Menon P; et al.: Autologous stem cell transplant for high-risk neuroblastoma: Achieving cure with low-cost adaptations. Pediatr Blood Cancer. 2020; 67: e28273. https://doi.org/10.1002/pbc.28273.
51. Herd F, Basta NO, McNally RJQ, Tweddle DA. A systematic review of re-induction chemotherapy for children with relapsed high-risk neuroblastoma. Eur Journal of Cancer. 2019; 111: 50-58.
52. Schmidt M, Hero B, Simon T. I-131-mIBG therapy in neuroblastoma: established role and prospective applications. Clin Transl Imaging. 2016; 4(2), 87–101.
53. Nguyen R, Dyer M. Chapter 3 - Neuroblastoma: Molecular Mechanisms and Therapeutic Interventions. In Ray S, ed. Academic Press. Neuroblastoma: Molecular Mechanisms and Therapeutic Interventions. Oxford (UK): Elsevier; 2019 p43-56.
54. Grewal S, Merchant T, Reymond R, McInerney M, Hodge C, Shearer P. Auditory late effects of childhood cancer therapy: a report from the Children's Oncology Group. Pediatrics. 2010;125(4): e938-e950.
55. Ehrhardt M, Ward Z, Liu Q, Chaudhry A, Nohria A, Border W. Cost-Effectiveness of the International Late Effects of Childhood Cancer Guideline Harmonization Group Screening Guidelines to Prevent Heart Failure in Survivors of Childhood Cancer. J Clin Oncol. 2020 Aug 14: JCO2000418. doi: 10.1200/JCO.20.00418

Stellenbosch University https://scholar.sun.ac.za

ACKNOWLEDGEMENTS

I would whole heartedly like to thank the following people and organizations that have contributed to the completion of this thesis:

My supervisor, Prof Mariana Kruger, for her insight into not only the academic and oncological aspects, but the potential of her student. Through mentorship, guidance and sincere friendship she trusted my vision, protected me from folly and assisted in creating a better understanding into the subject matter of neuroblastoma and the paediatric oncology processes… and myself. She initially advised on a maximum of five manuscripts but instead she let me write a book.

I acknowledge Ms Tonya Esterhuizen, Ms Kristien Wouters and Prof Ben Sartorius who performed the statistical analyses for the various articles, but most of all Tonya, who made the clinical world into statistical sense and the numbers of live a pleasurable experience.

The translations for various parts of the thesis were done by the Stellenbosch University Language Centre, Ms Gaëlle Dufour and Dr Machiel Van Den Akker.

The local investigators of each site and co-authors who supported and diligently worked on this project, advised on manuscript content and believed that the result would be worthy.

My colleagues at the Antwerp University Hospital, Koen Norga, Joris Verlooy and Machiel van den Akker, who afforded me the time to pursue this project, as well as enduring the challenges it brought, with their mature approaches and support.

The staff at Stellenbosch University, especially Anita Fourie, who assisted in managing various systems in order for me to complete this project.

The children and caregivers whose data are included in these analyses and have through their experience contributed to the world of medicine.

The South African Children's Cancer Study Group (SACCSG) and the South African Children's Tumour Registry (SACTR) for their support.

Funders of various aspects of the research including vzw Kinderkankerfonds, Belgium.

Most of all my deepest gratitude to:

My family, especially my partner Steve, my mother Tini and my brother Leon, for their patience, encouragement and understanding during the whole process.

My friends who thought I was insane, but kept putting more sugar in my tea at the Hatter's tea party… one in particular – Moira who marched me to the top of Mount Kilimanjaro to convince me I could do this.

And not least to…
Daniel – where the whole journey began.

Stellenbosch University https://scholar.sun.ac.za

AUTHORS AND AFFILIATIONS (at time of publication)

Büchner, Ané Paediatric Haematology and Oncology, Department of Paediatrics University of Pretoria, Steve Biko Academic Hospital, South Africa

Brink, Anita Department of Nuclear Medicine, Faculty of Health Sciences, University of Cape Town, Red Cross War Memorial Children's Hospital, South Africa

Burger, Celeste Department of Nuclear Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa

Du Plessis, Jan Department of Paediatrics Faculty of Health Sciences, University of the Free State, Division of Paediatric Haematology and Oncology, Universitas Hospital, South Africa

Engelbrecht, Gert Department of Nuclear Medicine, University of the Free State, Universitas Hospital, Bloemfontein, South Africa

Esterhuizen, Tonya Marianne Division of Epidemiology and Biostatistics, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

Geel, Jennifer Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Charlotte Maxeke Johannesburg Academic Hospital, South Africa

Hadley, GP Department of Paediatric Surgery, Faculty of Health Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, South Africa

Hendricks, Marc Haematology Oncology Service, Red Cross War Memorial Children's Hospital, Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Cape Town, South Africa

Janse van Vuuren, Magritha Drs B Vorster and M Janse van Vuuren Incorporated, Nuclear Physicians, Bloemfontein, South Africa

Kruger, Mariana Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, University of Stellenbosch, Tygerberg Hospital, Cape Town, South Africa

More, Stuart Department of Nuclear Medicine, Faculty of Health Sciences, University of Cape Town, Red Cross War Memorial Children's Hospital, South Africa

Naidu, Gita Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Chris Hani Baragwanath Academic Hospital, South Africa

Nyakale, Nosipho Department of Nuclear Medicine, Faculty of Health Sciences, University of KwaZulu-Natal, Inkosi Albert Luthuli Academic Hospital, Durban, South Africa

Stellenbosch University https://scholar.sun.ac.za

Poole, Janet Faculty of Health Sciences, Division of Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, University of the Witwatersrand, Charlotte Maxeke Johannesburg Academic Hospital, South Africa

Sartorius, Benn Discipline of Public Health Medicine, School of Nursing and Public Health, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa; Department of Health and Medical Sciences, University of Washington, Seattle, Unites States of America

Uys, Ronelle Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, University of Stellenbosch, Tygerberg Hospital, Cape Town, South Africa

van Emmenes, Barry Division of Paediatric Haematology and Oncology Hospital, Department of Paediatrics, Frere Hospital, South Africa

van Heerden, Jaques Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, University of Stellenbosch, Tygerberg Hospital, Cape Town, South Africa

Paediatric Haematology and Oncology, Department of Paediatrics Antwerp University Hospital, Antwerp, Belgium

van Zyl, Anel Paediatric Haematology and Oncology, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, University of Stellenbosch, Tygerberg Hospital, Cape Town, South Africa

Vermeulen, Johani Paediatric Haematology Oncology, Department of Paediatrics and Child Health, Port Elizabeth Provincial Hospital, Walter Sisulu University, Port Elizabeth, South Africa

Wouters, Kristien Clinical Trial Center, CRC Antwerp, Antwerp University Hospital, University of Antwerp, Edegem, Belgium

Stellenbosch University https://scholar.sun.ac.za

PUBLICATIONS (in order of publication)

Van Heerden, J, Hendricks, M, Geel, J, et al. Overall survival for neuroblastoma in South Africa between 2000 and 2014. Pediatr Blood Cancer. 2019; 66:e27944 https://doi.org/10.1002/pbc.27944

van Heerden, J., Kruger, M., Esterhuizen, T. et al. The importance of local control management in high-risk neuroblastoma in South Africa. Pediatr Surg Int. 2020; 36(1), 457–469. https://doi.org/10.1007/s00383-020-04627-x

Jaques Van Heerden, Jennifer Geel, Marc Hendricks, et al. The evaluation of induction chemotherapy regimens for high-risk neuroblastoma in South African children. Pediatric Hematology and Oncology. 2020; 37: 4(1), 300-313. https://doi.org/10.1080/08880018.2020.1717698

van Heerden J, Kruger M. Management of neuroblastoma in limited-resource settings. World J Clin Oncol 2020; 11(8): 0-0. https://dx.doi.org/10.5306/wjco.v11.i8.0000

SUBMITTED FOR PUBLICATION

Age at diagnosis as prognostic factor in South African children with neuroblastoma
Jaques van Heerden, Tonya M Esterhuizen, Marc Hendricks, Janet Poole, Ané Büchner, Gita Naidu, Jan du Plessis, Barry van Emmenes, Ronelle Uys, GP Hadley, Mariana Kruger

The association between tumor markers and mIBG scans in South African children with neuroblastoma
Van Heerden, Jaques; Kruger, Mariana; Esterhuizen, Tonya Marianne; Hendricks, Marc; Du Plessis, Jan; Engelbrecht, Gert; Janse van Vuuren, Magritha; van Emmenes, Barry; Uys, Ronelle; Burger, Celeste; Nyakale, Nozipho; More, Stuart; Brink, Anita.

Inequity in paediatric oncology in South Africa – the neuroblastoma case study
van Heerden, Jaques; Esterhuizen, Tonya Marianne; Kruger, Mariana

The implementation of a national paediatric oncology protocol for neuroblastoma in South Africa
van Heerden, Jaques; Kruger, Mariana.

MANUSCRIPT FORMAT

Reporting incidences of neuroblastoma in various resource settings
van Heerden, Jaques; Kruger, Mariana.

Stellenbosch University https://scholar.sun.ac.za

APPENDICES

Appendix A: PhD committee clearance

STELLENBOSCH UNIVERSITY
FACULTY OF MEDICINE AND HEALTH SCIENCES

3 July 2018

Dr Nicola Barsdorf
HREC
Tygerberg Campus

Dear Dr Barsdorf

APPLICATION ACCEPTED BY THE EVALUATION COMMITTEE: PHD IN PAEDIATRICS AND CHILD HEALTH BY DR JACQUES JOHAN VAN HEERDEN (22395814)

Please be informed that the candidate Dr Jacques Johan van Heerden's application has been approved by the Review Committee as indicated by the supporting document. The HREC representative was Prof H Simonds.

Kind regards

J.A. Chabilall

Jyothi Chabilall
Head: Doctoral Office

Stellenbosch University https://scholar.sun.ac.za

Appendix B: Ethical clearance

UNIVERSITEIT
STELLENBOSCH
UNIVERSITY

Approved

27/08/2018

Project ID #: 7714

HREC/UREC Reference #: S18/07/138 (PhD)

Title**: The management and outcomes of neuroblastoma in South Africa**

Dear Dr JAQUES VAN HEERDEN

The **Response to Modifications** received on 08/08/2018 was reviewed by members of the **Health Research Ethics Committee (HREC)** via Minimal Risk Review procedures on 27/08/2018 and was approved.

Please note the following information about your approved research protocol:

Protocol Approval Period: **This project has approval for 12 months from the date of this letter**.

Please remember to use your **Project ID(7714)** on any documents or correspondence with the HREC/UREC concerning your research protocol.

Translation of the consent document/s to the language applicable to the study participants should be submitted.

After Ethical Review:

Please note a template of the progress report is obtainable on https://applyethics.sun.ac.za/Project/Index/9995 and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Helene Visser at City Health (Helene.Visser@capetown.gov.za Tel:+27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.

For standard HREC forms and documents please visit: https://applyethics.sun.ac.za/Project/Index/9995

If you have any questions or need further assistance, please contact the HREC office at 021 938 9677.

Sincerely,

Franklin Weber

HREC Coordinator

Federal Wide Assurance Number: 00001372

Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2015 (Department of Health).

Stellenbosch University https://scholar.sun.ac.za

Appendix C: Amendment 1 clearance

UNIVERSITEIT
STELLENBOSCH
UNIVERSITY

Approval Letter
Amendment

18/06/2020

Project ID: 7714

Ethics Reference No: S18/07/138 (PhD)

Project Title: THE MANAGEMENT AND OUTCOMES OF NEUROBLASTOMA IN SOUTH AFRICA

Dear Dr Jaques Van Heerden

We refer to your amendment request dated 14/05/2020.

Please be advised that the Health Research Ethics Committee (HREC) reviewed and **approved** the amended documentation through an expedited review process.

Where to submit any documentation

Kindly note that the HREC uses an electronic ethics review management system, *Infonetica*, to manage ethics applications and ethics review process. To submit any documentation to HREC, please click on the following link: https://applyethics.sun.ac.za.

Please remember to use your project ID 7714 and ethics reference number S18/07/138 (PhD) on any documents or correspondence with the HREC concerning your research protocol.

Yours sincerely,

Mrs. Melody Shana
Coordinator: Health Research Ethics Committee 1

National Health Research Ethics Council (NHREC) Registration Number:

REC-130408-012 (HREC1)•REC-230208-010 (HREC2)

Federal Wide Assurance Number: 00001372
Office of Human Research Protections (OHRP) Institutional Review Board (IRB) Number:
IRB0005240 (HREC1)•IRB0005239 (HREC2)

The Health Research Ethics Committee (HREC) complies with the SA National Health Act No. 61 of 2003 as it pertains to health research. The HREC abides by the ethical norms and principles for research, established by the World Medical Association (2013). Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects;the South African *Department of Health (2006). Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (2nd edition)*; as well as the Department of Health (2015). Ethics in Health Research: Principles, Processes and Structures (2nd edition).

The Health Research Ethics Committee reviews research involving human subjects conducted or supported by the Department of Health and Human Services, or other federal departments or agencies that apply the Federal Policy for the Protection of Human Subjects to such research (United States Code of Federal Regulations Title 45 Part 46); and/or clinical investigations regulated by the Food and Drug Administration (FDA) of the Department of Health and Human Services.

Stellenbosch University https://scholar.sun.ac.za

Appendix D: Treating Neuroblastoma in South Africa SACCSG Neuroblastoma Study Protocol 2017 (SACCSG NB-2017)

TREATING NEUROBLASTOMA IN SOUTH AFRICA

SACCSG NEUROBLASTOMA STUDY PROTOCOL 2017

SACCSG NB-2017

SOUTH AFRICAN CHILDREN'S CANCER STUDY GROUP

Stellenbosch University https://scholar.sun.ac.za

STUDY PARTICIPANTS

PRINCIPAL INVESTIGATOR

JAQUES VAN HEERDEN, ANTWERP

SUPERVISOR FOR PHD AND CO-PRINCIPAL INVESTIGATOR

MARIANA KRUGER, STELLENBOSCH

SPECIALST DISCIPLINES CO-INVESTIGATORS AND CONTRIBUTORS

ANITA BRINK (NUCLEAR MEDICINE), RCWMCH, UCT, CAPE TOWN

LARRY HADLEY (PAEDIATRIC SURGERY), IALCH, UKZN, DURBAN

KOMALA PILLAY (ANATOMICAL PATHOLOGY), RCWMCH, UCT, CAPE TOWN

PAWEL SCHUBERT (ANATOMICAL PATHOLOGY), TYGERBERG, SU, CAPE TOWN

JEANETTE PARKES (RADIO-ONCOLOGY), GSH, UCT, CAPE TOWN

THURAN NAIKER (RADIO-ONCOLOGY), GSH, UCT, CAPE TOWN

MARC HENDRICKS (PAEDIATRIC ONCOLOGY), RCWMCH, UCT, CAPE TOWN

LERISKA HAUPT (HAEMATOLOGY), UOVS, BLOEMFONTEIN

LOCAL CO-INVESTIGATORS

Baragwanath Hospital POU – Dr Gita Naidu

Charlotte Maxeke Johannesburg Academic Hospital POU – Dr Janet Poole

Frere General Hospital POU – Dr Barry van Emmenes

Dr George Mukhari Academic Hospital – Dr Ele Madzhia

Inkosi Albert Luthuli Central Hospital – Dr Kamalina Coopasamy

Kimberley Provincial Hospital – Dr Liezl du Plessis

Pietermaritzburg Metropolitan Complex – Dr Beverley Neethling

Polokwane General Hospital POU- Dr Vuthilo Netshituni

Port Elizabeth General Hospital POU – Dr Johani Vermeulen

Red Cross War Memorial Children's Hospital POU – Dr Marc Hendricks

Steve Biko Academic Hospital POU – Dr Ané Buchner

Tygerberg General Hospital POU – Dr Anel van Zyl

Universitas Bloemfontein General Hospital POU – Dr Jan du Plessis

Stellenbosch University https://scholar.sun.ac.za

PARTICIPATING CENTRES

(In Alphabetical Order)

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL, UNIVERSITY OF WITWATERSRAND, JOHANNESBURG, GAUTENG, ZA

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL, UNIVERSITY OF WITWATERSRAND, JOHANNESBURG, GAUTENG, ZA

FRERE HOSPITAL, WALTER SISULU UNIVERSITY, EAST LONDON, EASTERN CAPE, ZA

GEORGE MUKHARI HOSPITAL, SEFAKO MAKGATHO HEALTH SCIENCES UNIVERSITY, GA-RANKUWA, NORTH WEST PROVINCE, ZA

INKOSI ALBERT LUTHULI CENTRAL HOSPITAL, UNIVERSITY OF KWA-ZULU NATAL, DURBAN, KWA-ZULU NATAL, ZA

KIMBERLEY PROVINCIAL HOSPITAL, UNIVERSITY OF THE FREESTATE, KIMBERLEY, NORTHERN CAPE, ZA

NELSON MANDELA CHILDREN'S HOSPITAL, UNIVERSITY OF WITWATERSRAND, JOHANNESBURG, GAUTENG, ZA

PORT ELIZABETH PRONVINCIAL HOSPITAL, WALTER SISULU UNIVERSITY, PORT ELIZABETH, EASTERN CAPE, ZA

PARKLANDS NETCARE PRIVATE HOSPITAL, DURBAN, KWA-ZULU NATAL, ZA

POLOKWANE PROVINCIAL HOSPITAL, UNIVERSITY OF LIMPOPO, POLOKWANE, LIMPOPO, ZA

RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL, UNIVERSITY OF CAPE TOWN, CAPE TOWN, WESTERN CAPE PROVINCE, ZA

STEVE BIKO ACADEMIC HOSPITAL, UNIVERSITY OF PRETORIA, TSHWANE, GAUTENG, ZA

TYGERBERG CHILDREN'S HOSPITAL, STELLENBOSCH UNIVERSITY, CAPE TOWN, WESTERN CAPE PROVINCE, ZA

UNIVERSITAS HOSPITAL, UNIVERSITY OF THE FREE STATE, BLOEMFONTEIN, ORANGE FREE STATE, ZA

PROTOCOL DEVELOPMENT

2017 JAQUES VAN HEERDEN, ANTWERP - DRAFT 1

2018 JAQUES VAN HEERDEN, ANTWERP - DRAFT 2

2018 JAQUES VAN HEERDEN, ANTWERP – DRAFT 3

Stellenbosch University https://scholar.sun.ac.za

TABLE OF CONTENTS

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

ABBREVIATIONS

ALK	Anaplastic lymphoma kinase
Amp	Amplification
ASR	Age standardised ratio
ATRX	Alpha Thalassemia/Mental Retardation Syndrome X-Linked
BuMel	Busulfan, Melphalan
CADO	Vincristine, Doxorubicine, Cyclophosphamide
CASP8	Caspase-8
CCG	Children's Cancer Group
CD	Cluster of differentiation
CE	Carboplatin, Etoposide
CEM	Carboplatin, etoposide and melphalan
CNS	Central nervous system
CT	Computer tomography
CTV	Clinical target volume
COG	Children's Oncology Group
COJEC	Carboplatin, Vincristine, Cisplatin, Etoposide and Cyclophosphamide
CRA	Cis-retinoic acid
CR	Complete response
CSR	Complete surgical resection
CyDEC	Cyclophosphamide, Doxorubicin, Etoposide and Carboplatin
CYDO	Cyclophosphamide, Doxorubicin
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
EBRT	External beam radiotherapy
EC	Extra copies
EF	Ejection fraction
EFS	Event free survival
EGFR	Epidermal growth factor receptor
EUNS	European Unresectable Neuroblatoma Study
FFPE	Frozen tissue block and matched paraffin embedded
FGF	Fibroblast growth factor
FH	Favourable histology

Stellenbosch University https://scholar.sun.ac.za

FISH	Fluorescent in vitro hybridization
GCSF	Granulocyte stimulating factors
GFR	Glomerular filtration rate
GTR	Gross total resection
GTV	Gross tumour volume
HSCT	Heamatopoietic stem cell transplantation
HIC	High income countries
HR	High risk
HR-SA	High risk South Africa
HVA	Homovanillic acid
ICR	Incomplete resection
IDRF	Image defined risk factors
IHC	Immune-histochemistry
IMRT	Intensity modulated radiotherapy
INPC	International Neuroblastoma Pathological Classification / Shimada classification
INRC	International Neuroblastoma Response Criteria
INRG	International Neuroblastoma risk group
INRGSS	International Neuroblastoma Risk Group staging system
IR	Intermediate risk
IR-SA	Intermediate risk – South Africa
LDH	Lactate dehydrogenase
LMIC	Low and middle income countries
LR	Low risk
LR-SA	Low risk – South Africa
MAT	Myelo-ablative therapy
MIBG	Meta-iodobenzylguanidine
MIC	Middle income country
MMP	Matrix metalloproteinases
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NA	Not amplified
NAdr	Noradrenalin
NB	Neuroblastoma
NI	Not involved
NPC-SA	Neuroblastoma Pathological Classification adapted for South Africa
NTKR	Neurotropic tropomyosin receptor kinase

Stellenbosch University https://scholar.sun.ac.za

OJEC/OPEC	Vincristine, Cisplatin, Etoposide, Cyclophosphamide, Vincristine, Carboplatin, Etoposide, Cyclophosphamide
OMAS	Opsoclonus/ Myoclonus Syndrome
OS	Overall survival
PCR	Polymerase chain reaction
PD	Progressive disease
PET	Positron emission tomography
PFS	Progression free survival
PR	Partial response
PRT	Proton beam radiotherapy
PhRT	Photon beam radiotherapy
PODC	Paediatric oncology in developing countries
POU	Paediatric oncology unit
PSR	Partial surgical resection
PTV	Pre treatment volume
RASSF1A	RAS-association domain family 1 isoform A
RECIST	Response Evaluation Criteria in Solid Tumors
SCH	Stem cell harvest
SD	Stable disease
SF	Shortening fraction
SIOP	International society of paediatric oncology
SIOPEN	SIOP European Neuroblastoma group
SACCSG	South African children cancer study group
SANARS	South African Neuroblastoma Adapted risk stratification
SIB	Simultaneous integrated boost
STR	Subtotal resection
TAMs	Tumour associated macrophages
TBI	Total body irradiation
UH	Unfavourable histology
UKCCSG	United Kingdom Childhood Cancer Study Group
VEGF	Vascular endothelial growth factor
VGPR	Very good partial response
VIP	Vaso-intestinal peptide
VLR	Very low risk
VMA	Vannilylmandelic acid

Stellenbosch University https://scholar.sun.ac.za

1. PREMISE

Neuroblastoma (NB) is a childhood malignancy with significant difference in prognostic outcomes between stages and biological characteristics, from a good prognosis in stage 1 disease and an especially poor prognosis in stage 4 disease [1,2,3], even in high-income countries (HICs). Without high intensity treatment, advanced stage disease has a poor outcome in low- and middle-income countries (LMICs), with treatment being palliative rather than curative [4,5].

It is the aim of the South African Children's Cancer Study Group (SACCSG) to launch a national multimodality treatment protocol for children diagnosed with NB. The protocol provides guidelines for standardised curative treatment for all patients, irrespective of risk assignment. It also provides guidelines for and documentation of palliative treatment options.

The joint national study of NB in South Africa will promote more accurate analysis of national data with regard to this rare childhood malignancy. It will form the basis for further study questions in improving the early diagnosis of and care for children with NB, especially children with advanced-stage disease.

2. BACKGROUND

Definition:

NB, along with Ganglioblastoma and Ganglioneuroblastoma, form a group of neural crest malignancies that mainly originate from the adrenal gland but also from the sympathetic chains. It extends from the base of the skull, down the upper neck through the thorax and abdomen to the coccyx, forming the unpaired coccygeal ganglion [3]. NB is the most common extracranial solid tumour in childhood [2]. It is an aggressive, metabolically active and complex disease that clinically mimics many other diseases. The heterogeneous pathophysiological presentation of neuroblastoma, paired with the diverse prognostic outcome in different stages of the disease, makes the treatment of this disease an oncological challenge [4,5]. Not only in low- and middle-income settings, where advanced disease is the norm, but also in high income countries, this tumour poses several clinical and management challenges [5].

Incidence and epidemiology:

The peak incidence is between 2–5 years of age in the sympathetic (adrenal) lineage of the neurological system [2,3]. Worldwide, NB accounts for more than 7% of childhood cancers, with an international prevalence of 1 in 7000 live births in developed countries [3]. It contributes to approximately 15% of all childhood cancer deaths [6], hence its label as one of the deadliest paediatric tumours.

Stellenbosch University https://scholar.sun.ac.za

A familial predisposition has been identified at an incidence of <5%. The penetrance follows an autosomal dominant pattern with incomplete penetrance [7]. Syndromes associated with NB include Beckwith-Wiedemann syndrome, von Recklinghausen syndrome, Hirschsprung's disease, Rubenstein-Taybi syndrome [7], Neurofibromatosis and Pheochromocytoma [8].

NB has a slight male predominance with a 1.2:1 ratio. NB has always been viewed as a rare malignancy in African children [9,10], yet statistical inaccuracy was always cited for the incidence. This difference is less obvious in reports from North America and the United Kingdom [11,12]. Internationally there is a predominance in Caucasian children but with higher-risk disease characteristics in other ethnicities such as black children and children from indigenous ethnicities [13].

Although conclusive epidemiological data regarding South African NB patients is not available, data from 1987 to 2007 has reported the estimated incidence in South Africa to be nearly 6% of all childhood malignancies [14].

Tumour biology and genetics:

NB has both germline and somatic mutations that are expressed in the clinical phenotype of the disease.

Germline mutations:

The aggressiveness of the tumour is determined by genetic changes. NB develops from a germline deletion at the 1p36 or 11q14-23 locus [15,16]. Yet a family history is only identified in 2% of patients, with heritable mutations being in the anaplastic lymphoma kinase (ALK) gene [17]. Another mutated gene found in the familial type NB is PHOX2B [18].

The development of NB is dependent on an accumulation of mutations where the pre-malignant cells rapidly replicate in proliferating tissue during embryogenesis in the central nervous system [19,20]. In NB the MYCN proto-oncogene is overexpressed in neurogenesis for the rapid expansion of progenitor cell populations [20,21]. Epidermal growth factor receptor (EGFR) gene and PHOX2B gene have also been associated with the development of NB and contributing to the development of oncogenic elements in the microenvironment of the tumours [20,22,23].

Somatic mutations:

The majority of NB cases are due to the development of somatic mutations. Sporadic mutations have been used in the prognostication, the identification of new targets for drug therapy as well as the prediction of clinical response due to drug-resistant genetic trades [24]. There is a paucity of mutations rather than a recurrent mutation of genes, suggesting an epigenetic regulation of gene expression [24] which explains the heterogeneous clinical presentation of the disease.

Two major subtypes of somatic mutations have been identified which have been linked to the development of favourable and unfavourable biological characteristics [25].

Type 1 is characterised by whole chromosomal gains and losses and few segmental alterations, which predispose to favourable biology like hyperdiploidy and MYCN-negative tumours [24]. These tumours express high levels of TrkA receptor [26].

Stellenbosch University https://scholar.sun.ac.za

Type 2 is characterised by segmental alterations of unbalanced deletions and alterations, with or without whole chromosome changes [24]. A further subtype 2A is mainly the 3p and 11q segmental deletions without MYCN amplification [24]. Subtype 2B is MYCN amplified with 1p deletion and unbalanced 17q gain, whilst expressing TrkB receptor. Subtype 2A is the most aggressive [24,27].

When NB cells acquire extra copies of the oncogene MYCN, it signifies more rapid tumour growth, which is associated with a resistance to treatment and a less favourable prognosis [19]. By comparison the NTRK1 gene (which synthesises the TrkA protein), when overactive in the cells of NB, portends a more favourable outcome [20].

Tumour genetics is also important in identifying genomic characteristics that lead to drug resistance and poor treatment response [30].

Tumour pathology:

The diagnosis of NB, a small blue round cell tumour, is made on the basis of pathology, which includes immunohistochemistry staining. The hallmark finding on pathology is Homer-Wright rosettes that stain positive for tyrosine hydroxylase, CD56 and synaptophysin [5,31]. The tumour histopathological behaviour varies from spontaneous regression and local maturation to aggressive proliferation of immature cells that disseminate and cause a high mortality rate [2,3]. By using the Modified Shimada or International Neuroblastoma Pathology Classification (INPC), classification histology is classified into favourable and unfavourable prognostic classifications [32].

The INPC system comprises three important aspects. This biologically based classification system takes age and morphologic features into consideration to prognosticate the outcomes of neuroblastic tumours [33,34,35,36]. This is achieved by using haematoxylin and eosin stains [34,35,36]. Firstly, the tumour is graded, distinguishing between poorly differentiated, differentiating and undifferentiated histology [34,35,36]. Secondly, it describes Schwann-like and stromal features of a spectrum between mature Ganglioneuroblastoma to immature NB [34,35,36]. Thirdly, the mitosis-karyorrhexis index of low, intermediate and high index is determined and included in the classification [34,35,36]. These aspects are then grouped to identify favourable and unfavourable histology utilised in the prognostication of NB in the INSS and the INRGSS classification [34,35,36].

The classical clumping of NB cells can also be found in metastatic bone marrow disease [32]. The hallmark rosette small, blue, round cells can be viewed under light microscopy. For the best yield, bilateral bone marrow aspirate and bone core biopsy are indicated [33,37,38]. Identifying the presence of NB cells in the bone marrow is the hallmark investigation for diagnosing stage 4 disease [33,37,38], and bone marrow is a ready source for genetic analysis such as MYCN determination [33,37,38].

In the micro-environment of the malignancy, tumour-associated macrophages (TAMs) can be seen [39,40]. This is due to the pro-inflammatory pathophysiology of the tumour to evade detection by the host immune response and to create chemotherapy resistance [39,40]. Immunohistochemistry to detect CD33, CD16, IL6 R, IL10 and FCGR3 is the main diagnostic tool for TAMs [39,40,41]. The TAMs in histological samples have been assessed in prognostication to have a poorer outcome [39,40]. The higher the infiltration with TAMs in NB, the poorer the prognosis, as opposed to osteosarcoma, where the higher the infiltration with TAMs, the better outcomes can be expected [41]. The postulated

Stellenbosch University https://scholar.sun.ac.za

pathogenesis in murine NB is the promotion of MYCN expression due to NB cell and macrophage interaction [42].

Furthermore, immature NB microenvironments are characterised by neovascularisation. Vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2), stimulators of angiogenesis, and tissue inhibitors of matrix metalloproteinases (MMPs) have been detected in NB tumours. High tumour vascularity correlates with unfavourable prognostic characteristics such as metastatic disease, MYCN amplification and unfavourable histology. The opposite is true for tumours with little vascularity. These tumours have favourable characteristics, such as a localised disease and favourable histology [43,44].

Clinical presentation:

The diagnostic dilemma lies in the diverse nature of the clinical presentation. Although nearly 65% of tumours present in the abdomen, other sites include the anatomical distribution of the sympathetic chain from the brain to the kidneys, including pelvic sites [2,3].

The most common clinical presentation is a local suprarenal mass originating from the adrenal glands [6], yet the features mostly associated with presentation are due to metastatic manifestations such as bilateral proptosis, racoon eyes, bone pain, pancytopaenia and constitutional symptoms (fever and loss of weight). NB primarily spreads to the bone marrow, with subsequent complete invasion of bone, lymph nodes and liver [3]. Other sites like the lungs, intra-abdominal seeding and brain have also been described [3].

Two paraneoplastic syndromes associated with NB are Vaso-intestinal peptide (VIP) syndrome and Opsoclonus/Myoclonus syndrome (OMAS) [6,45,46]. Both syndromes mimic common childhood illnesses like infective diarrhoea and intracranial pathology. Other neurological presentations include Horner syndrome, due to tumours in the neck [47], and compression associated with paraspinal symptomatology. Patients with stage 4S disease represent a special group in which, apart from the primary adrenal lesion, infants under 12 months of age may present with cutaneous lesions, bone marrow infiltration of less than 10% and/or liver lesions [3].

A simplified presentation of NB would classify the disease as either local disease, metastatic disease or stage 4S disease. The clinical presentation will then be dependent on the locality of the tumour from local pressure effects like paraspinal disease to a disseminated toxic presentation [5,6].

During growth, NB encases many vital structures like blood vessels, impairing the function of vital organs like the kidneys and the liver, necessitating monitoring of renal and liver functions during diagnostic and treatment phases [3,4,5]. Coagulopathy is frequently present in metastatic NB with hyper- or hypocoagulability as frequent findings [49]. Increased concentrations of fibrinopeptide A as an indicator of thrombin production, disseminated intravascular coagulation and bleeding tendencies due to infections could potentially complicate management, especially peri-surgical procedures [49, 50].

Screening:

As NB is a tumour of the sympathetic chain, predominantly of the adrenal glands, it excretes catecholamine hormones. Homovanillic acid (HVA) and vanillylmandelic acid (VMA) function as

Stellenbosch University https://scholar.sun.ac.za

specific tumour markers for diagnostic purposes and can be traced in an acidified urine sample. The same tumour markers can be used as a screening tool for monitoring treatment, at follow-up and in the event of a relapse [31].

Screening for NB in under-1-year-old infants [51]:

During the period 1984–2003 Japan screened 6-month-old infants for NB for VMA and HVA with high-performance liquid chromatography and published reports that there was a reduction in the death rates of NB due to screening. The natural evolution of NB tumour biology predicts that many tumours spontaneously regress before 1 year of age [51]. Thus, although death rates were increased, many unnecessary surgical interventions may have taken place on tumours that would have regressed [51].

In 2002, studies in Germany and Canada concluded that they could not reproduce the results from Japan [51]. They concluded that screening did not reduce the death rate in NB but did contribute to overdiagnosis of NB that would regress spontaneously and never have clinical significance [3].

In 2004 Japan reviewed their results and elected to stop the national screening for NB [51]. The tumours in infants with small, asymptomatic, low-stage adrenal NB, detected by screening or during prenatal ultrasound or incidental abdominal ultrasound examination, spontaneously regress [3].

In LMICs NB is underdiagnosed, especially in the first year of life, as children under 5 years more often present with infective and feeding problems or because NB is misdiagnosed for other sarcomas [52].

Laboratory evaluation and tumour markers:

VMA and HVA are the primary markers for NB diagnosis [53]. In addition, combined determination of normetanephrine with VMA (in children under 1 year of age) or normetanephrine with HVA (in children older than 1 year of age) produces an age-specific diagnostic sensitivity and specificity of 100% [53]. Other studies have suggested that either dopamine (DA) in combination with VMA and HVA [54] or noradrenaline (NAdr) [55] may be the preferred markers to determine the diagnosis. Zambrano et al. describe an age correlation similar to previous studies but include DA and NAdr in the 1-year age watershed [55].

Furthermore, the same group describe that unfavourable biology, like NMYC amplification, and unfavourable histology are associated with catecholamine level changes [55]. The study suggests that patients with stage 3 and 4 disease had increased ratios of catecholamines compared to patients with stage 1 and 2 disease [55] and, specifically, that patients with advanced disease had DA levels >2 times the upper limit of normal compared to only 8% of patients with low-stage disease [55]. Similar trends were shown in the two groups with regard to VMA: patients with stage 3 and 4 disease had VMA levels >10 times higher than the upper limit of normal compared to those patients with stage 1 and 2 disease, where the same phenomenon was seen in only one patient [55].

Lastly, chromogranin A may also be used as a tumour marker. It is an acidic protein that is stored with VMA and HMA in vesicles and is elevated in NB [56]. Hsiao et al. found that chromogranin A as a correlate for disease was 91% sensitive and 100% specific [56].

Bone marrow and bone are the most common sites of metastasis in NB. Microscopically, bone marrow aspirate with invaded malignant cells has the characteristic pattern of clusters or groups of cells with

Stellenbosch University https://scholar.sun.ac.za

granular chromatin and cytoplasmic projections that sometimes form a pseudorosette [57,58]. In the absence of a primary tumour the pathology, biological and genetic diagnosis of NB can be done on bone marrow [57,58].

Imaging:

Diagnosis and staging of tumours are important for the management, treatment response evaluation and follow-up of patients. Basic radiography like x-rays and abdominal ultrasound may be used to document thoracic and abdominal pathology [59], but it lacks the sensitivity to detect metastasis in the lymph nodes and lungs or to fully image the encasing nature of NB for staging and pre-operative planning [59]. A skeletal survey with radiographs for bone disease is possible but is less sensitive than a 99mTc phosphonate bone scan with 26% versus 59% of lesions identified for skeletal involvement [60,61].

To improve the sensitivity of 99mTc phosphonate bone scan, computerised tomography (CT) with intravenous contrast of neck, chest, abdomen and pelvis is advisable [62]. This also improves the evaluation of anatomic locations, image-defined risk factors (IDRF) for staging and surgery, and metastasis. CT is more sensitive for skeletal involvement than radiographic images [63,64]. Magnetic resonance imaging (MRI) is the study of preference for abdominal images, to evaluate soft tissue components as well as bone marrow infiltration [63,64]. MRI also provides less radiation exposure [64]. With suspected spinal involvement an MRI should be obtained to assess invasion of the neural foramina and spinal cord compression [63,64].

To aid in the diagnosis of NB the type 1 catecholamine transport uptake mechanism of the tumour cells has the unique ability to concentrate Iodine-123-metaiodobenzylguanidine [65]. Thus ^{123}I-MIBG is a valuable diagnostic tool to identify the presence of the tumour in the body. Furthermore, it can be used to stage NB. In a Cochrane review of 11 studies, the sensitivity of MIBG varied between 67%–100% [66]. In 20% of histologically proven NB the MIBG scan will be negative. Although MIBG has a specificity of 100% [62,65,66] some studies have shown these scans to illustrate less disease process than seen on CT scans [62,65]. Even with these limitations MIBG scans have a high sensitivity to detect post-treatment metastatic disease [67]. This characteristic is vital in the utilisation of reviewing treatment response [65].

Although ^{18}F-FDG Positive emission tomography (PET) scan is not considered the main nuclear diagnostic study, its utility in NB is well documented. It uses glucose receptor transport mechanisms in tumours to diagnose the presence of malignancies [67,68]. This is the reason why the specificity of 86% [67,68] is not as accurate as an MIBG-scan. It can concentrate radioactive isotopes in physiologically active sites, such as areas of inflammation and/or infection, giving a false positive reading [67.68]. MIBG-scans are still superior in terms of detecting stage 4 disease in bone and bone marrow due to their higher specificity and sensitivity in these tissues [66,67,68]

MIBG is not just used as a diagnostic test, as a tool to determine treatment response or as a treatment modality but can be applied in the determination of prognosis [69,70]. Both SIOPEN and the Children's Oncology Group (COG) have developed scoring methods, using MIBG in semi-quantitative methods. Both systems divide the body into segments. The Curie score (COG) divides the body into 9 segments (with 4 degrees), whereas the SIOPEN divides the body into 12 segments (with 6 degrees) [69,70]. The

Stellenbosch University https://scholar.sun.ac.za

Curie score was assessed as a prognostic marker for response and survival in NB with MIBG-avid disease. Patients with a Curie score greater than 2 after induction therapy had a significantly worse event-free survival (EFS) of 15% than those with scores less than 2, whose EFS was 45% [69].

Bleeker et al. studied the metastatic pattern of MIBG distribution in stage 4 NB and found two distinct patterns. The two patterns were a “limited and focal” pattern, found mainly in patients with amplified MYCN NB that correlated with prognosis, and an “extensive and diffuse” pattern, found mainly in patients with single-copy MYCN NB [71]. One implication is whether MYCN activity is responsible for the focal growth of metastatic lesions and determines the affected body segments [71].

In a study done by the German Pediatric Oncology Group the prognostic values of the Curie and SIOPEN scoring methods were compared. A Curie score of 2 or less and a SIOPEN score of 4 or less at diagnosis correlated with significantly better EFS and overall survival as compared with higher scores. After four cycles of induction, those with complete response on MIBG testing had a better outcome than those with residual uptake [70].

Staging and risk classification:

Management of the disease is based on risk stratification that is determined by clinical, pathological, biological and genetic features of each tumour. Two internationally recognised staging systems are the International Neuroblastoma Staging System (INSS) and the International Neuroblastoma Risk Group (INRG) [2,3]. The first main difference between the two staging systems is that INSS is a surgical outcome-based system and the INRG is a pre-surgical assessment predicated on imaging, bone marrow morphology and nuclear studies. The second difference is the inclusion of a very low risk group in the INGR classification [2,3]. The INSS has been used since 1986 with great variation in utilisation, since staging is dependent on the surgical skill [64]. It was reviewed in 1994 and it was determined that the diagnosis of NB could be made on histopathology of a specimen or cells in a bone marrow aspiration in combination with elevated catecholamine levels in the urine [72]. Furthermore, staging required bone marrow aspirate, CT scan and nuclear imaging [72]. It included the involvement of lymph nodes and tumours crossing the midline [64].

INRG utilises IDRFs such as the encasement, compression and infiltration of structures in local areas to define the staging [32,73]. It is better utilised as a pre-operative staging and aids in pre-operative planning, avoiding operative morbidity of vital structures [64]

Staging is performed by a combination of clinical examination and using various investigation modalities which include chest X-ray, abdominal ultrasound, CT, MRI and MIBG scan as well as bilateral bone marrow biopsies [5,62,74].

Owing to the diverse nature of this malignancy, many biochemical and biogenetic markers have been identified that contribute to the diagnosis and prognostication during treatment. Tumour markers associated with OS and EFS include MYCN copy number and proliferation, ploidy and deletion or loss of heterozygosity of chromosome 1p and gain of chromosome 17q [3,33]. These biological and genetic characteristics have been included in the risk stratification systems [2,3].

Stellenbosch University https://scholar.sun.ac.za

A third staging system was developed by SIOP-PODC. The aim was to develop a graduated staging and treatment protocol for resource- and expertise-limited settings [5]. This staging system provided a base for staging and diagnosis within the capabilities of various predetermined settings according to resources [5]. This staging system incorporates lactate dehydrogenase (LDH) and ferritin as surrogate prognostic markers in settings where advanced biological and genetic techniques are not available [5].

Prognostic considerations:

Age and stage are still the two most important prognostic factors [5,31,75]. Other prognostic factors that determine the outcome of treatment in NB are translocations, ploidy and the presence of MYCN. MYCN is an adverse prognostic marker in the NB context. In LMICs where either fluorescent in vitro hybridisation (FISH) or polymerase chain reaction (PCR) studies to detect the presence of MYCN are not available, ferritin and/or LDH can be used as surrogate markers to indicate an adverse prognosis [5,76].

MYCN amplification is a genomic expression in NB that is present in advanced disease and in rapidly progressive disease [77,78]. Prognostication with MYCN amplification has been found to be adverse and correlates with other poor prognostic markers such as advance disease, older age and unfavourable histology [77,78]. The percentage MYCN expression in stages 1 to 3 varies, but in stage 4 disease it peaks at 65% [77,78]. MYCN amplification is rarely seen in Ganglioneuroblastoma (3.1%) and almost never in Ganglioneuromas [77,78]. Furthermore, tumours are found with extra MYCN copies. This genomic aberration seems to carry a better prognosis than with MYCN amplification [77,78].

Treatment:

The treatment of NB is very diverse and thus necessitates a multidisciplinary team approach. It is essential that the planned treatment protocol include local disease control, e.g. surgery and radiotherapy [2,3]. Complete excision of the tumour is vital to cure. In many situations surgery is not a feasible therapeutic modality due to the encasing macroscopic nature of the tumour. When vital structures are encased, surgery becomes challenging, with a high surgical morbidity and mortality [2,3]. NB is very radiosensitive with a high proliferation index and is therefore sensitive to radiotherapy, which can be used if surgery cannot provide local control [79].

The treatment of NB was mainly spearheaded by two philosophies of the North American co-operatives (and the subsequent regions with twinning programs) versus the SIOP group which mainly originate in Europe [3,4,5].

The historical development of NB treatment has mainly been dictated by the risk group stratifications [80,81]. The risk groups not only determine the utilisation of the various treatment modalities but also the intensity of the chemotherapy used, for systemic treatment in the neo-adjuvant and adjuvant setting is predicated on all the risk factors. Each patient is grouped in a very low-, low-, intermediate- or high-risk group [3].

In the low- and intermediate-risk groups the main treatment modalities are chemotherapy, surgery and, to a lesser extent, radiotherapy [3,33,82]. The very-low-risk strategy employs a “wait-and-see” approach [3,33,82].

Stellenbosch University https://scholar.sun.ac.za

In the low-risk group patients, especially stage 1 treated for surgery alone without adverse prognostic markers, the 5-year survival nears 96% [2]. With the added treatment of chemotherapy and with stage 3 disease, the intermediate-risk group patients achieve a 5-year survival of 90% in both COG and SIOP studies [2]. It is the metastatic disease patients with high-risk grouping who fare poorer with 50%–60% 5-year survival in HICs and a 20% or less survival in LMICs [2,5].

The risk groups also determine the number of cycles and the duration of the chemotherapy drugs Cisplatin (P) or Carboplatin (J), Vincristine (V), Etoposide (E) and Cyclophosphamide (C) in regimes like OJEC/OPEC and rapid COJEC. Some co-operative groups advocate the inclusion of Adriamycin (A) or adaptions of the COG studies for the SIOP-PODC guidelines that advise Ifosfamide as part of the high-risk regimes [2,3,5].

In general, low-risk disease benefits from surgery, radiotherapy and chemotherapy, but high-risk disease necessitates increased treatment intensity. High-risk NB is treated with neoadjuvant chemotherapy (induction), surgery, radiotherapy and autologous transplant (consolidation), and immunotherapy with maturation therapy (maintenance) [43,79]. Due to varying resources the treatment in HICs and LMICs differs [5,35,79].

The induction phase aims to reduce tumour bulk and clear metastatic disease, especially bone marrow involvement, to under 2%–5% [3]. During this phase neo-adjuvant chemotherapy is also utilised to optimise the tumour site for surgical intervention. During the consolidation phase an autologous transplant after surgery with radiotherapy forms the main part of treatment. When there is no transplant option, radiotherapy and surgery consolidate treatment [35,82,83]. The goal of consolidation is to eliminate resistant tumour clones that were resistant to reduction phase treatment [3,35]. Thereafter minimal residual disease is treated with anti-GD2 therapy (immunotherapy), and maturation therapy of cis-retinoic acid (CRA) is the maintenance [35,79].

Observation only:

In patients younger than 6 months, especially perinatal patients, small adrenal masses found incidentally on ultrasound may safely be observed without obtaining a definitive histologic diagnosis and without surgery [3]. In the COG-ANBLOOP2, 81% of patients demonstrated spontaneous regression while avoiding surgical intervention. Only 19% ultimately underwent surgery with a 3-year EFS of 97.7% and OS of 100% [84].

Chemotherapy:

Chemotherapy administration is determined by risk group.

Low-risk disease:

In both POG and COG trials patients with local disease have excellent survival of up to 98% with surgery alone [85,86]. Even resected stage 1 tumours with residual disease with favourable biology (MYCN negative and favourable histology) have good outcomes, with EFS above 90% and OS of 99%–100% [85]. Stage 2 disease has lower outcomes of an EFS of 81% and OS of 98% [85]. This was especially true in patients with tumour MYCN amplification, older than 2 years of age and with either unfavourable histopathology or positive lymph nodes [85,86]. Yet these patients were salvageable with surgery and multimodal therapy.

Stellenbosch University https://scholar.sun.ac.za

As demonstrated in the COG-P9641 trial, chemotherapy in low-risk disease is restricted to patients with unfavourable biology such as MYCN-amplified stage 1 and 2 disease and MYCN non-amplified stage 2B disease older than 18 months who have unfavourable histology or diploid disease [86]. Unfavourable histology had significantly lower EFS of 72% and OS of 86%, as did diploid tumours with EFS of 75% and OS of 84% [3,80,81].

The outcome of patients with stage 2B diploid tumours with unfavourable histology was poor, with EFS of 54% and OS of 70%. There were no survivors in patients with additional 1p loss of heterozygosity and all in children older than 18 months of age [3,80,81]. Furthermore, chemotherapy is also reserved for low-risk patients who are symptomatic in cases with spinal cord compression and stage 4S respiratory compromise secondary to hepatic infiltration [3]. In the SIOPEN studies INES99.2 and INES99.3, good 2-year OS of 97.6% and EFS of 96.4% were obtained with surgery alone and carboplatin-and-etoposide-only chemotherapy for symptomatic patients or advancing disease [59].

Intermediate-risk disease:

The basis for treatment in intermediate disease relies strongly on tumour biology. The intermediate-risk group patients have three treatment strategies. Chemotherapy can be administered with or without surgery, infants can be operated on and then observed, and radiotherapy is only used in emergency situations [3,80].

Chemotherapy can be administered with or without surgery:

In the POG 8743 study, patients with intermediate risk disease were stratified by MYCN amplification and tumour cell ploidy [87]. Hyperdiploid tumours were treated with cyclophosphamide and doxorubicin, and diploid tumours received cisplatin and teniposide after an initial course of cyclophosphamide plus doxorubicin [87]. OS rates were 95% for hyperdiploid tumours and 52% for diploid tumours. MYCN copies and LDH levels during the same study augmented the hypothesis that adverse biology warranted more intense chemotherapy [87].

In CCG trials, patients were treated with a four-drug chemotherapy regimen, surgery, and local radiation to residual disease [8]. Non-MYCN-amplified tumours had a 3-year EFS rate of 93% and MYCN-amplified tumours had a 10% rate [88]. Whilst Japanese studies by Lehara et al. with 414 patients have indicated that MYCN-negative stage 3 patients can be treated with fewer cycles of chemotherapy [89]. It was during the COG-A3961 studies that it was determined that intermediate-risk patients with favourable biology did well with just four cycles of chemotherapy, and patients with unfavourable biology needed eight cycles [90] to prevent acute and long-term complications without compromising high cure rates [91]. In the COG-A3961 studies the 5-year OS in patients with favourable biologic features who received eight cycles of chemotherapy was 100% as compared with those who received four cycles with a 5-year OS of 96% [90]. The Japanese study distinguished between 6 cycles versus 8 cycles and mainly looked at MYCN amplification and surgical resectability for treatment determinants [89]. The NB95-S and NB97 studies from GPOH, SIOPEN and Euro-INF-NB-99 also proved that infants with favourable biology had a more than 90% survival rate with surgery and reduced chemotherapy treatment [92,93,94].

Stellenbosch University https://scholar.sun.ac.za

The current LINES study of the SIOPEN group bases surgical and chemotherapy treatment on the basis of tumour biology [95]. The current ANBL1232 North American trial is a response- and biology-based risk-factor guided therapy for non-high-risk NB [3].

Infants can be operated on and then observed:

Two separate European studies showed favourable outcomes in asymptomatic infants with stage 3 or 4 disease. This raised the question if surgery and observation was a viable option [92]. This is controversial as the view is not shared in North America.

In the SIOPEN study there was very little difference in the outcomes in early survival between the involvement of metastasis on bone scan with a 97% 2-year OS and a 100% OS without lesions [92]. The same was true between primary resection and without resection with a 2-year OS of 100% versus 97% respectively [92].

The NB95-S and NB97 German prospective studies with 340 infants concluded that the 3-year OS survival differed little between infants with unresected tumours with a 99% OS, infants with resected tumours with a 98% OS and infants receiving chemotherapy with a 95% OS [94].

A French study concluded the difference in survival of stage 4 patients to be the presence or absence in cortical bone changes on radiographic or scintigraphic evidence. The EFS was 90% for non-cortical bone involvement as opposed to 27% if there was bone involvement [96].

In the current LINES study, only infants with favourable biology without symptoms are observed. Children with INSS stage 1 NB, MYCN amplified, were previously observed according to the LNESG 1-2. In the LINES study they will receive adjuvant treatment [95].

Radiotherapy is only used in emergency situations or progression:

In The COG-A3961, unresectable tumours with favourable biology were spared radiotherapy as local treatment with favourable outcomes. Radiotherapy was limited to 2.5% of the 479 patients. The 3-year EFS of the whole group was 88%, and OS was 95% [90].

High-risk disease:

High-risk disease is defined as Stage 2A and 2B patients with MYCN amplification as well as unfavourable histology, Stage 3 disease with MYCN amplification regardless of age, and Stage 3 disease without MYCN amplification but with unfavourable histology. The Stage 4 disease with high risk classification is limited to the infants less than 1 year of age with MYCN amplification and Stage 4 patients over 1 year of age regardless of MYCN and histological status. Stage 4S infants with MYCN amplification are regarded as high risk [3,35,79].

Induction phase chemotherapy:

The development of high-risk NB treatment has been characterised by the delivery of increasingly intensive multimodality therapy. During induction, European approaches under the EU-20592 or CCLGNB-1990-11 studies have studied increasing rapid administration of maximum doses of chemotherapy. Originally chemotherapy was administered in 28 day-cycles, then 21-day cycles and currently every 10 days with rapid COJEC [97]. Rapid COJEC was devised as a protocol to increase the

Stellenbosch University https://scholar.sun.ac.za

treatment intensity by decreasing the chemotherapy-free periods. In the rapid COJEC protocol, chemotherapy is administered every 10 days, regardless of toxicity, as opposed to every 21 days. Although this ensures that a patient reaches the consolidation treatment phase sooner, it is with a greater burden of chemotherapy-induced toxicity [98]. Even though myelo-suppressive arms of vincristine, carboplatin and etoposide, or vincristine, cyclophosphamide and etoposide, and less myelo-suppressive arms of vincristine and cisplatin, are alternated, the incidence of febrile neutropenia and infectious complications during rapid COJEC induction is high [97].

In a Cochrane review there was no clear evidence of a difference between the OJEC/OPEC and rapid COJEC treatment groups in complete response, treatment-related mortality, overall survival and event-free survival [99] due to the limited number of studies. Yet prospective trials were advised due to the low level of evidence and the changes in risk classifications during the trial periods [99]. The review relied mainly on the CCLG-ENSG-5 randomised controlled trial of 262 patients with high-risk NB. In the study 132 patients were randomised to receive standard OPEC/COJEC and 130 patients to receive rapid COJEC induction chemotherapy [99]. Pearson et al., however, conclude in the study that there was an increasing difference in EFS after 3 years with the rapid COJEC induction protocol. With the same total dose, rapid COJEC at 10-day cycles had better EFS of 27% at 10 years versus the 21-day cycle at 18% [98]. The study postulates that the earlier start of myelo-ablative therapy (MAT) contributed to this outcome [100]. During European HR-NBL1/SIOPEN, patients were randomly assigned to primary prophylactic versus G-CSF. The patients with G-CSF had fewer febrile neutropenic episodes, days with fever and hospital- and antibiotic days [97].

North American approaches were based on treatment designed in Memorial Sloan Kettering hospital. The N6 protocol included doxorubicin during induction where SIOP did not include the drug [97]. With the 7 cycles in the N6 protocol, 87% of patients achieved either complete remission (CR) or very good partial response (VGPR) [101]. In the N7 protocol a reduction from 7 to 5 cycles of chemotherapy resulted in outcomes of 79% CR or VGPR and less toxicity [102]. Both studies were single-institution trials.

Currently SIOPEN is randomising a modified N7 induction protocol with Rapid COJEC in the HR-NBL-1.7/SIOPEN study [103]. The modified N7 induction is a dose-intensive induction chemotherapy regimen including high-dose cyclophosphamide plus doxorubicin or vincristine and high-dose cisplatin or etoposide [103]. The original regimen of 7 cycles was modified by reducing the number of cycles to 5 and lowering the vincristine dose. The use of G-CSF is included [103].

Consolidation phase chemotherapy:

In the development of consolidation in the CCG-3891 study, patients were randomised to receive a more intensive arm of consolidation therapy, which included MAT plus autologous bone marrow transplant, versus a less intensive arm of conventional-dose chemotherapy [104]. The same study introduced a randomisation of 13-CRA versus no CRA in patients who were disease free after consolidation [104]. The more intense treatment showed improved results with 5-year OS outcomes of 30% versus less than 20% for the non-intensive arm [104].

The modalities of surgery, radiotherapy and transplant are discussed as part of consolidation under the respective subheadings. A multimodal approach is of prognostic importance in the treatment of high-risk NB. If a patient only receives chemotherapy, surgery and radiotherapy, the 5-year

Stellenbosch University https://scholar.sun.ac.za

prognostication is 20%–30% [2]. With the inclusion of an autologous stem cell transplant as consolidation therapy, the OS is improved with approximately 20% in stage 4 patients [105,106]. The outcome with the drug repositioning of CRA is improved when used post transplantation [107]. The role in a non-transplant setting has not been established. CRA matures the rapidly dividing NB cells into more indolent ganglionic cells and plays a significant role in addressing minimal residual disease in patients who are in complete remission [5,107].

Stage 4S and infants:

Patients with stage 4S disease, despite tumour bulk, are associated with a favourable outcome [108]. Since patients are less than 1 year of age the potential risks of treatment must be weighed up against the benefits. With disease progression, intervention is important [86].

The patients who are very young and are symptomatic or have unfavourable biology do better with chemotherapy [86]. In the COG-P9641 the 5-year EFS was 63% and OS was 84% for infants with asymptomatic stage 4S NB treated with surgery alone, and the EFS was 95% and OS was 97% for infants treated with surgery and chemotherapy.

In symptomatic low-risk patients with stage 4S hepatic infiltration and respiratory distress as well as spinal cord compression, chemotherapy is indicated as an emergency measure according to the Philadelphia Score system [108]. Chemotherapy is administered in lower doses to prevent toxicity [86].

Surgery:

The goals of surgery are diagnostic and curative [3]. A biopsy for histological confirmation as well as genetic determination plays a primary role in surgery [3]. Resection of the tumour is vital to cure and determines the feasibility of transplant options [3]. In many situations surgery is not a feasible therapeutic modality due to the encasing macroscopic nature of the tumour. When vital structures are encased, surgery becomes challenging, with a high surgical morbidity and mortality [2,3]. Apart from resection, another important role of surgery is to evaluate the spread of the tumour. Evaluating the lymph nodes is important to promote maximum resection of the tumour [3,79].

The evidence regarding gross total resection is conflicting. Retrospective studies at Memorial Sloan Kettering have proven superior survival outcomes in complete surgical resections (CSR) versus partial surgical resections (PSR) in especially metastatic disease [109,110]. In the study, 1-, 2- and 3-year survival rates were compared between CSR and PSR, and survival rates were shown of 100% versus 77% 1-year survival, 80% versus 38% 2-year survival and 40% versus 15% 3-year survival [110]. Chamberlain et al. reproduced the conclusion that CSR was superior to PSR [111]. This was also proven in the stage 4 NB patients with BMAT involvement [111].

In contrast, the Great Ormond Street experience did not prove an advantage of CSR over PSR of the primary tumour [112]. A possible explanation is the MYCN proliferation positivity of the tumour. The survival outcomes of NB with MYCN proliferation were proportional to surgical outcomes from PSR to CSR [113].

In low-risk patients, tumours are evaluated for complete resection by excluding image-defined risk factors and the surgical ability of the surgeons [3]. Even in the presence of residual tumour, but in

Stellenbosch University https://scholar.sun.ac.za

favourable histology, outcomes with EFS are in excess of 90% and OS is 99% to 100% [3]. Patients need not undergo complete resection of disease to be cured by surgery alone [3]. Perez et al. conclude that patients with stage 1 and 2 NB have 98% survival with surgery alone as primary therapy, with supplemental treatment needed in 10% of stage 1 patients and 20% of stage 2 patients [85]. In stage 2 disease, unfavourable biology, positive lymph nodes or age over 18 months had a higher risk [85]. In stage 3 disease it was proven to be dependent on age, unfavourable biology, lymph node involvement and ferritin levels [91].

Matthey et al. proved that survival outcomes of more than 80% in stage 2 NB were independent of tumour biology and radiotherapy with surgery alone [85,114]. This provides evidence that even for high-risk stage 2 patients, there is no indication for radiotherapy [115]. Due to the encasement nature of NB around vital structures, surgical complications and morbidity can be up to 25% [110]. This is in relation to the aggressiveness of surgical intervention even after neo-adjuvant chemotherapy to shrink the tumour size [110,111]. Another factor, especially in stage 4S, is the surgical difficulty operating on children under the age of 1 year old [110,112].

Radiotherapy:

Neuroblastoma is very radiosensitive, with a high proliferation index. The role of radiotherapy has been investigated in emergency presentations, palliation and local and metastatic disease in relation to chemotherapy and surgery, as has the role of radiotherapy in the transplant setting [3,33].

In low-risk disease the application of radiotherapy is in local recurrence that does not respond to surgery and/or chemotherapy [33,85]. The second indication is where function of life is threatened during spinal compression or hepatomegaly-induced respiratory compromise [33,85].

For all other situations surgery alone is adequate, with specific-situation chemotherapy if needed. Added radiotherapy has no added survival benefit but does add to long-term complications [33,85,114].

The evidence for using radiotherapy in the intermediate-risk group is varied due to the inconsistency of trials over the years [3]. This makes comparing data a challenge and creates a difficulty in setting down recommendations [33,79]. What is clear is that stage 3 patients with residual tumour after surgery with unfavourable biology (non-hyperdiploidy, MYCN positive, unfavourable histology) have an increased chance of survival with radiotherapy. It is unclear if this translates to regional lymph node involvement. The COG A3961 study had a greater than 90% survival in the abdominal lymph node involved stage 3 group on only chemotherapy and surgery.

It is clear in all studies that radiotherapy plays a significant role in high-risk disease [33,79,91]. Originally total body irradiation (TBI) was used during transplant regimes [74], but it limited the dose toxicity of chemotherapy needed during myelo-ablation [79,116,117]. Although TBI became obsolete during older protocols, there is great evidence for the use of local radiotherapy, especially in bulky disease to prevent relapse [3,79]. Higher doses in two separate trials with doses up to 21 Gy on the pre-chemotherapy pre-surgery volume prevented high local relapse rates [33,115]. Local relapse rate is in the region of 70% with an MYCN amplified tumour [33,115]. Thus, local radiotherapy of 10 Gy above TBI is advised with MYCN disease [33,115].

Stellenbosch University https://scholar.sun.ac.za

Stage 4S disease is a very specific clinical entity. As these patients are less than 1 year of age, radiotherapy poses a significant problem due to size and physiology [79]. Thus, the routine use is discouraged. Indications for radiotherapy is in palliative situations or in life-threatening situations such as with tumour-induced hepatomegaly [3,79] that causes respiratory compromise, inferior vena cava obstruction, compromised renal perfusion, disseminated intravascular coagulation (DIC) or gastrointestinal obstruction [3,79]. Since most of these patients are 2–4 months of age at diagnosis, the risk of late effects and radiotherapy treatment benefits must be weighed.

The clinical picture of children with CNS metastasis is varied and the radiotherapy treatment unclear [79]. Primary CNS disease is rare. Yet CNS is the most common metastatic progression or relapse site [79]. In children older than 1 year this spread is mainly hematogenous spread, whereas in children less than 1 year it is due to non-hematogenous spread [79]. Prophylactic CNS radiotherapy is not indicated due to the rare nature of CNS metastasis [79], and the treatment guidelines for CNS relapse are unclear [79].

In palliative treatment situations, radiotherapy has a very specific role. Although systemic chemotherapy should always be started first in situations where function is threatened, for example vision with orbital metastasis, cord compression or in cases of severe pain, radiotherapy is effective as treatment [33,79]. Pain responds completely or partially in up to 60% of painful sites as well as nearly 60% soft tissue sites tumour reduction were noted in a study done by Duke [118]. Paulino describes successful treatment response rates to radiotherapy in various tissues that promoted positive palliative care [119].

Autologous transplantation:

High-risk disease further receives consolidation treatment consisting of a single or tandem myelo-ablative stem cell transplant, followed by treatment for minimal residual disease and cell maturation maintenance therapy [116]. With the inclusion of an autologous stem cell transplant as consolidation therapy, the OS is improved by approximately 20% in stage 4 patients from 20% with chemotherapy, radiotherapy and surgery to approximately 40% with MAT [106,120]. Individual co-operatives have had a 3-year EFS of more than 50% with tandem transplants, with low transplant mortality rates [115]. A COG group study proved 3-year EFS from diagnosis was 50% in the standard treatment group single transplant arm and 68% in the tandem MAT group. The 3-year OS rate was 69.1% in the standard treatment group compared to 74% in in the standard tandem group [121]. COG A3973 proved no survival benefit of purged cells during transplantation versus unpurged cells [115].

The main development with regard to transplant was the evaluation of the role of TBI as part of conditioning before haematopoietic stem cell transplantation (HSCT). TBI-containing regimens were developed to eradicate micro-metastases [117]. In a review of European transplant cases between 1978 and 2006, TBI regimens produced a significantly worse OS rate of 34% in comparison with an OS of 41% for regimens not including TBI [116,117]. A chemotherapy-only conditioning regimen was the standard of care. It has also been concluded that there was no survival benefit between autologous or allogeneic stem cell transplantation [116,117].

When comparing the results of the SIOPEN trial with previous COG research in which the 3-year EFS for the CEM group was 33% for the SIOPEN group, but 46% for the COG group, it was postulated that the rapid COJEC induction regimen used in the SIOPEN trial could have a negative interaction with

Stellenbosch University https://scholar.sun.ac.za

CEM [122,123]. The COG induction phase did not include carboplatin and had used lower doses of cisplatin and etoposide than the rapid COJEC regime [122,123].

In their own randomisation, SIOPEN studies have shown that in a cohort of 1577 patients randomised to receive either BuMel (busulfan and melphalan) or CEM (carboplatin, etoposide and melphalan) as conditioning therapy before HSCT, patients treated with BuMel had a significantly better outcome compared to those treated with CEM [116,117]. The EFS at 3 years was 49% (BuMel) versus 33% (CEM), and OS at 3 years was 60% versus 48% [116,117]. The study also concluded that overall BuMel was less toxic than CEM [116,117].

Maturation therapy:

The improvement of outcome with the medicine repositioning of CRA has been studied in the post-transplantation setting of high-risk and very-high-risk patients [124,125]. Two randomised controlled trials of high-risk patients receiving CRA post autologous transplant versus no CRA proved the benefit of maturation therapy. The UKCCSG group trial included 175 children from 10 countries, concluding with a 3-year EFS for patients receiving CRA, with 46% versus 43% patients receiving placebo [124]. A CCG study showed a 3-year EFS rate of 46% for patients assigned to receive CRA versus 29% for those assigned to receive no further therapy [126].

The role in a non-transplant setting has not been fully established. CRA matures the rapidly dividing NB cells into more indolent ganglionic cells and plays a significant role in addressing minimal residual disease in patients who are in complete remission [5,124]. CRA use outside an autologous transplant setting has more short-term survival benefits [78]. Yet by expert consensus it is recommended by the SIOP-PODC working group to included CRA as maintenance outside of a transplant setting [5, 127].

Biologicals and targeted therapy:

Post-consolidation therapy treatment is aimed at clearing minimal residual disease [6,72]. Targeted therapies have started playing an increasingly important role in this aspect of treatment. In the line of targeted therapies, anti-GD2 therapy is an immunotherapy which improved the OS to 60% 5-year survival [128]. Other targeted therapies for NB include ALK-inhibitors, which show great promise in pre-clinical trials [129]. A disialoganglioside that is expressed in NB is GD2. A targeted therapy of a chimeric murine-human antibody ch14.18 or anti-GD2 has been developed [72,128]. Initial trials have combined immunotherapy with transplantation [72,128] or added IL-2 for greater lymphocyte-mediated effect [72]. A COG phase I study is currently investigating anti-GD2-IL as first line relapse strategy [72,128].

Other:

A therapeutic approach for high-risk NB, especially refractory NB, is iodine-131 meta-iodobenzylguanidine (^{131}I-MIBG) therapy as targeted radiotherapy followed by an autologous HSCT [130]. Promising response rates of more than 30% were observed [130]. Currently SKION and COG are studying ^{131}I-MIBG in combination with myelo-ablative therapy and hematopoietic stem cell rescue in newly diagnosed high-risk patients [131].

Palliative strategies:

Stellenbosch University https://scholar.sun.ac.za

Metronomic therapy is a palliative strategy of the continuous administration of low doses of oral chemotherapy drugs designed to target the endothelial cells lining the blood vessels supplying tumour cells to modulate the micro-environment of the tumour for onco-static effect [132]. The efficacy of the continuous low-dose chemotherapy depends on the enhanced antiangiogenic and pro-apoptotic effects of some cytotoxic agents, in both dividing tumour cells and endothelial cells [132].

Furthermore, metronomic therapy stimulates both innate and acquired immunity, especially when in the T-lymphocytic range like NK-cells and Cytotoxic T-cells [132,133]. A substantial part of the onco-static effect is the promotion of dormancy of oncology cells [132,133].

This palliative strategy relies on drug repositioning for indications other than their original use [132,133]. Drugs like cyclo-oxygenase 2 (COX 2) inhibitors also show anti-tumour activity due to inhibition of angiogenesis [132,133]. A low dose of cyclophosphamide leads to a decrease in the number of circulating T-regulatory cells and directly inhibits T-regulatory cell function [132,133].

Some studies into the efficacy of metronomic therapy in children have been done. Fousseyni et al. conducted a study to evaluate the use of metronomic chemotherapy with vincristine, cyclophosphamide and methotrexate in 12 children with refractory cancer in Mali. No objective response was observed, but disease stabilisation was observed in 58% of the patients [133]. Wolter et al. and Xu et al. independently proved that propranolol induced apoptosis by inhibiting the growth of NB cells irrespective of MYCN status [134]. The anti-tumoural activity was dependent on inhibition of the β2 receptor, and that treatment resulted in activation of p53 and p73 signalling [134]. Higher mRNA levels of β2 adrenergic receptor correlate with improved patient survival. Propranolol leads to a slower tumour growth with a synergistic effect with COX-inhibitors and topo-isomerase inhibitors [134].

Late effects:

Acute and late chemotherapy and radiotherapy late effects are well documented. NB protocols contain doxorubicin and platin-based chemotherapy drugs like cisplatin and carboplatin. Doxorubicin has well-documented cardiotoxicity, whereas the platin-based chemotherapy has nephro- and ototoxic effects [135]. These acute effects must be monitored with serial cardiac echocardiograms, glomerular filtration rate (GFR) measurements and hearing screening tests respectively [136]. Other organ effects attributed to patients with radiation toxicity and transplant sequelae include endocrine dysfunctions [137]. Significant secondary malignancies attributed to etoposide treatment are leukaemias like AML and, in the long term, sarcomas and carcinomas [138].

Future research:

Current clinical trends are to identify a subgroup in the high-risk stratification of very high-risk stratification [139,140,141]. Patients who are considered as very high risk are high-risk patients who respond poorly to induction therapy [3] and patients with combined MYCN amplification and bone metastases [139]. Response to treatment has been associated with outcome. The persistence of NB cells in bone marrow or the persistence of a ^{123}I-MIBG-avid tumour measured as per the modified Curie score after induction chemotherapy is associated with a poor prognosis [96,142]. The pathological response of the tumour to chemotherapy has prognostic significance. An increase in

Stellenbosch University https://scholar.sun.ac.za

histologic differentiation of the primary tumour and decrease in mitosis are associated with improved outcomes [143].

When considering the micro-environment of NB tumours, irregularities in the morphological shape of blood vessels during neo-angiogenesis and with increased crosslinking and branching or stiff Ret Fs network predict a poorer outcome subgroup in the high-risk group NB [143]. By utilising 'omics-techniques, mRNA expression has aided in differentiating not only low-, intermediate- and high-risk tumours [144] more accurately than the current INSS [144], but also in differentiating the high-risk staging tumours that can be termed to be a very-high-risk group [144, 145]. Higher mRNA expression is associated with poorer outcomes [145]. Furthermore, genetics like the DKC1 gene are also associated with patients who respond poorly to induction and are thus a very-high-risk group [141].

The purpose is to identify a group of patients who will benefit from novel therapies [139] and predict patients with a resistance to therapy [140,141], especially in a group of patients where survival is poor even in HICs [139,140,141].

To be able to identify these novel therapies, new biological and genetic markers are being identified. With genome-wide sequencing of NB, a few molecular biological targets were identified [146]. Biological therapies with priority targets ALK, MEK, CDK4/6, MDM2, MYCN (druggable by BET bromodomain, aurora kinase, mTORC1/2), BIRC5 and checkpoint kinase 1 will potentially be developed [146]. A novel marker such as ALK has been identified in only 10% of NB and benefits a small percentage of patients for targeted therapy [146]. These drugs are currently not available in LMICs but might be considerations in the future.

Liquid biopsies test circulating tumour cells (CTCs) or circulating DNA (ctDNA) shed by the tumour in the blood or lymph [147]. It is a minimally invasive test to diagnose and monitor NB with the main aim of obtaining multiple time-independent samples to improve heterogenous pathophysiological diagnostics rather than a single point traditional biopsy [148]. Rapid DNA and RNA sequencing, as well as protein analysis with proteomics, will identify new treatments, diagnostics and diagnostic markers [144].

Considerations for LMICs:

The incidence and epidemiology of NB in HICs is well documented [7,149]. Due to limitations in resources that prevent accurate statistical reporting, incidence and epidemiology is still not completely documented in many LMICs [150]. Reliable paediatric cancer registries do not exist or are limited to single institutions, and this makes interpretation of data difficult. Yet, internationally, NB is the most common sympathetic tumour in children [149]. According to the world age-standardised rate (WSR), Caucasian incidence is higher than other ethnicities. Yet the WSR of 10.2 per million person-years in black children in the USA is in contrast with the WSR of 2.7 per million person-years in Sub-Saharan Africa, where there is a predominantly black population [149]. The highest incidence of all CNS tumours was noted in HICs, which is clearly related to the wide availability of diagnostic facilities. The lower incidence rates in LMICs is probably due to poor access to imaging facilities, prohibitive costs of diagnostics testing, delays in diagnosis and possibly under-diagnosis and lack of registration [149].

Stellenbosch University https://scholar.sun.ac.za

Thus far, epidemiological reporting of NB has been limited to single institutional publications [76,151]. Data from the South African Children's Tumour Registry published in 2015 reported an age-standardised ratio (ASR) of 3.1/1 000 000 for NB and 3.0/1 000 000 for NB and Ganglioneuroblastoma [14]. By institutional reporting, the outcomes for children diagnosed with NB are far poorer than other MICs and HICs [151].

Treatment in HICs aims at treatment-intense regimes with transplant and immunotherapy options [3]. In many LMICs, as is the case in South Africa, the treatment choices are limited to mainly chemotherapy, surgery and radiotherapy [5,152]. Due to advanced disease at diagnosis, tumours are often inoperable and treatment is therefore palliative. Other challenges for the management of NB include lack of surgical and radiotherapy skills or equipment, as well as lack of chemotherapy [5,152]. In settings where drug insecurity is high, many LMICs do not have even basic medicines for palliation [153]. Poor outcomes have necessitated the development of palliative strategies.

In LMICs, many factors play a role in the optimal care for patients with NB, such as stage of disease, nutritional status at diagnosis, family resources, belief systems and personal autonomy. Resources, drug security and expertise in institutions influence treatment decisions to a similar extent as treatment adherence and response to treatment. The ability of facilities to provide supportive care, in terms of antibiotics, intensive care and granulocyte-stimulating factors (G-CSF), influences decision-making in the intensity of treatment that patients receive [5,52].

In LMICs NB has a poor prognosis of less than 20% 5-year survival in advanced disease [52,152]. With the three modalities of chemotherapy, surgery and radiotherapy the prognosis varies from 96% 5-year OS for stage 1 to a dismal 20% 5-year OS for stage 4 [2,3,5], with limited data reporting the outcomes in South Africa to be lower than 20% [151].

Currently in South Africa, as in other LMICs, the management protocols used in the treatment of NB have been diverse and based on experience of paediatric oncologists [155]. As collaborative efforts, with data from multiple centres, promote more robust data [155], the SACCSG has committed to developing national protocols to harmonise treatment of patients with the same cancers. This will also assist as guidance to the planned National Health Insurance Plan currently being developed for South Africa.

Management choices:

The proposed multi-centre SACCSG's prospective treatment protocol aims to introduce a standard national management protocol for children treated for NB in South Africa to manage and treat NB patients uniformly, utilising the diagnostic and treatment modalities currently available nationally in an ethical and cost-effective manner. It also aims to accurately collect data and analyse the disease characteristics and treatment outcomes for South African patients to improve future outcomes.

The limitation of the INSS and INGR staging systems is that the determination of risk stratification in a health system, like in South Africa, with heterogeneous services, skills, resources and access to the diagnostic modalities utilised in the various staging systems, makes application challenging. The INSS system is a post-surgical risk classification [156]. In settings where predominant presentations are with advanced disease, where surgery is not the first line of treatment, the classification is of limited use. The INRG classification-based risk groups are based on imaging as a pre-surgical risk classification

Stellenbosch University https://scholar.sun.ac.za

[156]. Translational correlation and interpretation of various diagnostic tests versus specialised diagnostics have not been studied. This makes the interchangeable utilisation of diagnostics and interpretation of results according to set stratification systems difficult. Current INRG and COG risk-based classifications are dependent on ploidy and deletions such as 11q [2,3] with limitations for low-resource settings.

Due to the utilisation of different criteria according to institutional capabilities and preference, there are limitations to the standardisation of care, sharing national resources and the ethical distribution of resources. Thus, the risk classification for this protocol is based on the SIOP-PODC guidelines [5] and includes INSS and INRG risk classifications to support the capabilities of low- and high-resource units.

The diagnostic investigations are based on the standard of care in South Africa and include validated investigations described in the literature for accurate staging, risk classification, exclusion of co-morbid diseases and tumour-associated complications that will influence treatment decisions.

The recommendations regarding basic, advanced and nuclear imaging have been chosen to facilitate diagnostics as well as treatment response evaluations. The recommendations include the criteria based on the International Neuroblastoma Response Criteria (INRC) of 1993, the revised INRC criteria of 2017 and the soft tissue Response Evaluation Criteria in Solid Tumours (RECIST) to accommodate the heterogeneous availability of resources in various oncology units [156].

Although age and stage of disease are important prognostic factors, risk classification relies on pathology as well as molecular and genetic characteristics for risk stratification [2,3]. Treatment is increasingly guided by biological characteristics, as is evident from increased risk classification with unfavourable histology, MYCN amplification and targeted therapy such as anti-GD2 [72, 146]. Each risk group will be treated with individual protocols to address biological differences.

Protocols for low- and intermediate-risk disease have been selected to minimise toxicity (cytopenias, cardiotoxicity and renal and ototoxicity), whilst still basing treatment on symptomatic tumour presentations and/or prognostic biological pathology in line with North American and SIOPEN trials. Surgery is the standard of treatment in any setting [2,3]. With the INES99.2 and INES99.3 studies, good 2-year OS of 97.6% and EFS of 96.4% were obtained with surgery alone and carboplatin- and etoposide-only chemotherapy for symptomatic patients or advancing disease [59,157]. In these two studies, patients with poorer outcomes due to unfavourable histology are being treated as intermediary risk in more recent trials, as will the patients in the South African protocol.

Both SIOPEN and COG use doxorubicin-based protocols. The SIOPEN LINES study relies on histology, molecular techniques and genetics to divide the treatment into 10 groups [95]. The treatment ranges from observation only to chemotherapy, surgery and radiotherapy [95]. In low-resource settings, determining the genetic mutations and molecular aberrations might be delayed or not possible to determine. The COG-A3961 approach distinguishes between favourable and unfavourable histology as determinants for the chemotherapy protocol and duration [90]. As histological classification is essential for diagnosis, the approach is advised for treatment in South Africa.

For improved OS and EFS, independent of induction chemotherapy, patients must receive a gross total resection [109,110], local and/or metastatic radiotherapy plus a single or tandem autologous

Stellenbosch University https://scholar.sun.ac.za

transplant [121]. Further immunotherapy [128] and maturation therapy increases the OS [124]. No induction therapy has proved superior to obtain CR for high-risk patients [99]. Rapid COJEC only benefits EFS by ensuring patients are transplanted faster [99]. Yet rapid COJEC is more toxic with long hospital admissions for supportive treatment due to infections and cytopenias [99]. Infective complications of rapid COJEC due to neutropenia are decreased with the recommended inclusion of G-CSF [97]. G-CSF not only strains labour and financial resources but administering it is also painful to patients. An OJEC/OPEC or COJEC is advised to decrease resource burdens and lower toxicity. In the proposed protocol, OJEC/OPEC with a Carboplatin dose of 500mg/m^2 is advised versus a Carboplatin dose of 750mg/m^2 in COJEC to lessen nephrotoxicity [159]. Cumulative doses of carboplatin cause glomerular damage and loss of magnesium [159]. A non-Doxorubicin-based induction is preferable because CADO is reserved for bone marrow clearance post induction. A low cumulative dose of Doxorubicin is advised in the South African patient population due to complications of malnutrition and a risk of increased cardiotoxicity [136].

Topotecan in the treatment of NB has no indications for use in the South African essential medicines formulary [160]. This prevents availability of TVD in public hospitals in patients with a poor response to induction chemotherapy. Instead CADO is chosen as replacement.

For the patients who do qualify for autologous transplant, BuMel is recommended as conditioning before the transplant as it has proven survival benefit above CEM and less engraftment toxicity with OJEC/OPEC [121]. BuMel has less risk of severe mucositis and thus infectious complications. Yet a higher risk for sinusoidal obstruction syndrome has been documented [121].

For a metronomic strategy, a regime with increased immune-modulatory and anti-angioneogenesis activity is favoured above other regimes such as the Metro-Mali 1 [133], Metro-Mali 2 [161] or Metro-Mali 3, or COMBAT regime [162]. With vincristine, cyclophosphamide and methotrexate the Metro-Mali 1 study included 12 children with relapsing or refractory nephroblastoma, retinoblastoma or NB. Stable disease was observed in 58% for up to 6 months, and 50% were alive after 36 months [133]. In the Metro-Mali 2 study, solid tumours showed favourable responses to a combination of vincristine, cyclophosphamide, methotrexate and Valproic acid [161]. A single metastatic NB patient had a partial response and 36-month follow up on the regime [161]. The COMBAT trials have had mixed results with a combination of celecoxib and retinoic acid alternating with etoposide and temozolomide [162]. The outcomes were a 2-year EFS of 10% and a 2-year OS of 30% [162]. In NB, a vascular tumour, propranolol is a superior anti-angiogenic agent above Valproic acid [163], and propranolol is more easily available to reposition for metronomic purposes than Valproic acid. Valproic acid is an HDAC inhibitor, and its use in preclinical NB studies has suggested it increased CD133 cells [164]. These cells are less sensitive to chemotherapy treatment [164]. Propranolol inhibits growth, induces apoptosis and decreases proliferation in NB regardless of the MYCN status [165]. In xenografts it works synergistically with the topoisomerase I inhibitors [163].

The proposed study will be a multi-centre South African Children's Cancer Study Group treatment protocol in order to investigate the challenges of treating NB in South Africa and to treat all children with NB in a standard way with a prospective national management protocol.

As patients are accrued onto the study, potential risk factors for risk stratification will be prospectively identified in an open-label descriptive study. Changes in the treatment will follow to optimise care to

Stellenbosch University https://scholar.sun.ac.za

a primarily curative rather than palliative outcome and create new prospective study questions with improved outcomes.

3. AIM

The aim is to introduce a standard national management protocol for children treated for Neuroblastoma in South Africa with the aim to improve overall survival (OS):

- All children with neuroblastoma will be treated with a uniform treatment protocol.
- Current imaging and diagnostic tests will be utilised in a uniform manner to determine the optimal imaging and diagnostic tests necessary in a resource-limited setting.
- To accurately analyse the disease characteristics and the association with overall survival.

4. OBJECTIVE

4.1) Primary objectives:

- To implement a national prospective management protocol to improve OS and EFS at endpoint 2 years and 5 years post cessation of treatment.

4.2) Secondary objectives:

- To evaluate the prognostic significance of basic laboratory and imaging diagnostics in children treated for NB in South Africa using OS and EFS, as well as treatment response as predictors of outcome.
- To describe the epidemiological profile of South African Neuroblastoma patients.

5. METHODS

5.1) Study design:
The study will be an open label, prospective, multicentre, descriptive study.

5.2) Study size:

The expected sample size will be 35 – 40 new patients per year. This is an estimation based on recent statistics publish by the SACCSG [14]. The two year early outcomes aims to include 70 – 80 patients over this period.

Stellenbosch University https://scholar.sun.ac.za

5.3) Study period:

Recruitment will start in August 2018. The first evaluations will be during the 2 year early outcomes but the protocol will be a long term protocol to include 5 year outcomes.

5.4) Study setting:

All POUs treating patients with NB is eligible to participate in the study. All physicians within the Republic of South Africa will be invited to participate as a collaborative group to provide optimal treatment to children with NB.

5.5) Study population:

5.5.1) Inclusion criteria

- Newly diagnosed patients with NB between the ages of 0 and 18 years old at the start of treatment with:
  a) Biopsy proven NB or
  b) Radiological reported NB with positive urine catecholamines or BMAT with involvement of NB cells or
  c) BMAT with involvement of NB cells and a positive urine catecholamines (in cases where a biopsy is not possible, the primary tumour can't be visualised or radiological evidence is inconclusive)
  d) Relapsed stage 1A or 4s patients who are chemotherapy and radiotherapy naïve.
- With caregiver consent and patient assent (if older than 7 years of age).
- The patient is chemotherapy naïve.

5.5.2) Exclusion criteria

- There is no caregiver consent or assent in a child capable of providing assent.
- Patients that are not treatment naïve, in a relapse presentation or with disease progression.
- Patients who are unable to follow the protocol due to medical, social or geographical reasons. (These patients must still be documented and registered with the National Cancer Registry

6. PATIENT ENROLLMENT

All eligible patients will be enrolled when presenting to treatment centres by the primary physician(s) or medical practitioner (including nursing staff or auxiliary services). Parents or legal guardians will be asked to provide informed consent (please see Appendix A) to participate in the study. Where appropriate physicians will obtain signed assent from the patient older than 7 years of age (please see Appendix B).

Stellenbosch University https://scholar.sun.ac.za

Participation will include all phases of treatment from the diagnostic, treatment and follow up phases of the protocol. Patients will be followed for the duration of their therapy and for as long as is possible (ideally at least five years) following the completion of chemotherapy.

Informed consent and assent will be done in the participant's preferred language wherever possible or via an interpreter proficient in the local language. The interpreter should be oriented in a medical capacity to insure factual accuracy.

Participation is completely voluntary and participants will be free to withdraw their participation in the study at any time without disruption in medical care or digression from standard treatment. They will still be treated according to treatment protocol, but their data will not be included in data analysis.

7. DIAGNOSTIC EVALUATION

7.1) History and clinical examination

a) General History (including family history) and general examination.
   - Include
     - presenting complaints and duration
     - anamnesis and progression
     - previous medical and surgical history
     - family history, parental consanguinity, sibling history and familial malignancies
     - chronic illness, co-morbid diseases and current medication and traditional interventions
   - With attention to: weight loss, fever, night sweats, diarrhoea, nystagmus, bone pain, general pain hypertension, constipation, regional obstruction symptoms, mobility and bladder function. Note duration of these symptoms.

b) Complete general examination documenting:
   - Anthropometry (using SD-WHO charts) including weight for age, height for age, weight for height and head circumference.
   - Mid-upper arm circumference (MUAC) in children 6 months – 5 years of age.
   - Blood pressure.
   - Clinical features of the tumour laterality and size.
   - Size of the liver and masses
   - Lymphadenopathy.
   - Local pressure symptoms
   - Clinical symptoms and signs of metastases and complications of metastatic spread.
   - Neurological signs indicative of Opsocolnus/Myoclonus Syndrome [3].
   - Documentation of diarrhoea indicative of Vaso-Intestinal Peptide Syndrome [3].

Stellenbosch University https://scholar.sun.ac.za

- Skin lesions.
- Clinical indication for metastases i.e. skull nodules, racoon eyes, etc.
- Documentation of dysmorphisms and genetic or syndromic features.

c) The presence of life threatening symptoms (symptomatic tumour presentation)
   - Intraspinal neuroblastoma: symptoms of spinal cord compression or a spinal tumour component that occupies more than one third of the spinal canal.
   - Systemic symptoms:
     - Pain requiring opiate treatment
     - Gastrointestinal
       - Vomiting needing nasogastric/IV support
       - Weight loss >10% body weight
       - Diarrhoea with VIP does not respond to chemotherapy and is a definite indication for surgery
     - Respiratory
       - Respiratory distress without evidence of infection
       - Tachypnoea >60 bpm
       - Oxygen need or ventilatory support
     - Cardiovascular System
       - Hypertension
       - IVC compression +/- leg oedema
     - Renal - Impaired renal function, creatinine increased x 2 ULN
       - Poor urine output, less than 2mls/kg/day
       - Hydroureter/hydronephrosis
     - Hepatic
       - Abnormal liver function >2 ULN
       - Evidence of DIC
     - Neurological
       - Neurological sign indicative of pressure symptoms
       - Nystagmus

7.2) Laboratory Investigations

Laboratory investigations:
These standard investigations are for the evaluation of treatment eligibility, diagnostic evaluation and clinical evaluation of the disease process as well as co-morbid pathology.

- Full blood count including differential count
- Urea, creatinine and electrolytes, calcium, magnesium, phosphate and liver enzymes
- Clotting screen: INR, aPTT and fibrinogen (to exclude NB associated clotting disorders in preparation for invasive procedures).
- Non-specific tumour markers: Uric Acid and Lactate dehydrogenase (LDH) and Ferritin
- Urine analysis: Dipstix
- Urine catecholamines (24 hours urine collection or a spot sample x 3) must be done to confirm the diagnosis.

Stellenbosch University https://scholar.sun.ac.za

- HIV-test at diagnosis
- Lumbar puncture: only per indication with neurological signs

7.3) Radiological examination

Radiological evaluations must be done prior to the start of any treatment interventions and at the point of treatment response evaluation. Identifying diagnostic characteristics and image defined risk factors as well as measurements for treatment response should be recorded

Before chemotherapy and surgical treatment the following radiological investigations should be performed:

- Abdominal ultrasound (in the absence of CT or MRI facilities)
- Chest X-ray AP and lateral (AP only if a CT chest was done)
- CT chest
- CT abdomen or MRI abdomen
- Echocardiography to evaluate cardiac function for the eligibility to administer cardio-toxic chemotherapy drugs: Shortening fraction (SF) > 28%
- Clinical indications for brain and bone metastasis requires pathology directed imaging i.e. CT brain or X-ray of the bones
- A skeletal X-ray screening of the long bones is indicated in units where nuclear skeletal surveillance investigations are not available at the diagnostic phase.

7.4) Nuclear medicine studies

Nuclear investigations are important for the diagnosis, treatment response evaluation as well as toxicity monitoring in the treatment of NB.

For diagnostic purposes a ^{123}I-MIBG scan **and** bone scan should be done. With limitations in availability ^{123}I-MIBG scan is preferred above bone scan. Approximately 10% of NB are not ^{123}I-MIBG avid [3]. In this case a PET/CT-scan can be used to document the primary tumour and complete the skeletal survey with a bone scan. If a tumour is confirmed on PET/CT-scan all follow up scans must be a PET/CT-scan.

The same nuclear imaging test (excluding bone scan) used for diagnosis must be used to evaluate treatment response in the same patient.

Two different nuclear studies may not be done on the same day to prevent the photon energies of the images of different studies to overlap, leading to inaccurate reporting of studies.

Stellenbosch University https://scholar.sun.ac.za

7.4.1) Diagnosis and whole body survey:

^{123}I-MIBG scan:

The ^{123}I-MIBG imaging is the primary imaging for whole body imaging. All attempts should be made to use MIBG at diagnosis and for treatment response evaluation and should be restricted to nuclear medicine departments with appropriate facilities and staff experienced in paediatric imaging.

Indications:
Indications for diagnostic ^{123}I-MIBG imaging in Neuroblastoma (NB) include

- Diagnosis and confirmation in suspected NB
- When the initial diagnosis of was made from a metastatic site ^{123}I-MIBG imaging may be used to visualise a primary
- Staging of NB disease

Reporting:
All MIBG scans should be evaluated according to the modified Curie-score (please see Appendix Q) by a Nuclear Medicine physician.

Practical guidelines:

It is preferable to do a diagnostic ^{123}I-MIBG scan before the start of chemotherapy, but may be done up until a week after the start of the first dose of chemotherapy.

For re-assessment ^{123}I-MIBG scans should take place as close as possible before the next chemotherapy administration but not earlier than two weeks after a chemotherapy administration.

PET/CT-scan:

Indications:

- For the documentation of the primary tumour and complete the whole body imaging in a ^{123}I-MIBG non-avid tumour
- In patients where the primary is not found
- Assess treatment response
- Routine follow up in a suspected relapse setting if the restaging MIBG scan is negative

Practical guidelines:

The diagnostic PET/CT should be done before that start of chemotherapy.

For re-assessment PET/CT scans should take place as close as possible before the next chemotherapy administration but not earlier than two weeks after chemotherapy.

Bone scan:

In centres where ^{123}I-MIBG scan and/or PET/CT scan is not available a bone scan must be done as part of the whole body imaging.

If a bone scan is used for the whole body imaging the primary tumour must be well documented by CT or MRI scans.

Practical guidelines:

Stellenbosch University https://scholar.sun.ac.za

It is preferable to do a diagnostic bone scan before the start of chemotherapy, but may be done up until a week after the start of the first dose of chemotherapy to prevent flare phenomenon [166].

A bone scan may not be used for re-assessment. Even without a baseline ^{123}I-MIBG scan all reassessment nuclear skeletal screenings should be done by ^{123}I-MIBG scan.

7.4.2) Toxicity monitoring:

GFR:

The GFR should be recorded prior to commencing cisplatin chemotherapy and after every two cycles of cisplatin containing chemotherapy. The GFR should also be performed at the end of treatment.

7.5) Atypical presentations

In 10% of cases a primary can not be found – in which case diagnosis must be made on sites of the metastatic tumour. This can be done via the following diagnostic techniques:

- Bone marrow and/or trephine diagnosis of small blue round cell with positive u-catecholamine levels
- ^{123}I-MIBG positive with positive u-catecholamine levels
- PET-scan with positive u-catecholamine levels

7.6) Eligibility of platinum alkylators chemotherapy

a) For renal toxicity:

All children, irrespective of their chemotherapy regimen, should have a serum creatinine and calculated GFR performed at diagnosis and after every second course of chemotherapy.
All children receiving cisplatin containing regimens should have:

- a two plasma sample radioisotope GFR according to the EANM guidelines
- or a GFR using the bedside IDMS traceable Schwartz eGFR.

The Schwartz eGFR [167,168] may be used if a radio-isotope GFR cannot be performed at the participating institution. The GFR should be recorded prior to commencing cisplatin chemotherapy and after every two cycles of cisplatin containing chemotherapy. The GFR should also be performed at the end of treatment.

$$\text{Estimated Glomerular Filtration rate (eGFR) ml/minute/1.73m}^2 = \frac{\text{Height (cm) X 40}}{\text{Creatinine (µmol/L)}}$$

Stellenbosch University https://scholar.sun.ac.za

b) For ototoxicity:

All children receiving cisplatin containing regimens should have an qualitative audiogram done prior to commencing cisplatin chemotherapy and after every 2 cycles of cisplatin containing chemotherapy. This should be documented again at the end of treatment. See Appendix P for recommendations and the Boston Ototoxicity Scale [169].

8. PATHOLOGY EVALUATION AND CENTRAL REVIEW

References: [170-175]

From a histo-pathological point of view Neuroblastoma is a very heterogeneous tumour. Making a morphologic diagnosis and using histo-(cyto-)pathologic findings for prognostic purposes relies on diagnostics of the various relevant tumour areas for molecular, genetic or biological analyses and interpretation.

Relevant tumour material from several different tumour and nodular areas should preferably be taken (if feasible and/or possible) for accurate histologic and molecular, genetic and/or biological examination. The reason is due to tumour heterogeneities at the genetic level (e.g. for the MYCN and/or the chromosome 1p status) and/or at the histologic level (e.g. ganglioneuroblastoma and nodular subtype) both of which have prognostic implications. For this purpose, the paraffin embedded material is of great importance.

Tumour pathology will be reported in each centre according to a standardised pathology form for Neuroblastoma (see Appendix C) as well as biological testing will be done for prognostication.

In cases where the diagnosis is not conclusive and/or for standardised reporting quality assurance, central pathology review may be performed by a designated pathology group, made up of representative(s) from a designated Department(s) of Anatomical Pathology, NHLS Laboratories; with the lead pathologist being based at Red Cross War Memorial Children's Hospital. For this purpose a pathology block should/may be made available.

8.1) Tumour diagnosis:
There are **two requirements** needed for therapy in neuroblastoma:

1. Morphological diagnosis
2. Prognostication / molecular testing for:
    a. MYC-N *(mandatory)*
    b. Chromosome 1p status *(optional at this stage)*

8.2 Method of diagnosis:
The mode of diagnosis will depend on:

i. Access to interventional radiologist(s),
ii. Access to paediatric surgeon(s) and
iii. Theatre accessibility.

Stellenbosch University https://scholar.sun.ac.za

iv. Patient functional/clinical status

Biopsy of the primary tumour is preferable, but bone marrow and metastatic lesions are acceptable.

Methods:

The method of biopsy will depend on the access to:

i. Radiologist and radiological capabilities
ii. Location of tumour, tumour vascularity and accessibility to tumour via a percutaneous route
iii. Theatre facilities and accessibility.

Primary suggested method of biopsy:

1. Ultra-sound guided core needle biopsy (preferred method of choice).
2. Ultra-sounded guided Fine Needle Aspiration biopsy with cell block collection and onsite evaluation (ROSE) (alternative method)
3. Combination of methods 1 + 2 = would constitute the gold standard for obtain maximum/optimal material for initial diagnosis.

Additional secondary methods (not first choice):

1. Open biopsy / laparoscopic biopsy of primary tumour.
2. Bone marrow biopsy with bone marrow smears (unstained slides)
3. Ultra-sounded guided Fine Needle Aspiration (FNA) biopsy with cell block collection without onsite evaluation (ROSE) (alternative method).
4. Ultra-sound guided Fine Needle Aspiration biopsy with unstained smears made for molecular testing.
5. Sampling of metastatic lesions.

8.2) Ultrasound guided Core Needle Biopsy:

Ideally **more than two** samples should be taken for histopathological evaluation, and preferable four cores. Biopsy for histopathology should be sent to the laboratory in 10% buffered formalin.

If an excision or incisional biopsy is done enough tissue must be available for four samples whether it is from the primary tumour, metastatic tumour i.e. lymph node excision, liver biopsies or skin biopsies.

The tumour must always be handled by a pathologist in order to make a morphological diagnosis and provide a prognosis based on pathological findings. Another function of pathologists is to choose the relevant tumour areas for molecular-genetic/biological analyses.

During the handling of biopsy material, the pathologist or physician obtaining the biopsy should try to avoid artefacts due to crushing and squeezing. In addition, needle biopsies tend to stick to each other which may hamper the subsequent division of the material. Therefore, each biopsy should be kept in a separate tube in the above mentioned sterile solution.

The samples will be for the following purposes:

- Biologic and genetic studies
- Tumour banking

Treatment is determined by risk stratification. The information includes clinical information, imaging, biological and genetic testing. If tissue sampling is limited then diagnostic testing is more important than specialised testing.

8.3) Ultrasound Fine Needle Aspiration Biopsy

Stellenbosch University https://scholar.sun.ac.za

Ultrasound guided Fine Needle Biopsy (FNAB) is a sensitive and effective method for diagnosing neuroblastoma. Rapid on-site evaluation should be performed when using FNA for diagnostic purposes as the quality of material can be assessed.
FNAB alone is inadequate for the purposes of therapy, since additional molecular testing is needed. A cell block is the preferred method of choice to obtain material for immunocytochemical staining and molecular testing.
Unstained, air dried slides are also possible but not the preferred method of choice.

8.4) Open Biopsies:
In case of open biopsy, **at least two different areas** of the tumour should be biopsied by the surgeon. Specimen size should equal **at least 1cm³ (1cm x 1cm x 1cm)**.

8.5) Preparing samples for molecular testing:
Molecular testing can be performed on numerous specimen types and the method selected will depend on the method available to each laboratory:

1. Paraffin embedded (FFPE) tissue block – most widely used and preferred method of choice.
2. Cytology cell block in the form of paraffin embedded (FFPE) tissue block, on condition that it is adequately cellular.
3. Frozen tissue block.
4. Touch preparations

Make **10 touch preparations** from a freshly cut surface. The slides are air-dried and unfixed - which can be used for fluorescence based in situ hybridisation (FISH).

8.6) Tumour Material Obtained after Cytotoxic Therapy:
Sampling and sectioning of the tumour material in resected tumours or biopsies after cytotoxic therapy has been administered can be done following the same guidelines as for tumours resected or biopsied at diagnosis.
It is important that the percentage necrotic areas and viable tumour cells versus normal cells be reported. Furthermore, the morphologic changes induced by cyto-differentiation and maturation must also be reported.

8.7) Regional Lymph Node Examination:
Biopsy of regional nodes, when feasible, must be done despite their appearance. The histology report should include information on site and number of positive nodes, type of metastatic spread, i.e. presence of micrometastases (less than 2 mm), intranodal parcelled metastases, intranodal massive metastases, nodal metastases with extracapsular extension in localisations not adherent to the resected tumour specimen, and morphologic description of the tumour infiltrate.

8.8) Report:
For the purposes of standardisation, the pathology report must include the following [174]:

8.8.1) Immunohistochemistry:

Diagnostic confirmation

For histological diagnostics of a tumour and investigating the differential diagnosis the following immunohistological stains should be performed:

i. Synaptophysin
ii. NB84

Stellenbosch University https://scholar.sun.ac.za

iii. CD99

8.8.2) Diagnostic criteria [175]

- **Composed of neuroblasts exhibiting variable degrees of differentiation up to ganglion cells**
  - Neuroblasts
    - Small round nuclei with stippled ("salt and pepper") chromatin
    - Large cell/large nucleolar phenotype
      - Comprises approximately 8-10% of all neuroblastomas
      - Nuclei 1.5 to 2 times larger than those of typical neuroblastoma cells
      - 1-4 prominent nucleoli
      - Usually seen in the undifferentiated and poorly differentiated subtypes of neuroblastoma (see below)
      - Associated with MYCN amplification and a poor prognosis
    - Scant eosinophilic cytoplasm
    - Indistinct cell borders
  - Ganglion cells
    - Large round nuclei with prominent nucleoli
    - Abundant eosinophilic cytoplasm
    - Nissl substance in cytoplasm
      - Basophilic granules/bodies composed of endoplasmic reticulum
- **Background stroma also shows different levels of differentiation**
  - Schwannian stroma resembles collagen
    - Requires S100 stain (positive) for identification
  - Neuropil
    - Pink, fibrillary extracellular material
  - Dense lymphoid infiltrate occasionally present
    - Lymphoid follicles may be present
    - Often seen in patients with Opsoclonus-Myoclonus-Ataxia (OMA) [173] paraneoplastic syndrome
- Seven subtypes of neuroblastic tumors are recognized according to the degree of neuroblastic maturation and the amount of background schwannian stroma
  - **Neuroblastoma, undifferentiated**
    - Composed entirely of neuroblasts, no ganglion cell maturation
      - Immunohistochemistry is often required to confirm neuroblastic lineage
    - Schwannian stroma poor
      - Less than 50% of background stroma is schwannian
        - May be absent
  - **Neuroblastoma, poorly differentiated**
    - Predominantly neuroblasts, <5% maturing/mature ganglion cells
    - At least one focus of neuropil
    - Schwannian stroma poor
      - Less than 50% of background stroma is schwannian
        - May be absent
  - **Neuroblastoma, differentiating**
    - Predominantly neuroblasts but >5% maturing/mature ganglion cells
    - At least one focus of neuropil
    - Schwannian stroma poor
      - Less than 50% of background stroma is schwannian
        - May be absent

Stellenbosch University https://scholar.sun.ac.za

- **Ganglioneuroblastoma, nodular**
  - Predominantly maturing/mature ganglion cells but at least one well circumscribed nodule of residual neuroblasts
    - Neuroblast nodules may correspond to hemorragic nodules in gross specimen
  - Schwannian stroma rich
    - 50% or more of background stroma is schwannian
- **Ganglioneuroblastoma, intermixed**
  - Predominantly maturing/mature ganglion cells but at >1 focus of residual neuroblasts intermixed with ganglion cells
    - No distinct hemorragic nodules in gross specimen
  - Schwannian stroma rich
    - 50% or more of background stroma is schwannian
- **Ganglioneuroma, maturing**
  - Entirely composed of maturing and mature ganglion cells, no residual neuroblasts
    - No distinct hemorragic nodules in gross specimen
  - Schwannian stroma rich
    - 50% or more of background stroma is schwannian
- **Ganglioneuroma, mature**
  - Entirely composed of mature ganglion cells, no residual neuroblasts
    - No distinct hemorragic nodules in gross specimen
  - Schwannian stroma rich
    - 50% or more of background stroma is schwannian

8.8.3) Grading of the tumour – Differentiation for prognostication according to the Neuroblastoma Pathology classification adapted for South Africa without mitosis-karyorrhexis index (MKI):

Pathology is graded according to the adapted **International Neuroblastoma Pathology Classification.** The classification groups the tumour pathology into unfavourable (UH) and favourable histology (FH) based on only tumour subtype and differentiation as well as patient age.

Stellenbosch University https://scholar.sun.ac.za

Neuroblastoma Pathology classification [172] adapted for South Africa		**NPC-SA**
Neuroblastoma (Schwannian stroma-poor) • **Undifferentiated** • **Poor differentiated** • **Differentiating**		**Prognostic group**
<1.5 yrs	Poorly differentiated or differentiating	**FH**
1.5–5 yrs	Differentiating	**FH**
<1.5 yrs	Undifferentiated tumour	**UH**
1.5–5 yrs	Undifferentiated or poorly differentiated tumour	**UH**
≥5 yrs	All tumours	**UH**
Ganglioneuroblastoma intermixed (Schwannian stroma-poor)		**FH**
Ganglioneuroma intermixed (Schwannian stroma-dominant) • **Maturing** • **Mature**		**FH**
Ganglioneuroblastoma nodular (Schwannian stroma-rich/stroma-dominant and stroma-poor)		**UH**

Stellenbosch University https://scholar.sun.ac.za

8.8.4) Staging of the tumour

The INSS staging was developed as a post-surgical staging system and correlates with outcomes [72]. As surgical residual disease during local therapy is an important predictor of outcome the documentation of degree of resection is of vital importance.

International Neuroblastoma Staging System (INSS) [3]	
Stage 1	• Localized tumour with complete gross excision, with or without microscopic residual disease • Representative ipsilateral lymph nodes negative for tumour microscopically
Stage 2A	• Localized tumour with incomplete gross excision • Representative ipsilateral non-adherent lymph nodes negative for tumour microscopically.
Stage 2B	• Localized tumour with or without complete gross excision, with ipsilateral non-adherent lymph nodes positive for tumour. • Enlarged contralateral lymph nodes must be negative microscopically
Stage 3	• Unresectable unilateral tumour infiltrating across the midline, with or without regional lymph node involvement • Localized unilateral tumour with contralateral regional lymph node involvement • Midline tumour with bilateral extension by infiltration (unresectable) or by lymph node involvement. The midline is defined as the vertebral column. Tumours originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.
Stage 4	• Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs, except as defined for stage 4S.
Stage 4S	• Localized primary tumour, as defined for stage 1, 2A, or 2B, with dissemination limited to skin, liver, and/or bone marrow (by definition limited to infants younger than 12 months). • Marrow involvement should be minimal (i.e., <10% of total nucleated cells identified as malignant by bone biopsy or by bone marrow aspirate). More extensive bone marrow involvement would be considered stage 4 disease. • The results of the MIBG scan, if performed, should be negative for disease in the bone marrow.

Stellenbosch University https://scholar.sun.ac.za

8.8.5) MYCN

All diagnostic tumour samples should be sent for MYCN amplification determination or FISH studies

9. BONE MARROW ASPIRATE AND TREPHINE GUIDELINE

References: [176-180]

Bone marrow and bone are the most common site of metastasis in Neuroblastoma. Bone marrow aspirate and trephines (BMAT) will form part of the metastatic evaluation at diagnosis and treatment response evaluations.

9.1) Number of samples:

Bilateral BMATs should be done. Thus bone marrow aspiration and a representative bone core from two different sites should be collected.

9.2) Sample technique:
Bone marrow aspirate: A minimum of 10 slides per side should be prepared using separate syringes for each side. Aspirates from different sites should not be pooled that the heterogeneity of the Neuroblastoma cell infiltration can be recorded without underestimating the extent of bone marrow disease. Slides should be air-dried and stained using the May-Grünwald, Pappenheim or modified Wright stain for initial staging using cytological examination by light microscopy.

Bone core: A 1cm bone core per site should be sampled and should immediately be placed in fixative and decalcified. The fixed, decalcified biopsy should be embedded in paraffin, and a minimum of 2 separate slides should be stained with hematoxylin and eosin for histology and a minimum of 3 slides can be used for immunohistochemistry (IHC). More slides can be stained according to institution protocols.

9.3) Quality of the sample:
Bone marrow aspirate is considered representative for reporting when:

- There is >5% tumour cell infiltration.
- When infiltration with tumour is <5% then 3 out of the following 4 criteria must be fulfilled:
  1) The presence of particles with stromal cells (e.g. histiocytes, fibroblasts, or osteoblasts)
  2) The presence of megakaryocytes
  3) Erythroblasts are >20% of the nucleated cells
  4) Peripheral blood cells are within the range for age

A representative **trephine** should preferably contain red bone marrow parenchyma at a minimum length of 1 cm.

Stellenbosch University https://scholar.sun.ac.za

9.4) Pathology:
Morphology: The presence of tumour cell nests or clumps or rosettes of Neuroblastoma cells expressed as a percentage. Other specific features e.g. nuclear moulding or characteristic NB findings should be recorded.

Histology of trephine: Percentage of hematopoietic and tumour tissue and length of evaluable BM trephine recorded in mm.

Immunohistochemistry of bone marrow and trephine: At least 2 antibodies investigated in a minimum of 3 three sections. Antibodies that may be used are synaptophysin, tyrosine hydroxylase, chromogranin A, CD45 and CD 56. Immunohistochemistry staining should be done according to institutional protocols.

9.5) Reporting categories:
Bone marrow aspirate: Both left and right sided samples should be reported separately.
Categories: 0%, <5%, 5% to <20%, 20% to <50% and ≥50%. The bone marrow sample with the highest percentage of tumour infiltration is used.

Histology of trephine: Both left and right sided samples should be reported separately.
Surface area occupied by peripheral neuroblastic tumour as a percentage of bone marrow space and length of evaluable BM trephine in mm recorded.

Immunohistochemistry: Staining should be done according to institutional protocols but the most involved side should take preference for staining.
Surface area occupied by peripheral neuroblastic tumour as a percentage of bone marrow space and length of evaluable BM trephine in mm recorded.

9.6) Bone marrow and trephine treatment response assessment:
Treatment response should be document according to the International Neuroblastoma Response Criteria (INRC) revisions of 2012 (See Appendix D) [125]

In cases where the diagnosis is not conclusive or for standardised reporting quality assurance, central pathology review will be done at the Department of Anatomical Pathology, NHLS Laboratories, Red Cross War Memorial Children's Hospital. For these purposes slides should be made available.

10. BIOLOGICAL AND GENETIC MARKERS

All diagnostic tumour samples and/or positive bone marrow and/ or trephine samples should be tested for biological and genetic significant markers as per current standard of care:

1. Tumour sample:
    - MYCN FISH test for amplification of tumour
    - Ploidy (were possible)

Stellenbosch University https://scholar.sun.ac.za

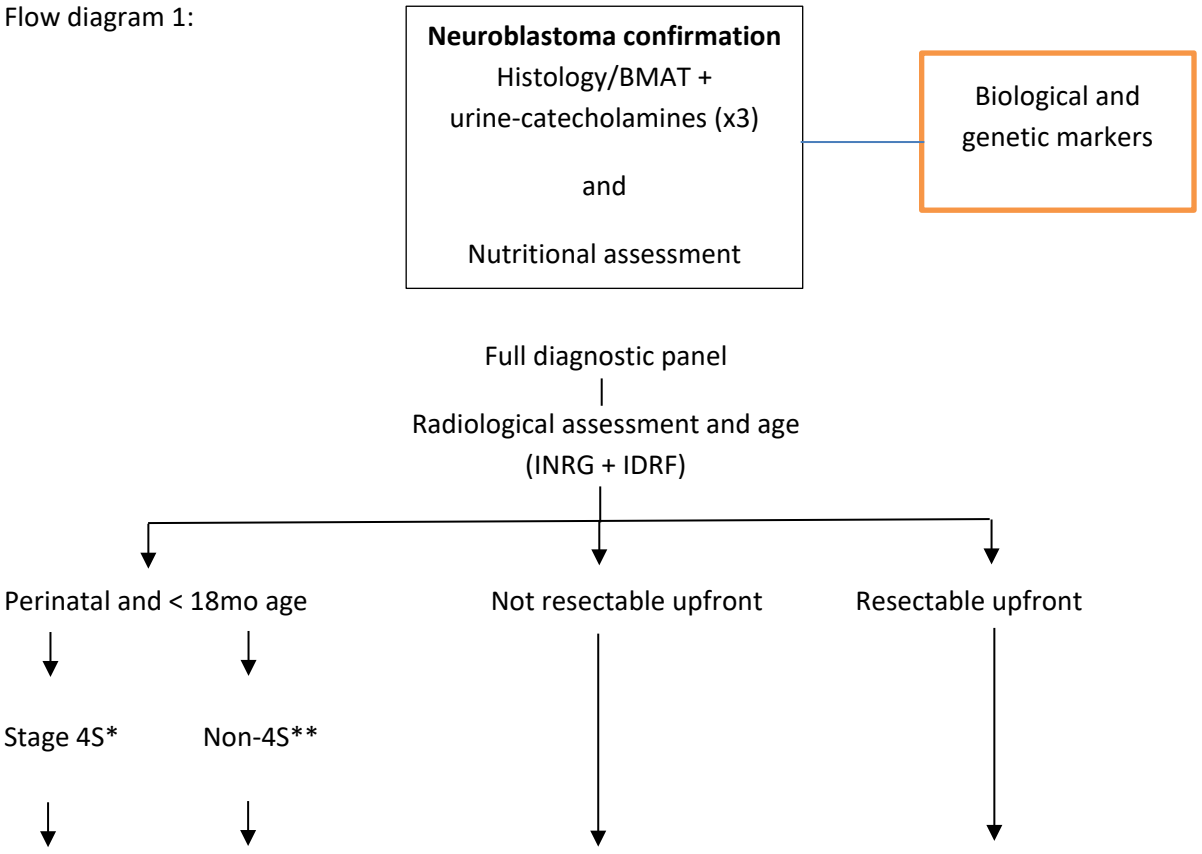
2. Analysis of Bone Marrow and Peripheral Blood (in patients with bone marrow infiltration where no tissue biopsy was done)
   - MYCN FISH test for amplification of tumour
   - Ploidy (were possible)

11. DEFINITIONS AND PRINCIPLES OF MANAGEMENT

The main aim of the diagnostic investigations is to document disease characteristics, evaluate the need for emergency interventions and risk stratify patients for treatment.

11.1) CURATIVE MANAGEMENT:

Flow diagram 1:



South African Neuroblastoma Adapted risk stratification (SANARS)

***Stage 4S - patients < 12 months with a primary adrenal lesion, cutaneous lesions, bone marrow infiltration of less than 10% and/or liver lesions.

** Non-4S - patients 0 – 18 months that does not define as Stage 4S.

Stellenbosch University https://scholar.sun.ac.za

After initial diagnosis and staging patients must be classified according to a risk classification based on imaging, initial surgery, age, histology and tumour markers.

11.1.1) Risk classifications:

The proposed South African Neuroblastoma Adapted risk stratification (SANARS) aims to provide a classification diagnosed via a medical approach, with the aid of the INRG staging, or a surgical diagnostic route, with the INSS staging system [2,3]. The degree of resection is also taken as risk factor by including the initial surgical status. Age is included due to the importance of age as risk factor, the definition of Stage 4s relying on age and that age plays an important part in the histological classification. MYCN is an important biological marker denoting a poor prognostic feature. It determines the difference between intermediate risk and high risk in stage 3, stage 4s and patients less than 18 months [5]. Histological confirmation is central to the diagnosis of NB and lends access to the degree of differentiation of the tumour. Histology, according to the Shimada classification [32], is validated prognostic factor that represents a biological factor in the risk classification, especially in POUs where MYCN is not available. Where MYCN is not available or the results are delayed, LDH and Ferritin are validated prognostic non-specific markers, easily determined in low resourced settings during diagnosis, that can be utilized as marker to distinguish between risk groups [5]. Stage 4s and patients under 18 months of age have good outcomes [86]. Symptomatic infants with hepatosplenomegaly, respiratory distress or coagulopathy fare poorer than asymptomatic infants [86]. The presence of symptoms increases the risk [5].

Clinical signs that define symptomatic tumours are the following:

- Deterioration of the general condition
- Feeding difficulties leading to weight loss
- Respiratory failure defined by oxygen requirement or carbon dioxide retention exceeding 60mmHg
- Circulatory failure defined by hypotension or hypertension according to the age specific blood pressure reference values
- Hepatic failure defined by grade 3 toxicity of bilirubin, fibrinogen, or thrombin time
- Renal failure defined by impaired blood urea or creatinine, new development of hydronephrosis or deteriorating pre-existent hydronephrosis
- Symptomatic or asymptomatic intraspinal involvement documented by MRI
- Failure of other organ systems.

Stellenbosch University https://scholar.sun.ac.za

Table 3: Proposed South African Neuroblastoma Adapted risk stratification (SANARS) [2,3,5,32]

INSS	Initial Surgical Status	INRG	Age (months)	LDH	Ferritin	MYCN	Histology	Risk Group
1	Resection > 50%	L1	>6mo	Any	Any	NA	Any	Low
1	Resection > 50%	L1	>6mo	Any	Any	Amp	Any	High
1	Observation	L1	<6mo	Any	Any	Any	Any	Very low (VLR)
2A	Resection >50% Asymptomatic	L1	Any	Any	Any	NA/EC/ Unknown	Any	Low
2B	Resection >50% Asymptomatic	L2	Any	Any	Any	NA/EC/Unknown	Any	Low
2A	Resection >50 % Symptomatic	L1	Any	Any	Any	NA/EC/ Unknown	FH	Intermediate
2A	Resection >50 % Symptomatic	L1	Any	Any	Any	NA/EC/Unknown	UH	High
2B	Resection >50% Asymptomatic	L2	Any	Any	Any	NA/EC/ Unknown	FH	Intermediate
2B	Resection >50 % Symptomatic	L2	Any	Any	Any	NA/EC/Unknown	UH	High
2A	Resection <50%	L1	Any	Any	Any	NA/EC/Unknown	FH	Intermediate
2A	Resection < 50%	L1	Any	Any	Any	NA/EC/Unknown	UH	High
2B	Resection <50%	L2	Any	Any	Any	NA/EC/Unknown	FH	Intermediate
2B	Resection < 50%	L2	Any	Any	Any	NA/EC/Unknown	UH	High
2A	Any resection	L1	Any	Any	Any	Amp	Any	High
2B	Any resection	L2	Any	Any	Any	Amp	Any	High
3		L2	<18mo	<750	<120	NA/EC/ Unknown	Any	Intermediate
3		L2	>18mo	<750	<120	NA/EC/ Unknown	FH	Intermediate
3		L2	>18mo	<750	<120	NA/EC/ unknown	UH	High
3		L2	Any	>750	>120	A/ Unknown	Any	High
4		M	<18mo	>750	>120	A/ Unknown	Any	High
4		M	<18mo	<750	<120	NA/EC/Unknown	Any	Intermediate
4		M	>18mo	Any	Any	Any	Any	High
4S	Asymptomatic	MS	<3mo	<750	<120	NA/ EC	FH	Low
4S	Asymptomatic	MS	3-12mo	<750	<120	NA/EC/Unknown	FH	Low
4S	Asymptomatic	MS	3-12mo	>750	>120	NA/Unknown	FH	Low
4S	Symptomatic	MS	3-12mo	<750	<120	NA/EC	FH	Low
4S	Asymptomatic	MS	3-12mo	Any	Any	NA/EC/Unknown	UH	Intermediate
4S	Symptomatic	MS	3-12mo	<750	<120	Unknown	Any	Intermediate
4S	Asymp/ Symp	MS	3-12mo	Any	Any	Amp	Any	High
4S	Symptomatic	MS	<3mo	Any	Any	Any	Any	High

Source documents:

Image Defined Risk Factor (IDRF) (Appendix F), International Neuroblastoma Risk Group (INRG)/ International Neuroblastoma Risk Group (INRG) Consensus pre-treatment staging system (INRGSS) (Appendix G), International Neuroblastoma Staging System (INSS) (Appendix H), International Neuroblastoma Pathological Classification (INPC) / Shimada classification (Appendix J), COG risk grouping (Appendix K),

Stellenbosch University https://scholar.sun.ac.za

Adapted Risk Stratification and Treatment Assignment for Neuroblastoma in LMIC (Appendix L) and the International Neuroblastoma Risk Group (INRG) Pretreatment Classification Schema for Stage 4S Neuroblastoma and Children's Oncology Group (COG) Neuroblastoma Stage 4S Group Assignment Schema Used for COG-P9641, COG-A3961, and COG-A3973 Studies.

11.1.2) Definitions of response to treatment:

The aim of the treatment response criteria is to ensure uniform assessment of tumour response. The criteria are based on the International Neuroblastoma Response Criteria (INRC) of 1993 [181], the revised INRC criteria of 2017 [182] and the soft tissue Response Evaluation Criteria in Solid Tumors (RECIST) to accommodate the heterogeneous availability of diagnostic and imagining techniques [183].

Measurement:

^{123}I-MIBG scan with CT or MRI- scan should be used as primary imaging techniques to evaluate soft tissue sites. FDG-PET can be used in MIBG- non-avid lesions. The measurement of non-nodal target lesions should be based on the longest single diameter whereas discrete lymph nodes are assessed using the short axis as a single dimension.

Response assessment should include anatomic imaging for primary and metastatic soft tissue disease, nuclear medicine imaging using ^{123}I-MIBG scan or FDG-PET scan for assessment of soft tissue and bone disease and bilateral bone marrow aspirates and trephine biopsies for assessment of marrow and skeletal disease.

Tissue biopsies may be used as an adjunct to verify the presence of metabolically active or viable Neuroblastoma or Ganglioneuroblastoma.

Urine catecholamine levels will be included to evaluate response but will only be used as supportive evidence in centres where other imaging techniques are not immediately available or delayed. Urine catecholamine levels lack of standardization in specimen collection and analysis and the influence of diet and sample handling have an effect on results.

Subsequent evaluations:

Subsequent evaluations of tumour response should always be done with the same imaging and diagnostic techniques used during the diagnostic phase to ensure comparable measurements and evaluations.

Anatomic imaging will not be used to evaluate osteo-medullary lesions, because these lesions may not shrink in size using CT/MRI scans even in the absence of residual viable tumour. Osseous lesions without a soft tissue mass are considered non-measurable

According to RECIST criteria. Extra-medullary soft tissue components of bone lesions that are measureable will be assessed using the same criteria used for other soft tissue sites.

Stellenbosch University https://scholar.sun.ac.za

Definitions:

Target lesions:
Defined as:

- Disease sites that meet criteria of measurable size
  - non-lymphoid soft tissue mass ≥10 mm in longest dimension or
  - lymph node ≥15mm in short axis

  AND

- as well as either uptake on MIBG (or FDG-PET scan for MIBG non-avid tumors)

  OR

- Biopsy positive for Neuroblastoma or Ganglioneuroblastoma

Non-target lesions:

Lesions that are considered to be active tumour sites but do not meet target lesion criteria for include cytology positive ascites, tumour positive cerebrospinal fluid, leptomeningeal tumour (meningitis carcinomatosis) and pleural effusion with positive cytology.

Discrete lymph node: Single lymph node that can be discretely identified measure by short axis.

The sum of diameters: The sum of the short axis of discrete lymph nodes added to the sum of the longest diameters of non–lymph node soft tissue metastases.

Conglomerate masses of non-discrete lymph nodes (i.e. multiple contiguous retroperitoneal nodes) will be measured using longest diameter.

Criteria:

- Primary (soft tissue) Tumour Response
- Tumour Response at Metastatic Soft Tissue and Bone Sites
- Bone Marrow Metastasis Response
- Determination of Overall Response

Abbreviations:
CR – Complete response; MD – Minimal disease; MR – Minimal response; NI – not involved;
PD – progressive disease; PR – partial response; VGPR – very good partial response;
SD – stable disease

Stellenbosch University https://scholar.sun.ac.za

Table 4: Primary (soft tissue) Tumour Response [181,182]	
Response	**Anatomic + ^{123}I-MIBG (FDG-PET) Imaging**
CR	Assessment with: • 10 mm residual soft tissue at primary site **AND** • Complete resolution of ^{123}I-MIBG or FDG-PET (for ^{123}I-MIBG-nonavid tumours) uptake at primary site. If no ^{123}I-MIBG or FDG-PET scan available: • 10 mm residual soft tissue at primary site **AND** • Urine catecholamine levels normal.
VGPR	Assessment with: • Primary tumour decreased by 90% to 99% **AND** • Urine catecholamine levels normal. Supporting evidence: • Residual ^{123}I-MIBG or FDG-PET uptake at primary site.
PR	Assessment with: • 30% decrease in longest diameter of primary site **AND** • ^{123}I-MIBG or FDG-PET uptake at primary site stable, improved, or resolved.
PD	Assessment with: • 20% increase in longest diameter taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study) **AND** • Minimum absolute increase of 5 mm in longest dimension
SD	Neither sufficient shrinkage for PR **nor** sufficient increase for PD at the primary site

Stellenbosch University https://scholar.sun.ac.za

Table 5: Tumour Response at Metastatic Soft Tissue and Bone Sites [181,182]	
Response	**Anatomic + ^{123}I-MIBG (FDG-PET) Imaging**
CR	Resolution of all sites of disease defined as: • Non-primary target and non-target lesions measure 10 mm **AND** • Lymph nodes identified as target lesions decrease to a short axis 10 mm **AND** • ^{123}I-MIBG uptake or FDG-PET (for ^{123}I-MIBG-non-avid tumours) uptake of non-primary lesions resolves completely If no ^{123}I-MIBG or FDG-PET scan available: • Resolution of all sites **AND** • Catecholamines normal.
VGPR	Assessment with: • No tumour **AND** • Catecholamines normal **AND** • Residual ^{123}I-MIBG or FDG-PET uptake at metastatic sites site.
PR	Assessment with: • >30% decrease in sum of diameters of non-primary target lesions compared with baseline **AND** • All of the following: - Non-target lesions may be stable or smaller in size AND - No new lesions AND - >50% reduction in MIBG absolute bone score (relative MIBG bone score > 0.1 to < 0.5) or > 50% reduction in number of FDG-PET–avid bone lesions
PD	Any of the following: • Any new soft tissue lesion detected by CT/MRI that is also MIBG avid or FDG-PET avid • Any new soft tissue lesion seen on anatomic imaging that is biopsied and confirmed to be Neuroblastoma or Ganglioneuroblastoma • Any new bone site that is MIBG avid • A new bone site that is FDG-PET avid (for MIBG non-avid tumours) **AND** has CT/MRI findings consistent with tumour **OR** has been confirmed histologically to be Neuroblastoma or Ganglioneuroblastoma • 20% increase in longest diameter taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study) **AND** minimum absolute increase of 5 mm in sum of diameters of target soft tissue lesions • Relative MIBG score > 1.2
SD	Neither sufficient shrinkage for PR nor sufficient increase for PD of non-primary lesions

Stellenbosch University https://scholar.sun.ac.za

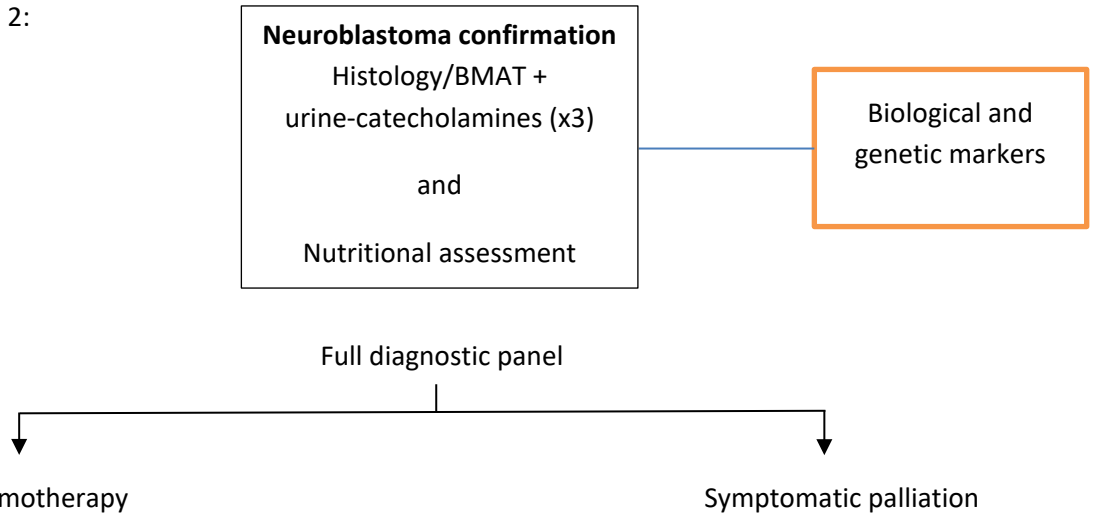
Table 6: Bone Marrow Metastasis Response [181,182]	
Response	**Cytology/Histology**
CR	Bone marrow with no tumour infiltration on reassessment, independent of baseline tumour involvement.
PD	Any of the following: • Bone marrow without tumour infiltration that becomes > 5% tumour infiltration on reassessment OR • Bone marrow with tumour infiltration that increases by > two fold and has > 20% tumour infiltration on reassessment.
MD	Any of the following: • Bone marrow with < 5% tumour infiltration and remains > 0 to < 5% tumour infiltration on reassessment **OR** • Bone marrow with no tumour infiltration that has < 5% tumour infiltration on reassessment **OR** • Bone marrow with > 20% tumour infiltration that.
SD	Bone marrow with tumour infiltration that remains positive with >5% tumour infiltration on reassessment but does not meet CR, MD, or PD criteria.

Table 7: Determination of Overall Response [181,182]	
Response	**Criterion**
CR	All components meet criteria for CR.
PR	PR in at least one component and all other components are either CR, MD (bone marrow), PR (soft tissue or bone), or NI; no component with PD.
MR	PR or CR in at least one component but at least one other component with SD; no component with PD.
SD	SD in one component with no better than SD or NI in any other component; no component with PD.
PD	Any component with PD.

Stellenbosch University https://scholar.sun.ac.za

11.2) PALLIATIVE MANAGEMENT:

Flow diagram 2:



* Document protocol

12. THERAPEUTIC ALGORITHMS

12.1) EMERGENCY PRESENTATIONS

12.1.1) Spinal cord compression

NB is the most common malignant cause of spinal cord or nerve root compression in the pediatric population with extension of the intervertebral foramina and/or the spinal canal being present in 10–15% of patients.

Since the survival is independent from the type of primary intervention [3], considerations in the choice for initial treatment modality are the risk group, the resectability of the tumour and the neurological symptomatology.

Chemotherapy is the treatment of choice above surgery in order to prevent long term spinal deformities. An osteoplastic laminectomy is the preferred surgical intervention. Radiotherapy has limited application in spinal cord compression.

Steroid therapy must be started immediately in conjunction with more definitive therapy.

Steroid therapy:
Indications for steroid therapy:

- Asymptomatic spinal canal involvement (threatening spinal cord compression)
- Acute symptomatic spinal cord compression
- Progressive symptomatic spinal compression
- In conjunction with spinal radiotherapy

Stellenbosch University https://scholar.sun.ac.za

Dose [184]:
Dexamethasone 2mg/kg/day in three divided doses IV or PO (with gastric protection therapy according to unit policies) with a taper period in relation to the duration of treatment.

Dexamethasone should be administered until asymptomatic or a maximum of 14 days by which time chemotherapy should have commenced. If surgery has taken place or chemotherapy has been started prior to 14 days, Dexamethasone must be tapered from the day of surgery or the last day of chemotherapy over 3 days.

Non-steroid therapy:
Indications for treatment in NB with spinal cord compression. Chemotherapy is prescribed according to risk group stratification.

Risk group	Clinical presentation	Treatment modality
High risk	• Asymptomatic • Longstanding neurological symptoms • Stable neurological symptoms	Chemotherapy before surgery and radiotherapy
High risk	• Acute neurological symptoms • Rapid neurological progression	Surgery before chemotherapy and radiotherapy
Intermediate risk	• Asymptomatic • Longstanding neurological symptoms • Stable neurological symptoms	Chemotherapy before surgery and radiotherapy
Intermediate risk	• Acute neurological symptoms • Rapid neurological progression	Surgery before chemotherapy and radiotherapy
Low risk	Primary resectable	Surgery
Low risk	Primarily not resectable	Chemotherapy

Radiotherapy:
Radiotherapy is not the main treatment of choice in spinal compression presentations in children. The reason is due to a higher rate of spinal deformities incurred with additional chemotherapy and future surgery.
In a setting where acute neurological symptoms and/or rapid neurological progression is present in the absence of available neuro-surgery, radiotherapy as primary treatment option is advisable.

Radiotherapy prescription:
- Emergency 2,5Gy x 4 days with dexamethasone cover 2mg/kg/day in three divided doses
- A maximum dose of 21Gy for any length of spinal cord is permitted

Stellenbosch University https://scholar.sun.ac.za

Other clinical presentations:
Biopsy:
When a biopsy is the only confirmatory test surgery can be considered as the primary treatment modality.

Severity of disease:
The choice of therapy is directed by the feasibility in terms of the clinical presentation.

12.1.2) Respiratory distress

The management of respiratory distress in patients with Neuroblastoma is determined by the following aspects:

- Anatomical site – intrathoracic tumour, cervical region (Horner syndrome), abdominal or critical hepatomegaly with diaphragmatic impingement.
- Resectability of the tumour
- Clinical presentation of the child

The following guidelines may be applied:

- Evaluate the need for cardio-respiratory support and initiate supportive measurements.
- Initiate diagnostic evaluation with the purpose of evaluating the possibility of resection as an emergency procedure and to exclude non-neuroblastoma malignancies such as lymphomas or other benign tumours.
- Evaluate the safest intervention such as surgical resection or chemotherapy.
- If dexamethasone is considered other malignancies such as lymphomas should be excluded first.

12.1.3) Abdominal obstruction

The management of intestinal obstruction in patients with Neuroblastoma is determined by the following aspects:

- Anatomical site – organ enlargement such as the liver or pelvic tumours causing obstruction
- Resectability of the tumour
- Clinical presentation of the child

The following guidelines may be applied:

- Evaluate the need for cardio-respiratory support and initiate supportive measurements such as nasogastric tubes and intravenous resuscitation.
- Evaluate pain management needs.
- Initiate diagnostic evaluation with the purpose of evaluating the possibility of resection as an emergency procedure and to excluded non-Neuroblastoma malignancies such as lymphomas or other benign tumours
- Evaluate the safest intervention such as surgical resection or chemotherapy.
- Consider stool softening treatment in cases of partial obstructions.

Stellenbosch University https://scholar.sun.ac.za

12.2 TREATMENT STRATEGIES

12.2.1) LOW RISK (LR-SA)

Patients under 6 months with FH, asymptomatic disease as well as completely resected FH, asymptomatic, MYCN non-amplified disease at any age can be observed. With progression and/ or symptomatic disease chemotherapy is indicated.

With progression surgery can be done upfront or after neoadjuvant chemotherapy if surgery is possible. If a response is achieved after 4 cycles, a further 3 cycles can be administered.

Re-evaluate

Cycle	1	2	3	4	5	6	7
Carboplatin	*	*	*	*	*	*	*
Etoposide	*	*	*	*	*	*	*

Regimen:

Low risk disease and symptomatic, MYCN non-amplified, favourable histology and progressing Stage 4S disease

- Chemotherapy is administered every 21 days for 4 cycles
- If the tumour response is favourable a further 3 cycles may be administered to a maximum of 7 cycles
- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L

Doses:

Carboplatin 600mg/m^2 IV over 2 hours x 1 day

Etoposide 175mg/m^2 IV over 4 hours x 1 day

Dose modifications:
Infants and low weight children:

- Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
- Infants with a weight equal and < 5kg:
- The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Carboplatin dose (mg) = Target AUC (5) X GFR (ml/min) + [0.36 X body weight (kg)]
over 2 hours x1/day

Etoposide 6.6mg/kg IV over 4 hours x 1 day

Stellenbosch University https://scholar.sun.ac.za

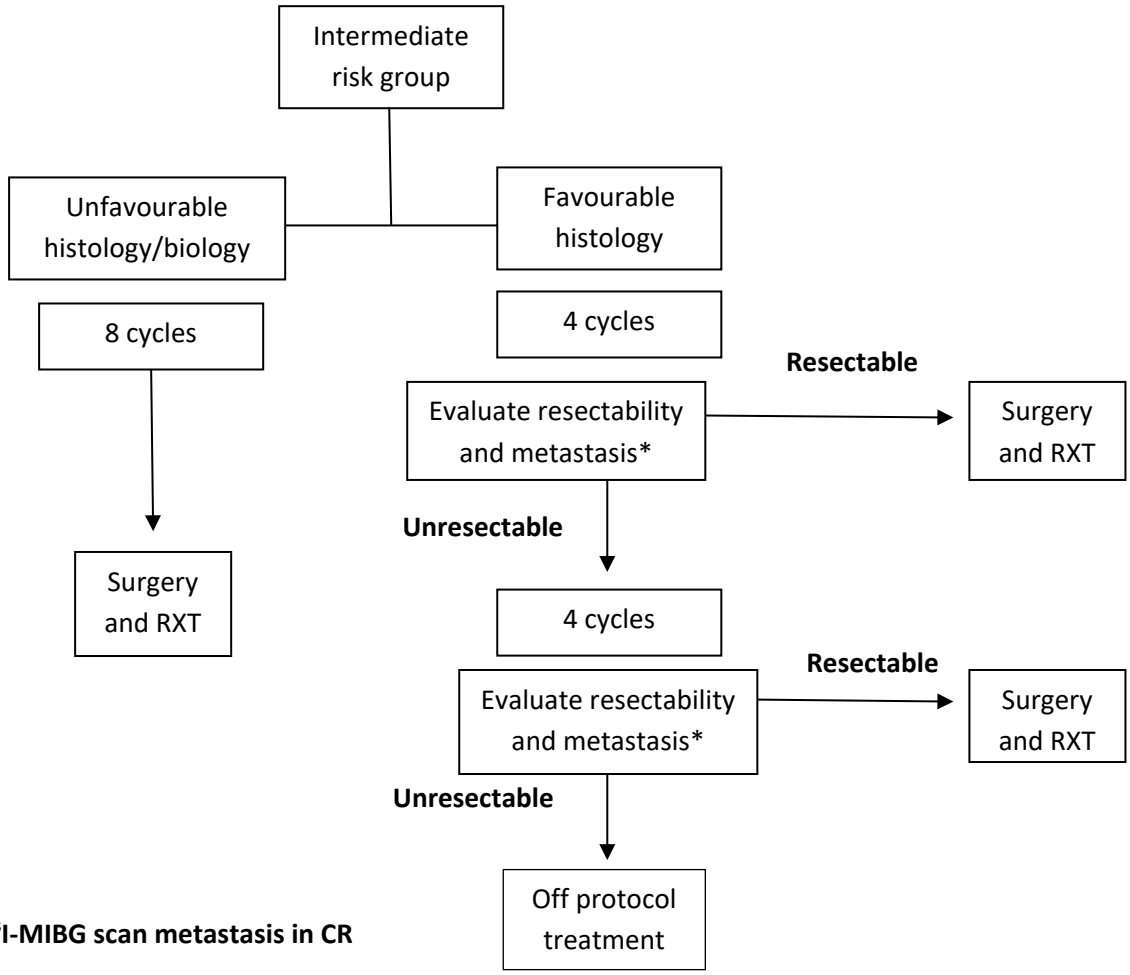
Precautions:

Infection support: Timely antimicrobial interventions according to standard guidelines

Nutritional support: Naso-gastric nutritional support must be started before starting chemotherapy and weekly measurements and monitoring must take place

12.2.2) INTERMEDIATE RISK (IR-SA)

Flow diagram 3: Intermediate disease is treated according to biological features.



***BMAT and ^{123}I-MIBG scan metastasis in CR**

Stellenbosch University https://scholar.sun.ac.za

Time point	Reason	Action
Induction		
After cycle 4	Evaluation for resectability and response of metastasis	**Resectable and metastatic CR** Proceed to surgery and complete induction post surgery
		Unresectable or metastatic < CR Continue induction and evaluate after cycle 7
After cycle 8	Evaluation for resectability and response of metastasis	**Resectable and metastatic CR** Proceed to surgery and radiotherapy
		Unresectable or metastatic <CR Off protocol

Chemotherapy:

Chemotherapy should be administered with the aim to resect the tumour. In favourable histology (FH) tumours evaluation for resectability may be done after 4 cycles. In unfavourable histology tumours (UH) evaluation should take place after 8 cycles

Cycle	1	2	3	4		5	6	7	8	
Carboplatin	*	*		*		*	*	*		
Etoposide	*		*	*		*		*		
Doxorubicin		*		*		*	*		*	
Cyclophosphamide		*	*				*		*	
Evaluate					*					*

Regimen:

- Chemotherapy is administered every 21 to 28 days for 4 or 8 cycles (according to treatment algorithm) if are neutrophils $\geq$ 1.0 x10^5/L and platelets $\geq$ 100 x 10^5/L and rising counts.

Stellenbosch University https://scholar.sun.ac.za

- Prophylactic G-CSF should be administered to patients < 3 months after every course at 5µg/kg SC.

Doses:

Carboplatin 560mg/m^2 IV over 2 hours x 1 day

Etoposide 120mg/m^2 IV over 4 hours x 3 day (360mg/m^2 x 1 day)

Doxorubicin 30mg/m^2 over 2 hours x 1 day

Cyclophosphamide 1000mg/m^2 over 2 hours x 1 days

Dose modifications:
Infants and low weight children:

- Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
- Infants with a weight equal and < 5kg:
- The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Carboplatin dose (mg) = Target AUC (5) X GFR (ml/min) + [0.36 X body weight (kg)]
over 2 hours x1/day

Etoposide 4mg/kg IV over 4 hours x 3 day

Doxorubicin 1mg/kg over 2 hours x1 day

Cyclophosphamide 33mg/kg over 2 hours x 1 days

Approach:

<u>Surgery and chemotherapy:</u>

If CR is possible surgery should be attempted.

- With R0 or R1 resection and FH – no chemotherapy and follow up
- With R2 resection or UH – 8 cycles of chemotherapy

If CR is not possible a biopsy should be done to determine FH/ UH. In the absence of histology tumours must be treated as UH.

Radiotherapy:

The indications for radiotherapy are the following:

- Progressive disease
- Life threatening symptoms without the possibility of surgery

Stellenbosch University https://scholar.sun.ac.za

Precautions:

Infection support: Timely antimicrobial interventions according to standard guidelines.

Re-evaluation

The following tests should be done at re-evaluation:

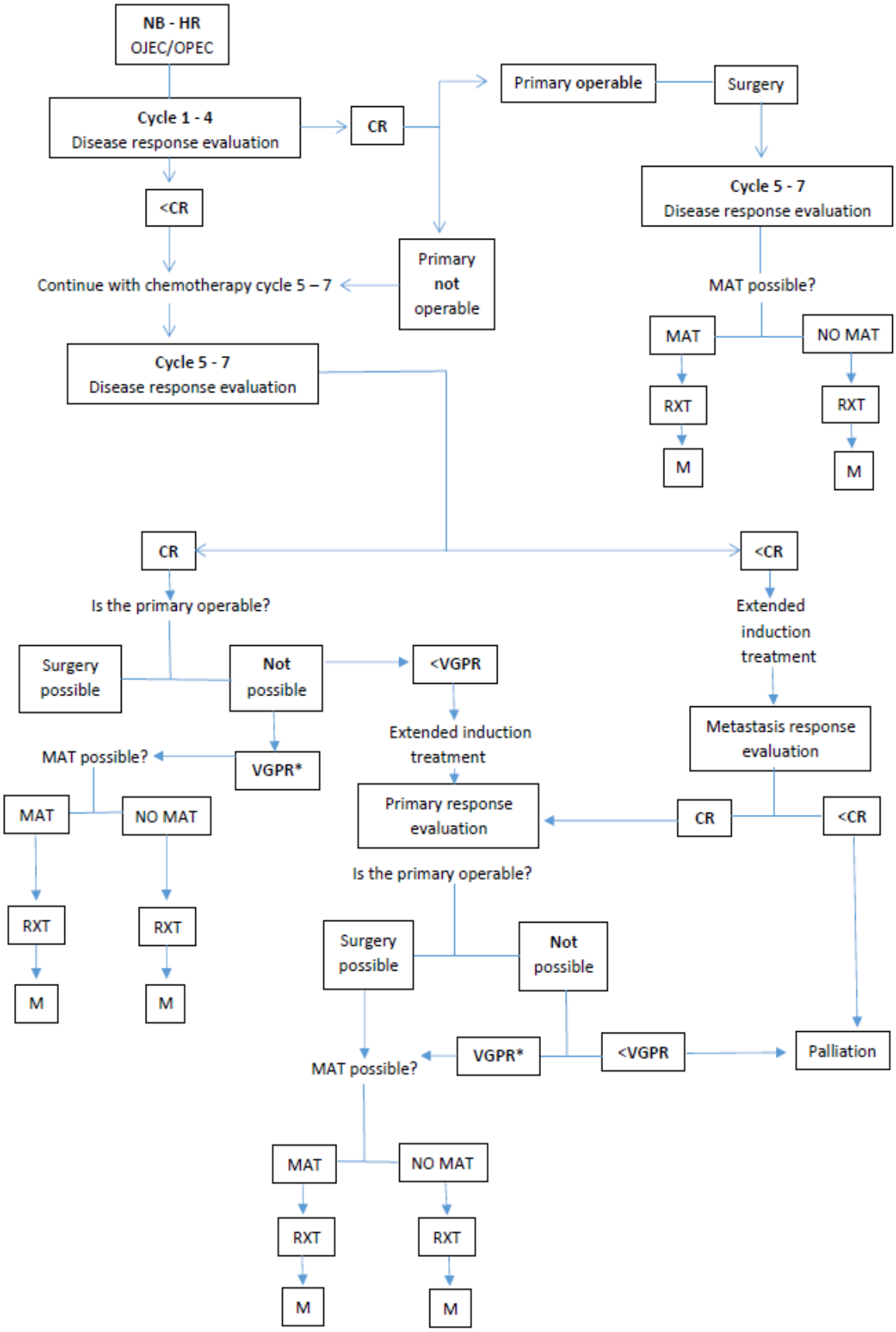
- Tumour markers
- Urine – catecholamine levels
- ^{123}I-MIBG scan (PET – scan in ^{123}I-MIBG non avid tumours or nuclear imaging test used during diagnosis)
- Bilateral BMAT (stage IV with bone marrow and skeletal involvement)
- Local evaluation with CT/MRI scan

12.2.3) HIGH RISK (HR-SA)

The treatment for high-risk disease should start with induction phase with an OJEC/OPEC regime. Treatment should take place as per flow diagram. If patients do not have a good response post induction a further extended induction should be given. Further treatment includes surgery, radiotherapy and possibly an autologous bone marrow transplant (MAT) in the consolidation phase. MAT is dependent on availability of transplant centres to do the transplant. Every HR patient should be discussed with a transplant centre regardless of the possibility of the transplant. All HR patients should receive radiotherapy and surgery for local measures during the consolidation phase. If surgery is not possible then radiotherapy must be done as a local measure. Maintenance therapy with Cis-retinoic acid follows the consolidation phase.

Stellenbosch University https://scholar.sun.ac.za

Flow diagram 4: Treatment of high risk disease



Stellenbosch University https://scholar.sun.ac.za

A) INDUCTION:

All patients diagnosed with high risk disease starts with OJEC/OPEC.

Cycle	1	2	3	4		5	6	7	
Vincristine	*	*	*	*		*	*	*	
Carboplatin	*		*			*		*	
Etoposide	*	*	*	*		*	*	*	
Cisplatin		*		*			*		
Cyclophosphamide	*	*	*	*		*	*	*	
Evaluate					*				*

Regimen:

- Chemotherapy is administered every 21 days for 7 cycles
- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L

Doses:

Vincristine 1.5mg/m^2 IV stat (max 2mg) x 1 day

Carboplatin 500mg/m^2 IV over 2 hours x 1 day

Etoposide 200mg/m^2 IV over 2-4 hours x 1 day in a 1:2 dilution

Cisplatin 20mg/m^2 IV over 3 hours x 4 days (Total 80mg)

Cyclophosphamide 600mg/m^2 over 1 hour x1 day

Dose modifications:

Infants and low weight children:

- Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
- Infants with a weight equal and < 5kg:
- The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Vincristine 0.05mg/kg IV stat (max 2mg) x 1 day

Carboplatin dose (mg) = Target AUC (5) X GFR (ml/min) + [0.36 X body weight (kg)]
over 2 hours x1/day

Etoposide 5.8mg/kg IV over 2-4 hours x 1 day

Cisplatin 2.6mg/kg IV over 24 hours x 1 day

Cyclophosphamide 35mg/kg over 1 hour x 1 day

Stellenbosch University https://scholar.sun.ac.za

Precautions:

Cisplatin administration:

- Prehydration for cisplatin

Infused at 200 ml/m²/hr for at least 3 hours with 0.9% sodium chloride with 20 mmol/l potassium chloride

- Posthydration for cisplatin

Infuse at 3 litres/m^2. To each litre add 10ml KCl (max 20ml), 5ml Ca gluconate (10% solution) or 250mg/L and 2ml (1-2g/L) $MgSO_4$. Post hydrate for 12 hours following each cisplatin dose.

- A target urine volume of 400ml/m^2/6h must be maintained

When a target volume is not reached administer hydrochlortiazide (HCTZ) 1mg/kg/dose PO/IV 2x/d max 50mg per dose [185].

Infection support:

- Timely antimicrobial interventions according to standard guidelines

Toxicity Monitoring

- A GFR by radio-nucleotide measurement (and calculated GFR) should be done prior to commencing cisplatin chemotherapy, after every 2 cycles and again at the end of treatment.
- An audiogram should be done prior to commencing cisplatin chemotherapy, after every 2 cycles and again at the end of treatment.

B) <u>TUMOUR RESPONSE EVALUATION</u> [181-183]

Response to treatment has been associated with outcome. The tumour response to chemotherapy should take place at pre-determined points in the treatment. Response assessment should include anatomic imaging for primary and metastatic soft tissue disease, nuclear medicine imaging for assessment of soft tissue and bone disease and bilateral bone marrow aspirates and trephine biopsies for assessment of marrow disease.

Tissue biopsies may be used as an adjunct to verify the presence of viable neuroblastoma or ganglioneuroblastoma that is evaluable for response.

Urine catecholamine levels will be used to evaluate response as supporting evaluation especially in units where the availability of other tests are limited, because of a lack of standardization in specimen collection and analysis and the influence of diet on results positive urine catecholamine level s alone are not indicative of treatment response failure.

Stellenbosch University https://scholar.sun.ac.za

The following tests must be done at re-evaluation:

- Tumour markers
- Urine – catecholamine levels
- ^{123}I-MIBG scan (PET – scan in ^{123}I-MIBG non avid tumours or nuclear imaging test used during diagnosis)
- Bilateral BMAT (stage IV)
- Local evaluation with CT/MRI scan

Time point	Reason	Action
Induction		
After cycle 4 OJEC/OPEC	Evaluation for resectability	**Resectable** Proceed to surgery and complete induction post surgery
		Unresectable Continue induction and evaluate after recycle 7
	If MAT option available	If BMAT CR first opportunity for stemcell harvest
After cycle 7 OJEC/OPEC	Evaluation for resectability and MAT (MAT option available)	**Resectable** Proceed to surgery
		Second opportunity for stemcell harvest Proceed to MAT
	Evaluation for resectability and MAT (MAT option not available)	**Resectable** Proceed to surgery and radiotherapy
		Unresectable Continue to extended induction
Extended induction		
After cycle 4 CADO	Evaluation for resectability and MAT (MAT option available)	**Resectable** Proceed to surgery
		Third opportunity for stemcell harvest Proceed to MAT
	Evaluation for resectability and MAT (MAT not option available)	**Resectable** Proceed to surgery and radiotherapy
		Unresectable Continue to radiotherapy
***Post transplant**		Continue to radiotherapy and maintenance
Post radiotherapy		Continue to maintenance
End of treatment		Continue to long term follow up

Stellenbosch University https://scholar.sun.ac.za

B) <u>EXTENDED INDUCTION</u>

Extended induction is a CADO-based regime where 4 cycles should be administered and a disease response re-evaluated. Extended induction is for children with a disease response < CR after cycle 4 AND 7 of OJEC/OPEC (see diagram).

Cycle	**Day**	**1**	**2**	**3**	**4**
Vincristine	1	*	*	*	*
Doxorubicin	1	*	*	*	*
Cyclophosphamide	2-6	*	*	*	*

Regimen:

- Chemotherapy is administered every 21 days for 4 cycles
- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L

Doses:

Vincristine 1.5mg/m^2 IV stat (max 2mg) x 1 day

Doxorubicin 35mg/m^2 IV in 5% DW over 30 minutes x 1 day

Cyclophosphamide 350mg/m^2 PO x 5 days

Dose modifications:
Infants and low weight children:

- Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
- Infants with a weight equal and < 5kg:
- The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Vincristine 0.05mg/kg IV stat (max 2mg) x 1 day

Doxorubicin 1.2mg/kg IV in 50ml 5% Dextrose over 1 hour

Cyclophosphamide 12mg/kg PO x 5 days

Precautions:

Infection support: Timely antimicrobial interventions according to standard guidelines

Stellenbosch University https://scholar.sun.ac.za

C) CONSOLIDATION

Consilidation consists of three parts.

1. Surgery (if it is possible to perform)
2. Radiotherapy
3. Myelo-ablative therapy (MAT) or autologous stemcell transplantation (if the option is available to the patient)

1) SURGICAL GUIDELINES [186-199]

Surgery plays many roles in the management of Neuroblastoma including biopsy, surgical resection, second look surgery as well as surgical interventions in emergency clinical presentations.

Neuroblastoma either requires upfront surgery, if complete resection is possible, or delayed surgery after induction chemotherapy for the purpose of cyto-reduction which may facilitate complete resection at a later stage.

Aims of surgery:

- Assist in the diagnosis of Neuroblastoma by providing sufficient histological material for basic and advanced histo-pathological, cytological, molecular and genetic diagnosis.
- Intervene in the acute treatment of emergency presentations to aid further treatment and prevent long term co-morbidities.
- Definitive local treatment by ensuring, within non-maleficent measures, to achieve complete surgical excision via:
    - Primary resection
    - Post induction chemotherapy resection
    - Second look surgery for the purpose of complete resection

Surgical technique:
Both laparotomy and laparoscopy are acceptable as long as all oncological principals are adhered to.

Suggested techniques:
Abdominal tumours
A transverse laparotomy incision is recommended which can be extended to a thoraco-abdominal approach if needed.

Pelvic tumours
A midline incision is the access of choice. A combined laparotomy and posterior sagittal approach may be required for some low lesions.

Cervical and thoracic tumours
It is suggested that thoracic lesions may require a double thoracotomy over a distance of up to 3 intercostal spaces. A soft tissue incision or a thoraco-abdominal approach is recommended if the lesion is just above the diaphragm.

Stellenbosch University https://scholar.sun.ac.za

For lesions in the apex of the thorax and/or thoracic inlet require alternative approaches may be considered

Timing of surgery:
Primary surgical interventions:

- Upfront resection takes place if the primary tumour is resectable and there are no metastases
- After induction chemotherapy if surgical indications are met. In HR disease after cycles 4 of OJEC/OPEC, completed OJEC/OPEC and extended induction are opportunities for resection if tumours are resectable.

Primary and metastatic tumours:

- Surgery may happen synchronous
- Or in stages

Considerations for second-look surgery are to obtain a complete resection after MAT.

The role of image defined risk factors (IDRF) (see Appendix F):
IDRF is a guideline of the documentation of the pre-treatment extent of disease. It also guides the surgeons in the pre-operative evaluation of resectability of tumours in preventing surgical co-morbidity and minimizing subtotal resections.

IDRF and INRG staging should be done by the MDT which at minimum should include a radiologist, surgeon and treating physician.

INSS surgical definitions:

Table 8: Terminology related to the degree of the surgical resection [186]

Level of resection	Description of resection
Complete surgical resection (CR)	Macroscopic total removal of all visible tumour and nearby abnormal lymph nodes
Near-complete gross resection (NC-GR)	Resection of tumour leaving a minimal macroscopic residue
Gross total resection (GTR)	Removal of >90 % of the visible tumour

Stellenbosch University https://scholar.sun.ac.za

Incomplete resection (ICR)	
Subtotal resection (STR)	Removal of >50 % but <90 % of the visible tumour
Less than STR	Removal of <50 % of the visible tumour/ biopsy

Surgical and pathological resection margins:

Table 9: Classification of margins [186]		
Classification	Meaning	
R0	Resection for cure or complete remission	CR
R1	Microscopic residual tumour	NC-GR
R2	Macroscopic residual tumour	GTR or ICR

In the South African context, the aim for surgery should be an **R0 resection** or an **R1 resection (with radiotherapy)** to ensure local control.

An **R2** resection with a GTR is only indicated for emergency purposes, with control for symptoms or when second look surgery is an option with the aim for CR.

Indications for surgical procedures:

a) At presentation

- Resection: L1 by INGR – local tumour: IDRF negative
- Biopsy only: L2 by INGR – local tumour: IDRF positive
  M or MS by INGR – if the local tumour is IDRF negative a primary resection may be termed as an excision biopsy
- Observation only: An adrenal mass in selected infants under 90 days of age at presentation < 5cm
- With symptomatic primary tumour where surgery is preferred above chemotherapy.

b) Excision of primary mass should be attempted

- Persistent or enlarging adrenal mass in infants under 90 days, following a period of observation, provided the tumour is IDRF negative. In IDRF positive infants resection may still be recommended if resection of the primary tumour suggests that the risk to life or a major functional loss is less than leaving residual disease. With a positive IDRF the aim should be GTR without a threat to life even if residual tumour is left behind.
- Enlarging renal mass at presentation in infants under 90 days of age, provided the tumour is IDRF negative. In IDRF positive infants resection may still be recommended after a failed attempt to cyto-reduce the tumour with chemotherapy if the evaluation of the primary

Stellenbosch University https://scholar.sun.ac.za

tumour suggests that the risk to life or a major functional loss, is less risk than leaving residual disease.

- With L2 tumours
    - In patients **under** 18 months old at presentation, where the IDRF become negative after chemotherapy or natural involution
    - In patients **over** 18 months old at presentation
        - With differentiating tumours which became IDRF negative after chemotherapy
        - With undifferentiated or poorly differentiated tumours irrespective of IDRF status
        - With tumours of unknown differentiation irrespective of IDRF status after chemotherapy
    - In IDRF positive tumours, regardless of differentiation, even after chemotherapy, resection may still be recommended if the evaluation of the primary tumour suggests that the risk to life or a major functional loss, is less risk than leaving residual disease or the complications with persisting symptoms due to tumour effect.
- With bone, lung and/or central nervous system metastatic tumours (M) of children under 18 months of age at presentation, with no evidence of metastatic residual (liver excluded) irrespective of IDRF status.
- Ms tumours of children under 18 months of age at presentation that still progress or are symptomatic after chemotherapy. Surgery must only be attempted if a GTR is possible without a threat to morbidity or mortality.

c) Other:

- In cases where repeat histology is of value
- For symptom control during palliation

Contra-indications to surgery:

- M tumours in children who still have metastatic disease, other than resectable liver or lung metastases, at the end of induction chemotherapy.
- L2 tumours with IDRF after chemotherapy still encasing major blood vessels [200].

Biopsy:

Neuroblastoma is a very heterogeneous tumour with multiple clinical presentations. Surgery plays a very important role in the diagnosis, staging and treatment of NB. Initially if the presentation does not necessitate emergency intervention due to compression or obstruction, a core biopsy is important to provide enough tissue sample for diagnostic confirmation and biologic testing.

The biopsy sampling must be done by an experienced clinician, preferably under ultrasound guidance, with sedation or under anaesthetics within local protocols. The biopsy must be done in a safe and effective manner especially in the neck and thoracic region close to vital structures.

All retroperitoneal tumours must be biopsied **retroperitoneally**. This prevents upstaging to stage III due to the creation of a biopsy tract through the peritoneal cavity or causing peritoneal leakage. If a biopsy is done from any other site except a posterior entry, the tumour is upstaged to stage III.

Stellenbosch University https://scholar.sun.ac.za

Surgical resection:

Only 20-40% of children present with local disease and most cases can not be resected as a primary treatment modality. The pathophysiological nature of NB is to grow intimately with vital structures like blood vessels and nerves which present great challenges during resection. If initial resection is attempted the surgeon must ensure that there is no metastatic disease present and local resection must be done with the aim as little co-morbidity and collateral damage as possible to vital structures.

The aim is complete resection (CR) especially in stage I – III tumours. In stage IV tumours either CR or gross total resection (GTR) of 95% is recommended. The COG A3973 study concluded that even GTR of >90% ensures a better survival [188]. A poorer prognosis is likely with a gross total resection of <90%. Secondary to surgical resection age and tumour biology play the most important prognostic roles in the prognosis of NB.

All studies include that metastatic control is more important than resection [196,198].

Local relapse plays a major role in mortality especially in high risk patients. Local relapse rates of 20 - 80% have been recorded even with the adjunct of radiotherapy and myeloablative therapy (MAT) when ICR was done.

The debate of resectability is of importance even in the advent of myeloablative therapy and immunotherapy. Yet when these modalities are not available, as in LMIC setting, resectibility plays an even more important role in the prevention of relapse [199]. This is important in the extent of surgical resection when different treatment modalities are involved:

- When there is a possibility for MAT complete surgical resection should not include nephrectomy unless renal function is not salvageable.
- A nephrectomy during second look surgery to achieve CSR **after** a MAT is advised.
- When there is no possibility for MAT complete surgical resection, including nephrectomy, should be considered.

For patients with stage I and stage II disease the cure rate reaches 85 – 90% [3]. Complete, margin free resection ensures the need for less intensive chemotherapy without radiotherapy. In stage III patients obtaining CR ensures a better survival in patients with especially unfavourable tumour biology [192, 193].

During resection the following should be done:

- inspection of and biopsy of any ABNORMAL appearing peritoneal surfaces.
- inspection of and biopsy of any ABNORMAL appearing lymph nodes.
- inspection of and biopsy of any ABNORMAL appearing omentum.
- inspection of and biopsy of any ABNORMAL appearing contralateral adrenal glands
- in stage I – III a CR must be done
- in stage IV tumours GTR of the primary tumour >90% without collateral damage to vital structures or organs and resection of metastasis.

There is no indication for surgical resection in a stage IV patient if there are still distant metastases present. Surgery is purely palliative.

Stellenbosch University https://scholar.sun.ac.za

However if a CR of distant metastases (medically or surgically) have been achieved, surgery to the primary tumour is indicated if CR of the **metastases** is proven with:

- bilateral bone marrow aspirate and trephine biopsy AND
- bone scan or ^{123}MIBG scan

Even with high intensity treatment such as autologous transplants or immunotherapy, less than GTR doesn't provide any survival benefit and debulking has no role to play in a LMIC setting.

Second look surgery:
Neo-adjuvant chemotherapy and radiotherapy may cause significant interval regression of primary tumour. Surgery may be applied to ensure a complete response. In prognostic terms this improves survival. Performing surgery after chemotherapy may improve the ease of resectability and decreases surgical complications.

Second look surgery is a possibility post MAT to achieve CSR in metabolically active tumours but should only done by experienced surgeons. Referral to surgical centers with the adequate experience to perform the surgery.

Lymph nodes adjacent or adherent to primary tumours are of uncertain prognostic implication. During surgery all attempts must be made to sample these nodes for radiotherapy purposes. If a lymph node is clinically pathological, it must be removed and evaluated for histology.

Unnecessary aggressive surgical techniques to ensure gross total resection are not warranted, especially is the intervention will cause significant co-morbidity.

In dumbbell tumours surgery is a two stage procedure where the extra-spinal component of the tumour is removed before the extradural, intra-spinal component.

Emergency procedures:

a) Spinal cord compression

With spinal cord compression it is important to note the duration of the symptoms and if the clinical situation is life threatening. If the symptoms are longstanding and not life threatening, chemotherapy and/or radiotherapy is the treatment of choice for the cyto-reduction of the tumour followed by surgery.

Surgery is not the primary treatment choice for decompression due to severe late effects from the surgery but a laminectomy is performed first even if an osteoplastic laminotomy minimizes deformities.

If with chemotherapy/ and or radiotherapy the symptoms resolve and the extraspinal component becomes resectable i.e. IDRF negative then a surgical resection of the extraspinal component should take place. There is no indication to surgically resect the residual spinal canal component of a dumbbell tumour.

b) Respiratory distress

Stellenbosch University https://scholar.sun.ac.za

Rapidly enlarging tumours due to liver metastases in stage 4S patients compromise respiratory function with pressure on the diaphragm or compression on the inferior vena cava. The primary treatment of choice is chemotherapy and radiotherapy but if reduction is slow a silastic sheet may be inserted in the abdominal wall. Resection can be done if the patient is still symptomatic after chemotherapy and radiotherapy intervention.

Major surgical complications:

- Serious haemorrhage due to the vascularity of the tumours >30% blood volume.
- Vascular injury with tissue loss
- Nerve damage and spinal cord injury
- Organ failure
- Surgical inoperability

Post-surgery complications:

After surgery there are common complications that need to be monitored.

a) Bleeding

Neuroblastomas surround the major abdominal vessels like the coeliac trunk and the superior mesenteric artery. Major vessel injuries can occur during resection.
A hallmark of NB is the vascular nature due to angioneogenesis. This predisposes to bleeding during surgery. This predisposes the patient to post surgical bleeding as well.

b) Diarrhoea [187]
In 30% of patients intractable diarrhoea follows retroperitoneal nerve injury postoperatively. Large volumes of diarrhoea may lead to intravenous support of the patient.

c) Adrenal insufficiency

Various studies documenting endocrinological late effects in patients treated for Neuroblastoma and nephroblastoma have been published. This includes decreased levels of glucocorticoid and mineralcorticoid steroids. Late effects such as decreased bone density, disregulation in glucose metabolism and blood pressure instabilities are a few. In later life fertility and metabolic syndromes are important co-morbidities. These are inevitable if both adrenals are resected.

2) RADIOTHERAPY GUIDELINES

RATIONALE FOR RADIOTHERAPY (RXT)
Neuroblastoma is a radiosensitive disease. However routine use of radiotherapy was not practiced historically in European trials. American trials in neuroblastoma used radiotherapy routinely and local relapse rates were noted to be lower in these series'. However, the trials used varying staging systems, indications, doses and systemic treatment, so that the role of radiotherapy remains unclear. Radiotherapy was routinely introduced into SIOPEN protocols in 2002. Dosage and indications, however, remain the subject of clinical trial protocols.

Stellenbosch University https://scholar.sun.ac.za

The indications for radiotherapy (RT) in NB are varied. It has a place in emergency treatment, local primary control, metastatic treatment and palliative treatment.

INDICATIONS FOR RADIOTHERAPY IN LOCALISED NB:

Neuroblastoma is defined by SIOPEN as low, intermediate or high risk, and overall treatment is determined based on these:

Low risk disease:

RT generally not indicated except for:

- In clinical presentations where function is threatened and no improvement is seen with chemotherapy:
    - Acute spinal cord compression
    - Respiratory compromise with tumour induced hepatomegaly, if chemotherapy is ineffective.

Intermediate risk disease:

Only selected patients in this group should receive radiotherapy:

- Localised unresectable, or residual disease (stage L2) or
- Patient>18 months or
- Poorly or undifferentiated histology

High risk:

- Stage 4 (Stage M) >1 year old with good response and limited disease
- Stage 2,3 (stage L2) MYCN amplified, any age

Stage 4S:

- For palliation
- In life threatening or function-limiting situations e.g. tumour induced hepatomegaly causing respiratory compromise, inferior vena cave obstruction, compromised renal perfusion, DIC or gastro intestinal obstruction that does not respond to chemotherapy.

Technique of radiotherapy:

The radiotherapy dose and volume is determined by risk group, surgical outcome and options for transplant.

Equipment and Technique:

In younger children sedation maybe required for the simulation and treatment and when Paediatric anaesthetists are available as a resource, the treatment is stream lined.

Ideally, patients should be CT planned (ideal slice thickness 3mm-5mm). If not available, then virtual simulation or conventional simulation is acceptable. Accurate positioning is necessary.

Linac radiotherapy with an energy of >4 MV is suggested with a 6MV usually sufficing for planning. Cobalt radiotherapy may also be used if Linac not available.

Stellenbosch University https://scholar.sun.ac.za

Patient should be positioned supine in a reproducible and comfortable position and a 3-point set up used.

For metastases, relevant anatomical areas may require a foam or thermoplastic cast.

Timing of radiotherapy:

a**) In patients without an option of myelo-ablative therapy (MAT):**

- **Resectable tumours**:
  Radiotherapy is started after surgical recovery, usually at 2 weeks but no later than 30 days after surgery
- **Non-resectable tumours**:
  Radiotherapy must still be considered to the primary tumour and metastatic sites even without surgical resection in the following situations:
  - If a tumour is irresectable after induction chemotherapy and radiotherapy is logistically possible after evaluation of the primary tumour suggests that the risk to life or major functional loss, is less from radiotherapy than from the disease OR for persisting symptoms due to tumour effect.
  - If there is limited metastatic tumour, and radiotherapy is logistically possible.

b) In patients with an option of MAT:

Radiotherapy will be given to the initial diagnostic primary tumour site as well as to limited post induction metastatic sites after MAT and according to suggested radiotherapy dosages the interval should be greater than 60 days but before 90 days post transplantation in order to prevent chemotherapy-induced radio-toxicity.

Radiotherapy of primary site:

Volumes are based on post-chemotherapy, pre-surgical disease as seen on MRI/CT.

Technique:

Planning may be done by simulation, virtual simulation or 3-D planning depending on site-specific availability. Planning with 3-D allows for DVH evaluation of tumour and critical organs at risk (OAR).

Volumes for 3-D CRT:

Gross tumour volume (GTV):

- Pre-surgery/ post- neo-adjuvant chemotherapy volumes are used, taking original tumour volumes into account.
- If post resection, then there is no GTV, but tumour bed (TB) is determined using pre-op scans and clips, as well as operative notes and histology.

Clinical target volume (CTV)

- This is a margin that is added to the GTV or tumour bed, and accounts for possible microscopic tumour extension.

CTV= GTV+ 1cm margin

PTV (Planning target volume)

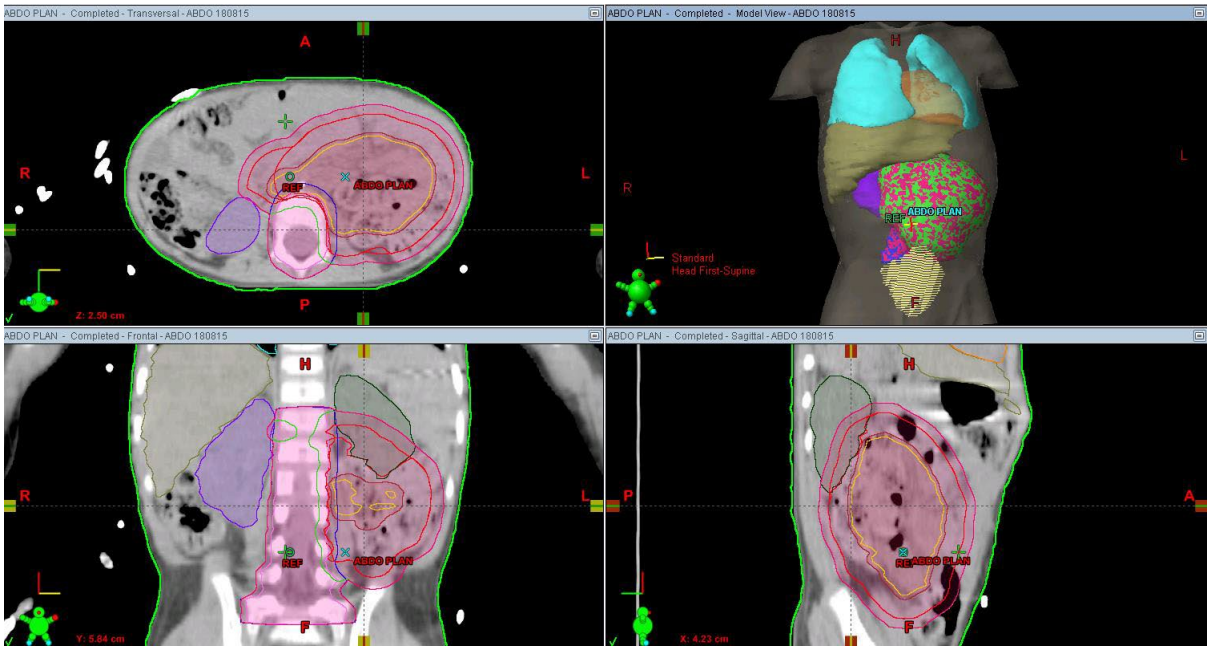
Stellenbosch University https://scholar.sun.ac.za

This is a margin that is added to account for physiological motion and set up error (this is institutional).

PTV= CTV + 1 cm

In addition, in growing children, bones must be symmetrically irradiated and therefore the entire vertebral column must be delineated, and a margin added for set-up/movement. This is PTV2, and should be added to the main PTV:

<u>Total PTV= PTV1 + PTV2</u>



- OAR are delineated
- For neuroblastoma this includes:
    - Kidneys (R&L)
    - Liver
    - Spinal cord
    - Ovaries/testes

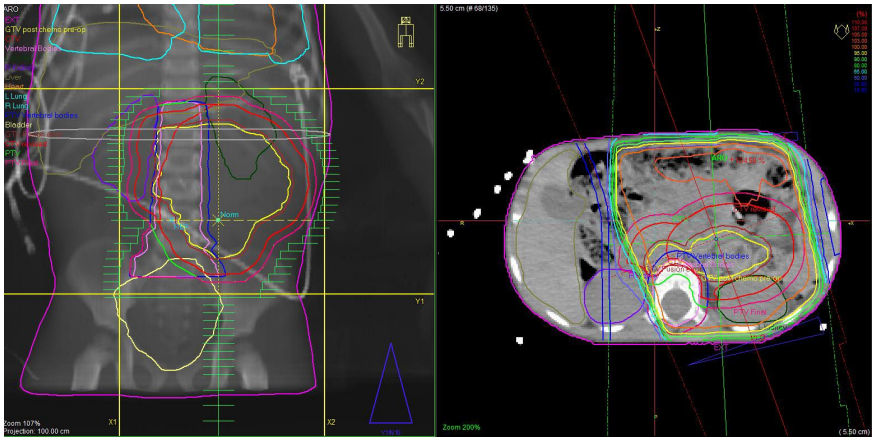
<u>Field arrangement</u>

Field arrangement is frequently AP/PA or opposing oblique fields in order to minimise kidney/ovarian dose.

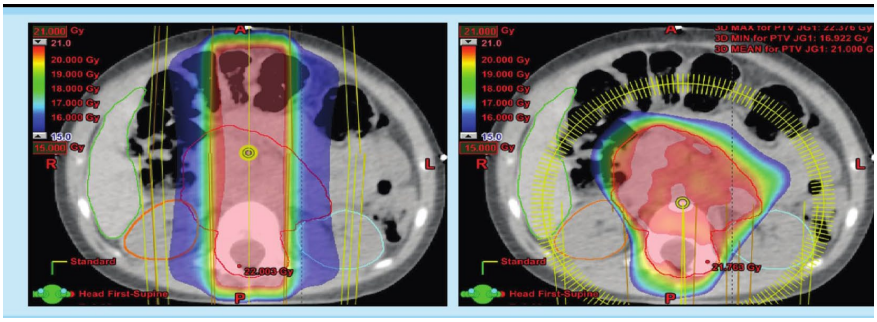
Differential field weighting may be used if planned.

Kidney shielding is used to keep kidneys within tolerance (see below).

Stellenbosch University https://scholar.sun.ac.za



If volumetric modulated arc therapy (VMAT) is available, this may be used in order to allow better tumour volume coverage [201].



If planning is done with simulation alone:
Clips are identified and pre-op scans/op note/pathology are used to localise tumour bed.
A margin of 1-2cm around tumour bed is give, incorporating the entire width of the vertebral column.

AP/PA fields are delivered with shielding to kidneys after tolerance reached.

Dose:

1. **After complete resection (CR):**

1.5 Gy x 14#= 21 Gy in 5x per week (lower dose per # because bowel in field)

Dose is prescribed to either mid-plane (central) dose (if simulated), to ICRU point (=100%) if 3-D planned radiotherapy, or to mean tumour dose for VMAT prescriptions.
Radiotherapy should be delivered in less than 21 days.

2. **For incomplete surgical resection:**

1.8 Gy x 12 #= 21.6 Gy, 5x per week

Stellenbosch University https://scholar.sun.ac.za

A boost may be added as per new SIOPEN protocols but this is not yet standard practice
Boost 1.8 Gy x 8#= 14,4 Gy (8 fractions) to any gross residual tumour
Total dose =36 Gy in 20 fractions.
A simultaneous integrated boost (SIB) may be planned with a maximum daily dose limited to 2.1 Gy.

Table 10: Radiotherapy doses			
Normal dose by site	**Target volume**	**Dose/Fraction**	**Number of fractions**
Primary site 21.6 Gy	PTV1	1.8 Gy	12
Primary site boost 14.4 Gy	PTV2	1.8 Gy	8
Metastatic site 21.6 Gy		1.8 Gy	12

3. Tumour response with limited surgical possibility:
Patients with a CR or VGPR on induction chemotherapy or with IDRF that prevents surgical intervention should still receive radiotherapy for local control as for incomplete surgical resection.

Stage 4S hepatic RXT (Functional impairment):

- A dose of 2-6 Gy (2-4 fractions) to whole liver (1.5 Gy x3# = 4.5 Gy on 3 consecutive days)
- Allow for 2-3 weeks to assess response to RXT. If needed the dose may be repeated to a max cumulative dose of 12 Gy.
- Primary (Asymptomatic Stage 4S – no radiotherapy)

Metastatic sites:
Post induction chemotherapy MIBG non-active sites – no RT
Post induction chemotherapy oligo metastases: MIBG active sites – 21,6 Gy (in 12 fractions)

Palliative RT (Pain relief):
Small fields – 16-20 Gy (4-5 Gy per fraction)
Large fields – 20-30 Gy (2-3 Gy per fraction)
Alternative is 8 Gy single fraction for patients with limited life expectancy. Response is good but lesion may return sooner than with greater total dose.

Spinal compression:

- Emergency 2,5 Gy x 4= 20Gy over 4 days with dexamethasone cover 2 mg/kg TDS [184]
- A maximum dose of 21Gy for any length of spinal cord is permitted

Other sites:
If the gonads are in the field a dose <5 Gy is permitted

<u>Dose Modifications for OAR (as per COG guidelines)</u>

Peritoneal Cavity
Dose to the contralateral kidney must be kept below 1500 cGy.

Stellenbosch University https://scholar.sun.ac.za

Thorax
No more than 1500 cGy shall be given if two-thirds (2/3) or more of the lung volume must be included in the target volume.

Liver
No more than 1500 cGy shall be given if two-thirds (2/3) or more of the liver volume must be included in the target volume.

3) MYELO-ABLATIVE THERAPY GUIDELINES AND STEM CELL HARVEST [103]

An autologous transplant as myelo-ablative therapy consolidates the treatment of Neuroblastoma (NB) after the induction phase and local control of the tumour. An autologous transplant improves the OS by up to 20% 5-year survival [126]. There is no literature available for the experience of autologous transplants in South Africa.

Indications for an autologous transplant:

1. The patient is a high-risk NB patient
   - All non-stage IV high risk NB patients
   - Stage IV high risk NB patients are selected by treatment response and clinical elligibility
2. The patient must not have any co-morbidities that are a contra-indication for an autologous transplant.
3. A patient must have received a gross total resection (>90% resection) **or** the tumour must have CR or VGPR with neo-adjuvant chemotherapy and surgery is not possible due to an inoperable small residual tumour mass.
4. The patient must have a complete remission of all distal metastasis (according to INSS criteria).
   - This must be proven by an I^{123} MIBG for skeletal response
   - **and** cytomorphological and histological bilateral bone marrow and trephine response (negative BM and trephine involvement).
5. The patient must have adequate post transplantation follow-up options.

Timing of Peripheral Stem Cell Harvest (SCH):

The disease response status should be evaluated before performing the PBSC harvest procedure. There are various time points for stem cell collection. The recommendation is to start early with harvest efforts. The first harvest option is following recovery from aplasia after the end of induction (Rapid COJEC or OJEC/OPEC) **before** surgery and radiotherapy.

Patients achieving CR at metastatic sites on MIBG or bone scan **and** no positive bone marrow aspirate and trephine on **bilateral** biopsy will be eligible for peripheral stem cell harvest.

- Mobilisation of stem cells, with a protocol according to institutional preference, may take place after cycle 4 of OJEC/OPEC or post surgery.
- Steady state mobilisation (defined as >1,0 neutrophils and >100 platelets x 109/l) after induction chemotherapy but prior to surgery.

Stellenbosch University https://scholar.sun.ac.za

A minimum of 10-20 CD34+ cells/µL are required to start the first leukapheresis. The number of collections depends on the quantity of peripheral blood stem cells harvested, evaluated by the number of CD34 positive cells.

The aim of the peripheral SCH:

A sufficient number of blood progenitors must be collected by apheresis to allow safe and prompt haematological recovery following the high dose chemotherapy of a BuMel regimen and autograft.

- Dose of progenitor cells:
  CD34+ cells is $\geq$ 2-3 x 10^6 CD34 positive cells/kg for one BuMel (or Melphalan based protocol)
- A circulating level still higher than 10 - 20 CD34 positive cells per µL the leukapheresis procedure should be started
- A second procedure on the next day is recommended, if the amount of first apheresis is near by or less than 2-3 x 10^6 CD34 positive cells/kg.
- The total harvest should always be divided into minimum two bags. The amount of dimethyl sulphoxide (DMSO) should not be more than 1g/kg body weight (bw) for one reinfusion per day to avoid toxicity.
- There is no ideal timing for PBSC harvest in patients with metastatic NB, except that early collection increases the risk of tumour cell contamination and later ones of poor progenitor cell collection. The monitoring of both tumour cell contamination and progenitors in the graft.

Timing of autologous transplant (MAT):

Autologous transplant should be done after surgery of the tumour. The BuMel myelo-ablative regime has proven to have a superior outcome above other conditioning regimes in NB.
The BuMel MAT regime consists of Busulfan and Melphalan followed by a reinfusion of stem cells.

The BuMel MAT - regimen
The regime consists of intravenous administration of Busulfan as a two-hour infusion every 6 hours over 4 (or 5) consecutive days through a central venous catheter only. After a day of rest it is followed by an intravenous infusion of Melphalan.

In case of body weight below 12kg the Melphalan dose calculation is recommended to be dosed by per kg.
For Infants with a weight < or equal to 5 kg, a further 1/3 reduction is advised.

After the chemotherapy the stem cells are infused on the following day.

The dosage:
Peripheral stem cells
A minimum of 2 - 5 x 10^6 CD34 cells/kg should be used.
A maximum of 10 x 10^6 CD 34 cells/kg should be used.
Bone marrow collection

Stellenbosch University https://scholar.sun.ac.za

A minimum of 3 x 10^8 mononuclear bone marrow cells/kg or > 8 x 10^4 CFU-GM/kg should be used.

Timing:

Reinfusion of stem cells will be infused intravenously on Day 0 with specified rest, according to the MAT regimen, following completion of chemotherapy, within 1½ hours of thawing.

BUMEL-regime:

DRUG	DOSE DAY	-8	-7	-6	-5	-4	-3	-2	-1	0
Busulfan	An IV dose every 6 hours for 16 doses: < 9 kg: 1.0mg/kg 9 kg to < 16 kg: 1.2 mg/kg 16 kg to 23 kg: 1.1 mg/kg >23 kg to 34 kg: 0.95 mg/kg >34 kg: 0.8 mg/kg		**	****	****	****	**			
Melphalan	IV: 140mg/m^2 over 15min not before 24 hours after busulfan								*	
Hydration	3L/m^2 (125ml/hr) till 24 hours after melphalan infusion		→							→
Clonazapam	0.025 – 0.1 mg/kg/day IV: total dose as a continuous infusion PO: divided in 3 doses/day Dose can be reduced if the child is drowsy		→							→
			***	***	***	***	***	***	***	***
Ursodiol	300 mg/m²/day PO 150 mg/m²/day PO (Day -8 till day 80)	*	*	*	*	*	*	*	*	*
Stem cells	As per regime dose									*

Supportive treatments:

- Anti-emetics Ondansetron 5mg/m² (0.15mg/kg) [185] every 12 hours PO or IV as anti-emetic (maximum single dose 8mg)
- Adequate hydration is crucial prior to and following chemotherapy especially prior to Melphalan administration due to bladder irritation from high urine concentrations of the drug.
- Minimal urine output immediately prior to and 24 hours following Melphalan administration should be more than 90 ml/m²/hr. To achieve this urine output, give IV hydration at 125 ml/m²/hr.
- Ursodiol: The administration twice per day during the entire prophylactic period, even in the case of mucositis, from day -8 until day 80 post stem cell reinfusion is of major importance.
- G-CSF 5µg/kg/day IV will be given daily beginning on Day +5. G-CSF will continue until a stable increase of WBC > 5 x 10^9/l or ANC >0.5 x 10^9/l
- All blood products (packed red blood cells, platelets) must be irradiated with and be leucocyte depleted (ideally CMV negative). It is recommended that patients receive red packed blood cells to maintain haemoglobin > 8.0g/dl.

Stellenbosch University https://scholar.sun.ac.za

- Stop Co-trimoxazole prophylaxis from day 0 until at least day +10 or until WBC ³1.0 x 109/l.
- Prophylactic antifungal treatment with Ketoconazole, Itraconazole or Fluconazole should be avoided, because of the increased risk of VOD with these drugs in particular in association with Busulfan. For proven fungal infection Amphotericin is advised.
- Antibiotics and antivirals should be given in line with the institutional policy whenever Indicated.

D) MAINTENANCE or MATURATION THERAPY

Cis-retinoic acid (CRA) is used as maintenance therapy with the aim of maturing remaining undifferentiated cells.

Regime:
CRA is started no sooner than 14 days after radiotherapy to avoid radiotherapy related toxicity.

CYCLE	DAY 1 - 14	D 15 - 28
	CRA	REST

Starting Criteria:

- Neutrophils > 500 Platelets > 50 Hemoglobin > 8 g/dl
- Normal ALT and AST
- Normal renal function
- Normal serum calcium and urate
- Triglycerides must be < 2 x normal

Starting Dose (if > 12kg):
CRA 80 mg/m^2 PO 2x/d

Cycles
6 cycles will be administered
Each cycle consists of CRA D1-14 and then resting D15-28
Dose adjustments:

With the following criteria CRA must be stopped and reintroduced at 60mg/m^2 PO 2x/d

- Hemoglobin < 8 g/dl
- Platelets < 25
- Neutrophils < 500
- AST or ALT > 5 x normal
- Total bilirubin > 1.5 x normal
- Hypercalcaemia
- Erythema multiforme
- Severe vomiting and/or abdominal pain
- Urethritis and/or dysuria
- Severe cheilitis
- Severe conjunctivitis
- Severe headache or vertigo
- Persistent muscle cramps requiring sustained symptomatic care

Stellenbosch University https://scholar.sun.ac.za

After completion of 6 cycles management must continue according to the long term follow up schedule.

12.2.4) STAGE 4S

Patients with Stage 4S NB is a unique clinical entity that should be managed different from stage 4 NB. If a patient < 1 year presents with Neuroblastoma but do not fulfil the diagnostic criteria for 4S the standard NB protocol must be followed with appropriate dose modifications.

Diagnostic criteria:

- Age < 1 year of age **and**
- Meets the diagnostic criteria for neuroblastoma **and**
- Spread to the skin, liver or bone marrow (<10%), but **not to** bone

Management:

The majority of patients with stage 4S neuroblastoma do not require therapy. There are three patient groups who are at an increased mortality risk:

- symptomatic due to an evolving hepatomegaly or organ compromise
- tumours with unfavourable biology (pathology and genetics)
- patients less than 2 months of age

Patients are treated according to risk classification.

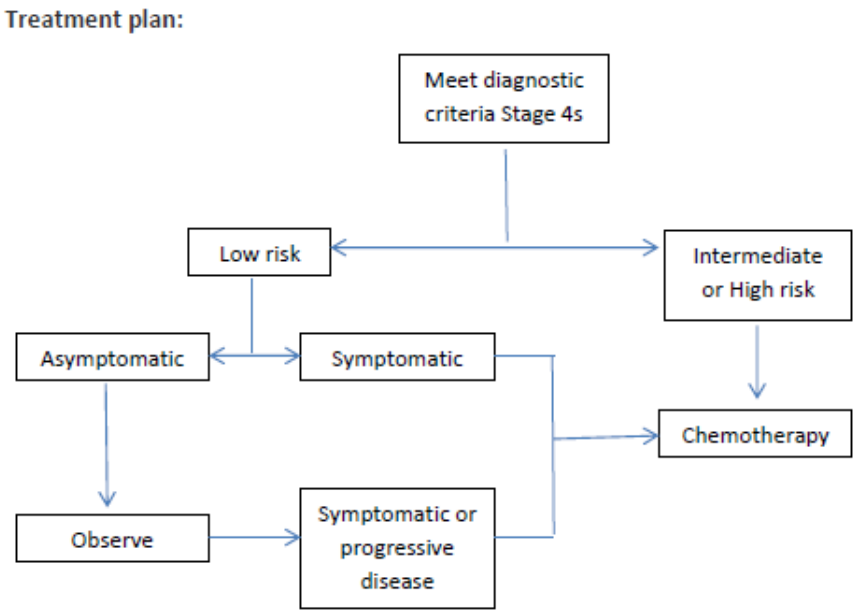
Table 11: Proposed South African Neuroblastoma Adapted risk stratification (SANARS) [2,3,5,32]

INSS	Initial Clinical Status	INRG	Age (months)	LDH	Ferritin	MYCN	Histology	Risk Group
4S	Asymptomatic	MS	<3mo	<750	<120	NA/ EC	FH	Low
4S	Asymptomatic	MS	3-12mo	<750	<120	NA/EC/ Unknown	FH	Low
4S	Symptomatic	MS	3-12mo	<750	<120	NA/EC	FH	Low
4S	Asymptomatic	MS	3-12mo	<750	<120	NA/EC/Unknown	UH	Intermediate
4S	Symptomatic	MS	3-12mo	<750	<120	Unknown	Any	Intermediate
4S	Asym/ symp	MS	3-12mo	Any	Any	Amp	Any	High
4S	Symptomatic	MS	<3mo	Any	Any	Any	Any	High

Modified from the International Neuroblastoma Risk Group (INRG) Pretreatment Classification Schema for Stage 4S Neuroblastoma and Children's Oncology Group (COG) Neuroblastoma Stage 4S Group Assignment Schema Used for COG-P9641, COG-A3961, and COG-A3973 Studies

Stellenbosch University https://scholar.sun.ac.za

Flow diagram 5: Treatment of Stage 4s disease



Guidelines of symptomatic stage 4s disease:

Progression in stage 4s NB is common. Clinical deterioration will need chemotherapy intervention. Especially infants under 3 months of age are at high risk of mortality without chemotherapy intervention.

Clinical signs that indicate progressive disease are the following:

- Deterioration of the general condition
- Feeding difficulties leading to weight loss
- Respiratory failure defined by oxygen requirement or carbon dioxide retention exceeding 60mmHg
- Circulatory failure defined by hypotension or hypertension according to the age specific blood pressure reference values
- Hepatic failure defined by grade 3 toxicity of bilirubin, fibrinogen, or thrombin time according to the NCI-CTC toxicity criteria [202]
- Renal failure defined by impaired blood urea or creatinine, new development of hydronephrosis or deteriorating pre-existent hydronephrosis
- Symptomatic or asymptomatic intraspinal involvement documented by MRI
- Failure of other organ systems.

Indications for chemotherapy treatment is based on the Philadelphia score system. Chemotherapy should be started on a Philadelphia score of **2 or more**.

Stellenbosch University https://scholar.sun.ac.za

Table 12: Philadelphia score system [203]		
Clinical entity	**Grade**	**Score**
GI-tract		
Emesis of > 10% of intake	Mild	1
Repeated emesis requiring IV fluids	Severe	2
Respiratory compromise		
Tachypnoe over 60/min and O2 supplementation	Mild	1
Need for CPAP or mechanical ventilation	Severe	2
Venous return		
Leg oedema	Mild	1
Leg oedema with scrotal a/o sacral oedema	Severe	2
Renal		
Oliguria with output < 2 ml/kg/hr	Mild	1
Oliguria with signs of renal failure, rising ur/kreat	Severe	2
Hepatic		
Thrombocytopenia/DIC/Platelet count < 50x109/l	Severe	2
	Total	

In infants under 3 months of age should be started on the high-risk protocol and infants of 3 months and more should start with the CE-regime. If there is no clinical response on the CE regime the infant must be considered high risk.

Chemotherapy:

CE regimen

Drug	**Dose**	**Days**
Carboplatin	6.6 mg/kg	D1-3
Etoposide	5 mg/kg	D1-3

Repeated after 21 days for 4 cycles

Follow up:

Up to 3 years of age

At every visit the following investigations should be done:

- Clinical examination for new masses
- BP
- FBC, U&E, creatinine, LDH, Uric acid and ALP
- CXR and abdominal US every visit (abdominal US only in abdominal and pelvic primary)
- U-catecholamine
- Skeletal survey is only advised if the clinical information indicates an MIBG scan or bone scan

The follow up schedule:
End of treatment

Stellenbosch University https://scholar.sun.ac.za

3 monthly for 1 year
Then 6 monthly for 1 year
Then yearly thereafter

12.2.5) PERINATAL NEUROBLASTOMA

In NB there is a high rate of spontaneous regression of small tumours in infants. Several oncology study groups have reported the spontaneous regression of larger tumours that were observed after diagnostic biopsies or PR.

Therefore, it is safe to observe perinatal tumours. Tumours included are the following:

- Patients < 6 months
- Asymptomatic
- Non-stage 4 or stage 4S – thus a negative skeletal screening
- Tumour size of smaller than 5cm x 5cm x 5cm (75ml)
- Restricted to the adrenal glands – no suspicious lymph nodes or extra adrenal extension
- Not crossing the midline
- Chemotherapy and radiotherapy naïve

Observation schedule and tests:

Period in weeks	**0**	**3**	**6**	**12**	**18**	**30**	**42**	**66**	**90**
Abdominal U/S	X	X	X	X	X	X	X	X	X
U-catecholamines	X	X	X	X	X	X	X	X	X
Abdominal CT/MRI	X			X					X

Method:

- Abdominal CT/ MRI is done for accurate staging
- U-catecholamine levels and abdominal U/S is done for monitoring purposes
- If a tumour increases by 50% or the u-catecholamine levels increase by 50% the tumour is resected immediately
- If a tumour becomes symptomatic the tumour is resected immediately
- A tumour is monitored at first in 3 weekly visits. After a tumour is stable for two consecutive visits – visits are scheduled 6 weekly. After two stable visits – visits are scheduled 12 weeks apart until 90 weeks.
- If tumour increases but not > 50% visits are scheduled at 3 weeks
- After 90 weeks the patient is managed according to the standard post treatment follow up.

Criteria for active treatment:

1) Evidence of persistent tumour growth

- A >50% increase in the volume of the mass **or**
- A >50% increase in either VMA or HVA, neither returns to baseline/nadir within 12 weeks, and the value is above the upper limit of normal for that metabolite **or**
- The VMA/HVA <0.5 (and HVA greater than upper limit of normal) and does not increase above 0.5 within 6 weeks **or**
- Progression, secondary malignancy, or metastasis

Stellenbosch University https://scholar.sun.ac.za

2) Symptomatic tumour

When a tumour becomes symptomatic

- the tumour must be resected and managed according to risk group
- if the tumour is unresectable the low-grade chemotherapy can be administered before resection. After resection the patient will be managed according to risk group.

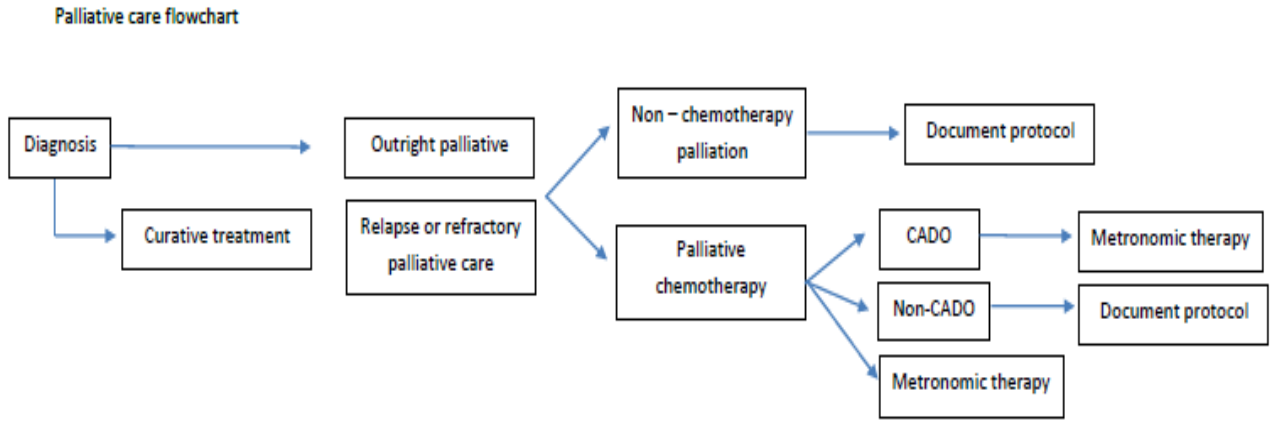
12.2.6) PALLIATIVE STRATEGIES

If the treatment intent is palliative the following should be documented

- The reasons for the palliative decision must be documented
- The clinical symptoms that have to be treated
- The modality of palliative intervention
  - Palliative chemotherapy/ metronomic therapy/ radiotherapy/ surgery
  - Non-chemotherapy symptomatic palliation
- Which protocol was used

Even if a palliative approach is chosen a full diagnostic work-up must be done (within reasonable limitations of the patient's presentation).

Flow diagram 6: Palliative strategy



Palliative strategies can be chemotherapy related or non-chemotherapy related (supportive).

Stellenbosch University https://scholar.sun.ac.za

CADO regime:

Cycle	Day	1	2	3	4	5	6	7	8	9	10
Vincristine	1	*	*	*	*	*	*	*	*	*	*
Doxorubicin	1	*	*	*	*	*	*	*	*	*	*
Cyclophosphamide	2-6	*	*	*	*	*	*	*	*	*	*

Regimen:

- Chemotherapy is administered every 21 days for 10 cycles

(**Note** that if CADO is used for palliation post treatment with an extended induction of CADO or post IR-SA to adapt number of cycles to a max of max tolerated dose of 300mg/m^2)

- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L

Doses:

Vincristine 1.5mg/m^2 IV stat (max 2mg) x 1 day

Doxorubicin 35mg/m^2 IV in 5% DW over 30 minutes x 1 day

Cyclophosphamide 350mg/m^2 PO x 5 days

Dose modifications:
Infants and low weight children:

- Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
- Infants with a weight equal and < 5kg:
- The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Vincristine 0.05mg/kg IV stat (max 2mg) x 1 day

Doxorubicin 1.2mg/kg IV in 50ml 5% Dextrose over 1 hour

Cyclophosphamide 12mg/kg PO x 5 days

Precautions:

Infection support: Timely antimicrobial interventions according to standard guidelines.

Nutritional support: Naso-gastric nutritional support must be started before starting chemotherapy and weekly measurements and monitoring must take place.

Stellenbosch University https://scholar.sun.ac.za

12.2.7) METRONOMIC THERAPY GUIDELINES

The metronomic therapy guidelines should be used according to POUs management resources as an optional treatment option during palliation.

The aim of metronomic therapy is in the palliative, relapse or refractory setting as a low dose continuous chemotherapy option to modulate the micro-environment of the tumour.

Metronomic therapy (M-NB-SA)

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14…**
Propranolol	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cyclophosphamide	*		*		*		*		*		*		*	
Vinorelbine	*	*	*		*	*	*		*	*	*		*	*

Regimen:

Palliative, relapse or refractory setting

- Chemotherapy is administered after cardiac function screening
- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L

Doses:

Propranolol 3mg/kg PO daily (see precautions)

Cyclophosphamide 30mg/m^2 PO daily – 1 week on, 1 week off

Vinorelbine 15mg/m^2 PO daily – 3 weeks on, 1 week off

Alternative to oral Vinorelbine:

If oral vinorelbine is not available the suggested replacement is intravenous vinblastine

Vinblastine 3mg/m^2 IV over 30 min x 1 day – monthly.

Dose modifications:

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14…**
Vinblastine	*				*				*				*	

Infants and low weight children:
Body weight below 12kg should be dosed according to their weight (kg): no dose reductions

Stellenbosch University https://scholar.sun.ac.za

Infants with a weight equal and < 5kg: no dose reductions

Precautions:

- Before starting Propranolol, cardiac function should be evaluated with echocardiogram and ECG.
- Administration starts with 1mg/kg/d. With stable blood pressures according to age and glucose levels daily doses can be increased by 1mg/kg/d till 3mg/kg/d [185]

13. DOSE MODIFICATIONS FOR TOXICITY

13.1) NEPHROTOXICITY

In patients with pelvic tumours with renal failure from obstructive uropathy, carboplatin can still be administered according to GFR measurements. It can also be administered if renal failure is severe enough to require dialysis.

A recommendation for adults is as follows:

Table 13: Adjustment of carboplatin dose according to BSA (mg/m^2) in renal failure

Cr Cl (ml/min)	Starting dose (mg/m^2)
>60	No dose reduction
41-59	250
16-40	200
<15	No information available

Cr Cl = N (Sex) X (140-Age) X weight (kg) / serum creatinine (micromoles/l), where N = 1.04 for males and 1.23 for females.

Stellenbosch University https://scholar.sun.ac.za

The recommendation for adjustment of cisplatin dose in adults with renal impairment is shown in table 14.

TABLE 14: Adjustment of cisplatin dose according to BSA (mg/m^2) in renal failure	
Cr Cl (ml/min)	**Dose (mg/m^2)**
>60	100%
45-59	75% of cisplatin dose or change to carboplatin if available.
<45	Hold cisplatin or delay with additional fluids or change to carboplatin if available.

Cr Cl = N (Sex) X (140-Age) X weight (kg) / serum creatinine (micromoles/l), where N = 1.04 for males and 1.23 for females.

13.2) MYELOTOXICITY

Every cycle of chemotherapy is given 21 days apart. An absolute neutrophil count of 1 X 10^9/L and platelets of >100 X 10^9/L is required. If the count is not sufficiently recovered by day 21, the patient should be rested until count recovery occurs, usually less than a week.

13.3) OTOTOXCITY

In children with hearing impairment secondary to cisplatin treated with cisplatin-containing regimens, carboplatin can be substituted on day 1 (AUC 5), except in children who have already been upgraded to the HR regimens for poor response to carboplatin at first evaluation according to the SIOP Boston Ototoxicity Scale (see Appendix N). A change from carboplatin to cisplatin is warranted if the hearing impairment is grade 3 or more.

13.4) CARDIOTOXICITY

All patient should have a cardiac evaluation with an echocardiogram (other test as per unit protocols) before the start of chemotherapy.

Schedule:

- Before the start of chemotherapy and end of chemotherapy regimens (even non-Doxorubicin containing regimens)
- Doxorubicin containing regimens:
  - Before the start of a CADO regime and after every 6^{th} cycle and 8^{th} cycle
  - At cumulative dose of $200mg/m^2$ and the maximum cumulative dose of $280mg/m^2$
  - Symptoms of cardiac toxicity

Stellenbosch University https://scholar.sun.ac.za

Cumulative doses:

IR-SA: Total 150mg/m^2 (5 x 30mg/m^2)

HR-SA extended induction: Total 140mg/m^2 (4 x 35mg/m^2)

CADO: 6^{th} cycle (or extended HR-SA + 2 cycles): 210mg/ m^2, 8^{th} cycle (or extended HR-SA + 4 cycles): 280mg/m^2

Minimum requirements during echocardiogram studies:

- Left ventricular functioning:
  - Shortening fraction (SF)
  - Ejection fraction (EF)
- Anatomical defects/ physiological that prevent chemotherapy administration due to the risk of cardiomyopathy

Normal pre-chemotherapy cardiac function:
SF > 28%
EF > 50%

Management of cardiotoxicity:

Grade 0 toxicity:	Normal No action
Grade 1 toxicity:	Asymptomatic decline of resting SF or EF of $\geq$10% but <20% of baseline value. Wait 1 week and re-evaluate if not improved omit single dose anthracycline. Echocardiogram before next planned dose.
Grade 2 toxicity:	Asymptomatic decline of resting SF or EF $\geq$20% but <25% of baseline value. Omit next anthracycline. Echocardiogram before next planned dose.
Grade 3 toxicity:	Responsive cardiac failure or decline of resting SF or EF $\geq$ 25% Requiring therapy and definitely stop anthracyclines
Grade 4 toxicity:	Severe cardiac failure requiring intensive care Stop anthracyclines

With symptomatic toxicity consult a paediatric cardiologist and/or evaluate for supportive drug treatment according to cardiac function.

Stellenbosch University https://scholar.sun.ac.za

14. DOSE MODIFICATIONS FOR INFANTS

Infants of less than 6 months and low weight children:

- Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
- Infants with a weight equal and < 5kg:
  The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Per chemotherapy drug:

Please see section 12 as per risk stratification

Carboplatin:

Carboplatin dose calculations are done using the Calvert formula modified for the use in children:

Carboplatin dose (mg) = Target AUC (5) X GFR (ml/min) + [0.36 X body weight (kg)]

For AUC 5 maximum carboplatin dose should be capped at 750mg. Paediatric dosing tables are available for comparison.

Cisplatin:

See individual treatment schedule.

Vincristine:

See individual treatment schedule.

Doxorubicin:

See individual treatment schedule.

Cyclophosphamide:

See individual treatment schedule.

Stellenbosch University https://scholar.sun.ac.za

15. END OF TREATMENT EVALUATIONS

This should consist of the following:

- A clinical examination
- Nutritional assessment
    - Anthropometry and
    - MUAC measurements
    - Biochemical evaluation
- Appropriate tumour markers to document normal values
    - U-catecholamines
    - LDH, Ferritin, ESR
- Imaging with an appropriately comparable modality (chest X Ray, abdomino-pelvic ultrasound, CT or MRI)
- Skeletal survey: MIBG scan and/or bone scan
- Bone marrow biopsy is not routinely advised but should be performed only if an abnormal full blood count cannot be explained by any other phenomenon e.g. bleeding.

16. LONG TERM FOLLOW UP

At every visit the following investigations should be done:

- Clinical examination for new masses
- BP
- FBC, U&E, creatinine, LDH, Uric acid and ALP
- CXR and abdominal US every visit (abdominal US only in abdominal and pelvic primary)
- U-catecholamine
- Skeletal survey is only advised if the clinical information indicates an MIBG scan or bone scan

The follow up schedule:
End of treatment
3 monthly for 2 years
Then 6 monthly for 3 years
Then 6 monthly for 3 years
Then yearly thereafter

Stellenbosch University https://scholar.sun.ac.za

17. STATISTICAL CONSIDERATIONS

17.1) Overview:

The hypothesis of the study that will be addressed is an improved survival outcome with the national protocol and identification of treatment risk factors as well as a correlation between clinical aspects and special investigations. The aim of the statistical design and analysis of this study is to evaluate a response-based approach to therapy of NB in childhood.

17.2) Accrual and study duration:

Based on accrual rates from the South Africa Children's Tumour Registry [14] the anticipated contribution will be a minimum 35-40 patients per year with an expected 70 -80 patients over 2 years and 200 patients over 5 years.

The duration of the study will be to adequately study 2 year and 5 year survival outcomes with the following study end points:

- **Efficacy endpoints**

  The endpoint for analysis is event-free survival (EFS) and overall survival (OS). For the palliative group progression free survival (PFS) will be a primary endpoint.

  This will include the minimum time from study entry, time of response assessment or treatment failure i.e. disease progression, disease recurrence, biopsy positive residual after completion of all protocol therapy, occurrence of a second malignant neoplasm, or death from any cause.

- **Toxicity endpoints**

  The primary endpoint for toxicity analysis will be occurrence of any mayor toxicity that prevents further oncological management or cause a toxic death. Toxicity will be defined according to The National Cancer Institute Common Terminology criteria for Adverse events version 5.0 [202].

17.3) Data collection:

Data will be collected by each individual unit and collated in a central, anonymous database managed by the principle investigator.

17.4) Data analysis:

- Statistics will be done by a qualified statistician associated with the University of the primary investigators
- Data will be analysed using Stata 13.1 SE (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.).
- Continuous variables will be summarised using mean and standard deviation (SD). If any continuous variables are skewed, then medians and interquartile ranges will be

Stellenbosch University https://scholar.sun.ac.za

presented instead. Categorical data will be presented using frequency tables. Association between two categorical variables will be assessed using the standard Pearson's chi-square ($\chi 2$) test. If expected cell count in the cross tabulation contains fewer than 5 observations (sparse numbers) then the Fisher's exact test will be employed. The standard t-test will be used to compare the mean of continuous explanatory variables by dichotomous outcome classification (e.g. mortality or remission). If the normality assumption is not upheld, then the non-parametric equivalent Wilcoxon rank-sum test (Mann Whitney U test) will be used instead. Comparison of means by 3 or more groups (e.g. risk classification of low, intermediate, high) will be performed using one-way analysis of variance (ANOVA) with post hoc pairwise comparison using Bonferroni correction. If the data are not normal, then the Kruskal-Wallis equality-of-populations rank test will be used instead. Time to event (survival) analysis will also be employed.

- Kaplan-Meier survival curves will be developed and differences by groups assessed using a log rank test. This will be extended to a multivariable proportional hazards (Cox) regression model to adjust for confounding factors and test for potential interactions. The proportional hazards assumption of the model will be assessed to confirm adequacy. If this assumption is not upheld, then a semi-parametric and/or parametric survival model will be used instead. An adjusted p-value of <0.05 will be considered statistically significant.

18. DISSEMINATION PLAN

- The information will be used in the PhD thesis of the principal investigator
- The aim is to publish findings with the PhD student as first author in collaboration with participating members of the SACCSG.
- The principal investigator will present the information at international and national meetings under the SACCSG.

19. FUNDING

The treatment is current standard treatment available in all government and private sector hospitals. No additional funding is required as it is in line with the current standard of care, although there is currently not a uniform treatment protocol, but the same medicines are used in the current treatment protocols of international origin.

Stellenbosch University https://scholar.sun.ac.za

20. CONFLICT OF INTEREST

The investigators wish to declare no conflict of interest in terms of this study.

21. ETHICAL CONSIDERATIONS

All research conducted during this study will be done according to the benchmarks for research in LMIC [204].

Collaborative partnership:
The research is a national, multi departmental study which includes multiple hospital sites. The study is a joint partnership between pediatric oncologists, radiologists, surgeons, nuclear medicine physicians as well as staff active in the care of patients and diagnostics such as laboratories.

Social value:
The proposed management protocols will be sustainable treatment interventions. Future suggestions for management adaptations will benefit the South African population and needs.

Scientific validity:
The risk of intervention and scientific validity is according to acceptable standard medical care for all children diagnosed with NB. All treatments and palliation will be standard of international care with practical application to South African health care guidelines and resource restraints.

Study results will be given to participating parties and the data will be discussed in academic meetings and congresses, thesis purposes and published in peer reviewed journals.

Fair selection of study population:
All patients diagnosed with NB will be invited to participate in the study as part of a fair selection process.

Favourable risk benefit ratio:
Untreated NB is a terminal disease. The aim of the study is to improve outcomes as well as improve palliative care for patients without further treatment options. All interventions will only be done to benefit the patient and treatment above the negative effects of the treatment or interventions on the patient.

Independent review:
Applications for ethical approval will be submitted at the Health Research Evaluation Committee of respective universities attached to participating POUs. The ethical approval from respective Departments of Health will be obtained. The study will be open for regular review or audits.

Stellenbosch University https://scholar.sun.ac.za

Informed consent:
Written consent will be obtained from participant's caretakers as well as assent in children older than 7 years old. Assent from children below 7 years of age will not be taken, however the willingness to participate in the management will be taken into consideration. Participation in the study is voluntary. Participants or their parents may withdraw at any stage of the study. All consent and assent will be obtained according to age, education and language sensitive requirements.

Respect for the recruited participants and study communities:
Where there is the possibility of discomfort or emotional distress all efforts will be made to minimize the discomfort without hazardous effects.

Each patient will receive a unique study number. A list linking the name and the unique study number will be kept separate in the individual POU and not be used in the analysis. The data will be collected prospectively and analysed without the name or any identifiable data of the individual patient to ensure confidentiality.

The electronic information will be kept in each POU on a password protected computer that will only be accessible by the staff participating in the study. The main data base will be kept by the principle investigator at Grey's Hospital on a password protected computer.

Human tissue and biological samples will be collected and treated according to the ethical guidelines of the ethical committees of participating Universities and guidelines of the Health Professions Council of South Africa.

All interventions and management recommendations will be done with consideration to social and cultural aspects of each patient.

All investigator are GCP compliant.

22. APPENDICES

Appendix A: Informed Consent

Appendix B: B1 Informed Assent Age 7-13

B2 Informed Assent Age 13-16

Appendix C: Pathology Form

Appendix D: Bone Marrow aspirate and trephine form

Appendix E: Patient Clinical Record Form (CRF)

Appendix F: Image Defined Risk Factor (IDRF)

Appendix G: International Neuroblastoma Risk Group (INRG) Consensus pre-treatment staging system (INRGSS)

Appendix H: International Neuroblastoma Staging System (INSS)

Stellenbosch University https://scholar.sun.ac.za

Stellenbosch University https://scholar.sun.ac.za

24. REFERENCES

1. Smith, A; Altekruse, S; Adamson, P; et al. Declining childhood and adolescent cancer mortality. Cancer 2014;120 (16): 2497-506.
2. National Cancer Institute: Surveillance, Epidemiology and End Results Database http://seer.cancer.gov (Accessed Nov 2015);
3. National Cancer Institute: Neuroblastoma http://www.cancer.gov/types/neuroblastoma (Accessed Nov 2015);
4. Bhatnagar, S; Sarin, Y. Neuroblastoma: A Review of Management and Outcome. Indian J Pediatr 2012; 79 (6):787-792;
5. Parikh, N; Howard, S; Chantada, G; Israels, T; Khattab, M; Alcasabas, P; Lam, C; Faulkner, L; Park, J; London, W; Matthay, K: SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for neuroblastoma in low- and middle-income settings. Pediatr Blood Cancer 2015; 62:1305-1316.
6. Maris, J; Hogarty, M; Bagatell, R; Cohn, S.: Neuroblastoma. Lancet 2007; 369: 2106-20.
7. Heck, J; et al.: The epidemiology of Neuroblastoma: a review. Paediatric and Perinatal Epidemiology 2009; 23: 125–143.
8. Lanzkowsky, P. Manual of Pediatric Hematology and Oncology 5th Edition. Cambridge: Academic Press, Elsevier. 2011.p671-672.
9. Miller, R. No neuroblastoma in Zaire. Lancet 1989; 2(8669): 978–9.
10. Parkin, D. Cancer in Africa: Epidemiology and Prevention IARC Scientific Publications; 2003 No.153, IARC, Lyon, France.
11. Ries, L; et al. Cancer Incidence and Survival among Children and Adolescents: United States SEER Program 1975–1995, National Cancer Institute, SEER Program. NIH Pub 1991; No. 99-4649 Bethesda, MD.
12. Stiller, C; et al. Childhood cancer and ethnic group in Britain: a United Kingdom Children's Cancer Study Group (UKCCSG) study. Br J Cancer 1991; 64(3): 543–8.
13. Latorre, V; Diskin, S; Diamond, M; et al.: Replication of neuroblastoma SNP association at the BARD1 locus in African-Americans. Cancer Epidemiol Biomarkers Prev 2012; 21(4): 658-663.
14. Stefan, D; Stones, D; Wainright, R; Kruger, M; Davidson, A; Poole, J; Hadley, G; Forman, D; Colombet, M; Steliarova-Foucher, E. Childhood cancer incidence in South Africa, 1987–2007. S Afr Med J 2015; 105(11): 939-947.
15. Mossé, Y; Greshock, J; King, A; et al.: Identification and high-resolution mapping of a constitutional 11q deletion in an infant with multifocal neuroblastoma. Lancet Oncol 4 (12): 769-71, 2003.
16. Satgé D, Moore SW, Stiller CA, et al.: Abnormal constitutional karyotypes in patients with neuroblastoma: a report of four new cases and review of 47 others in the literature. Cancer Genet Cytogenet 147 (2): 89-98, 2003.
17. Mossé, Y; Laudenslager, M; Longo, L; et al.: Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 455 (7215): 930-5, 2008.
18. Mossé, Y; Laudenslager, M; Khazi, D; et al.: Germline PHOX2B mutation in hereditary neuroblastoma. Am J Hum Genet 75 (4): 727-30, 2004.

Stellenbosch University https://scholar.sun.ac.za

19. Sanai, N; Alvarez-Buylla, A; Berger, M. Mechanisms of disease: neural stem cells and the origin of gliomas. The New England Journal of Medicine 2005. 353(8): 811–822.
20. De Weille, J. On the genesis of Neuroblastoma and Glioma. International Journal of Brain Science. Volume 2014. 1-4.
21. Melotte, V; Qu, X; Ongenaert, M et al. The N-myc downstream regulated gene (NDRG) family: diverse functions, multiple applications. The FASEB Journal 2010, 24(11) 4153–66.
22. Jiang, M; Stanke, J and Lahti, J. The connections between neural crest development and Neuroblastoma. Current Topics in Developmental Biology vol. 94, chapter 4, page 77–127, Elsevier, New York, NY, USA, 2011.
23. Mohlin, S; Wigerup, C; P˚ahlman, S. Neuroblastoma aggressiveness in relation to sympathetic neuronal differentiation stage. Seminars in Cancer Biology 2011, 21(4): 276–282.
24. Pizzo PA, Poplack DG, editors. Neuroblastoma, Chapter 30 p773-774. Principles and practice of pediatric oncology, 7th edition. Philadelphia: Lippincott-Williams and Wilkens, 2015.
25. Mosse, Y; Diskin, S; Wasserman, N; Rinaldi, K; Attiyeh, E; Cole, K; Jagannathan, J; Bhambhani, K; Winter, C; Maris, J. Neuroblastomas have distinct genomic DNA profiles that predict clinical phenotype and regional gene expression. Genes Chromosom. Cancer 2007; 46: 936–949.
26. Kogner, P; et al.: Coexpression of Messenger RNA for TRK Protooncogene and Low Affinity Nerve Growth Factor Receptor in Neuroblastoma with Favorable Prognosis. Cancer Res 1993; (53) (9): 2044-2050.
27. Nakagawara, A; Azar, C; Scavarda, N; Brodeur, G. Expression and function of TRK-B and BDNF in human neuroblastomas. Molecular and Cellular Biology. 1994;14(1):759-767.
28. Tang, X; et al: The MYCN enigma: significance of MYCN expression in Neuroblastoma. Cancer Res 2006; 66: 2826-33.
29. Lastowska; M, Cullinane, C; Variend, S; et al.: Comprehensive genetic and histopathologic study reveals three types of neuroblastoma tumors. J Clin Oncol 2001; 19 (12): 3080-90.
30. Pinto, N; Gamazon, E; Antao, N; Myers, J; et al. Integrating Cell-Based and Clinical Genome-Wide Studies to Identify Genetic Variants Contributing to Treatment Failure in Neuroblastoma Patients. Clinical Pharmacology & Therapeutics 2014; 95: 644–652.
31. Cohn, S; Diagnosis and classification of Small round-cell tumors of childhood. American Journal of Pathology 1999; 155(1): 2181–2182.
32. Brodeur, G; Seeger, R; Barrett, A; et al.: International criteria for diagnosis, staging, and response to treatment in patients with neuroblastoma. J Clin Oncol 1988; 6 (12): 1874-81.
33. Pizzo PA, Poplack DG, editors. Neuroblastoma, Chapter 30 p886-922. Principles and practice of pediatric oncology, 6th edition. Philadelphia: Lippincott-Williams and Wilkens, 2011.
34. Peuchmaur, M; d'Amore, E; Shimada, H. Revision of Neuroblastoma International Classification. Cancer 2003; 98(10):2274-2281
35. Shimada, H; Ambros, I et al. The International Neuroblastoma Pathology Classification (the Shimada system). Cancer Jul 1999; 15;86(2): 364-72.
36. Shimada, H; Umehara, S et al. International Neuroblastoma Pathology Classification for the prognostic evaluation of patients with peripheral neuroblastic tumours. Cancer Nov 2001; 92(9): 2451-2461.
37. Cheung, I; Barber, D; Cheung, n. Detection of microscopic Neuroblastoma in marrow by histology, immunocytology, and reverse transcription-PCR of multiple molecular markers. Clin Cancer Res 1998; 4:2801-2805.

Stellenbosch University https://scholar.sun.ac.za

38. Seeger, R; Reynolds, C; Gallego, R, et al. Quantitative tumour cell content of bone marrow and blood as a predictor of outcome in stage IV Neuroblastoma: a Children's Cancer Group Study. J Clin Oncol 2000; 18:4067-4076.
39. Bingle, L; Brown, J; Lewis, E. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol. 2002 Mar;196(3):254-65.
40. Asgharzadeh, S; Salo, J; Ji, L; Oberthuer, A; et al. Clinical significance of tumor-associated inflammatory cells in metastatic Neuroblastoma. J Clin Oncol 2012 Oct;30(28):3525-32.
41. Buddingh, E; Kuijjer, M; Lankester, A; Cleton-Jansen, A. Tumor-Infiltrating Macrophages Are Associated with Metastasis Suppression in High-Grade Osteosarcoma: A Rationale for Treatment with Macrophage Activating Agents. Clin Cancer Res April 15, 2011; 17:2110.
42. Hadjidaniel, M; et al. Abstract 3668: Tumor-associated macrophages (TAMs) promote murine neuroblastoma tumor growth through upregulation of MYC and independent of IL6 expression. Proceedings: AACR Annual Meeting 2014; April 5-9, 2014; San Diego, CA. Cancer Res Oct 2014; 74: 3668.
43. Ribatti, D; Marimpietri, D; Pastorino, F; Brignole, C; Nico, B; Vacca, A; Ponzoni; M. Angiogenesis in neuroblastoma. Ann N Y Acad Sci. 2004; 1028:133-42.
44. Choudhury, S; Karmakar, S; Banik, N; Ray, S. Targeting Angiogenesis for Controlling Neuroblastoma. Journal of Oncology 2012, Article ID 782020, 15 pages, 2012.
45. Bourdeaut, F; de Carli, E; Timsit, S; et al.: VIP hypersecretion as primary or secondary syndrome in neuroblastoma: A retrospective study by the Société Française des Cancers de l'Enfant (SFCE). Pediatr Blood Cancer 52 (5): 585-90, 2009.
46. Matthay, K; Blaes, F; Hero, B; et al.: Opsoclonus myoclonus syndrome in neuroblastoma a report from a workshop on the dancing eyes syndrome at the advances in neuroblastoma meeting in Genoa, Italy, 2004. Cancer Lett 228 (1-2): 275-82, 2005.
47. Mahoney, N; Liu, G; Menacker, S; et al.: Pediatric horner syndrome: etiologies and roles of imaging and urine studies to detect neuroblastoma and other responsible mass lesions. Am J Ophthalmol 142 (4): 651-9, 2006.
48. Mossé, Y; Greshock, J; King, A; et al.: Identification and high-resolution mapping of a constitutional 11q deletion in an infant with multifocal neuroblastoma. Lancet Oncol 4 (12): 769-71, 2003.
49. Scott, J; Morgan, E. Coagulopathy of disseminated neuroblastoma. The Journal of Pediatrics 1983; 103(2): 219-222.
50. Dippenaar, A. Bleeding in children with cancer. CME 2010; 28(7): 343-347.
51. Tsubono, Y; Hisamichi, S. Letter to editor: A Halt to Neuroblastoma Screening in Japan. 2004 May; N Engl J Med; 350: 2010-2011.
52. Stiller, C; Parkin, D. International variations in the incidence of Neuroblastoma. Int J Cancer 1992; 52(4): 538–43.
53. Monsaingeon, M; Perel, Y; Simonnet, G; Corcuff, J. Comparative values of catecholamines and metabolites for the diagnosis of neuroblastoma. European Journal of Pediatrics. 2003: 162(6), 397-403.
54. Volker Strenger, V; Kerbl, R; Dornbusch, H; Ladenstein, R; Ambros, P; Ambros, I; Urban, C. Diagnostic and prognostic impact of urinary catecholamines in neuroblastoma patients. Pediatric Blood and Cancer 2007; 48(5): 504-509.
55. Zambrano, E; Reyes-Múgica, M. Hormonal activity may predict aggressive behavior in neuroblastoma. Pediatr Dev Pathol. 2002; 5(2):190-9.

Stellenbosch University https://scholar.sun.ac.za

56. Hsiao, R; Seeger, R; Yu, A; O'Connor, D. Chromogranin A in children with Neuroblastoma: Serum Concentration Parallels Disease Stage and Predicts Survival. J. Clin. Invest. 1990; 85: 1555-1559.
57. Papac, R. Bone marrow metastasis: a review. Cancer 1994; 74(9): 2403-2413.
58. Burchill, S; Beiske, K; Shimada, H; Ambros, P; Seeger, R; Tytgat, G; Brock, P; Haber, M; Park, J; Berthold, F. Recommendations for the standardization of bone marrow disease assessment and reporting in children with neuroblastoma on behalf of the International Neuroblastoma Response Criteria Bone Marrow Working Group. Cancer 2017; 123(7):1095-1105.
59. De Bernardi, B; Mosseri, V; Rubie, H; et al. Treatment of localised resectable neuroblastoma. Results of the LNESG1 study by the SIOP Europe Neuroblastoma Group. Br J Cancer 2008; 99:1027–1033.
60. Howman-Giles, R; Gilday, D; Ash, J. Radionuclide skeletal survey in neuroblastoma. Radiology 1979; 131:497–502.
61. Baker, M; Siddiqui, A; Provisor, A; Cohen, M. Radiographic and scintigraphic skeletal imaging inpatients with neuroblastoma: Concise communication. J Nucl Med 1983; 24:467–469.
62. Kushner, B. Neuroblastoma: A disease requiring a multitude of imaging studies. J Nucl Med. 2004 Jul;45(7):1172-88.
63. Papaioannou, G; Hugh, K. Neuroblastoma in childhood: review and radiological findings. Cancer Imaging (2005) 5, 116–127.
64. Dumba, M; Jawad, N; McHugh, K. Neuroblastoma and nephroblastoma: a radiological review. Cancer Imaging (2015) 15:5.
65. Katzenstein, H; Cohn, S; Shore, R et al. Scintigraphic Response by 123I-Metaiodobenzylguanidine Scan Correlates with Event-Free Survival in High-Risk Neuroblastoma. J Clin Oncol 2004; 22:3909-3915.
66. Bleeker, G; Tytgat, G; Adam, J; Caron, H; Kremer, L: Hooft, L; van Dalen, E. 123I-MIBG scintigraphy and 18F-FDG-PET imaging for diagnosing neuroblastoma (Review). The Cochrane Database of Syst Rev 2015;9.
67. Bleeker, G; van Eck-Smit, B; Zwindermann, K; et al. MIBG scans in patients with stage 4 neuroblastoma reveal two metastatic patterns, one is associated with MYCN amplification and in MYCN-amplified tumours correlates with a better prognosis. Eur J Nucl Med Mol Imaging (2015) 42:222–230.
68. Fawzy, M; Moussa, E; Hamoda, A; Zaher, A; Hassanain, A; Azmy, S. PET/CT and MIBG scans in diagnosis and management of Neuroblastoma.Journal of solid tumours 2015; 2(5):32-40
69. Yanik, G; Parisi, M; Shulkin, B; et al.: Semiquantitative mIBG scoring as a prognostic indicator in patients with stage 4 neuroblastoma: a report from the Children's oncology group. J Nucl Med 2013; 54 (4): 541-8.
70. Decarolis, B; Schneider, C; Hero, B; et al.: Iodine-123 metaiodobenzylguanidine scintigraphy scoring allows prediction of outcome in patients with stage 4 neuroblastoma: results of the Cologne interscore comparison study. J Clin Oncol 2013; 31 (7): 944-51.
71. Bleeker, G; van Eck-Smit, B; Zwinderman, K; et al. MIBG scans in patients with stage 4 neuroblastoma reveal two metastatic patterns, one is associated with MYCN amplification and in MYCN-amplified tumours correlates with a better prognosis. Eur J Nucl Med Mol Imaging 2015; (42) 2:222-230.

Stellenbosch University https://scholar.sun.ac.za

72. Weinstein, J; Katzenstein, H; Cohn, S. Advances in the diagnosis and treatment of Neuroblastoma. The Oncologist. 2003; 8:278-292.
73. Monclair T, Brodeur GM, Ambros PF, et al.: The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. J Clin Oncol 27 (2): 298-303, 2009.
74. Parikh, N; Sholler, G; Roberts, S; Howard, S; Shochat, S; Haase, G; Borker, A; Kurkure, P; Sachdeva, A; Matthay, K. Advancing diagnosis and treatment of neuroblastoma for children In Low income countries (LIC) via web-based tumor board platform. In: Robert Arceci JMD, Ph. D, Peter E, Newburger MD, editors. Auckland, New Zealand: Wiley Periodicals Inc; 2011. p. 733.
75. Cotterill, S; Pearson, A; Pritchard, J; et al.: Clinical prognostic factors in 1277 patients with neuroblastoma: results of The European Neuroblastoma Study Group 'Survey' 1982-1992. Eur J Cancer 36 (7): 901-8, 2000.
76. Hesseling PB, Ankone K, Wessels G, Schneider JW, Du Plessis, Moore L. Neuroblastoma in Southern Africa: Epidemiological features, prognostic factors and outcome. Ann Trop Paediatr 1999;19: 357–363.
77. Bagatelle, R; Beck-Popovic, M; London, W; Zhang, Y; et al. Significance of MYCN Amplification in International Neuroblastoma Staging System Stage 1 and 2 Neuroblastoma: A Report from the International Neuroblastoma Risk Group Database. Journal of Clinical Oncology 2009; 27 (3): 365-370.
78. Brodeur, G. Neuroblastoma: Biological insights into a clinical enigma. Nature Reviews: Cancer March 2003;3: 203-2016.
79. Halperin, E; Constine, L; Tarbell, N; Kun, L. Neuroblastoma, Chapter 6 p108-136. Pediatric Radiation Oncology, 5th edition. Philadelphia: Lippincott-Williams and Wilkins, 2011.
80. Maris, J; Hogarty, M; Bagatell, R; Cohn, S. Seminar: Neuroblastoma. Lancet. 2007; 369:2106-20.
81. Park, J; et al. Neuroblastoma: Biology, prognosis and treatment. Pediatr Clin North Am. 2008; 55:97-120.
82. Kushner, B; Wolden, S; LaQuaglia, M; et al. Hyperfractionated Low-Dose Radiotherapy for High-Risk Neuroblastoma After Intensive Chemotherapy and Surgery. JCO Jun 2001; 19(11): 2821-2828.
83. George, S; Tucker, E; Chesler, L. High-Risk Neuroblastoma: Current and Future Therapeutic Strategies. Ann Pediatr Child Health 2015; 3(8):1086-1091.
84. Nuchtern, J; London, W; Barnewolt, C; et al.: A prospective study of expectant observation as primary therapy for neuroblastoma in young infants: a Children's Oncology Group study. Ann Surg 2012; 256 (4): 573-80.
85. Perez, C; Matthay, K; Atkinson, J; et al. Biologic variables in the outcome of stages I and II neuroblastoma treated with surgery as primary therapy: a Children's Cancer Group study. J Clin Oncol 2000; 18:18–26.
86. Strother, D; London, W; Schmidt, M; et al.: Outcome after surgery alone or with restricted use of chemotherapy for patients with low-risk neuroblastoma: results of Children's Oncology Group study P9641. J Clin Oncol 2012; 30 (15): 1842-8.
87. Bowman, L; Castleberry, R; Cantor, A; et al. Genetic staging of unresectable or metastatic neuroblastoma in infants: a Pediatric Oncology Group study. J Natl Cancer Inst 1997; 89:373–380.

Stellenbosch University https://scholar.sun.ac.za

88. Schmidt, M; Lukens, J; Seeger, R; et al. Biologic factors determine prognosis in infants with stage IV neuroblastoma: a prospective Children's Cancer Group study. J Clin Oncol 2000; 18:1260–1268.
89. Iehara, T; Hamazaki, M; Tajiri, T; et al.: Successful treatment of infants with localized neuroblastoma based on their MYCN status. Int J Clin Oncol 2013; 18 (3): 389-95.
90. Baker, L; Schmidt, M; Cohn, S; et al.: Outcome after reduced chemotherapy for intermediate-risk neuroblastoma. N Engl J Med 2010; 363 (14): 1313-23.
91. Matthay, K; Perez, C; Seeger, R; et al. Successful treatment of stage III neuroblastoma based on prospective biologic staging: a Children's Cancer Group study. J Clin Oncol 1998; 16:1256–1264.
92. Rubie, H; De Bernardi, B; Gerrard, M; et al.: Excellent outcome with reduced treatment in infants with nonmetastatic and unresectable neuroblastoma without MYCN amplification: results of the prospective INES 99.1. J Clin Oncol 2011; 29 (4): 449-55.
93. Kohler, J; Rubie, H; Castel, V; et al.: Treatment of children over the age of one year with unresectable localised neuroblastoma without MYCN amplification: results of the SIOPEN study. Eur J Cancer 2013; 49 (17): 3671-9.
94. Hero, B; Simon, T; Spitz, R; et al.: Localized infant neuroblastomas often show spontaneous regression: results of the prospective trials NB95-S and NB97. J Clin Oncol 2008; 26 (9): 1504-10.
95. Neuroblastoma LINES Study, SIOP.
96. Minard' V; Hartmann, O; Peyroulet, M; et al.: Adverse outcome of infants with metastatic neuroblastoma, MYCN amplification and/or bone lesions: results of the French society of pediatric oncology. Br J Cancer 2000; 83(8): 973-9.
97. Greengard, E; et al.: Treatment of high-risk neuroblastoma in children: recent clinic trial results. Clin. Invest. 2013; 3(11): 1071–1081.
98. Ladenstein, R; Valteau-Couanet,D; Brock,P; Yaniv, I; Castel,V. Randomized Trial of Prophylactic Granulocyte Colony-Stimulating Factor During Rapid COJEC Induction in Pediatric Patients With High-Risk Neuroblastoma: The European HR-NBL1/SIOPEN Study. JCO 2010; 28(21): 3516-3524.
99. Peinemann, F; Tushabe, D; Berthold, F. Rapid COJEC versus standard induction therapies for high-risk Neuroblastoma. Cochrane Database of Systematic Reviews 2013, Issue 10.
100. Pearson, A; Pinkerton, C; Lewis, I; Imeson, J; Ellershaw, C; Machin, D; et al. High-dose rapid and standard induction chemotherapy for patients aged over 1 year with stage 4 neuroblastoma: a randomised trial. Lancet Oncology 2008; 9(3): 247–56.
101. Kushner, B; Laquaglia, M; Bonilla, M; et al. Highly effective induction therapy for stage 4 neuroblastoma in children over 1 year of age. J. Clin. Oncol 1994; 12(12): 2607–2613.
102. Kushner, B; Kramer, K; Laquaglia, M; Modak, S; Yataghene, K; Cheung, N. Reduction from seven to five cycles of intensive induction chemotherapy in children with high-risk neuroblastoma. J. Clin. Oncol 2004; 22(24): 4888–4892.
103. HR-NBL-1.7/SIOPEN study
104. Matthay, K; Reynolds, C; Seeger, R et al: Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: A Children's Oncology Group Study. J Clin Oncol 2009; 27: 1007– 1013.
105. George, S; Tucker, E; Chesler, L. High-Risk Neuroblastoma: Current and Future Therapeutic Strategies. Ann Pediatr Child Health 2015; 3(8):1086-1091.

Stellenbosch University https://scholar.sun.ac.za

106. Berthold, F; Boos, J; Burdach, S; Erttmann, R; Henze, G; Hermann, J; Klingebiel, T; Kremens, B; Schilling, F; Schrappe, M; Simon, T; Hero, B. Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: A randomised controlled trial. Lancet Oncol 2005; 6: 649–658.

107. Kohler, J; Imeson, J; Ellershaw, C; Lie, S.: A randomized trial of 13-Cis retinoic acid in children with advanced neuroblastoma after high-dose therapy. British Journal of Cancer 2000; 83(9): 1124–1127.

108. Nickerson, H; Matthay, K; Seeger, R; et al.: Favorable biology and outcome of stage IV-S neuroblastoma with supportive care or minimal therapy: a Children's Cancer Group study. J Clin Oncol 2000; 18 (3): 477-86.

109. Mullassery, D; Farrelly, P; Losty, P. Does aggressive surgical resection improve survival in advanced stage 3 and 4 neuroblastoma? A systematic review and meta-analysis. Pediatr Hematol Oncol 2014, 31 (8): 703-16.

110. La Quaglia, M; Kushner, B; Su, W; Heller, G; Kramer, K; Abramson, S; Rosen, N; Wolden, S; Cheung, N. The impact of gross total resection on local control and survival in high-risk neuroblastoma. J Pediatr Surg.2004 Mar;39(3):412-7.

111. Chamberlain, R; Quinones, R; Dinndorf, P;et al. Complete surgical resection combined with aggressive adjuvant chemotherapy and bone marrow transplantation prolongs survival in children with advanced Neuroblastoma. Ann Surg Oncol 1995; 2:93-100.

112. Kiely, E. The surgical challenge of Neuroblastoma. J Pediatr Surg 1994; 29:128-133.

113. Nakagawara, A; Ikeda, K; Yokoyama, T; et al. Surgical aspects of MYCN oncogene amplification of NB. Surgery 1988;104:34-40.

114. Matthay, K; Sather, H; Seeger, R; Haase, G; Hammond, G. Excellent outcome of stage II neuroblastoma is independent of residual disease and radiation therapy. JCO 1989; 7(2): 236-244.

115. Haas-Kogen, D; Swift, P; Selch, M;et al. Impact for radiotherapy for high-risk Neuroblastoma: a Children's Cancer Group study. Int J Radiat Oncol Biol Phys 2003; 56:28-39.

116. Grupp, S; Asgerzadeh, S; Gregory, Y. Neuroblastoma: Issues in transplantation. Biol Blood Marrow Transplant. Jan 2012; 18(1 Suppl): S92–S100.

117. Ladenstein, R; Pötschger, U; Hartman, O; Pearson, A; et al. 28 years of high-dose therapy and SCT for Neuroblastoma in Europe: lesson from more than 4000 procedures. Bone Marrow Transplantation 2008; 41, S118- S127.

118. Halperin, E; Cox, E. Radiation therapy in the management of neuroblastoma: the Duke University Medical Center experience 1967-1984. Int J Radiat Oncol Biol Phys. Oct1986; 12(10):1829-37.

119. Paulino, A. Palliative radiotherapy in children with Neuroblastoma. Pediatr Hematol Oncol. 2000; 20(2):111-7.

120. Pritchard, J; Cotterill, S; Germond, S; Imeson, J; de Kraker, J; Jones, D; High dose melphalan in the treatment of advanced neuroblastoma: Results of a randomised trial (ENSG-1) by the European Neuroblastoma Study Group. Pediatr Blood Cancer 2005; 44:348–357.

121. Park JR, Kreissman SG, London WB, et al. A phase III randomized clinical trial (RCT) of tandem myeloablative autologous stem cell transplant (ASCT) using peripheral blood stem

Stellenbosch University https://scholar.sun.ac.za

cell (PBSC) as consolidation therapy for high-risk neuroblastoma (HR-NB): A Children's Oncology Group (COG) study. J Clin Oncol. 2016; 34 (suppl; abstr LBA3).

122. Ladenstein, R; et al. J Clin Oncol. 2011;29 (suppl; abstr 2).
123. Park, J; et al. J Clin Oncol. 2016;34 (suppl; abstr LBA3).
124. Kohler, J; Imeson, J; Ellershaw, C; Lie, S.: A randomized trial of 13-Cis retinoic acid in children with advanced neuroblastoma after high-dose therapy. British Journal of Cancer 2000; 83(9): 1124–1127.
125. Cash, T; Alazraki, A; Qayed, M; Katzenstein, H. Prolonged Isotretinoin in Ultra High-Risk Neuroblastoma. J Pediatr Hematol Oncol. 2017; 39(1):e33-e35.
126. Matthay, K; Villablanca, J; Seeger, R; Stram, D; Harris, R; Ramsay, N; Swift, P; Shimada, H; Black, C; Garrett, M; Brodeur, M; Gerbing, R; Reynolds, C. Treatment of high risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-Cis-retinoic acid. N Eng J Med 1999; 341: 1165–1173.
127. Matthey, K. Treatment options for high risk Neuroblastoma in LMIC. Presented at: SIOP Africa; 5-7 April 2017; Marrakech, Morocco.
128. Matthey, K; et al: Promising therapeutic targets in Neuroblastoma. Clin Cancer Res 2012; 18(10): 2740-53.
129. Xue, C; et al: Small molecule drugs and targeted therapies for Neuroblastoma. www.intechopen.com (accessed Nov 2015).
130. Matthay, K; Quach, A; Huberty, J; et al: Iodine-131-metaiodobenzylguanidine double infusion with autologous stem-cell rescue for neuroblastoma: A New Approaches to Neuroblastoma Therapy phase I study J Clin Oncol 2009; 27: 1020– 1025.
131. de Kraker, J; Hoefnagel, K; Verschuur, A; et al.: Iodine-131-metaiodobenzylguanidine as initial induction therapy in stage 4 neuroblastoma patients over 1 year of age. Eur J Cancer 2008; 44:551-556.
132. Bahl, A; Bakhashi, S. Metronomic Chemotherapy in Progressive Pediatric Malignancies: Old Drugs in New Package. Indian J Pediatr. 2012 April; Special article.
133. Fousseyni, T; Diawana, M; Pasquier, E; André, N. Children treated with metronomic chemotherapy in a low-income country: METRO-MALI-01. J Pediatr Hematol Oncol. 2011; 33:31–4.
134. Xu, T; Xiao, X; Zheng, S; Zheng, J; Zhu, H; Ji, Y; Yang, S. Antiangiogenic effect of propranolol on the growth of the neuroblastoma xenografts in nude mice. Pediatr Surg. 2013 Dec;48(12):2460-5.
135. Karasawa T, Steyger PS. An integrated view of cisplatin-induced nephrotoxicity and ototoxicity. Toxicol Lett 2015; 237(3): 219-27.
136. Wallace, K. Doxorubicin-Induced Cardiac Mitochondrionopathy. Pharmacology & Toxicology 2003; 93: 105–115.
137. Cohen, L; et al. Late effects in children treated with intensive multimodal therapy for high-risk neuroblastoma: High incidence of endocrine and growth problems. Bone Marrow Transplantation 2014; 49, 502–508.
138. Federico, S; Allewelt, H; et al. Subsequent Malignant Neoplasms in Pediatric Patients Initially Diagnosed with Neuroblastoma. J Pediatr Hematol Oncol. 2015; 37(1): e6–e12.
139. Saarinen-Pihkala, U; Jahnukainen, K; Wikström, S; Koivusalo, A; Karikoski, R; Sariola, H; Hovi, L. Ultrahigh-risk group within the high-risk neuroblastoma category. J Pediatr Hematol Oncol 2013; 35(6):e254-9.

Stellenbosch University https://scholar.sun.ac.za

140. von Stedingk, K; De Preter, K; Vandesompele, J; Noguera, R; Øra, I; Koster, J; Versteeg, R; Påhlman, S; Lindgren, D; Axelson, H. Individual patient risk stratification of high-risk neuroblastomas using a two-gene score suited for clinical use. Int J Cancer. 2015; 137(4): 868-77.
141. Tadeo, I; Berbegall, A; Castel, V; Garcı´a-Miguel, P; Callaghan, R; Påhlman, S; Navarro, S; Noguera, R. Extracellular matrix composition defines an ultra-high-risk group of neuroblastoma within the high-risk patient cohort. British Journal of Cancer (2016) 115, 480–489.
142. Burchill, A; Lewis, J; Abrams, K; et al.: Circulating neuroblastoma cells detected by reverse transcriptase polymerase chain reaction for tyrosine hydroxylase mRNA are an independent poor prognostic indicator in stage 4 neuroblastoma in children over 1 year. J Clin Oncol 19 (6): 1795-801, 2001.
143. George, E; Perez-Atayde, A; Yao, X; et al.: Tumor histology during induction therapy in patients with high-risk neuroblastoma. Pediatr Blood Cancer 59 (3): 506-10, 2012.
144. Kumar, H; Zhong, X; Rescorla, R; Hickey, R; Malkas, L; Sandoval, J. Proteomic approaches in Neuroblastoma: a complementary clinical platform for the future. Exper Rev. Proteomics 6(4), 387-394 (2009).
145. Viprey, V; Gregory, W; Corrias, M; et al. Neuroblastoma mRNAs predict outcome in children with Stage 4 Neuroblastoma: A European HR-NBL1/SIOPEN Study. J Clin Oncol. 2014 Apr; 32(10): 1074-1084.
146. Moreno, L; Curon, H; et al.: Accelerating drug development for neuroblastoma - New Drug Development Strategy: an Innovative Therapies for Children with Cancer, European Network for Cancer Research in Children and Adolescents and International Society of Paediatric Oncology Europe Neuroblastoma project. Expert Opin Drug Discov 2017; 12(8): 801-811.
147. Friedlander, T; et al. Looking back, to the future of circulating tumor cells. Pharmacology & Therapeutics 2014; 142 (3): 271-280.
148. Huetter, S; Walder; D, Brunner; C et al. Genome-wide analysis of liquid biopsies reveals a novel layer of tumorheterogeneity in neuroblastoma. [abstract]. In: Proceedings of the AACR Special Conference on Advances in Pediatric Cancer Research: From Mechanisms and Models to Treatment and Survivorship; 2015 Nov 9-12; Fort Lauderdale, FL. Philadelphia (PA): AACR; Cancer Res 2016;76(5 Suppl): Abstract nr B49.
149. Steliarova-Foucher, E; et al.: International incidence of childhood cancer, 2001–10: a population-based registry study. Lancet Oncol 2017; 18: 719–31.
150. Kruger M, Hendricks M, Davidson A, Stefan CD, van Eyssen AL, Uys R, van Zyl A, Hesseling P. Childhood cancer in Africa. Pediatr Blood Cancer 2014; 61(4): 587-92.
151. Hadley, G; Van Heerden, J. High-risk neuroblastoma in a sub-Saharan African country: telling it like it is. Trop Doct. 2017; 47(4):370-374.
152. Magrath, I; Steliarova-Foucher, E; Epelman, S; Ribeiro, R; Harif, M; Li, C; Kebudi, R; Macfarlane, S; Howard, S.: Paediatric cancer in low-income and middle-income countries. Lancet Oncol 2013;14: e104–e116.
153. Logie, D; Harding, R. An evaluation of a morphine public health programme for cancer and AIDS pain relief in Sub-Saharan Africa. BMC Public Health 2005; (5)82.
154. Parkin, D; Stiller, C; Draper, G; Bieber, C; The international incidence of childhood cancer. Int J Cancer 1988; 42:511–520.

Stellenbosch University https://scholar.sun.ac.za

155. Salman, Z; Kababri, M; Hessissen, L. An Intensive Induction Protocol for High Risk Neuroblastoma in Morocco. Journal of Global Oncology 2016 2:3 suppl, 80s-81s.
156. Monclair, T; Brodeur, G; Ambros, P; et al.: The International Neuroblastoma Risk Group (INRG) Staging System: An INRG Task Force Report. Journal of Clinical Oncology 2009; 27:2, 298-303.
157. Rubie, H; Michon, J; Plantaz, D; et al. Unresectable localized neuroblastoma: improved survival after primary chemotherapy including carboplatin and etoposide. Neuroblastoma Study Group of the Societe Francaise d'Oncologie Pediatrique (SFOP). British journal of cancer. 1998; 77(12):2310-7.
158. Yáñez et al. Two independent epigenetic biomarkers predict survival in neuroblastoma. Clinical Epigenetics (2015) 7:16 DOI 10.1186/s13148-015-0054-8
159. English, M; Skinner, R; Pearson, A; et al. Dose-related nephrotoxicity of carboplatin in children. British Journal of Cancer (1999) 81(2), 336–341.
160. http://www.health.gov.za/index.php/essential-drugs-programme-edp (Accessed February 2018)
161. Traore, F; Togo, B; Pasquier, E; Dembélé, A; André, N. Preliminary evaluation of children treated with metronomic chemotherapy and valproic acid in a low‐income country: Metro‐Mali‐02. Indian Journal of Cancer 2013; 50(3): 250-253.
162. Pasquier et al. Moving Forward with Metronomic Chemotherapy Translational Oncology 2011; 4: 203–211.
163. Wolte, J; Wolter, N; et al. Anti-tumor activity of the beta-adrenergic receptor antagonist propranolol in neuroblastoma. Oncotarget. 2014; 15;5(1):161-72.
164. Khalil, M; Hraběta, J; Groh, T; Procházka, P; Doktorová, H; Eckschlager, T. Valproic Acid Increases CD133 Positive Cells that Show Low Sensitivity to Cytostatics in Neuroblastoma. PLoS ONE 2016; 11(9): e0162916. doi:10.1371/journal.pone.0162916.
165. Pasquier et al. Moving Forward with Metronomic Chemotherapy Translational Oncology 2011; 4: 203–211.
166. Cook, Gary J.R. et al. The role of nuclear medicine in monitoring treatment in skeletal malignancy. Seminars in Nuclear Medicine 2001; 31 (3): 206 – 211.
167. Schwartz, G; Feld, L; Langford, D. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. J Pediatr 1984; 104(6): 849-54.
168. Schwartz, G; Gauthier, B. A simple estimate of glomerular filtration rate in adolescent boys. J Pediatr 1985; 106(3): 522-6.
169. Brock, P; Knight, K; Freyer, D; Campbell, K; Steyger, P; Blakley, B; Rassekh, S; Chang, K; Fligor, B; Rajput, K; Sullivan, M; Neuwelt, E. Platinum-induced ototoxicity in children: a consensus review on mechanisms, predisposition, and protection, including a new International Society of Pediatric Oncology Boston ototoxicity scale. J Clin Oncol 2012; 30(19): 2408-17.
170. Shimada, H; Ambros, I; Dehner, L; Hata, J; Joshi, V; Roald, B; Stram, D; Gerbing, R; Lukens, J; Matthay, K; Castleberry, R. The International Neuroblastoma Pathology Classification (the Shimada system). Cancer. 1999; 86(2):364-72.
171. Shimada, H; Umehara, S; Monobe, Y; Hachitanda, Y; Nakagawa, A; Goto, S; Gerbing, R; Stram, D; Lukens, J; Matthay, K. International neuroblastoma pathology classification for prognostic evaluation of patients with peripheral neuroblastic tumors: a report from the Children's Cancer Group. Cancer. 2001; 92(9): 2451-61.

Stellenbosch University https://scholar.sun.ac.za

172. Brodeur, G; Pritchard, J; Berthold, F; et al. Revisions of the International Criteria for Neuroblastoma Diagnosis, Staging, and Response to Treatment. Journal of Clinical Oncology 1993; 11(8): 1466-1477.
173. Cooper, R; Khakoo, Y; Matthay, K; et al. Opsocolonus-Myoclonus-Ataxia Syndrome in Neuroblastoma: Histopathologic Features - A Report from the Children's Cancer Group. Medical and Pediatric Oncology 2001; 36: 623-629.
174. Tornoczky, T; Semjen, D; Shimada, H; et al. Pathology of Peripheral Neuroblastic Tumors: Significance of Prominent Nucleoli in Undifferentiated/Poorly Differentiated Neuroblastoma. Pathology Oncology Research 2007;13(4):269-275.
175. Gray, K. Stanford School of Medicine. Surgical Pathology Criteria. www.surgpathcriteria.stanford.edu (Accessed January 2017).
176. Ambros, P; Mehes, G; Ambros, I; Ladenstein, R. Disseminated tumor cells in the bone marrow - chances and consequences of microscopical detection methods. Cancer Lett. 2003;197(1-2):29-34.
177. Aronica, P; Pirrotta, V; Yunis, E; Penchansky, L. Detection of neuroblastoma in the bone marrow: biopsy versus aspiration. J Pediatr Hematol Oncol. 1998;20(4):330-4.
178. Krishnan, C; Twist, C; Fu, T; Arber, A. Detection of Isolated Tumor Cells in Neuroblastoma by Immunohistochemical Analysis in Bone Marrow Biopsy Specimens Improved Detection with Use of β-Catenin. Am J Clin Pathol 2009; 131:49-57.
179. Burchill, S; Beiske, K; Shimada, H; Ambros, P; Seeger, R; Tytgat, G; Brock, P; Haber, M; Park, J; Berthold, F. Recommendations for the standardization of bone marrow disease assessment and reporting in children with neuroblastoma on behalf of the International Neuroblastoma Response Criteria Bone Marrow Working Group. Cancer 2017; 123(7):1095-1105.
180. Me´hes, G; Luegmayr, A; Kornmüller, R; et al. Detection of disseminated tumor cells in neuroblastoma: 3 log improvement in sensitivity by automatic immunofluorescence plus FISH (AIPF) analysis compared with classical bone marrow cytology. AmJ Pathol 2003; 163:393-399.
181. Brodeur, G; Pritchard, J; Berthold, F; Carlsen, N; Castel, V; Castelberry, R; et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncology 1993;11(8):1466– 77.
182. Park, J; Bagatell, R; Cohn, S; Pearson, A; Villablanca, J; Berthold, F; Burchill, S; Boubaker, S; McHugh, K; Nuchtern, J; London, J; Seibel, N; Lindwasser, O; Maris, J; Brock, P; Schleiermacher, G; Ladenstein, R; Matthay, K; Valteau-Couanet, D. Revisions to the International Neuroblastoma Response Criteria: A Consensus Statement from the National Cancer Institute Clinical Trials Planning Meeting. J Clin Oncol 2017; 35(22):2580-2587.
183. Eisenhauer; E, Therasse, P Bogaert, J; Schwartz, L; Sargent, D; Ford, R; Dancey, J; Arbuck, S; Gwyther, S; Mooney, M; Rubinstein, L; Shankar, L; Dodd, L; Kaplan, R; Lacombe, D; Verweij, J. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). European Journal of Cancer 2009; 45(2): 228-247.
184. Boogerd, W; van der Sande, J. Diagnosis and treatment of spinal cord compression in malignant disease. Cancer Treatment Rev. 1993; 19: 129.
185. https://www.kinderformularium.nl Accessed Dec 2017.
186. Kiely E. A technique for excision of abdominal and pelvic neuroblastoma. Ann Royal College Surg Engl 2007; 98(4):342–348.

Stellenbosch University https://scholar.sun.ac.za

187. Rees, H; Markley, M; Kiely, E; Pierro, A; Pritchard, J. Diarrhea after resection of advanced abdominal neuroblastoma: a common management problem. Surgery 1998; 123(5):568-572.

188. Von Allmen, D; Davidoff, M; London, W; Van Ryn, M; Haas-Kogan, D; Kreissman, S; Khanna, G; Rosen, N; Park, J; La Quaglia, M. Impact of Extent of Resection on Local Control and Survival in Patients from the COG A3973 Study with High-Risk Neuroblastoma. JCO Journal of Clinical Oncology 2017; 35(2): 208-216.

189. Bernardi, B; Mosseri, V; Rubie, H; et al. Treatment of localised resectable neuroblastoma. Results of the LNESG1 study by the SIOP Europe Neuroblastoma Group. Br J Cancer. 2008; 99:1027–1033.

190. Perez, C; Matthay, K; Atkinson, J; et al. Biologic variables in the outcome of stages I and II neuroblastoma treated with surgery as primary therapy: a children's cancer group study. J Clin Oncol. 2000; 18:18–26.

191. Haase, G; Atkinson, J; Stram, D; Lukens, J; Matthay, K. Surgical management and outcome of locoregional neuroblastoma: comparison of the Childrens Cancer Group and the international staging systems. J Pediatr Surg. 1995; 30:289–294.

192. Modak, S; Kushner, B; LaQuaglia, M; Kramer, K; Cheung, N. Management and outcome of stage 3 neuroblastoma. Eur J Cancer. 2009; 45:90–98.

193. Powis, M; Imeson, J; Holmes, S. The effect of complete excision on stage III neuroblastoma: a report of the European Neuroblastoma Study Group. J Pediatr Surg. 1996; 31:516–519.

194. Schweinitz, D; Hero, B; Berthold, F. The impact of surgical radicality on outcome in childhood neuroblastoma. Eur J Pediatr Surg. 2002; 12:402–409.

195. Allmen, D; Grupp, S; Diller, L; et al. Aggressive surgical therapy and radiotherapy for patients with high-risk neuroblastoma treated with rapid sequence tandem transplant. J Pediatr Surg. 2005; 40:936–941.

196. Matsumura, M; Atkinson, J; Hays, D; et al. An evaluation of the role of surgery in metastatic neuroblastoma. J Pediatr Surg. 1988; 23:448–453.

197. Browne, M; Kletzel, M; Cohn, S; Seshadri, R; Reynolds, M. Excellent local tumor control regardless of extent of surgical resection after treatment on the Chicago Pilot II protocol for neuroblastoma. J Pediatr Surg. 2006; 41:271–276.

198. Salim, A; Mullassery, D; Barry Pizer, B; McDowell, H; Losty, P. Neuroblastoma: A 20-year experience in a UK regional centre. Pediatr Blood Cancer 2011; 57:1254–1260

199. Englum, B; Rialon, K; Speicher, P; Gulack, B; Driscoll, T; Kreissman, S; Rice, H. Value of surgical resection in children with high-risk neuroblastoma. Pediatric Blood & Cancer 2015; 62(9): 1529-1535.

200. Monclair, T; Mosseri, V; Cecchetto, G; De Bernardi, B; Michon, J; Holmes, K. Influence of image-defined risk factors on the outcome of patients with localised neuroblastoma. A report from the LNESG1 study of the European International Society of Paediatric Oncology Neuroblastoma Group. Pediatr Blood Cancer 2015; 62: 1536–1542.

201. Gains et al. Intensity-modulated arc therapy to improve radiation dose delivery in the treatment of abdominal Neuroblastoma. Future Oncology 2013; 9: 439-449.

Stellenbosch University https://scholar.sun.ac.za

202. National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm (Accessed August 2017).
203. Hsu LL, Evans AE, D'Angio GJ. Hepatomegaly in neuroblastoma stage 4s: criteria for treatment of the vulnerable neonate. Med Pediatr Oncol. 1996; 27:521-8.
204. Emmanuel, E; et al. What Makes Clinical Research in Developing Countries Ethical? The Benchmarks of Ethical Research. Perspective 2004; 189: 930-93.

Stellenbosch University https://scholar.sun.ac.za

APPENDIX A

INFORMED CONSENT

Information Sheet and Consent to Participate in Research

Date_____

Dear parent /caregiver

You are being invited to consider participating in a study that involves research on behalf of your child (name of patient)_____ The aim and purpose of this research is to offer all children across South Africa standardised treatment according to the nature of their cancer and to use this information for research purposes. In your child's case it is a cancer that originates from the adrenal glands and the main nerve that supplies the adrenal gland called Neuroblastoma.

The study does not have any randomisation which means that you will not be assigned different treatments by chance but rather your child will receive what is considered to be standard of care according to the risk group of your child's clinical presentation. In Neuroblastoma this might include surgery with or without intensive chemotherapy, radiotherapy and a bone marrow transplant.

The study is expected to enrol 200 patients over 5 years and patients will be enrolled across the country at 11 different sites in both private and state hospitals where children with cancer are cared for. After 2 years an interim evaluation of the information will be done. The purpose of the study is to provide uniform care to all children with Neuroblastoma and to improve the outcomes of children with these cancers. None of the procedures that your child undergoes will be experimental.

Stellenbosch University https://scholar.sun.ac.za

Tissue obtained during diagnosis might be sent to other medical facilities for a second opinion and if needed retested to confirm the diagnosis or determine further information regarding your child's cancer. With this testing you will not incur additional costs.

You are free to withdraw from the study at any point. Your child's care will be no different whether you choose to participate in the study or not or once your child is part of the study, stay in the study or to leave. The intention of the study is to follow your child's progress through the treatment and then after treatment to at least five years from the end of therapy. This is to ensure that any complications which may arise at a later stage can be detected early and that we can intervene.

At this point the study is not externally funded and all therapy will be for according to the determinations of state payment scales or according to private medical funding if you belong to a private medical aid. The study itself does not incur any additional costs to you.

The study may involve no additional risk or discomfort outside of what would reasonably be expected in this instance. The treatment plan which involves surgery and in some cases chemotherapy or bone marrow transplant will be explained to you, you will receive a copy of the chemotherapy protocol as well as drug information sheets which explain possible drug complications (attached). Disclose in full any appropriate alternative procedures and treatment etc. that may serve as possible alternate options to study participation.

This study has been ethically reviewed and approved by an ethical committee:

_____

With approval number_____

In the event of any problems or concerns/questions you may contact the researcher at Jaques.vanheerden@uza.be or Human Research Ethics Committee contact details as follows:

RESEARCH ETHICS ADMINISTRATION

Address

Tel:

Stellenbosch University https://scholar.sun.ac.za

Fax:
Email:

It important for you to know that:

1. That participation in this research is completely voluntary.
2. That you may withdraw your participation at any point.
3. That in the event of refusal/withdrawal of participation you will not incur any penalty or loss of treatment or other benefit to which they are normally entitled.
4. No additional costs (outside of what would normally be expected) will be incurred by you as a result of participation in the study.
5. There are no financial incentives or reimbursements for participation in the study.
6. All your medical information will be completely confidential. All data will be de-identified using numerical descriptors. All data will be entered into a central database which will only be accessible to your local physician and the study co-ordinator / principal investigator.
7. After a certain period of time data will be analysed to assess whether the standardised treatment approach has improved survival of patients.
8. Information gathered from this study will be used to improve the care provided for children with Neuroblastoma across South Africa.

I (Name of caregiver) _____ have been informed about the study entitled:

Treatment of Neuroblastoma in South Africa, SACCSG NB-2017

By (Name of researcher) _____

I understand the purpose and procedures of the study.

I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

Stellenbosch University https://scholar.sun.ac.za

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at_____:

RESEARCHER

Principal Investigator

Room Paediatric Haematology Oncology Service, Koningin Mathilde Mother and Child Hospital, 10 Wilrijk Street, Edegem, Belgium, 2650

Tel +32 3 821 3000, jaques.vanheerden@uza.be

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

RESEARCH ETHICS ADMINISTRATION

Address

Tel:

Fax:

E-mail:

_____ _____

Signature of Participant Date

_____ _____

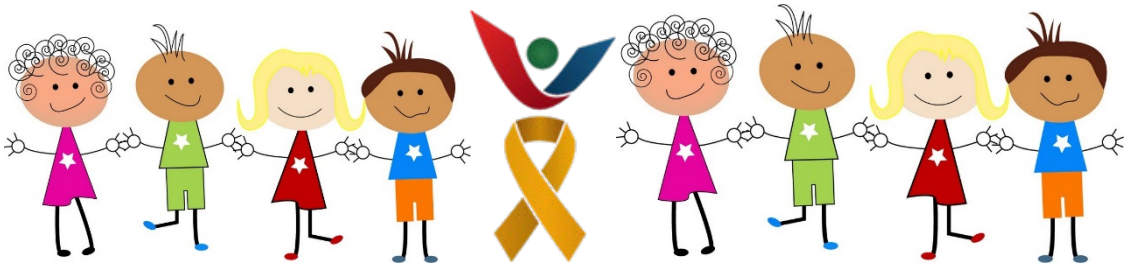
Signature of Witness Date

_____ _____

Signature of Translator Date

APPENDIX B

Stellenbosch University https://scholar.sun.ac.za



INFORMED ASSENT

Date:_____

Do you know what RESEARCH is?

Research is something we do to find new knowledge about the way things (and people) work. We use research projects or studies to help us find out more about diseases or illness. Research also helps us to find better ways of helping, or treating children who are sick.

What are we doing this research project?

I want to invite you to be part of a study that will treat children with your illness. We then use this information to learn more about your illness.

Why have I been invited to take part in this research project?

You have an illness called neuroblastoma and it is something growing in your body that should not be there. The treatment is given to stop the growth. This means we want to make you better.

Who is doing the research?

Doctor Jaques van Heerden from the Department of Paediatrics, Koningin Matilde Mother and Child Hospital in Belgium and Doctor _____, who is treating you.

What will happen to me in this study?

The treatment involves (taking blood, operations and medication) to make you better.

- The different drugs and their names are Carboplatin and/or Cisplatin, Etoposide, Vincristine and Cyclophosphamide. Sometimes we use Doxorubicin as well.

- How it is given? After applying some local anaesthetic to your skin (cream or patch) we will insert a needle in your arm on day 1 to give you the drugs through a drip which is a long plastic line attached to a bag of fluid. You will not feel the medication running

Stellenbosch University https://scholar.sun.ac.za

in apart from it being a little cold. It will not make your arm feel strange or sore. Sometimes this medication can make you feel nauseas or cause vomiting. To prevent that we will give you some medication in the drip before the chemotherapy starts to stop you from feeling nauseas and vomiting.

- The chemotherapy will take a few hours to run into your body.

Can anything bad happen to me?

You may have problems due to the drugs, which the doctors will help to manage. You need to inform the doctors if there is a problem so that they can help you. The problems are:

- Nausea and vomiting and being unable to eat. We will give you tablets or medicine in the drip to help with this problem.
- You may develop a fever and need antibiotics because the chemotherapy may lower your white blood cells. Your white blood cells are in your blood and are the soldiers of your body which fight infections.
- Tiredness, which is due to low red blood cells. Red blood cells are found in the blood and carry the oxygen from the lungs to the rest of the body. You may need a red blood cell transfusion.
- Bleeding due to low platelets. Platelets are found in the blood and normally form a plug to stop bleeding. You will know it as the scab that forms on a skin wound.
- Hair loss. The drugs cause the hair to stop growing. You will notice that your hair will begin to fall out about 2-3 weeks into chemotherapy. Fortunately, the hair will grow back normally after the drugs are stopped.
- Some of the medication can cause you not to hear well and therefore you will regularly tested for hearing.

Can anything good happen to me?

The medicine we give you will hopefully make your cancer small enough to remove so that you will be healthy again. It will also mean that once you feel better you will be able to see friends, start playing sport and be able to go back to school.

Stellenbosch University https://scholar.sun.ac.za

Will anyone know I am in the study?

The data will be kept confidential, which means nobody but the treating doctor will know who you are. Your name will not appear on the data sheet where we look whether you have become better.

Who can I talk to about the study?

You can speak to anyone but your doctor or your parents may be the best people to start off with. Feel free to ask any questions at any time. Sometimes writing down questions can help us to remember what to ask.

What if I do not want to do this?

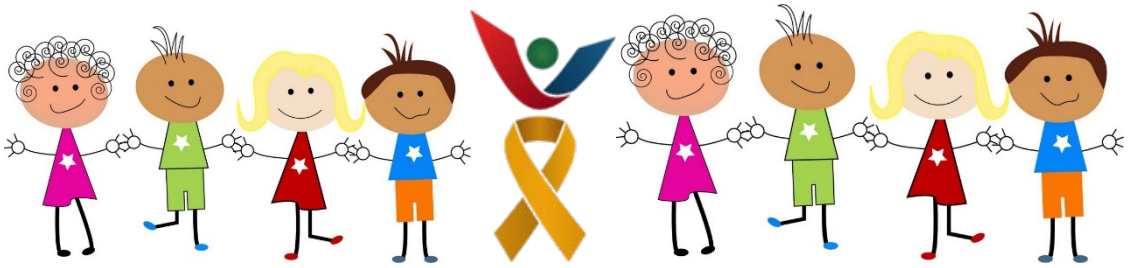
You get to choose whether or not you want to be in the study. If you say “yes” we can collect your information about how you respond to the treatment. We will use this information to help other children with the same cancer. If you say “no” you will still get the same treatment and care from us.

I understand that by signing I do not give up my legal rights.

Ethics Review

The (Name of the Research Ethics Committee) makes sure that the doctor researchers will look after your best interest read this study.

Stellenbosch University https://scholar.sun.ac.za



Permission

Do you understand this research study and are you willing to take part in it?

YES	NO

Do you understand that information about your illness will be used?

YES	NO

Has the researcher answered all your questions?

YES	NO

Do you understand that you can pull out of the study at any time?

YES	NO

_____ _____

Signature or thumb print of Child Date

_____ _____

Signature of Doctor Date

_____ _____

Signature of Witness Date

_____ _____

Signature of Witness (or translator) Date

Stellenbosch University https://scholar.sun.ac.za

SOUTH AFRICAN CHILDREN'S CANCER STUDY GROUP	SACCSG NB-2017	**APPENDIX C** PATHOLOGY FORM

Patient Identifier	**SACCSG No.** ☐☐☐☐☐
	Centre:

Date of biopsy ☐☐ ☐☐ ☐☐☐☐ (D D M M Y Y Y Y)

Pathology number(s): _____

_____

Biopsy taker:_____

Caring physician:_____

Pathologist:_____

Date of receipt:_____

Date of report:_____

Clinical context, relevant clinical history, including immunosuppression status:

Indication for investigation Primary diagnosis:

Staging □ Review □

Specimen type:

Excision biopsy □ Needle core biopsy □

Other biopsy (specify)_____

Specimen description:

Site: _____

Size(s): _____x _____ x _____mm **Weight(s):** _____ g

Macroscopic description:

Stellenbosch University https://scholar.sun.ac.za

Microscopic description:

International Neuroblastoma Pathology Classification:

Extent of the invasion:

Surgical edges:

Vascular invasion:

Tumour necrosis (%):

Calcifications:

Lymph node status:

Immunohistochemistry:				
Diagnostic:	**Pos/Neg**	**%**	**Differential diagnosis:**	**Pos/Neg**
Synaptophysin			CD45RB	
NB84			CD 20	
CD99			CD3	
			Myogenin	
Other:			Desmin	
CD 31			Keratin	
CD 34			HMB45	
CD 68			S100	
			WT1	

Provisional diagnosis:

Tumour type:

ICD-10 :_____

ICD-O morphology code: ___ ___ ___•___

(If diagnosis is incomplete/uncertain, provide reasons):

Summary (any additional comments):

Signed out by: **Date:**

Sent for central review: Yes ☐ No ☐ Reason:_____

Stellenbosch University https://scholar.sun.ac.za

SOUTH AFRICAN CHILDREN'S CANCER STUDY GROUP	**SACCSG NB-2017**	**APPENDIX D1** BONE MARROW AND TREPHINE FORM

Patient Identifier	**SACCSG No.** ☐☐☐☐☐
	Centre:

Date of diagnostic BMAT ☐☐☐☐☐☐☐☐ (D D M M Y Y Y Y)

Lab number(s):
_____

_____

Biopsy taker:_____

Caring physician:_____

Laboratory hematologist:_____

Date of receipt:_____

Date of report:_____

Clinical context, relevant clinical history, including immunosuppression status:

Indication for investigation Primary diagnosis:

Staging ☐ Review ☐ Specify timepoint of review:_____

Specimen site (Please indicate site AND left or right):

Specimen dimensions:

Specimen details: Aspirate ☐ Trephine ☐ Trephine imprint ☐

Aspirate: Number of slides evaluated:

Trephine: Measurments: _ _ _ _ _ _ _ _ _ _ cm Quality:

Nature of BMAT:

Diagnosis ☐ Induction ☐ Post induction ☐ Post extended induction ☐

End of treatment ☐ Relapse ☐ Other: Please specify_____

Stellenbosch University https://scholar.sun.ac.za

<table>
<tr><td colspan="2">Microscopic description: Aspirate

Normal cell features:

Pathology:

% Infiltration:
0% ☐
<5% ☐
5% to <20% ☐
20% to <50% ☐
>50% ☐</td><td>Microscopic description: Trephine</td></tr>
<tr><td colspan="2">Immunohistochemistry

Diagnostic: Pos/Neg</td><td>Ploidy:

MYCN:</td></tr>
<tr><td>Synaptophysin</td><td></td><td rowspan="7">H and E stain:
Description:</td></tr>
<tr><td>Tyrosine hydroxylase</td><td></td></tr>
<tr><td>Chromogranin A</td><td></td></tr>
<tr><td>CD 45</td><td></td></tr>
<tr><td>CD 56</td><td></td></tr>
<tr><td>Other: Specify</td><td></td></tr>
<tr><td colspan="3">Provisional diagnosis:</td></tr>
<tr><td colspan="3">Summary (any additional comments):</td></tr>
</table>

Signed out by: **Date:**

Stellenbosch University https://scholar.sun.ac.za

Appendix D2

International Neuroblastoma Respons Criteria:

Abbreviations:
CR – Complete response; MD – Minimal disease; MR – Minimal response; NI – not involved;
PD – progressive disease; PR – partial response; VGPR – very good partial response;
SD – stable disease

Definitions:
Target lesions: Disease sites that meet criteria of measurable size (non-lymphoid soft tissue mass ≥10 mm in longest dimension or lymph node ≥15mm in short axis) as well as either uptake on MIBG (or FDG for MIBG non-avid tumors) OR biopsy positive for neuroblastoma or ganglioneuroblastoma

Non-target lesions: Lesions that are considered to be active tumor sites but do not meet target lesion criteria including leptomeningeal tumour, tumour in cerebrospinal fluid, ascites, and pleural effusion cytology.

Primary (soft tissue) Tumour Response	
Response	**Anatomic + ^{123}I-MIBG (FDG-PET) Imaging**
CR	Assessment with: • 10 mm residual soft tissue at primary site **AND** • Complete resolution of ^{123}I-MIBG or FDG-PET (for ^{123}I-MIBG-nonavid tumours) uptake at primary site. If no ^{123}I-MIBG or FDG-PET scan available: • 10 mm residual soft tissue at primary site **AND** • Catecholamines normal.
VGPR	Assessment with: • Primary tumour decreased by 90% to 99% **AND** • catecholamines normal. Supporting evidence: • Residual ^{123}I-MIBG or FDG-PET uptake at primary site.
PR	Assessment with: • 30% decrease in longest diameter of primary site **AND** • ^{123}I-MIBG or FDG-PET uptake at primary site stable, improved, or resolved.
PD	Assessment with: • 20% increase in longest diameter taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study) **AND**

Stellenbosch University https://scholar.sun.ac.za

	• Minimum absolute increase of 5 mm in longest dimension
SD	Neither sufficient shrinkage for PR **nor** sufficient increase for PD at the primary site

Tumour Response at Metastatic Soft Tissue and Bone Sites	
Response	**Anatomic + ^{123}I-MIBG (FDG-PET) Imaging**
CR	Resolution of all sites of disease defined as: • Non-primary target and non-target lesions measure 10 mm **AND** • Lymph nodes identified as target lesions decrease to a short axis 10 mm **AND** • ^{123}I-MIBG uptake or FDG-PET (for ^{123}I-MIBG-non-avid tumours) uptake of non-primary lesions resolves completely If no ^{123}I-MIBG or FDG-PET scan available: • Resolution of all sites **AND** • Catecholamines normal.
VGPR	Assessment with: • No tumour **AND** • Catecholamines normal **AND** • Residual ^{123}I-MIBG or FDG-PET uptake
PR	Assessment with: • >30% decrease in sum of diameters of non-primary target lesions compared with baseline **AND** • All of the following: - Non-target lesions may be stable or smaller in size AND - No new lesions AND - >50% reduction in MIBG absolute bone score (relative MIBG bone score > 0.1 to < 0.5) or > 50% reduction in number of FDG-PET–avid bone lesions
PD	Any of the following: • Any new soft tissue lesion detected by CT/MRI that is also MIBG avid or FDG-PET avid • Any new soft tissue lesion seen on anatomic imaging that is biopsied and confirmed to be Neuroblastoma or Ganglioneuroblastoma • Any new bone site that is MIBG avid • A new bone site that is FDG-PET avid (for MIBG non-avid tumours) **AND** has CT/MRI findings consistent with tumour **OR** has been confirmed histologically to be Neuroblastoma or Ganglioneuroblastoma • 20% increase in longest diameter taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest

Stellenbosch University https://scholar.sun.ac.za

	on study) **AND** minimum absolute increase of 5 mm in sum of diameters of target soft tissue lesions • Relative MIBG score > 1.2
SD	Neither sufficient shrinkage for PR nor sufficient increase for PD of non-primary lesions

Bone Marrow Metastasis Response	
Response	**Cytology/Histology**
CR	Bone marrow with no tumour infiltration on reassessment, independent of baseline tumour involvement.
PD	Any of the following: • Bone marrow without tumour infiltration that becomes > 5% tumour infiltration on reassessment OR • Bone marrow with tumour infiltration that increases by > two fold and has > 20% tumour infiltration on reassessment.
MD	Any of the following: • Bone marrow with < 5% tumour infiltration and remains > 0 to < 5% tumour infiltration on reassessment **OR** • Bone marrow with no tumour infiltration that has < 5% tumour infiltration on reassessment **OR** • Bone marrow with > 20% tumour infiltration that.
SD	Bone marrow with tumour infiltration that remains positive with >5% tumour infiltration on reassessment but does not meet CR, MD, or PD criteria.

Determination of Overall Response	
Response	**Criterion**
CR	All components meet criteria for CR.
PR	PR in at least one component and all other components are either CR, MD (bone marrow), PR (soft tissue or bone), or NI; no component with PD.
MR	PR or CR in at least one component but at least one other component with SD; no component with PD.
SD	SD in one component with no better than SD or NI in any other component; no component with PD.
PD	Any component with PD.

Stellenbosch University https://scholar.sun.ac.za

Reference:

1. Brodeur, G; Pritchard, J; Berthold, F; Carlsen, N; Castel, V; Castelberry, R; et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *J Clin Oncology* 1993;11(8):1466– 77

2. Park, J; Bagatell, R; Cohn, S; Pearson, A; Villablanca, J; Berthold, F; Burchill, S; Boubaker, S; McHugh, K; Nuchtern, J; London, J; Seibel, N; Lindwasser, O; Maris, J; Brock, P; Schleiermacher, G; Ladenstein, R; Matthay, K; Valteau-Couanet, D. Revisions to the International Neuroblastoma Response Criteria: A Consensus Statement From the National Cancer Institute Clinical Trials Planning Meeting. J Clin Oncol 2017; 35(22):2580-2587.

3. Eisenhauer; E, Therasse, P Bogaert, J; Schwartz, L; Sargent, D; Ford, R; Dancey, J; Arbuck, S; Gwyther, S; Mooney, M; Rubinstein, L; Shankar, L; Dodd, L; Kaplan, R; Lacombe, D; Verweij, J. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). European Journal of Cancer 2009; 45(2): 228-247

SOUTH AFRICAN CHILDREN'S CANCER STUDY GROUP	**SACCSG** **NB-2017**	**APPENDIX E** PATIENT INFORMATION

Patient Identifier: **Date of birth:**_____ **Age (months):**_____		**Centre:**	.....
SACCSG NO.	**NB -**	**Responsible Physician:**	.....
Sex:	**M** ☐ **F** ☐	**Date of diagnosis:**	D D M M Y Y Y Y

Demographics:
Race: African ☐ Coloured ☐ Caucasian ☐ Indian ☐ Other (specify)_____

Anthropometry:
Weight-for-age(kg)_____ WHO SD _____
MUAC(cm)_____
Height-for-age (cm)_____ WHO SD _____
Weight-for-height_____ WHO SD_____

Co-morbidities:
HIV: Negative ☐ Positive ☐ Unknown ☐
Newly diagnosed ☐ Treatment initiated ☐ Treatment not initiated ☐
On treatment ☐
Tuberculosis:
Negative ☐ Positive ☐ Unknown ☐
Newly diagnosed ☐ Treatment initiated ☐ Treatment not initiated ☐
On treatment ☐

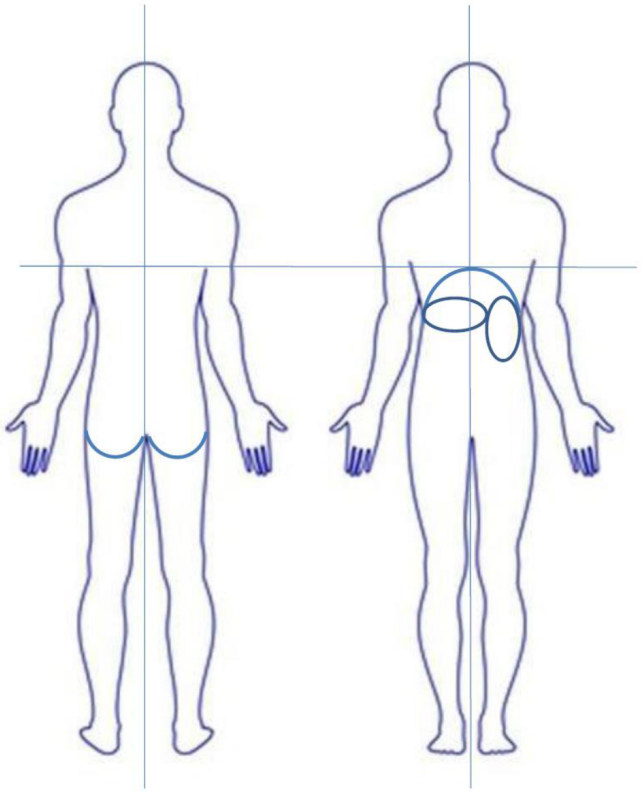
Staging: (Use Appropriate appendix for staging and complete) Please circle:

INSS: 1 2A 2B 3 4 4s

Stage 4s sites of metastasis:_____

INRG: L1 L2 M MS **SANAS:** VLR LR IR HR

Stellenbosch University https://scholar.sun.ac.za



Please indicate locally involved site(s) on diagram
Diffuse involvement:
Bone (specifiy):
Bone marrow (specify):

Staging done by (multiple options possible):

Clinical exam

Chest X-ray

Abdominal U/S

CT chest

CT abdomen

MRI abdomen

BMAT

MIBG scan

Bone scan

Other
(specify)_____
_____
_____
_____
_____
_____

Presenting symptoms (more than one is possible):

Abdominal mass/ hernia / swelling/ ascites / pain/ distention	☐
Loss of weight /anorexia	☐
Fever	☐
Sweating/ night sweats	☐
Bone pain/ skull lesions / bone mass / skull mass / Joint	☐
Proptosis/ eye swelling	☐
Urinary retention/ unable to urinate	☐
Constipation/ faecal incontinence	☐
Breathing difficulty/ inability to breathe	☐
Unable to walk	☐
Back pain/ mass	☐
Diarrhoea	☐
Bleeding	☐
Neurological signs	☐
Blind/ Horner Syndrome/ decreased vision	☐

Stellenbosch University https://scholar.sun.ac.za

Hypertension ☐
Pallor ☐
Infections ☐
Other (Specify):_____

Diagnostic (values):
Non-specific tumour markers
LDH: _____ Ferritin:_____
Specific tumour markers:
MYCN: Amp ☐ NA ☐ EC ☐

Diagnostic (values):
Creatinine: _____ Calculated GFR:_____
AST: _____
ALT: _____
ALP: _____
GGT:

Urine studies:
Catecholamines: Raised: ☐ Normal: ☐
Indicate raised values (according to institutional reporting):_____
_____
_____

Imaging results at diagnosis: Anatomy, IDRF and measurements

Chest X-ray: Mediastinal mass: _____

Lymphadenopathy:_____

Metastasis_____

Abdominal U/S:_____

_____

CT chest: _____

_____

CT/MRI Abdomen:_____

Toxicity screens:

Audiogram quantitive results:

Audiogram qualitative results:

Echo cardiogram: SF:

Other:

Stellenbosch University https://scholar.sun.ac.za

Appendix F

Image Defined Risk Factor (IDRF)

IDRFs include the following:

- Ipsilateral tumour extension within two body compartments: neck and chest; chest and abdomen; abdomen and pelvis.
- Infiltration of adjacent organs/structures: pericardium, diaphragm, kidney, liver, duodeno-pancreatic block, mesentery.
- Encasement of major vessels by tumour: vertebral artery, internal jugular vein, subclavian vessels, carotid artery, aorta, vena cava, major thoracic vessels, branches of the superior mesenteric artery at its root and the coeliac axis, iliac vessels.
- Compression of trachea or central bronchi.
- Encasement of brachial plexus.
- Infiltration of port hepatic or hepato-duodenal ligament.
- Infiltration of the costo-vertebral junction between T9 and T12.
- Tumour crossing the sciatic notch.
- Tumour invading renal pedicle.
- Extension of tumour to base of skull.
- Intraspinal tumour extension such that more than one-third of the spinal canal is invaded, leptomeningeal space is obliterated, or spinal cord MRI signal is abnormal.

APPENDIX G

International Neuroblastoma Risk Group Staging System (INRGSS)

STAGE	DESCRIPTION
L1	Localized tumor not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment
L2	Locoregional tumor with presence of one or more image-defined risk factors
M	Distant metastatic disease (except stage MS)
MS	Metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow

IMPORTANT: Patients with multifocal primary tumors should be staged according to the greatest extent of disease as defined in the table.

Stellenbosch University https://scholar.sun.ac.za

Appendix H

International Neuroblastoma Staging System (INSS)

Stage 1	• Localized tumour with complete gross excision, with or without microscopic residual disease • Representative ipsilateral lymph nodes negative for tumour microscopically
Stage 2A	• Localized tumour with incomplete gross excision • Representative ipsilateral non-adherent lymph nodes negative for tumour microscopically.
Stage 2B	• Localized tumour with or without complete gross excision, with ipsilateral non-adherent lymph nodes positive for tumour. • Enlarged contralateral lymph nodes must be negative microscopically
Stage 3	• Unresectable unilateral tumour infiltrating across the midline, with or without regional lymph node involvement • Localized unilateral tumour with contralateral regional lymph node involvement • Midline tumour with bilateral extension by infiltration (unresectable) or by lymph node involvement. The midline is defined as the vertebral column. Tumours originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.
Stage 4	• Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs, except as defined for stage 4S.

Stellenbosch University https://scholar.sun.ac.za

Stage 4S	Localized primary tumour, as defined for stage 1, 2A, or 2B, with dissemination limited to skin, liver, and/or bone marrow (by definition limited to infants younger than 12 months). Marrow involvement should be minimal (i.e., <10% of total nucleated cells identified as malignant by bone biopsy or by bone marrow aspirate). More extensive bone marrow involvement would be considered stage 4 disease. The results of the MIBG scan, if performed, should be negative for disease in the bone marrow.

Stellenbosch University https://scholar.sun.ac.za

Appendix I

Neuroblastoma Pathological Classification (NPC-SA)

adapted for South Africa

International Neuroblastoma Pathology classification adapted for South Africa		INPC-SA
Neuroblastoma (Schwannian stroma-poor) • **Undifferentiated** • **Poor differentiated** • **Differentiating**		**Prognostic group**
<1.5 yrs	Poorly differentiated or differentiating	**FH**
1.5–5 yrs	Differentiating	**FH**
<1.5 yrs	Undifferentiated tumour	**UH**
1.5–5 yrs	Undifferentiated or poorly differentiated tumour	**UH**
≥5 yrs	All tumours	**UH**
Ganglioneuroblastoma intermixed (Schwannian stroma-poor)		**FH**
Ganglioneuroma intermixed (Schwannian stroma-dominant) • **Maturing** • **Mature**		**FH**
Ganglioneuroblastoma nodular (Schwannian stroma-rich/stroma-dominant and stroma-poor)		**UH**

FH – Favourable histology, UH – Unfavourable histology, * NPC-SA adapted from INPC

Stellenbosch University https://scholar.sun.ac.za

Appendix J

International Neuroblastoma Pathological Classification (INPC)/Shimada classification

International Neuroblastoma Pathology classification		Original Shimada classification	Prognostic group
Neuroblastoma (Schwannian stroma-poor) • **Undifferentiated** • **Poor differentiated** • **Differentiating**		**Stroma-poor**	**FH**
<1.5 yrs	Poorly differentiated or differentiating & low or intermediate MKI tumour		**FH**
1.5–5 yrs	Differentiating & low MKI tumour		**FH**
<1.5 yrs	a) undifferentiated tumour b) high MKI tumour		**UH**
1.5–5 yrs	a) undifferentiated or poorly differentiated tumour b) intermediate or high MKI tumour		**UH**
≥5 yrs	All tumours		**UH**
Ganglioneuroblastoma intermixed (Schwannian stroma-poor)		**Stroma-rich Intermixed**	**FH**
Ganglioneuroma intermixed (Schwannian stroma-dominant) • **Maturing** • **Mature**		**Well differentiated Ganglioneuroma**	**FH**
Ganglioneuroblastoma nodular (Schwannian stroma-rich/stroma-dominant and stroma-poor)		**Stroma-rich Nodular**	**UH**

Stellenbosch University https://scholar.sun.ac.za

Appendix K

COG Risk grouping:

INSS	Age	MYCN	Shimada	DNA ploidy	Risk group
1	Any	Any	Any	Any	Low
2A/2B	<365d	Any	Any	Any	Low
	>365d	Nonamplified	Any	-	Low
	>365d	Amplified	FH	-	Low
	>365d	Amplified	UH	-	High
3	<365d	Nonamplified	Any	Any	Intermediate
	<365d	Amplified	Any	Any	High
	>365d	Nonamplified	FH	-	Intermediate
	>365d	Nonamplified	UH	-	High
	>365d	Amplified	Any	-	High
4	<548d	Nonamplified	Any	Any	Intermediate
	<548d	Amplified	Any	Any	High
	>548d	Any	Any	-	High
4S	<365d	Nonamplified	FH	>1	Low
	<365d	Nonamplified	Any	1	Intermediate
	<365d	Nonamplified	UH	Any	Intermediate
	<365d	Amplified	Any	Any	High

Low risk

- All children who are Stage 1
- Any child who is Stage 2A or 2B and younger than age 1
- Any child who is Stage 2A or 2B, older than age 1, whose cancer has *no* extra copies of the *MYCN* gene
- Any child who is Stage 4S (younger than age 1), whose cancer has favorable histology, is hyperdiploid (excess DNA) and has *no* extra copies of the *MYCN* gene

Intermediate risk

- Any child who is Stage 3, younger than age 1, whose cancer has *no* extra copies of the *MYCN* gene
- Any child who is Stage 3, older than age 1, whose cancer has *no* extra copies of the *MYCN* gene and has favorable histology (appearance under the microscope)
- Any child who is Stage 4, younger than age 1, whose cancer has *no* extra copies of the *MYCN* gene
- Any child who is Stage 4S (younger than age 1), whose cancer has *no* extra copies of the *MYCN* gene and has normal DNA ploidy (number of chromosomes) and/or has unfavorable histology

High risk

- Any child who is Stage 2A or 2B, older than age 1, whose cancer has extra copies of the *MYCN* gene
- Any child who is Stage 3, younger than age 1, whose cancer has extra copies of the *MYCN* gene
- Any child who is Stage 3, older than age 1, whose cancer has extra copies of the *MYCN* gene
- Any child who is Stage 3, older than 18 months of age, whose cancer has unfavorable histology

Stellenbosch University https://scholar.sun.ac.za

- Any child who is Stage 4, whose cancer has extra copies of the *MYCN* gene regardless of age
- Any child who is Stage 4 and older than 18 months
- Any child who is Stage 4 and between 12 and 18 months old whose cancer has extra copies of the *MYCN* gene, unfavorable histology, and/or normal DNA ploidy (a DNA index of 1)
- Any child who is Stage 4S (younger than age 1), whose cancer has extra copies of the *MYCN* gene

Appendix L

Adapted Risk Stratification and Treatment Assignment for Neuroblastoma in LMIC

INSS	Initial Status	Risk Group	Age (yr)	LDH	Ferritin	MYCN Rx
1	Resection	Low	0.5-21	Any	Any	Any
1	Observation	VLR	< 0.5	Any	Any	Any
2A/2B	Resection >50% asymptomatic	Low	Any	Any	Any	Unknown
2A/2B	Resection 50% symptomatic	Intermediate	Any	Any	Any	NA/ Unknown
2A/2B	Resection <50% asymptomatic	Intermediate	Any	Any	Any	NA/ Unknown
2A/2B	Any resection	High	Any	Any	Any	Amplified
3		Intermediate	<1.5	<750	<120	NA/Unknown
3		Intermediate	>1.5	<750	<120	NA/Unknown
3		High	Any	>750	>120	NA/Unknown
4		High	<1.5	>750	>120	NA/Unknown
4		Intermediate	<1.5	<750	<120	NA/Unknown
4		High	>1.5	Any	Any	Any
4S	Asymptomatic	Low	<1	<750	<120	NA/Unknown
4S	Symptomatic	Intermediate	<1	<750	<120	NA/Unknown
4S	Asymptomatic	High	<1	Any	Any	A

VLR - very low risk **LR** - low risk **IR** - intermediate risk; **HR** - high risk; **A -** Amplified; **NA -** Non-Amplified; **Unk -** Unknown.

Stellenbosch University https://scholar.sun.ac.za

APPENDIX M

Proposed South African Neuroblastoma Adapted risk stratification (SANARS)								
INSS	**Initial Surgical Status**	**INRG**	**Age (months)**	**LDH**	**Ferritin**	**MYCN**	**Histology**	**Risk Group**
1	Resection > 50%	L1	>6mo	Any	Any	NA	Any	Low
1	Resection > 50%	L1	>6mo	Any	Any	Amp	Any	High
1	Observation	L1	<6mo	Any	Any	Any	Any	Very low (VLR)
2A	Resection >50% Asymptomatic	L1	Any	Any	Any	NA/EC/ Unknown	Any	Low
2B	Resection >50% Asymptomatic	L2	Any	Any	Any	NA/EC/Unknown	Any	Low
2A	Resection >50 % Symptomatic	L1	Any	Any	Any	NA/EC/ Unknown	FH	Intermediate
2A	Resection >50 % Symptomatic	L1	Any	Any	Any	NA/EC/Unknown	UH	High
2B	Resection >50% Asymptomatic	L2	Any	Any	Any	NA/EC/ Unknown	FH	Intermediate
2B	Resection >50 % Symptomatic	L2	Any	Any	Any	NA/EC/Unknown	UH	High
2A	Resection <50%	L1	Any	Any	Any	NA/EC/Unknown	FH	Intermediate
2A	Resection < 50%	L1	Any	Any	Any	NA/EC/Unknown	UH	High
2B	Resection <50%	L2	Any	Any	Any	NA/EC/Unknown	FH	Intermediate
2B	Resection < 50%	L2	Any	Any	Any	NA/EC/Unknown	UH	High
2A	Any resection	L1	Any	Any	Any	Amp	Any	High
2B	Any resection	L2	Any	Any	Any	Amp	Any	High
3		L2	<18mo	<750	<120	NA/EC/ Unknown	Any	Intermediate
3		L2	>18mo	<750	<120	NA/EC/ Unknown	FH	Intermediate
3		L2	>18mo	<750	<120	NA/EC/ unknown	UH	High
3		L2	Any	>750	>120	A/ Unknown	Any	High
4		M	<18mo	>750	>120	A/ Unknown	Any	High
4		M	<18mo	<750	<120	NA/EC/Unknown	Any	Intermediate
4		M	>18mo	Any	Any	Any	Any	High
4S	Asymptomatic	MS	<6mo	<750	<120	NA/EC	FH	Low
4S	Asymptomatic	MS	<12mo	<750	<120	NA/EC/ Unknown	FH	Low
4S	Symptomatic	MS	<12mo	<750	<120	NA/EC	FH	Low
4S	Asymptomatic	MS	<12mo	<750	<120	NA/EC/Unknown	UH	Intermediate
4S	Symptomatic	MS	<12mo	<750	<120	Unknown	Any	Intermediate
4S	Asym/ symp	MS	<12mo	Any	Any	Amp	Any	High

Stellenbosch University https://scholar.sun.ac.za

Appendix N1

BUSULFAN (Bu)

Also called Busulfex, Myleran. Busulfan can be given by mouth and intravenous injection or infusion.

Following a dose of Busulfan, some patients may experience:

a. Immediate:

1. Nausea and vomiting (in the first 3 - 5 days).
2. Diarrhea (in the first 3 - 5 days).
3. Poor appetite.

b. Delayed:

1. Hair loss.
2. Mouth sores.
3. Low blood counts.
4. Liver problems.
5. Convulsions.
6. Increase risk of secondary cancers.
7. Infertility.

c. Less frequent side effects:

1. Discoloration of the skin, especially in the creases of the hands and nail beds.
2. Skin rash
3. Itching

Contact your doctor if you notice:

1. Fever
2. Nausea (interferes with ability to eat and unrelieved with prescribed medication).
3. Vomiting (vomiting more than 4-5 times in a 24 hour period).
4. Diarrhea (4-6 episodes in a 24-hour period).
5. Unusual bleeding or bruising
6. Black or tarry stools, or blood in your stools
7. Blood in the urine
8. Pain or burning with urination
9. Extreme fatigue (unable to carry on self-care activities)
10. Dizziness
11. Mouth sores (painful redness, swelling or ulcers)
12. Yellowing of the skin or eyes
13. Swelling of the abdomen
14. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.
15. Any other problem or have questions.

Special instructions:

1. Leaking of this drug outside the vein may cause pain and skin damage similar to a burn. Contact your doctor IMMEDIATELY if pain, redness or swelling occurs near the injection site.

Monitoring:

1. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.

Stellenbosch University https://scholar.sun.ac.za

Appendix N2

CARBOPLATIN

Carboplatin is usually given by intravenous infusion.

Following a dose of carboplatin, some patients may experience:

a. Immediate:

1. Nausea and vomiting

b. Delayed:

1. A drop in blood cell counts
2. Taste changes

c. Less frequent side effects:

1. Burning sensation at the injection site
2. Abdominal pain
3. Diarrhea
4. Constipation
5. Mouth sores
6. Infection
7. Sensory loss, numbness and tingling, and difficulty in walking may last for at least as long as therapy is continued.
8. Central neurotoxicity: dizziness, confusion, visual changes, ringing in the ears.
9. Kidney problems
10. Liver problems
11. Allergic reaction may occur with itching, rash, shortness of breath or dizziness.

Contact your doctor if you notice:

1. Fever of 38°C or higher
2. Severe nausea and vomiting
3. Bruising or bleeding
4. Any other problems or have questions

Monitoring:

1. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.
2. In addition, regular tests of kidney function will be done to make sure that no damage is detectable and it is safe to give more doses.

Stellenbosch University https://scholar.sun.ac.za

Appendix N3

CISPLATIN

A platinum compound. Cisplatin is only given by intravenous infusion.

Following a dose of cisplatin, some patients may experience:

a. Immediate:

1. Nausea and vomiting. This may begin within 2 hours and last for from one to several days.
2. Loss of appetite
3. Diarrhoea

b. Delayed:

1. Low blood counts
2. Kidney problems
3. Hearing loss and/or ringing in the ears.
4. Low magnesium, low calcium, low potassium

c. Less common side effects:

1. Hair loss
2. Liver problems
3. Peripheral sensory loss
4. Taste changes

Contact your doctor if you notice:

1. A fever of 38°C or higher
2. Nausea (interferes with ability to eat and unrelieved with prescribed medication)
3. Vomiting (vomiting more than 4-5 times in a 24 hour period)
4. Diarrhea (4-6 episodes in a 24 hour period)
5. No urine output in a 12 hour period
6. Blood in the urine
7. Pain or burning with urination
8. Unusual bleeding or bruising
9. Black or tarry stools, or blood in your stools or urine
10. Extreme fatigue (unable to carry on self-care activities)
11. Swelling, redness and pain in one leg or arm and not the other
12. Yellowing of the skin or eyes
13. Mouth sores (painful redness, swelling or ulcers)

Special instructions

1. Abundant fluid intake prior to and after cisplatin administration and frequent voiding of large volumes of urine is extremely important to protect against kidney damage.
2. Take anti-nausea medications as directed.
3. If needed, magnesium tablets or syrup should be taken as directed.

Monitoring:

1. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.
2. In addition, regular tests of hearing and of kidney function will be done to make sure there is no significant damage to the hearing or to the kidneys, and that it is safe to give more doses.

Stellenbosch University https://scholar.sun.ac.za

Appendix N4

13-CIS-RETINOIC ACID (CRA)

Also called Accutane. CRA can only be given by mouth.

Following a dose of CRA, some patients may experience:

a. Immediate:

1. Allergic reaction

b. Delayed:

1. Headache
2. Fever
3. Dry skin
4. Dry mucous membranes (mouth, nose)
5. Bone pain
6. Nausea and vomiting
7. Rash
8. Mouth sores
9. Itching
10. Sweating
11. Eyesight changes

c. Less frequent side effects:

1. Back pain
2. Pain in muscles and joints
3. Abdominal pain
4. Poor appetite
5. Dizziness
6. Drowsiness
7. Insomnia
8. Anxiety
9. Numbness and tingling of hands and feet
10. Weakness
11. Depression
12. Hair loss (thinning)
13. Dry eyes, sensitivity to light (see eye problems)
14. Decreased night vision, which may persist after treatment is stopped
15. Swelling of the feet or ankles
16. Low blood counts. Your white and red blood cells and platelets may temporarily decrease. This can put you at increased risk for infection, anemia and/or bleeding.
17. Abnormal blood tests: increased triglyceride, cholesterol and/or blood sugar levels.
18. Increases in blood tests measuring liver function

Contact your doctor is you notice:

1. Shortness of breath, wheezing, difficulty breathing, closing up of the throat, swelling of facial features, hives (possible allergic reaction)
2. Fever of 38°C or higher, chills (possible signs of infection)
3. Having thoughts or feeling like you may want to harm yourself or others
4. Difficulty breathing, sudden weight gain, swelling, vision changes
5. Severe abdominal pain

Stellenbosch University https://scholar.sun.ac.za

6. Black or tarry stools, or blood in your stools or urine
7. Unusual bleeding or bruising
8. Abdominal cramping
9. Nausea (interferes with ability to eat and unrelieved with prescribed medications)
10. Vomiting (more than 4-5 episodes within a 24-hour period)
11. Diarrhea (more than 4-6 episodes in a 24-hour period)
12. Mouth sores (painful redness, swelling or ulcers)
13. Extreme fatigue (inability to perform self-care activities)
14. Anxiety, changes in thinking or mood, confusion, difficulty concentrating or trouble sleeping, aggressive or violent behavior, or suicidal thoughts.
15. Depressed (interfering with your ability to carry on your regular activities)
16. Ringing in the ears, problems with hearing
17. Yellowing of the skin or eyes

Special instructions:

1. Before starting CRA treatment, make sure you tell your doctor about any other medications you are taking (including prescription, over-the-counter, vitamins, herbal remedies, etc.).
2. Do not take vitamin A supplements with CRA. Taking both together may increase your chance of getting side effects.
3. Do not receive any kind of immunization or vaccination without your doctor's approval while taking CRA.
4. Inform your health care professional if you are pregnant

Monitoring:

1. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.

Stellenbosch University https://scholar.sun.ac.za

Appendix N5

CYCLOPHOSPHAMIDE (CY)

Also called Cytoxan. Cyclophosphamide can be given by intravenous injection, infusion or by mouth.

Following a dose of Cyclophosphamide, some patients may experience:

a. Immediate:

1. Nausea and vomiting
2. Loss of appetite

b. Delayed:

1. Low blood counts
2. Loss of hair
3. Discolouration of the nails
4. Infertility

c. Less frequent side effects:

1. Mouth sores
2. Bladder irritation
3. Secondary malignancies

Contact your doctor is you notice:

1. Fever above 38°C.
2. Nausea (interferes with ability to eat and unrelieved with prescribed medication).
3. Vomiting (vomiting more than 4-5 times in a 24 hour period).
4. Diarrhoea (4-6 episodes in a 24-hour period).
5. Unusual bleeding or bruising
6. Black or tarry stools, or blood in your stools.
7. Blood in the urine.
8. Pain or burning with urination.
9. Extreme fatigue (unable to carry on self-care activities).
10. Mouth sores (painful redness, swelling or ulcers).

Monitoring:

1. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.

Stellenbosch University https://scholar.sun.ac.za

Appendix N6

DOXORUBICIN (DOX)

This is a red coloured drug which can only be given by intravenous injection.

Following a dose of Doxorubicin, some patients may experience:

a. Immediate:

1. Nausea and vomiting in the afternoon following the injection
2. Pink, orange and red coloured urine on the night, or the day following the injection
3. A low grade fever
4. Rash or urticarial (itchy red spots) at the injection site immediately after the dose is given
5. Burning at the site of infusion

b. Delayed:

1. Drop in blood cell counts, 7 to 10 days after treatment is started.
2. Mouth ulcers.
3. Hair loss (2 to 3 weeks after treatment).

c. Less frequent side effects:

1. Heart damage (rare - see below).
2. Darkening of the skin

Contact your doctor if you notice:

1. Mouth ulcers
2. Fever of 38°C or higher
3. Any other problems or questions

Special instructions:

Leaking of this drug outside the vein may cause pain and skin damage similar to a burn. Contact your doctors immediately if pain, redness or swelling occurs near the injection site.

Monitoring:

1. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.
2. In addition, regular tests of heart contraction and function will be done to check that no damage is detectable and that it is safe to give more doses. The total dose is limited to minimize any risk of heart damage.

Stellenbosch University https://scholar.sun.ac.za

Appendix N7

ETOPOSIDE (VP-16)

VP-16 can be given by intravenous infusion

Following a dose of VP-16, some patients may experience:

a. Immediate:

1. Nausea, vomiting and loss of appetite

b. Delayed:

1. A drop in blood cell count
2. Infertility
3. Low blood pressure
4. Metallic taste in the mouth
5. Secondary malignancies

c. Less frequent side effects:

1. Mouth ulcers
2. Diarrhoea
3. Hair loss
4. Possible allergic reactions during infusion (fever, rash, shortness of breath, dizziness due to lowered blood pressure).

Contact your doctor if you notice:

1. Fever of 38°C or higher
2. Severe nausea or vomiting
3. Any other problems or have questions

Special instructions:

1. In addition to capsules, VP-1 6 injection vials can sometimes be given by mouth. To minimise the unpleasant taste, the drug should be mixed into a glass of fruit juice.

Monitoring:

1. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.

Stellenbosch University https://scholar.sun.ac.za

Appendix N8

MELPHALAN (Mel)

Also called Alkeran. Melphalan can be given by mouth or intravenous injection or infusion.

Following a dose of Melphalan, some patients may experience:

a. Immediate:

1. Nausea and vomiting (in the first 3 - 5 days).
2. Diarrhea (in the first 3 - 5 days).
3. Poor appetite.
4. Allergic reaction.

b. Delayed:

1. Hair loss.
2. Mouth sores.
3. Low blood counts.
4. Kidney problems.
5. Heart arrhythmias
6. Increase risk of secondary cancers.
7. Infertility.

c. Less frequent side effects:

1. Abdominal cramps

Contact your doctor is you notice:

Immediately:

1. Shortness of breath,
2. Wheezing,
3. Difficulty breathing,
4. Closing up of the throat,
5. Swelling of facial features,
6. Hives
7. Fever

Other reasons to contact your doctor:

1. Nausea (interferes with ability to eat and unrelieved with prescribed medication).
2. Vomiting (vomiting more than 4-5 times in a 24 hour period).
3. Diarrhea (4-6 episodes in a 24-hour period).
4. Unusual bleeding or bruising
5. Black or tarry stools, or blood in your stools
6. Blood in the urine
7. Pain or burning with urination
8. Extreme fatigue (unable to carry on self-care activities)
9. Dizziness
10. Mouth sores (painful redness, swelling or ulcers)
11. Yellowing of the skin or eyes
12. Swelling of the abdomen
13. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.
14. Any other problem or have questions.

Stellenbosch University https://scholar.sun.ac.za

Special instructions:

1. Leaking of this drug outside the vein may cause pain and skin damage similar to a burn. Contact your doctor IMMEDIATELY if pain, redness or swelling occurs near the injection site.

Monitoring:

1. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.

Stellenbosch University https://scholar.sun.ac.za

Appendix N9

VINCRISTINE (VCR)

Also called Oncovin or Pericristine. Vincristine can only be given by intravenous injection or infusion.

Following a dose of Vincristine, some patients may experience:

a. Immediate:

1. Aches and pains in the limbs, jaw and/or abdomen, developing a 3 to 5 days after an injection.
2. Constipation (3 to 5 days after an injection).

b. Delayed:

1. Hair loss (2 to 3 weeks after a dose).
2. Muscle weakness (noted as difficulty walking up stairs or getting up off the floor), tingling pain in the hands, fingers and toes.

c. Less frequent side effects:

1. Abdominal cramps.
2. Difficulty walking.

Contact your doctor is you notice:

1. The patient has not moved his or her bowels for greater than 36 hours in the 2 to 7 days following a dose, or complains of abdominal pain or diarrhoea.
2. The patient has severe tingling in the fingers, decreased manual dexterity, muscle weakness or jaw pain.
3. Severe nausea or vomiting.
4. Any other problem or have questions.

Special instructions:

1. Leaking of this drug outside the vein may cause pain and skin damage similar to a burn. Contact your doctor IMMEDIATELY if pain, redness or swelling occurs near the injection site.
2. Medication may be prescribed by your doctor after a dose of vincristine to prevent constipation.

Monitoring:

1. Regular blood counts and other tests will be done to check on possible side effects and treatment will be adjusted accordingly.

Stellenbosch University https://scholar.sun.ac.za

Appendix O1:

CE (LR-SA)

Re-evaluate ⇩

Cycle	1	2	3	4	5	6	7
Carboplatin	*	*	*	*	*	*	*
Etoposide	*	*	*	*	*	*	*

Regimen:

Low risk, symptomatic and progressing Stage 4S disease

- Chemotherapy is administered every 21 days for4 cycles
- If the tumour response is favourable a further 3 cycles may be administered
- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L

Doses:

Carboplatin 600mg/m^2 IV over 2 hours x 1 day

Etoposide 175mg/m^2 IV over 4 hours x 1 day

Dose modifications:

Infants and low weight children:
Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
Infants with a weight equal and < 5kg:
The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Carboplatin dose (mg) = Target AUC (5) X GFR (ml/min) + [0.36 X body weight (kg)]
over 2 hours x1/day

Etoposide 6.6mg/kg IV over 4 hours x 1 day

Precautions:

Infection support: Timely antimicrobial interventions according to standard guidelines

Stellenbosch University https://scholar.sun.ac.za

Appendix O2

Intermediate risk (IR-SA):

Cycle	**1**	**2**	**3**	**4**		**5**	**6**	**7**	**8**	
Carboplatin	*	*		*		*	*	*		
Etoposide	*		*	*		*		*		
Doxorubicin		*		*		*	*		*	
Cyclophosphamide		*	*				*		*	
Evaluate					*					*

Regimen:

- Chemotherapy is administered every 21 days for 8 cycles
- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L
- Prophylactic G-CSF is standardly administered to patients < 3 months after every course at 5µg/kg IV/ SC

Doses:

Carboplatin 560mg/m^2 IV over 2 hours x 1 day

Etoposide 120mg/m^2 IV over 4 hours x 3 day

Doxorubicin 30mg/m^2 over 2 hours x 1 day

Cyclophosphamide 1000mg/m^2 over 2 hours x 1 days

Dose modifications:

Infants and low weight children:
Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
Infants with a weight equal and < 5kg:
The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Carboplatin 18mg/kg IV over 2 hours x 1 day

Etoposide 4mg/kg IV over 4 hours x 3 day

Doxorubicin 1mg/kg over 2 hours x1 day

Stellenbosch University https://scholar.sun.ac.za

Cyclophosphamide 33mg/kg over 2 hours x 1 days

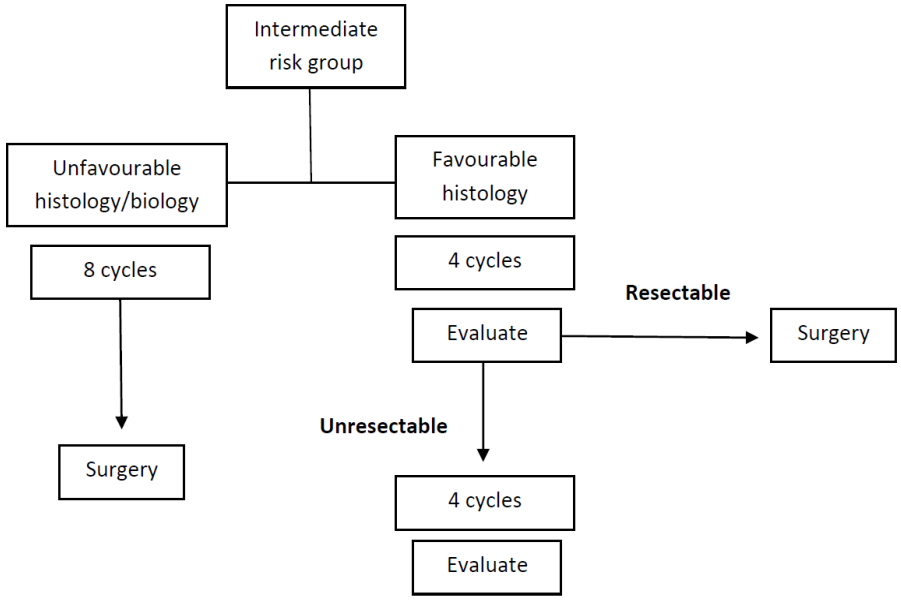
Approach:

Surgery and chemotherapy:

If CR is possible surgery should be attempted.

- With R0 or R1 resection and FH – no chemotherapy and follow up
- With R2 resection or UH – 8 cycles of chemotherapy

If CR is not possible a biopsy should be done to determine FH/ UH. In the absence of histology tumours must be treated as UH.



Radiotherapy:

The indications for radiotherapy are the following:

- Progressive disease
- Life threatening symptoms without the possibility of surgery

Precautions:

Infection support:

- Timely antimicrobial interventions according to standard guidelines

Nutritional support

- Naso-gastric nutritional support must be started before starting chemotherapy and weekly measurements and monitoring must take place

Stellenbosch University https://scholar.sun.ac.za

Appendix O3

OJEC/OPEC (HR-SA)

Cycle	**1**	**2**	**3**	**4**		**5**	**6**	**7**	
Vincristine	*	*	*	*		*	*	*	
Carboplatin	*		*			*		*	
Etoposide	*	*	*	*		*	*	*	
Cisplatin		*		*			*		
Cyclophosphamide	*	*	*	*		*	*	*	
Evaluate					*				*

Regimen:

- Chemotherapy is administered every 21 days for 7 cycles
- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L

Doses:

Vincristine 1.5mg/m^2 IV stat (max 2mg) x 1 day

Carboplatin 750mg/m^2 IV over 2 hours x 1 day

Etoposide 175mg/m^2 IV over 4 hours x 1 day

Cisplatin 80mg/m^2 IV over 24 hours x 1 day

Cyclophosphamide 1200mg/m^2 over 1 hour x1 day

Dose modifications:

Infants and low weight children:
Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
Infants with a weight equal and < 5kg:
The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Vincristine 0.05mg/kg IV stat (max 2mg) x 1 day

Carboplatin 25mg/kg IV over 2 hours x 1 day

Stellenbosch University https://scholar.sun.ac.za

Etoposide 5.8mg/kg IV over 4 hours x 1 day

Cisplatin 2.6mg/kg IV over 24 hours x 1 day

Cyclophosphamide 35mg/kg over 1 hour x 1 day

Precautions:

Cisplatin administration:

- Prehydration for cisplatin

  Infused at 200 ml/m²/hr for at least 3 hours with 0.9% sodium chloride with 20 mmol/l potassium chloride

- Posthydration for cisplatin

  Infuse at 3 litres/m^2. To each litre add 10ml KCl (max 20ml), 5ml Ca gluconate (10% solution) or 250mg/L and 2ml (1-2g/L) $MgSO_4$. Post hydrate for 12 hours following each cisplatin dose.

- A target urine volume of 400ml/m^2/6h must be maintained

Infection support:

- Timely antimicrobial interventions according to standard guidelines

Nutritional support

- Naso-gastric nutritional support must be started before starting chemotherapy and weekly measurements and monitoring must take place

Stellenbosch University https://scholar.sun.ac.za

Appendix O4

CIS-RETINOIC ACID (CRA)

Regime:
CRA is started no sooner than 14 days after radiotherapy to avoid radiotherapy related toxicity.

CYCLE	DAY 1 - 14	D 15 - 28
	CRA	REST

Starting Criteria:

- Neutrophils > 500 $\times 10^9$/L, Platelets > 50 $\times 10^9$/L, Hemoglobin > 8 g/dl
- Normal ALT and AST
- Normal renal function
- Normal serum calcium and urate
- Triglycerides must be < 2 x normal

Starting Dose (if > 12kg):
CRA 80 mg/m^2 PO 2x/d

Cycles
6 cycles will be administered
Each cycles consists of CRA D1-14 and then resting D15-28
Dose adjustments:

With the following criteria CRA must be stopped and reintroduced at 60mg/m^2 PO 2x/d

- Hemoglobin < 8g/dl
- Platelets < 25 $\times 10^9$/L
- Neutrophils < 500 $\times 10^9$/L
- AST or ALT > 5 x normal
- Total bilirubin > 1.5 x normal
- Hypercalcaemia
- Erythema multiforme
- Severe vomiting and/or abdominal pain
- Urethritis and/or dysuria
- Severe cheilitis
- Severe conjunctivitis
- Severe headache or vertigo
- Persistent muscle cramps requiring sustained symptomatic care

After completion of 6 cycles management must continue according to the long term follow up schedule.

Stellenbosch University https://scholar.sun.ac.za

Appendix O5

The BuMel MAT - regimen

The dosage:

Peripheral stem cells

A minimum of 3 - 5 x 10^6 CD34 cells/kg should be used.
A maximum of 10 x 10^6 CD 34 cells/kg should be used.

BUMEL-regime:

DRUG	DOSE DAY	-8	-7	-6	-5	-4	-3	-2	-1	0
Busulfan	An IV dose every 6 hours for 16 doses: < 9 kg: 1.0mg/kg 9 kg to < 16 kg: 1.2 mg/kg 16 kg to 23 kg: 1.1 mg/kg >23 kg to 34 kg: 0.95 mg/kg >34 kg: 0.8 mg/kg		**	****	****	****	**			
Melphalan	IV: 140mg/m^2 over 15min not before 24 hours after busulfan								*	
Hydration	3L/m^2 (125ml/hr) till 24 hours after melphalan infusion		→	→	→	→	→	→	→	→
Clonazapam	0.025 – 0.1 mg/kg/day IV: total dose as a continuous infusion PO: divided in 3 doses/day Dose can be reduced if the child is drowsy		→	→	→	→	→	→	→	→
			***	***	***	***	***	***	***	***
Ursodiol	300 mg/m²/day PO 150 mg/m²/day PO (Day -8 till day 80)	*	*	*	*	*	*	*	*	*
Stem cells	As per regime dose									*

The regime consists of intravenous administration of Busulfan as a two-hour infusion every 6 hours over 4 (or 5) consecutive days through a central venous catheter only. After a day of rest it is followed by an intravenous infusion of Melphalan.

In case of body weight below 12kg the Melphalan dose calculation is recommended to be dosed by per kg.
For Infants with a weight < or equal to 5 kg, a further 1/3 reduction is advised.

After the chemotherapy the stem cells are infused on the following day.

Supportive treatments:

- Anti-emetics Ondansetron 5mg/m² every 12 hours PO or IV as anti-emetic (maximum single dose 8mg)

Stellenbosch University https://scholar.sun.ac.za

- Adequate hydration is crucial prior to and following chemotherapy especially prior to Melphalan administration due to bladder irritation from high urine concentrations of the drug.
- Minimal urine output immediately prior to and 24 hours following Melphalan administration should be more than 90 ml/m²/hr. To achieve this urine output, give IV hydration at 125 ml/m²/hr.
- Ursodiol: The administration twice per day during the entire prophylactic period, even in the case of mucositis, from day -8 until day 80 post stem cell reinfusion is of major importance.
- G-CSF 5µg/kg/day IV will be given daily beginning on Day +5. G-CSF will continue until a stable increase of WBC > 5 x 10^9/l or ANC >0.5 x 10^9/l
- All blood products (packed red blood cells, platelets) must be irradiated with and be leucocyte depleted (ideally CMV negative). It is recommended that patients receive red packed blood cells to maintain haemoglobin > 8.0g/dl.
- Stop Co-trimoxazole prophylaxis from day 0 until at least day +10 or until WBC ³1.0 x 109/l.
- Prophylactic antifungal treatment with Ketoconazole, Itraconazole or Fluconazole should be avoided, because of the increased risk of VOD with these drugs in particular in association with Busulfan. For proven fungal infection Amphotericin is advised.
- Antibiotics and antivirals should be given in line with the institutional policy whenever Indicated.

Stellenbosch University https://scholar.sun.ac.za

Appendix O6

CADO

Cycle	Day	1	2	3	4	5	6	7	8	9	10
Vincristine	1	*	*	*	*	*	*	*	*	*	*
Doxorubicin	1	*	*	*	*	*	*	*	*	*	*
Cyclophosphamide	2-5	*	*	*	*	*	*	*	*	*	*

Regimen:

- Chemotherapy is administered every 21 days for 10 cycles
- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L

Doses:

Vincristine 1.5mg/m^2 IV stat (max 2mg) x 1 day

Doxorubicin 35mg/m^2 IV in 5% DW over 30 minutes x 1 day

Cyclophosphamide 350mg/m^2 PO x 5 days

Dose modifications:

Infants and low weight children:
Body weight below 12kg should be dosed according to their weight (kg) instead of their body surface area (m^2) according to the known formula of 30 kg= 1 m^2.
Infants with a weight equal and < 5kg:
The dose in mg/kg **with** a further 1/3 dose reduction is advised.

Vincristine 0.05mg/kg IV stat (max 2mg) x 1 day

Doxorubicin

Cyclophosphamide mg/kg PO x 5 days

Precautions:

Infection support:

- Timely antimicrobial interventions according to standard guidelines

Nutritional support

- Naso-gastric nutritional support must be started before starting chemotherapy and weekly measurements and monitoring must take place

Stellenbosch University https://scholar.sun.ac.za

Appendix O7:

Metronomic therapy (M-NB-SA)

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14…**
Propranolol	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Cyclophosphamide	*		*		*		*		*		*		*	
Vinblastine	*	*	*	*	*		*		*		*		*	

Regimen:

Palliative, relapse or refractory setting

- Chemotherapy is administered after cardiac function screening
- Chemotherapy is administered with bone marrow recovery on rising counts of neutrophils of 1.0 x10^5/L and platelets of 100 x 10^5/L

Doses:

Propranolol 3mg/kg PO daily (see precautions)

Cyclophosphamide 30mg/m^2 PO daily – 1 week on, 1 week off

Vinblastine 3mg/m^2 IV over 30 min x 1 day – weekly for 5 weeks, then once a month

Dose modifications:

Infants and low weight children:
Body weight below 12kg should be dosed according to their weight (kg): no dose reductions
Infants with a weight equal and < 5kg: no dose reductions

Precautions:

- Before starting Propranolol cardiac function should be evaluated with echocardiogram and ECG.
- Administration starts with 1mg/kg/d. With stable blood pressures according to age and glucose levels daily doses can be increased by 1mg/kg/d till 3mg/kg/d

Stellenbosch University https://scholar.sun.ac.za

Appendix P

SIOP Boston Ototoxicity Scale	
Grade	**Parameter**
0	< 20 dB HL at all frequencies
1	>20dB HL(i.e. 25 dB HL or greater) SNHL above 4,000 Hz(i.e. 6 or 8kHz)
2	>20dB HL SNHL at 4,000Hz and above
3	>20dB HL SNHL at 2,000 Hz or 3,000Hz and above
4	>40dB HL (i.e. 45dB HL or more) SNHL at 2,000 Hz and above
NOTE: scale is based on sensorineural hearing thresholds in dB hearing level (HL; bone conduction or air conduction with a normal tympanogram). Bone conduction thresholds are used to determine the grade in the case of abnormal tympanometry and/or suspected conductive or mixed hearing loss. Even when the tympanogram is normal, bone conduction is strongly recommended at the single frequency that is determining the ototoxicity grade to fully confirm that the hearing loss at that frequency is sensorineural. Temporary, fluctuating conductive hearing loss due to middle ear dysfunction or wax impaction is common in the paediatric population, and decreases in hearing thresholds that include conductive hearing losses do not reflect ototoxicity to the cochlear. **SNHL** – sensorineural hearing loss	

Diagnostic Paediatric Protocol

Infants younger than 6 months of age:

- Child and family history
- Electrophysiological measure of threshold such as ABR using frequency specific stimuli,
- Diagnostic OAEs,
- Assessment of middle-ear functioning,
- Acoustic reflex thresholds,
- Observation of the infant's behavioural response to sound,

Stellenbosch University https://scholar.sun.ac.za

- Parental report of emerging communication and auditory behaviours.

Infants and toddlers between 6 through 36 months of age:

- Child and family history
- Diagnostic OAEs,
- Assessment of middle-ear functioning,
- Acoustic reflex thresholds,
- Ear-specific behavioural response audiometry according to the child's developmental age (visual reinforcement or conditioned play audiometry),
- Speech detection and recognition measures,
- Parental report of auditory and visual behaviours,
- Screening of communication and language milestones.

Children 36 months and older:

- Child and family history
- Ear-specific behavioural response audiometry according to the child's developmental age (visual reinforcement or conditioned play audiometry),
- Speech detection and recognition measures,
- Parental report of auditory and visual behaviours,
- Screening of communication and language milestones.
- Diagnostic protocols for electro-acoustic and electro-physiologic test-procedures for the paediatric population

Emittance measurements:

Tympanometry

Appropriate measures of middle-ear functioning include tympanometry with high frequency probe tones of 660 or 1000 Hz, but preferably 1000 Hz, for children younger than 8 months of age corrected for prematurity. For children 9 months and older conventional 226 Hz probe tone tympanometry can be used. Pressure sweep should be positive to negative from +200 to - 400 daPa.

Acoustic Reflexes

Acoustic reflexes in children younger than 8 months (corrected for prematurity) must be determined using a 1000Hz probe tone. A conventional probe tone of 226 Hz can be used in children 8 months and older. The stimuli to be used can vary from broadband noise as a general screening stimulus to

Stellenbosch University https://scholar.sun.ac.za

frequency specific tones from 500, 1000, 2000 and 4000 Hz. Measurements should be made contralaterally and/or ipsilaterally as time permits

Diagnostic Oto-acoustic emission:

Transient Evoked (TE) or Distortion Product (DP) oto-acoustic emissions (OAE) may be used for diagnostic testing. Click stimuli can be used for the TEOAE measurements and DPOAE measurements should be conducted from 750 – 8000 Hz.

Auditory evoked potentials:

Auditory brainstem response

Auditory brainstem response (ABR) measurements are the most widely used auditory evoked potential test for estimating hearing loss. The following minimum protocol is recommended for ABR testing for infants and young children:

- Frequency-specific measurements using toneburst (TB) ABR must be conducted to provide threshold information across the frequency spectrum (At least between 500 – 4000 Hz)
- A click stimulus may be used for assessing neurological integrity, cross-checking results, and for monitoring the cochlear microphonic response.
- A cochlear microphonic response must be evaluated by changing stimulus polarity at high intensities for children with absent or abnormal ABR waves. If supra-aural earphones are used the stimulus artefact may obscure the cochlear microphonic response and therefore insert earphones are recommended.
- If conductive hearing loss is suspected bone conduction ABR measurements are recommended to provide frequency-specific bone-conduction thresholds. Auditory steady-state response

The auditory steady-state response (ASSR) is a useful measure for frequency-specific threshold estimations in infants and children. The following minimum protocol is recommended for ASSR testing in infants:

- Frequency specific tones modulated between 70 – 110 Hz should be used for assessing young infants preferably between 500 – 4000 Hz.
- If extended periods of averaging is not utilized at low intensities the technique cannot differentiate between mild hearing loss and normal hearing thresholds in infants and children.
- Since it does not provide information regarding neural integrity it should be used in combination with at least one ABR measurement (a click or

Stellenbosch University https://scholar.sun.ac.za

high-frequency toneburst). This will ensure an indication of neural integrity as determined by the ABR waves or lack thereof in addition to a test for a cochlear microphonic response when waves are absent or abnormal.

- Bone conduction ASSR measurements often result in artefactual responses and is not recommended as a clinical technique at this stage.

Referral protocol upon diagnosis of a hearing loss:

- All children (regardless of age) MUST be referred to Hi-Hopes upon diagnosis of a permanent hearing loss (SNHL or CHL). A detailed audiogram must accompany the referral, and placed in the Hi-Hopes referral box.
- All children under the age of 3 with a confirmed permanent hearing loss (SNHL or CHL) MUST be referred to the Carel du Toit CHAT Centre, regardless of the caregiver's financial situation, or the presence of multiple pathologies.
- All children up until Grade 3 with a confirmed permanent hearing loss (SNHL or CHL) MUST be referred to Carel du Toit School for a school-based assessment.

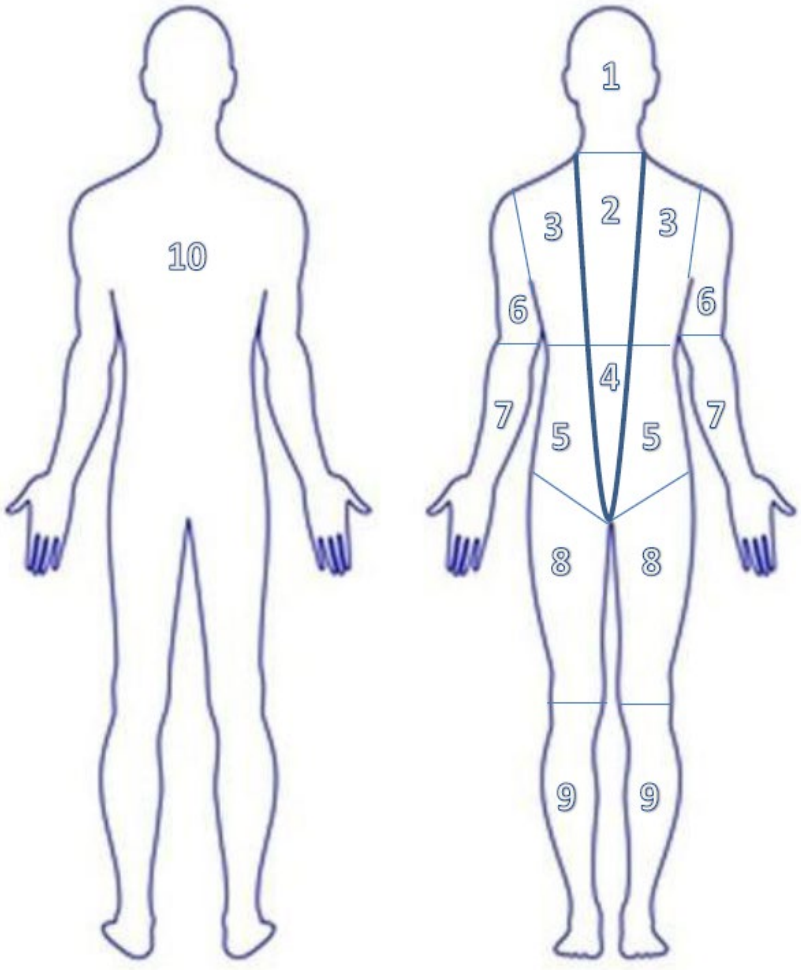
With kind permission from Silvia Kuschke, Chief Audiologist, Red Cross War Memorial Children's Hospital, Rondebosch, Cape Town, South Africa

Stellenbosch University https://scholar.sun.ac.za

Appendix Q

MODIFIED CURIE-SCORE

The scoring system divides the skeleton into 9 compartments.
A 10th compartment represents the soft tissue lesions.



Scoring of MIBG scan
Level of lesion certainty:

0 = unknown
1 = possible
2 = probable
3 = definite

Extent of uptake:

0 = no uptake/ no foci per segment
1 = one focal lesion per segment
2 = more than one focal lesion per segment
3 = diffuse involvement (≥50% of segment involved)

Intensity of uptake:

0 = no sites of uptake
1 = doubtful uptake
2 = obvious but mild uptake
3 = obvious and intense uptake

Soft-tissue lesions scoring system:

0 = no MIBG involvement
1 = one MIBG-avid soft-tissue lesion present
2 = more than one MIBG-avid soft-tissue lesion present
3 = MIBG avidity in a soft-tissue lesion that occupies 50% of the chest or abdomen

*Maximum attainable score is 30
*Focal uptake: uptake with clearly defined margins
* Diffuse uptake: is indistinguishable from background

Stellenbosch University https://scholar.sun.ac.za

Location/ segment involved	Level of certainty	Number of lesions in the segment	Extent of involvement and score	Level of certainty	Intensity of uptake
Head					
Cervico-thorasic spine					
Ribs/sternum/ scapula					
Lumbosacral spine					
Pelvis					
Upper arms					
Forearm and hands					
Upper legs/ Thighs					
Lower legs/ feet					
Soft tissue involvement					
				Score	

PATTERN OF UPTAKE

Focal metastases
(Clear margins distinguishable from the background and/or located to a single region) ☐

or

Diffuse metastases
(No clear margins and/or spread throughout body segments) ☐

Stellenbosch University https://scholar.sun.ac.za

APPENDIX R:

Philadelphia score system for symptomatic stage 4s Neuroblastoma

Clinical signs that indicate progressive disease are the following:

- Deterioration of the general condition
- Feeding difficulties leading to weight loss
- Respiratory failure defined by oxygen requirement or carbon dioxide retention exceeding 60mmHg
- Circulatory failure defined by hypotension or hypertension according to the age specific blood pressure reference values
- Hepatic failure defined by grade 3 toxicity of bilirubin, fibrinogen, or thrombin time according to the NCI-CTC toxicity criteria
- Renal failure defined by impaired blood urea or creatinine, new development of hydronephrosis or deteriorating pre-existent hydronephrosis
- Symptomatic or asymptomatic intraspinal involvement documented by MRI
- Failure of other organ systems.

Indications for chemotherapy treatment is based on the Philadelphia score system. Chemotherapy should be started on a Philadelphia score of **2 or more**.

Philadelphia score system		
Clinical entity	**Grade**	**Score**
GI-tract		
Emesis of > 10% of intake	Mild	1
Repeated emesis requiring IV fluids	Severe	2
Respiratory compromise		
Tachypnoe over 60/min and O2 supplementation	Mild	1
Need for CPAP or mechanical ventilation	Severe	2
Venous return		
Leg oedema	Mild	1
Leg oedema with scrotal a/o sacral oedema	Severe	2
Renal		
Oliguria with output < 2 ml/kg/hr	Mild	1
Oliguria with signs of renal failure, rising ur/creat	Severe	2
Hepatic		
Thrombocytopenia/DIC/Platelet count < 50x109/l	Severe	2
	Total	

Stellenbosch University https://scholar.sun.ac.za

Appendix E: A retrospective analysis of the management and outcome of Neuroblastoma (NB) in South African Children 2000-2014

A retrospective analysis of the management and outcome of Neuroblastoma (NB) in South African Children 2000-2014

STUDY TEAM

JAQUES VAN HEERDEN, PIETERMARITZBURG
MARIANA KRUGER, STELLENBOSCH

STATISTICS
BENN SARTORIUS, DURBAN

Participants
SOUTH AFRICAN CHILDREN'S STUDY GROUP (SACCSG)

Stellenbosch University https://scholar.sun.ac.za

INDEX

Stellenbosch University https://scholar.sun.ac.za

ABBREVIATIONS

ALK	Anaplastic lymphoma kinase
CT	Computer tomography
COG	Children's Oncology Group
COJEC	Cyclophosphamide, Vincristine, Carboplatin, Etoposide, Cisplatin
EFS	Event free survival
FISH	Fluorescent in situ hybridization
HIC	High income countries
HVA	Homovanillic acid
IDRF	Image defined risk factors
INPC	International neuroblastoma pathologic classification
INRG	International neuroblastoma risk group
INSS	International neuroblastoma staging system
LDH	Lactate dehydrogenase
LMIC	Low- and middle-income countries
MIBG	Meta-iodobenzylguanidine
MKI	Mitotic Kyrotosis Index
MRI	Magnetic resonance imaging
MUAC	Mid upper arm circumference
NB	Neuroblastoma
OJEC	Vincristine, Carboplatin, Etoposide, Cyclophosphamide
OPEC	Vincristine, Cisplatin, Etoposide, Cyclophosphamide
OMAS	Opsoclonus/ Myoclonus Syndrome
OS	Overall survival
PCR	Polymerase chain reaction
PODC	Paediatric oncology in developing countries
POU	Paediatric oncology unit
SIOP	International Society of Paediatric Oncology
SACCSG	South African Children Cancer Study Group
TSF	Triceps skin fold
VIP	Vaso-intestinal peptide
VMA	Vannilylmandelic acid

Stellenbosch University https://scholar.sun.ac.za

BACKGROUND

Worldwide neuroblastoma accounts for more than 7% of childhood cancers but contributes to approximately 15% of all childhood cancer deaths [1]. Available data regarding the incidence in South Africa estimates it to be nearly 6% of all childhood malignancies reported from 1987 to 2007 [2].

Neuroblastoma (NB) is a neural crest malignancy that mainly originates from the adrenal glands. It is the most common extracranial solid tumour in childhood [3]. The heterogeneous pathophysiological presentation of neuroblastoma, paired with the diverse prognostic outcome in different stages of the disease, makes the treatment of this disease an oncological challenge [3,4]. The problem of diagnosis is in the diverse nature of the clinical presentation. The peak incidence is between 2 – 5 years of age in the sympathetic (adrenal) lineage of the neurological system [3,4]. Although nearly 65% of tumours present in the abdomen other, sites include the anatomical distribution of the sympathetic chain from the brain to the kidneys and includes pelvic sites [4].

The most common clinical presentation is an abdominal mass [1] yet the most common features of presentation are due to metastatic manifestations such as bilateral proptosis, racoon eyes, bone pain, pancytopaenia and constitutional symptoms (fever and loss of weight). Two paraneoplastic syndromes associated with NB are Vaso-intestinal peptide (VIP) syndrome and Opsoclonus/Myoclonus syndrome (OMAS) [1,5,6]. Other neurological presentations are Horner syndrome, due to tumours in the neck [7], and paraspinal symptomatology. In stage 4s infants may present with cutaneous lesions [4]. A simplified presentation of NB would classify the disease as either local disease, metastatic disease or stage 4s disease. The clinical presentation will then be dependent on the locality of the tumour from local pressure effects like paraspinal disease to a disseminated presentation [1,8].

Two internationally recognised staging systems are the International Neuroblastoma Staging System (INSS) and the International Neuroblastoma Risk Group (INRG). The main difference between the two staging systems is that INSS is a surgical outcome-based system and the INRG is a pre-surgical assessment predicated on imaging, bone marrow morphology and nuclear studies. INRG utilises image defined risks factors (IDRF) such as the encasement, compression and infiltration of structures in local areas to define the staging [9,10]. The staging is performed by clinical examination and using various investigation modalities which includes chest X-ray, abdominal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), meta-iodobenzylguanidine (MIBG) scan as well as bilateral bone marrow biopsies [8,11].

Stellenbosch University https://scholar.sun.ac.za

The diagnosis of NB, a small blue round cell tumour, is made on the basis of pathology which includes immunohistochemistry staining. The hallmark finding on pathology is Homer-Wright rosettes that stain positive for tyrosine hydroxylase, CD56 and synaptophysin [8,12]. The tumour histopathological behaviour varies from spontaneous regression, local maturation or to aggressive proliferation of immature cells that disseminate and cause a high mortality rate [3,4]. By using the Shimada or INPC classification histology is classified into favourable and unfavourable prognostic classifications [9]. The classical clumping of neuroblastoma cells can also be found in the bone marrow in metastatic disease [9].

The aggressiveness of the tumour is determined by genetic changes. NB develops from a germline deletion at the 1p36 or 11q14-23 locus [13,14]. A family history is only identified in 2% of patients with heritable mutations in the anaplastic lymphoma kinase (ALK) gene [15]. Another mutated gene found in the familial type neuroblastoma is PHOX2B [16]. When NB cells have extra copies of the oncogene MYCN; it signifies a more rapid tumour growth and the tumour potentially become resistant to treatment [17]. In contrast the NTRK1 gene (which synthesises the TrkA protein) is often overactive in the cells of NB with a more favourable outcome [18].

As NB is a tumour of the sympathetic chain, predominantly of the adrenal glands, it excretes catecholamine hormones. Homovanillic acid (HVA) and vannilylmandelic acid (VMA) are the two main hormones that function as specific tumour markers for diagnostic purposes which can be traced in an acidified urine sample. The same tumour markers can be used as a screening tool for treatment monitoring and follow up for relapse [12].

Age and stage are still the two most important prognostic factors [8,19,20]. Other prognostic factors that determine the outcome of treatment in NB are translocations, ploidy and the presence of MYCN. MYCN is an adverse prognostic marker specific to NB. In LMIC where either fluorescent in vitro hybridization (FISH) or polymerase chain reaction (PCR) studies to detect the presence of MYCN are not available, ferritin and/or lactate dehydrogenase (LDH) may be used as surrogate markers to indicate an adverse prognosis [8,21].

The treatment of NB is very diverse which necessitates a multidisciplinary team approach. It is essential that the planned treatment protocol include local disease control e.g. surgery. Complete excision of the tumour is vital to cure. In many situations surgery is not a feasible therapeutic modality due to the encasing macroscopic nature of the tumour. When vital structures are encased surgery becomes challenging with a high surgical morbidity and mortality [3,4]. NB is very radio-sensitive with

Stellenbosch University https://scholar.sun.ac.za

a high proliferation index and therefore sensitive to radiotherapy, which can be used if surgery cannot provide local control.

The chemotherapy used, for systemic treatment, in the neo-adjuvant and adjuvant setting is predicated on risk stratification. Taking in consideration all the risk factors each patient is grouped in a low, intermediate and high-risk group. The risk groups determine the amount of cycles and duration the chemotherapy drugs Cisplatin (P) or Carboplatin (J), Vincristine (V), Etoposide (E) and Cyclophosphamide (C) in regimes like OJEC/OPEC and rapid COJEC. Some co-operative groups advocate the inclusion of adriamycin or adaptions of the COG studies for the SIOP-PODC guidelines that advise ifosfamide as part of the high-risk regimes [3,4,8].

In the low risk group patients, especially stage I treated with surgery alone without adverse prognostic markers, the 5-year survival nears 96% in HIC [3]. With the added treatment of chemotherapy and with stage III disease, the intermediate group patients achieve a 5-year survival of 90% in both COG and SIOP studies [3]. It is the metastatic disease patients with high risk grouping that fare poorer with 50 – 60% 5-year survival. If a patient only receives chemotherapy, surgery and radiotherapy the 5-year prognostication is 20 – 30% [3]. With the inclusion of an autologous stem cell transplant as consolidation therapy, the OS is improved with approximately 20% in stage IV patients [22,23]. The outcome with the drug repositioning of Cis-retinoic acid is improved when used post transplantation [24]. The role in a non-transplant setting has not been established. Cis-retinoic acid matures the rapidly dividing neuroblastoma cells into more indolent ganglionic cells and plays a significant role in addressing minimal residual disease in patients who are in complete remission [8,24].

Other therapies include therapeutic MIBG scans which work on the premise of neurotrophin receptor specific affinity as a form of targeted therapy in relapse and refractory settings. The response rates in patients are approximately 15% and 30% respectively [25,26]. MIBG therapy poses great logistic challenges to administer significant doses of radio-isotopes into children [25,26]. In the line of targeted therapies anti-GD2 therapy is an immunotherapy which improved the OS to 60% 5 year survival [27]. Other targeted therapies for NB include ALK-inhibitors which has great promise in pre-clinical trials [28].

In LMIC the treatment choices are limited to mainly chemotherapy, surgery and radiotherapy. Due to advanced disease at diagnosis, tumours are often inoperable and treatment is therefore palliative. Other challenges for the management of neuroblastoma include lack of surgical and radiotherapy skills or equipment, as well as lack of chemotherapy [8,29]. In settings where drug insecurity is high many LMIC do not have even basic medicines for palliation.

Stellenbosch University https://scholar.sun.ac.za

In LMIC many factors play a role in the optimal care of patients with neuroblastoma, such as stage of disease, nutritional status at diagnosis, family resources, belief systems and personal autonomy. Resources, drug security and expertise in institutions influence treatment decisions to a similar extent as treatment adherence and response to treatment. The ability for facilities to provide supportive care in terms of antibiotics, intensive care and granulocyte stimulating factors (GCSF), influences decision making in the intensity of treatment that patients receive [8].

Most published data is from high income countries (HIC) where the five year survival is around 60% for all stages, including advanced stage of the disease [29,31]. In contrast low- and middle-income countries (LMIC) neuroblastoma has a poor prognosis of approximately 20% 5-year overall survival (OS) in advanced disease [29,30]. With the three modalities of chemotherapy, surgery and radiotherapy the prognosis varies from 96% 5-year OS for stage I to a dismal 20% 5-year OS for stage IV [3,4,8]. In LMIC these three modalities are the only modalities available. In some centres not all the modalities are available. This reserves the intensive treatment of NB with autologous transplants, immunotherapy and MIBG therapy for HIC, patients with private funds or medical insurance.

Currently in South Africa, the management protocols used in the treatment of NB have been varied and based on the experience of paediatric oncologists. As collaborative efforts, with data from multiple centres, promotes more robust data, the SACCSG has committed to developing national protocols to treat patients with the same malignancies in a homogenous manner.

The proposed study will be a multi-centre South African Children's Cancer Study Group review in order to investigate the challenges of treating neuroblastoma in South Africa and will inform the development of a prospective national management protocol to ensurehigh quality, uniform treatment for all children treated with NB in South Africa.

AIM

To determine the incidence and investigate the management of neuroblastoma in South Africa.

OBJECTIVES

Primary objectives:

1. To determine the incidence and epidemiology of NB in South Africa
2. To determine the outcome of children treated for NB in South Africa.

Stellenbosch University https://scholar.sun.ac.za

Secondary Objectives:

1. To evaluate outcomes linked to the different chemotherapy protocols used in the treatment of children with NB in South Africa.
2. To analyse data for the development of future treatment protocols.
3. To retrospectively evaluate the predictive value of clinical symptomatology and special investigations in prognostication in NB with the help of multivariate analysis.

METHODOLOGY

Design:

The study will be a retrospective, multi-centre, patient record review of all children treated for neuroblastoma in South Africa and the primary outcome will be evaluated at two time points to identify early survivors and defining cure with:

a) two-year overall survival (OS) and event free survival (EFS).

b) five-year overall survival (OS) and event free survival (EFS).

Setting:

All POUs in South Africa treating children with NB will be invited to participate in the study.

Paediatric Oncology Units are:

University of Cape Town
- Groote Schuur Hospital, Cape Town
- Red Cross War Memorial Children's Hospital, Cape Town
-Rondebosch Hospital, Cape Town

University of the Freestate
- Universitas Hospital, Bloemfontein

University of KwaZulu-Natal
- Inkosi Albert Luthuli Central Hospital, Durban
- Pietermaritzburg Metro Complex, Pietermaritzburg

University of Pretoria
-Steve Biko Academic Hospital, Pretoria
- Unitas Hospital, Pretoria

University of Limpopo
- Polokwane Provincial Hospital, Polokwane

Sefako Makgatho Health Sciences University
- George Mukhari Hospital, Ga-Rankuwa

University of Stellenbosch

Stellenbosch University https://scholar.sun.ac.za

- Tygerberg Hospital, Stellenbosch

Walter Sisulu University

-Frere Hospital, East London

-Port Elizabeth Provincial Hospital, Port Elizabeth

University of Witwatersrand

-Chris Hani Baragwanath Academic Hospital, Johannesburg

-Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg

-Wits Donold Gordon Medical Centre, Johannesburg

Private units

-Parklands Netcare Hospital, Durban

Sample:

Inclusion criteria:

All patients ≤ 18 years treated for NB from 2000 – 2014

a) Biopsy proven NB or

b) Radiological reported neuroblastoma with positive u-catecholamine or

c) BMAT with involvement of NB and a positive u-catecholamine (in cases where a biopsy of the primary tumour is not possible or radiological evidence is inconclusive)

Exclusion criteria:

1) Patients with indeterminate or inconclusive histology results.
2) All patients who completed treatment before 2000.
3) All patients who have **not** completed first line treatment by 31 December 2014.

Sample size:

The sample size depends on the number of patients treated in participating POUs. Literature suggests 35 to 40 NB cases per year [2]. Statistics will be calculated on a minimum of 10 years' data.

A two-sided logrank test with an overall sample size of 350 subjects (~35 per year x 10 years) achieves 80% power at a 0.05 or 5% significance level to detect a minimum hazard ratio of 1.4 when the control group hazard rate is a hazard ratio of 1.00. If it is anticipated that the proportion of subjects having the event during the study is 0.50 or 50% for the lower risk group and 0.70 or 70% for the higher risk group then a two-sided test of whether the hazard ratio is one with an overall sample size of 350 subjects (~35 per year x 10 years) achieves 80% power at a 0.05 or 5% significance level when the

Stellenbosch University https://scholar.sun.ac.za

hazard ratio is approximately 1.47 (i.e. minimum detectable hazard ratio). These results assume that the hazard ratio is constant throughout the study and that Cox proportional hazards regression is used to analyze the data [32,33].

Data collection:

1) Data will be collected at all POUs in South Africa with patients that meet the study criteria – see Appendix F for data sheet.
2) Data will be collected by the investigators into an Excel® spreadsheet which will be identical for all POU.
3) Institutions will assign a numerical identifier to each case in order to blind the principal investigator.
4) All data collection will be centralised at Grey's Hospital, Pietermaritzburg.

Analysis:

1) Data will be analysed using Stata 13.1 SE (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.).
2) Continuous variables will be summarised using mean and standard deviation (SD). If any continuous variables are skewed then medians and interquartile ranges will be presented instead. Categorical data will be presented using frequency tables. Association between two categorical variables will be assessed using the standard Pearson's chi-square ($\chi 2$) test. If expected cell count in the cross tabulation contains fewer than 5 observations (sparse numbers) then the Fisher's exact test will be employed. The standard t-test will be used to compare the mean of continuous explanatory variables by dichotomous outcome classification (e.g. mortality or remission). If the normality assumption is not upheld, then the non-parametric equivalent Wilcoxon rank-sum test (Mann Whitney U test) will be used instead. Comparison of means by 3 or more groups (e.g. risk classification of low, intermediate, high) will be performed using one-way analysis of variance (ANOVA) with post hoc pairwise comparison using Bonferroni correction. If the data are not normal then the

Stellenbosch University https://scholar.sun.ac.za

Kruskal-Wallis equality-of-populations rank test will be used instead. Time to event (survival) analysis will also be employed.

3) Kaplan-Meier survival curves will be developed and differences by groups assessed using a log rank test. This will be extended to a multivariable proportional hazards (Cox) regression model to adjust for confounding factors and test for potential interactions. The proportional hazards assumption of the model will be assessed to confirm adequacy. If this assumption is not upheld then a semi-parametric and/or parametric survival model will be used instead. An adjusted p-value of <0.05 will be considered statistically significant.

Budget:

As this is a retrospective record review no additional cost will be incurred above the standard care of treatment currently received in South Africa.

DISSEMINATION OF INFORMATION

- The aim is to publish results in a major international journal, e.g. Pediatric Blood and Cancer or Journal of Clinical Oncology for peer review and/or degree purposes.
- An abstract will be submitted for oral presentation at the SIOP international 2017 meeting.
- Data will be presented in academic forums at local institutions.

CONFLICT OF INTEREST

The investigators wish to declare no conflict of interest in terms of this study.

ETHICAL CONSIDERATIONS

- Confidentiality and anonymity will be guaranteed by numerical identifiers in the database both for institutions and patients.
- All data will be securely stored in locked premises and password protected computers. Only the investigator will have access to both the premises and computer.
- Ethical and scientific approval for the study will be sought by all participating POU under the guidelines and regulations of local academic universities and HREC.

Stellenbosch University https://scholar.sun.ac.za

REFERENCES

1. Maris, J; Hogarty, M; Bagatell, R; Cohn, S.: Neuroblastoma. Lancet 2007; 369: 2106-20
2. Stefan, D; Stones, D; Wainright, R; Kruger, M; Davidson, A; Poole, J; Hadley, G; Forman, D; Colombet, M; Steliarova-Foucher, E. Childhood cancer incidence in South Africa, 1987 – 2007. S Afr Med J 2015;105(11):939-947
3. National Cancer Institute: Surveillance, Epidemiology and End Results Database http://seer.cancer.gov (Accessed Nov 2015)
4. National Cancer Institute: Neuroblastoma http://www.cancer.gov/types/neuroblastoma (Accessed Nov 2015)
5. Bourdeaut, F; de Carli, E; Timsit, S; et al.: VIP hypersecretion as primary or secondary syndrome in neuroblastoma: A retrospective study by the Société Française des Cancers de l'Enfant (SFCE). Pediatr Blood Cancer 52 (5): 585-90, 2009.
6. Matthay, K; Blaes, F; Hero, B; et al.: Opsoclonus myoclonus syndrome in neuroblastoma a report from a workshop on the dancing eyes syndrome at the advances in neuroblastoma meeting in Genoa, Italy, 2004. Cancer Lett 228 (1-2): 275-82, 2005.
7. Mahoney, N; Liu, G; Menacker, S; et al.: Pediatric horner syndrome: etiologies and roles of imaging and urine studies to detect neuroblastoma and other responsible mass lesions. Am J Ophthalmol 142 (4): 651-9, 2006.
8. Parikh, N; Howard, S; Chantada, G; Israels, T; Khattab, M; Alcasabas, P; Lam, C; Faulkner, L; Park, J; London, W; Matthay, K: SIOP-PODC adapted risk stratification and treatment guidelines: Recommendations for neuroblastoma in low- and middle-income settings. Pediatr Blood Cancer 2015; 62:1305-1316
9. Brodeur, G; Seeger, R; Barrett, A; et al.: International criteria for diagnosis, staging, and response to treatment in patients with neuroblastoma. J Clin Oncol 6 (12): 1874-81, 1988.
10. Monclair T, Brodeur GM, Ambros PF, et al.: The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. J Clin Oncol 27 (2): 298-303, 2009.
11. Parikh, N; Sholler, G; Roberts, S; Howard, S; Shochat, S; Haase, G; Borker, A; Kurkure, P; Sachdeva, A; Matthay, K. Advancing diagnosis and treatment of neuroblastoma for children In Low income countries (LIC) via web-based tumor board platform. In: Robert Arceci JMD, Ph. D, Peter E, Newburger MD, editors. Auckland, New Zealand: Wiley Periodicals Inc; 2011. p. 733.
12. Cohn, S; Diagnosis and classification of Small round-cell tumors of childhood. American Journal of Pathology, Vol. 155, No. 1, July 1999
13. Mossé, Y; Greshock, J; King, A; et al.: Identification and high-resolution mapping of a constitutional 11q deletion in an infant with multifocal neuroblastoma. Lancet Oncol 4 (12): 769-71, 2003.
14. Satgé D, Moore SW, Stiller CA, et al.: Abnormal constitutional karyotypes in patients with neuroblastoma: a report of four new cases and review of 47 others in the literature. Cancer Genet Cytogenet 147 (2): 89-98, 2003.
15. Mossé, Y; Laudenslager, M; Longo, L; et al.: Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 455 (7215): 930-5, 2008.
16. Mossé, Y; Laudenslager, M; Khazi, D; et al.: Germline PHOX2B mutation in hereditary neuroblastoma. Am J Hum Genet 75 (4): 727-30, 2004.
17. Tang, X; et al: The MYCN enigma: significance of MYCN expression in Neuroblastoma. Cancer Res 2006; 66: 2826-33.
18. Lastowska; M, Cullinane, C; Variend, S; et al.: Comprehensive genetic and histopathologic study reveals three types of neuroblastoma tumors. J Clin Oncol 19 (12): 3080-90, 2001.
19. Cotterill, S; Pearson, A; Pritchard, J; et al.: Clinical prognostic factors in 1277 patients with neuroblastoma: results of The European Neuroblastoma Study Group 'Survey' 1982-1992. Eur J Cancer 36 (7): 901-8, 2000.

Stellenbosch University https://scholar.sun.ac.za

20. Hesseling PB, Ankone K, Wessels G, Schneider JW, Du Plessis, Moore L. Neuroblastoma in southern Africa: Epidemiological features, prognostic factors and outcome. Ann Trop Paediatr 1999; 19:357–363.
21. Cangemi, G; Reggiardo, G; Barco, S; Barbagallo, L; Conte, M; D'Angelo, P; Bianchi, M; Favre, C; Galleni, B; Melioli, G; Haupt, R; Garaventa, A; Corrias, M. Prognostic value of ferritin, neuron-specific enolase, lactate dehydrogenase, and urinary and plasmatic catecholamine metabolites in children with neuroblastoma. Onco Targets Ther 2012; 5:417–423.
22. Berthold, F; Boos, J; Burdach, S; Erttmann, R; Henze, G; Hermann, J; Klingebiel, T; Kremens, B; Schilling, F; Schrappe, M; Simon, T; Hero, B. Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: A randomised controlled trial. Lancet Oncol. 2005; 6:649–658.
23. Pritchard, J; Cotterill, S; Germond, S; Imeson, J; de Kraker, J; Jones, D; High dose melphalan in the treatment of advanced neuroblastoma: Results of a randomised trial (ENSG-1) by the European Neuroblastoma Study Group. Pediatr Blood Cancer. 2005; 44:348–357.
24. Kohler, J; Imeson, J; Ellershaw, C; Lie, S.: A randomized trial of 13-Cis retinoic acid in children with advanced neuroblastoma after high-dose therapy. British Journal of Cancer. 2000; 83(9), 1124–1127.
25. Polishchuk, A; Dubois, S; Haas-Kogan, D; et al.: Response, survival, and toxicity after iodine-131-metaiodobenzylguanidine therapy for neuroblastoma in preadolescents, adolescents, and adults. Cancer 117 (18): 4286-93, 2011.
26. Matthay, K; Yanik, G; Messina, J; et al.: Phase II study on the effect of disease sites, age, and prior therapy on response to iodine-131-metaiodobenzylguanidine therapy in refractory neuroblastoma. J Clin Oncol 25 (9): 1054-60, 2007.
27. Matthey, K; et al: Promising therapeutic targets in Neuroblastoma. Clin Cancer Res 2012 May 15;(18)10: 2740-53.
28. Xue, C; et al: Small molecule drugs and targeted therapies for Neuroblastoma. www.intechopen.com (accessed Nov 2015)
29. Magrath, I; Steliarova-Foucher, E; Epelman, S; Ribeiro, R; Harif, M; Li, C; Kebudi, R; Macfarlane, S; Howard, S.: Paediatric cancer in low-income and middle-income countries. Lancet Oncol. 2013; 14:e104–e116.
30. Parkin, D; Stiller, C; Draper, G; Bieber, C; The international incidence of childhood cancer. Int J Cancer. 1988; 42:511–520.
31. Stiller, C; Parkin, D; International variations in the incidence of neuroblastoma. Int J Cancer. 1992; 52:538–543.
32. Schoenfeld, David A. 1983. 'Sample Size Formula for the Proportional-Hazards Regression Model', Biometrics, Volume 39, Pages 499-503.

Stellenbosch University https://scholar.sun.ac.za

Appendix A

International Neuroblastoma Staging System (INSS)

Stage 1	• Localised tumour with complete gross excision, with or without microscopic residual disease • Representative ipsilateral lymph nodes negative for tumour microscopically
Stage 2A	• Localised tumour with incomplete gross excision • Representative ipsilateral non-adherent lymph nodes negative for tumour microscopically.
Stage 2B	• Localised tumour with or without complete gross excision, with ipsilateral non-adherent lymph nodes positive for tumour. • Enlarged contralateral lymph nodes must be negative microscopically
Stage 3	• Unresectable unilateral tumour infiltrating across the midline, with or without regional lymph node involvement • Localised unilateral tumour with contralateral regional lymph node involvement • Midline tumour with bilateral extension by infiltration (unresectable) or by lymph node involvement. The midline is defined as the vertebral column. Tumours originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.
Stage 4	• Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs, except as defined for stage 4S.
Stage 4S	Localised primary tumour, as defined for stage 1, 2A, or 2B, with dissemination limited to skin, liver, and/or bone marrow (by definition limited to infants younger than 12 months). Marrow involvement should be minimal (i.e., <10% of total nucleated cells identified as malignant by bone biopsy or by bone marrow aspirate). More extensive bone marrow involvement would be considered stage 4 disease. The results of the MIBG scan, if performed, should be negative for disease in the bone marrow.

Stellenbosch University https://scholar.sun.ac.za

Appendix B

International Neuroblastoma Risk Group (INRG)

International Neuroblastoma Risk Group Pre-treatment Classification Scheme							
INGR stage	**Age (months)**	**Histologic classification**	**Grade of tumour differentiation**	**MYCN**	**11q Aberration**	**Ploidy**	**Pre-treatment risk group**
L1/L2		GN maturing GN intermixed					A – very low
L1		Any except GN maturing GN intermixed		N/A			B – very low
L2	<18	Any except GN maturing GN intermixed		Amp			K – high
				N/A	No		D – low
	>18	GNB nodular neuroblastoma	Differentiating		Yes		G –intermediate
				N/A	No		E – low
			Poorly differentiated or undifferentiated		Yes		H –intermediate
				N/A			H – intermediate
M	<18			Amp			N – High
	<12			N/A		Hyper	F – Low
	12 to <18			N/A		Diploid	I – Intermediate
	<18			N/A		Diploid	J –Intermediate
	>18			Amp			O – High
MS	<18						P – High
				N/A			C – very low
							Q – High
				Amp			R – High

Appendix C

Image Defined Risk Factor (IDRF)

IDRF include the following:

- Ipsilateral tumour extension within two body compartments: neck and chest; chest and abdomen; abdomen and pelvis.
- Infiltration of adjacent organs/structures: pericardium, diaphragm, kidney, liver, duodeno-pancreatic block, mesentery.
- Encasement of major vessels by tumour: vertebral artery, internal jugular vein, subclavian vessels, carotid artery, aorta, vena cava, major thoracic vessels, branches of the superior mesenteric artery at its root and the coeliac axis,

Stellenbosch University https://scholar.sun.ac.za

iliac vessels.

- Compression of trachea or central bronchi.
- Encasement of brachial plexus.
- Infiltration of port hepatic or hepato-duodenal ligament.
- Infiltration of the costo-vertebral junction between T9 and T12.
- Tumour crossing the sciatic notch.
- Tumour invading renal pedicle.
- Extension of tumour to base of skull.
- Intraspinal tumour extension such that more than one-third of the spinal canal is invaded, leptomeningeal space is obliterated, or spinal cord MRI signal is abnormal.

L1	Localised tumour not involving vital structures as defined by the list of IDRF and confined to one body compartment.
L2	Loco-regional tumour with presence of one or more IDRF
M	Distant metastatic disease (except MS).
MS	Metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow. (Stage 4S)

Stellenbosch University https://scholar.sun.ac.za

Appendix D

International Neuroblastoma Pathological Classification (INPC)/Shimada classification

International Neuroblastoma Pathology classification		Original Shimada classification	Prognostic group
Neuroblastoma (Schwannian stroma-poor) • **Undifferentiated** • **Poor differentiated** • **Differentiating**		**Stroma-poor**	**FH**
<1.5 yrs	Poorly differentiated or differentiating & low or intermediate MKI tumour		**FH**
1.5–5 yrs	Differentiating & low MKI tumour		**FH**
<1.5 yrs	c) undifferentiated tumour d) high MKI tumour		**UH**
1.5–5 yrs	c) undifferentiated or poorly differentiated tumour d) intermediate or high MKI tumour		**UH**
≥5 yrs	All tumours		**UH**
Ganglioneuroblastoma intermixed (Schwannian stroma-poor)		**Stroma-rich Intermixed**	**FH**
Ganglioneuroma intermixed (Schwannian stroma-dominant) • **Maturing** • **Mature**		**Well differentiated Ganglioneuroma**	**FH**
Ganglioneuroblastoma nodular (Schwannian stroma-rich/stroma-dominant and stroma-poor)		**Stroma-rich Nodular**	**UH**

FH – Favourable histology, UH – Unfavourable histology

Stellenbosch University https://scholar.sun.ac.za

Appendix E

COG Risk grouping:

INSS	Age	MYCN	Shimada	DNA ploidy	Risk group
1	Any	Any	Any	Any	Low
2A/2B	<365d	Any	Any	Any	Low
	>365d	Non-amplified	Any	-	Low
	>365d	Amplified	FH	-	Low
	>365d	Amplified	UH	-	High
3	<365d	Non-amplified	Any	Any	Intermediate
	<365d	Amplified	Any	Any	High
	>365d	Non-amplified	FH	-	Intermediate
	>365d	Non-amplified	UH	-	High
	>365d	Amplified	Any	-	High
4	<548d	Non-amplified	Any	Any	Intermediate
	<548d	Amplified	Any	Any	High
	>548d	Any	Any	-	High
4S	<365d	Non-amplified	FH	>1	Low
	<365d	Non-amplified	Any	1	Intermediate
	<365d	Non-amplified	UH	Any	Intermediate
	<365d	Amplified	Any	Any	High

Low risk

- All children who are Stage 1
- Any child who is Stage 2A or 2B and younger than age 1
- Any child who is Stage 2A or 2B, older than age 1, whose cancer has *no* extra copies of the *MYCN* gene
- Any child who is Stage 4S (younger than age 1), whose cancer has favourable histology, is hyperdiploid (excess DNA) and has *no* extra copies of the *MYCN* gene

Intermediate risk

- Any child who is Stage 3, younger than age 1, whose cancer has *no* extra copies of the *MYCN* gene
- Any child who is Stage 3, older than age 1, whose cancer has *no* extra copies of the *MYCN* gene and has favourable histology (appearance under the microscope)
- Any child who is Stage 4, younger than age 1, whose cancer has *no* extra copies of the *MYCN* gene
- Any child who is Stage 4S (younger than age 1), whose cancer has *no* extra copies of the *MYCN* gene and has normal DNA ploidy (number of chromosomes) and/or has unfavourable histology

High risk

- Any child who is Stage 2A or 2B, older than age 1, whose cancer has extra copies of the *MYCN* gene
- Any child who is Stage 3, younger than age 1, whose cancer has extra copies of the *MYCN* gene
- Any child who is Stage 3, older than age 1, whose cancer has extra copies of the *MYCN* gene
- Any child who is Stage 3, older than 18 months of age, whose cancer has unfavourable histology
- Any child who is Stage 4, whose cancer has extra copies of the *MYCN* gene regardless of age

Stellenbosch University https://scholar.sun.ac.za

- Any child who is Stage 4 and older than 18 months
- Any child who is Stage 4 and between 12 and 18 months old whose cancer has extra copies of the *MYCN* gene, unfavourable histology, and/or normal DNA ploidy (a DNA index of 1)
- Any child who is Stage 4S (younger than age 1), whose cancer has extra copies of the *MYCN* gene

Appendix F

If any aspect is not done please fill **NotD**

Data sheets codes:

Information	Classification	Code
Source ID code:		
Sex:	Female Male	F M
Race: (Statistical purposes)	African Indian Mixed race (Coloured) White Other (Specify)	A I M W O
Date of birth (DOB): yy/mm/dd		
Age (months):		
Date of diagnosis (DOD): yy/mm/dd		
Anthropometry (values): Weight Height/length BMI (Will be determined by the PI) MUAC TSF (Triceps skin fold)	(Will be determined by the PI)	
Presenting symptoms:		

Stellenbosch University https://scholar.sun.ac.za

Bone pain:	No Yes	N Y
Clinical skull metastasis:	No Yes	N Y
Racoon eyes:	No Yes	N Y
Duration of symptoms: (From onset of disease related symptoms till diagnosis)	Days (State value i.e. 1 week = 7 days)	
Hypertension	No Yes	N Y
Paraneoplastic syndromes (ParaNS):	OMAS (Opsoclonus Myoclonus Syndrome) VIP (Vaso Intestinal Peptide Syndrome) Other (Specify)	O V
Emergency	Spinal compression Hepatic pain Respiratory distress Horner Syndrome/ Airway compromise Intestinal obstruction Other (Specify)	Sp L R HS IO
HIV status	Positive Negative Unknown	P N U
TB status	Positive – New Positive – on treatment Negative Unknown	PN PT N U

Stellenbosch University https://scholar.sun.ac.za

Other infectious diseases:		
Co-morbid disease or Syndromes (Sxd) (Specify):		
Site of primary	Adrenal Abdominal (other than adrenal) Chest Neck Primary not found Other (Specify)	Ad Am C N PNF
Stage (INSS):	Please see appendix A	
Stage (INRG):	Please see appendix B	
Image defined risk factors (IDRF):	Please see appendix C	
Sites of metastasis:	Bone (Specify) Bone marrow Intra abdominal Liver Local invasion Lungs Lymphnodes (Specify) Paraspinal Skull Skin	BT BM Am L I R N Sp Sl Sn
Cytopaenia:	Anaemia Leucopaenia Trombocytopaenia Bicytopaenia Pancytopaenia	A L T e.g. AT / AL/ LT P

Stellenbosch University https://scholar.sun.ac.za

LDH (value):		
Ferritin (value):		
ESR (value):		
Haptoglobin (value):		
ALP (value):		
Bloods at diagnosis (value): HCO3 Potassium Magnesium		
U-catecholamine State raised: R Not raised: N Example: HVA R or HVA N	HMA VMA Other	
CXR:	Lung parenchyma Paraspinal Other (Specify)	LP PS
Skull XR involvement:	Coin lesions Oribital lesions Other (Specify)	CL OR
Other X-rays: bone involvement	Describe main areas	
ABD U/S:	Adrenal Other (Specify)	Adr
CT scan/MRI: (Example for adrenal primary with liver and node involvement: Adr, L, M Or	Primary: Adrenal Non adrenal Unkown Lymphnodes:	 Adr Non U LN

Stellenbosch University https://scholar.sun.ac.za

Paraspinal primary with local infiltration Non, I)	Local infiltration: Metastasis liver	I M
CT scan bone involvement:	Specify sites	
MIBG scan: Pre-treatment Reassessment End of treatment	Bone Marrow involvement: Bone involvement (sites):	
Bone scan: Pre-treatment Reassessment End of treatment	Describe main areas	
BMAT involved pre-treatment:	No Yes	N Y
BMAT involved reassessment:	No Yes Not applicable (non-stage 4)	N Y A
BM aspirate pre-treatment:	No Yes	N Y
% involvement pre-treatment	Packed Moderate	P M
BM aspirate reassessment:	No Yes Not applicable (non-stage 4)	N Y A
% involvement reassessment	Packed Improved Clear	P I C

Stellenbosch University https://scholar.sun.ac.za

Trephine pre-treatment:	No Yes	N Y
Trephine reassessment:	No Yes Not applicable (non-stage 4)	N Y A
Histology (INPC):	Favourable: Unfavourable:	F U
MYCN amplification:	Negative Extra copies Positive	N EC P
Ploidy (DNA index):	Diploid Hyperdiploid	D H
Genetics markers:	1p 11q	1p 11q
Risk classification: (INSS or INRG)	Very low risk Low risk Intermediate risk High risk	VLR LR IR HR
Treatment intent from onset:	Cure Palliative	C P
Chemotherapy (Regimes):		
Chemotherapy adapted or interrupted	No Yes	N Y
Reason:	Death Abandonment of treatment Tumour related complications Medical complication	D A T M

Stellenbosch University https://scholar.sun.ac.za

	Other (Specify)	
Surgery:	Complete resection: Incomplete resection: No surgery:	C I N
Radiotherapy:	Describe areas	
Remission:	No Yes	N Y
Autologous transplant (Auto Tx):	No Yes	N Y
Conditioning regime:		
Toxicity: Neutropaenia Anaemia Thrombocytopaenia Ototoxicity Renal toxicity Neurotoxicity Chemical cystitis Fanconi Syndrome Other	Describe presence during induction treatment thus Rapid COJEC and OJEC/OPEC or institutional induction when other protocols used)	
Palliation (please state): Chemotherapy and type Other		
Infections (Infx): Documented microbes	Blood culture Urine Other (Specify)	
Outcomes:	Remission (<5 years post last treatment)	R

Stellenbosch University https://scholar.sun.ac.za

	Relapse	
	Primary site	PR
	Metastatic site	MR
	Alive (>5 years post last treatment)	A
	Dead	D
Lost to follow up (LTF):	No	N
	Yes	Y
Date last seen (DLS): yy/mm/dd		
Date of Death: (DODx): yy/mm/dd		
Reason for death	Disease	D
	Toxicity (Specify)	T
	Disease related mortality (Specify)	
	Other (Specify)	
Port in situ/ Hickman	No	N
	Yes	Y

Stellenbosch University https://scholar.sun.ac.za

Appendix F: The association between tumour markers and ^{123}I-MIBG scans in neuroblastoma in low resource settings

The association between tumour markers and ^{123}I-MIBG scans in neuroblastoma.

Principal investigator

Jaques van Heerden, Antwerpen, Belgium

Co-Principal investigator:

Mariana Kruger, Stellenbosch, South Africa

Local co-investigators:

Baragwanath Hospital POU – Drs Gita Naidu en Lizette Louw

Charlotte Maxeke Johannesburg Academic Hospital POU – Dr Jennifer Geel

Frere General Hospital POU – Dr Barry van Emmenes

Polokwane General Hospital POU- Dr Vuthilo Netshituni

Port Elizabeth General Hospital POU – Dr Johani Vermeulen

Red Cross War Memorial Children's Hospital POU – **Dr Anita Brink**

Steve Biko Academic Hospital POU – Dr Ané Buchner

Tygerberg General Hospital POU – Dr Anel van Zyl

Universitas Bloemfontein Genereal Hospital POU – Dr Jan du Plessis and Dr Gert Engelbrecht

Inkosi Albert Luthuli Central Hospital – Dr Nozipho Nyakala

Stellenbosch University https://scholar.sun.ac.za

INDEX

Stellenbosch University https://scholar.sun.ac.za

ABBREVIATIONS

ALK	Anaplastic lymphoma kinase
BMAT	Bone Marrow aspirate and trephine
CT	Computer tomography
COG	Children's Oncology Group
CSR	Complete surgical resection
DIC	Disseminated intravascular coagulation
EBRT	External beam radiotherapy
EFS	Event free survival
EGFR	Epidermal growth factor receptor
FISH	Fluorescent in vitro hybridization
GaTATE	Gallium-68 DOTA-Octreotate
GC-MS	Gas chromatography spectrometry
HIC	High income countries
HVA	Homovanillic acid
IDRF	Image defined risk factors
IMRT	Intensity modulated radiotherapy
INRG	International Neuroblastoma risk group
INSS	International Neuroblastoma staging system
IORT	Intra-operative radiotherapy
ITCC	Innovative Therapies for Children with Cancer
LDH	Lactate dehydrogenase
LMIC	Low- and middle-income countries
MIBG	Meta-iodobenzylguanidine
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NANT	New Approaches to Neuroblastoma Treatment Consortium
NA	Noradrenalin
NB	Neuroblastoma
OMAS	Opsoclonus/ Myoclonus Syndrome
OS	Overall survival
PCR	Polymerase chain reaction
PET	Positron emission tomography
PFS	Progression free survival
PRT	Proton beam radiotherapy
PhRT	Photon beam radiotherapy
PODC	Paediatric oncology in developing countries
POU	Paediatric oncology unit
PSR	Partial surgical resection
SIOP	International society of paediatric oncology
SIOPEN	SIOP European Neuroblastoma group
SACCSG	South African children cancer study group
TAMs	Tumour associated macrophages
VIP	Vaso-intestinal peptide
VMA	Vannilylmandelic acid

Stellenbosch University https://scholar.sun.ac.za

PREMISE

Neuroblastoma (NB) is a childhood malignancy with a poor prognosis, especially stage 4 disease [1,2,3], even in high income countries (HIC). Without high intensity treatment advanced stage disease has a poor outcome in low- and middle-income countries (LMIC) with treatment being palliative rather than curative [4,5].

The heterogeneous clinical presentation of NB and the diverse pathophysiological disease characteristics makes staging and treatment challenging [5,6].

Yet Neuroblastoma has a diverse pathophysiology that includes metabolic, inflammatory and genetic components [7,8] which has not been fully studied in a Sub-Saharan context.

SUBJECT REVIEW

Definition:

Neuroblastoma commonly presents in children with a peak incidence between 2 – 10 years of age [2,3]. The primary lesions develop along the sympathetic chain and the adrenal glands, yet up to 60% of children present with primary metastatic disease [10].

The most common clinical presentation is an abdominal mass [4] and features of metastatic manifestations such as bilateral proptosis, racoon eyes, bone pain, pancytopaenia and constitutional symptoms (fever and loss of weight). In stage 4s infants may present with cutaneous lesions [9]. A simplified presentation of NB would classify the disease as either local disease, metastatic disease or stage 4s disease. Metastatic disease involves primarily the bone and bone marrow [3,9]. In stage 4s, apart from the primary lesion, infants of under 12 months may present with cutaneous lesions, bone marrow infiltration of less than 10% and/or liver lesions [3, 9].

Pathophysiology:

The development of NB is dependent on accumulation of mutations where the pre-malignant cells rapidly replicate in proliferating tissue during embryogenesis in the central nervous system [11,12]. In NB the NMYC proto-oncogene has traditionally been overexpressed in neurogenesis for the rapid expansion of progenitor cell populations [12,13]. Epidermal growth factor receptor (EGFR) gene and PHOX2B gene has also be associated with the development of NB [12,14,15].

The diagnosis and staging of NB require many non-invasive and invasive investigations. The gold standard for the diagnosis of NB is histology [16,17]. The diagnosis of NB, a small blue round cell tumour, is made on the basis of pathology which includes immunohistochemistry staining. The hallmark finding on pathology is Homer-Wright rosettes that stain positive for tyrosine hydroxylase, CD56 and synaptophysin [5,18]. The tumour's histopathological behaviour varies from spontaneous regression, local maturation or to aggressive proliferation of immature cells that disseminate and cause a high mortality rate [3,4]. The Modified Shimada or International Neuroblastoma Pathology Classification (INPC) histology classification system is used not only for diagnosis but for the distinction into favourable and unfavourable prognostic classifications [19].

Stellenbosch University https://scholar.sun.ac.za

For staging purposes, it is necessary to investigate the potential NB infiltration into the bone and bone marrow via a bone marrow aspirate and bone core trephine (BMAT) [20]. Before nuclear medicine imaging, bone marrow aspirate was the gold standard for diagnosing metastasis. Yet BMATs are site specific investigations [20]. An Iodine-123-Meta-iodobenzylguanidine (^{123}I-MIBG) scan is more reliable because the whole body is screened for metastasis [21, 22]. The limitation of ^{123}I-MIBG scan is that it can't detect small clusters of cells like with BMATs [20, 21, 22]. Many studies have compared ^{123}I-MIBG scan to BMAT for the diagnosis of metastasis, and in the monitoring of treatment response and diagnose relapse [19,23]. Hero et al had proven in a study with 367 unselected stage 4 NB patients, treated according to the German cooperative trial NB90 at diagnosis, ^{123}I-MIBG scan was positive in 306 patients (92%), borderline in seven patients (2%), and negative in 19 patients (6%) whilst bone marrow aspirates were cytologically positive in 292 patients (84%) and negative in 57 patients (16%) [24]. This study concluded that low frequency ^{123}I-MIBG -scan might be false negative and that a supplemental bilateral bone marrow was still important [24].

Nuclear medicine and imaging in management:

Two isotopes ^{123}I-MIBG and ^{131}I-MIBG are selectively taken up by tumour cells of sympatho-adrenal origin [25, 26, 27]. To aid in the diagnosis and detect metastases of NB the type 1 catecholamine transport uptake mechanism of the tumour cells has the unique ability to concentrate especially ^{123}I-MIBG [28]. In a Cochrane review of 11 studies the sensitivity of MIBG varied between 67 -100% [29]. In 20% of histologically proven NB the MIBG scan may be negative. The lower radiation dose and optimal imaging characteristics of ^{123}I-MIBG in comparison to ^{131}I-MIBG, makes ^{123}I-MIBG the preferred radiopharmaceutical for imaging of Neuroblastoma [30]. Even with a high sensitivity and specificity in 10% of Neuroblastomas MIBG is not taken up because of a low expression of the norepinephrine transporter [31].

Other staging imaging studies include magnetic resonance imaging (MRI), computer tomography (CT) and positron emission tomography (PET) scans. The information is important for anatomic imaging such as determination of size of primary tumours, extent of regional disease and in detecting distant metastases [32]. This is reflected in the INRG image defined risk factors [33]. MRI and CT detect the encasement of vital structures and involvement of cortical bone metastases in NB. There were many limitations including availability, radiation doses and detection is limited to the actual fields scanned. CT and MRI have sensitivities of 43% and 97% respectively for detecting stage 4 disease and specificities are reported to be 97% and 88% respectively. Yet combined with ^{123}I-MIBG scans the sensitivity and specificity of disease detection in Neuroblastoma increase to 99% and 95% respectively [32]. Thus, metabolic and pathophysiological aspects for the detection of metastases are combined [32] with a high sensitivity to detect post treatment metastatic disease [34]. This characteristic is vital in the utilization of reviewing treatment response [28].

^{123}I-MIBG scan is not just used as a diagnostic test or a tool to evaluate treatment response but can assist in determination of prognosis. [35,36] Both International Society of Paediatric Oncology European Neuroblastoma group (SIOPEN) and Children's Oncology Group (COG) developed scoring methods using ^{123}I-MIBG in semi-quantitative methods to use as a prognostic marker for response and survival in Neuroblastoma with ^{123}I-MIBG -avid disease.

Stellenbosch University https://scholar.sun.ac.za

Both scoring systems divide the body into segments. The Curie score (developed by COG) divides the body into 9 segments (with four degrees of nuclear differentiation) where the SIOPEN scoring system divides the body into 12 segments (with six degrees of nuclear differentiation) [35,36].

In terms of prognostication patients with a Curie score greater than 2 after induction therapy, had a significantly worse 3 years event-free survival (EFS) of 15% than those with scores less than 2 of 45% [35]. The German Pediatric Oncology Group investigated the prognostic value of both the Curie and SIOPEN scoring method, as well as comparing them. Both scoring systems proved good prognostication tools for outcome [36]. A Curie score of 2 or less and a SIOPEN score of 4 or less at diagnosis correlated to significantly better with event free survival (EFS) and overall survival (OS) as compared with higher scores. After four cycles of induction, those with complete response on MIBG testing had a better outcome than those with residual uptake [36].

The limitation of the Curie score and the SIOPEN score is that both do not take in consideration the collection of radio-isotopes in the soft tissue. Subsequently the Curie score includes a 10^{th} segment for soft tissue involvement and is known as the modified Curie score [36]. The modified Curie score not only takes in account the bone and bone marrow distribution but soft tissue involvement as well without losing prognostic relevance [36].

Yanik and colleagues studied 280 patients with stage 4 neuroblastoma who were on Children Oncology Group (COG) protocol A3973. They reported that in 52 patients with a Curie score > 2 had a decreased event free survival (EFS) compared to the 185 patients those with Curie scores ≤ 2 after induction therapy with 3-year EFS of 15.4% ± 5.3% vs. 44.9% ± 3.9% (p = 0.001) [35]. In the same study tumour reduction of more than 50% in the Curie score from diagnosis to after induction had a much better survival when compared to those with less than 50% reduction [35]. Katzenstein et al studied 29 patients with NB to determine the prognostic significance of ^{123}I-MIBG scan scores [28]. By using a scoring scheme that divided the skeleton into 10 segments it was concluded that the post induction scan predicted prognosis as ^{123}I-MIBG score ≥ 3 after induction therapy was associated with a significantly worse event free survival [28].

Perel et al reported that an abnormal post induction chemotherapy ^{123}I-MIBG scan was associated with a poor outcome [25]. However, Andrich et al did not find post therapy ^{123}I-MIBG imaging findings to be predictive of prognosis [37]. It is important to note that residual ^{123}I-MIBG avid disease on the post induction chemotherapy ^{123}I-MIBG scan predicts poor outcome after allogenic stem cell transplant [22] and relapse after high dose therapy with peripheral blood stem cell rescue, local radiotherapy, and cis-retinoic acid [28].

Two distinct metastatic patterns of ^{123}I-MIBG distribution in stage 4 NB where found by Bleeker et al [34]. The two patterns were a “limited and focal” pattern found mainly in patients with amplified MYCN Neuroblastoma that correlated with prognosis and an “extensive and diffuse” pattern found mainly in patients with single copy MYCN Neuroblastoma [34]. One question is whether MYCN activity is responsible for the focal growth of metastatic lesions and determines the affected body segments [34]. Patients with MYCN amplified tumours and those with focal lesions had an improved event free survival (EFS) and overall survival (OS) than those in the other metastatic groups with 5-year EFS

Stellenbosch University https://scholar.sun.ac.za

respectively [34]. Exclusively focal pattern of uptake was not associated with a better overall survival in patients with MYCN amplified disease [34].

In low- and middle-income countries the data on the role of ^{123}I-MIBG scans is very limited. This is possibly due to the scarcity of nuclear imaging facilities, as well as logistic problems in obtaining radio-pharmaceuticals [38].

Although ^{18}F-fluorodeoxyglucose (FDG)- Positron emission tomography (PET) scans is not considered the main nuclear diagnostic investigation, its utility in NB is well documented. It uses glucose receptor transport mechanisms in tumours to diagnose the presence of malignancies [38]. This is the reason why the specificity of 86% [39, 40] is not as accurate as an ^{123}I-MIBG -scan. ^{18}F-FDG PET is taken up in areas of infection and inflammation and has high normal physiological uptake in certain organs which may lead to false positive results [39, 40].

A Cochrane review concluded that in patients with a negative ^{123}I-MIBG scan, it is advisable to perform a second confirmatory test such as ^{18}F-FDG-PET/CT [41]. Yet diagnostic accuracy of ^{18}F-FDG-PET/CT in these cases have not been determined [41]. Comparative studies between ^{18}F-FDG-PET/CT and ^{123}I-MIBG SPECT/CT scintigraphy imaging for detecting a neuroblastoma tumour and its metastases are limited [41].

Role of tumour markers in management:

The presence of tumours in the body can be detected by non-imaging modalities. Tumour markers are substances found in tissue, blood or body fluids that may indicate the presence of cancer in the body. They may be derived from normal cells or cancer cells and can be genetic, hormonal, immune or inflammatory in nature [5,42]. Non-specific tumour markers are produced either by the tumour or by the body in response to the cancer but may be produced in benign conditions where high numbers of cells is generated [43].

Two non-specific tumour markers of prognostic significance are Lactate dehydrogenase (LDH) and Ferritin [5]. In the International Neuroblastoma risk group (INRG) database 959 patients were identified with an increased diagnostic LDH and 666 patients were identified to have an increased pre-treatment Ferritin. Analysis showed that a Ferritin level between 90-140ng/ml (median 120ng/ml) and an LDH level between 580–1000IU/L (median of 750IU/L) had prognostic significance [5]. Ferritin had a 5-year EFS of 77% versus 61% for associated with a value of lower than 96 ng/ml versus a value higher than 96 ng/ml ($P<0.001$) [5]. Similarly, the 5-year EFS for patients with an LDH less than 580 U/L versus a value higher than 580 U/L was 78% versus 67% ($P<0.0001$) [5].

MYCN is a diagnostic tumour marker of NB [3]. MYCN is a V-myc myelocytomatosis (viral-related oncogene) neuroblastoma derived gene and is amplified in 20% of NB tumours and denotes a poor prognosis [43]. In NB it is linked to increased angiogenesis and vascularization, decreased apoptosis, the ability to escape immune surveillance, increased tumorigenic proliferation and ability to metastasise due to the presence of MYCN [43]. MYCN amplification is a genomic expression in NB that is present in advanced disease and in rapidly progressive disease [44,45]. Prognostication with MYCN amplification has been found to be adverse and correlates with other poor prognostic markers such as advance disease, older age and unfavourable histology [44,45]. The percentage MYCN expression in stages 1 to 3 varies but in stage 4 disease has peaks up to 65% [41,42]. Furthermore, we

Stellenbosch University https://scholar.sun.ac.za

find tumours with extra MYCN copies. This genomic aberration seems to carry a better prognosis than with MYCN amplification [44,45].

In LMIC where either fluorescent in vitro hybridization (FISH) or polymerase chain reaction (PCR) studies to detect the presence of MYCN is not available, ferritin and/or LDH can be used as surrogate markers to indicate an adverse prognosis [5,47].

As NB is a tumour of the sympathetic chain, predominantly of the adrenal glands, it excretes catecholamine hormones. Homovanillic acid (HVA) and vannilylmandelic acid (VMA) are the two main hormones that function as specific tumour markers for diagnostic purposes which can be traced in an acidified urine sample. The same tumour markers can be used as a screening tool for treatment monitoring and follow up for relapse [18].

Vanillylmandelic (VMA) and homovanillic acids (HVA) are the best markers [48]. In addition, combined determination of normetanephrine with vanillylmandelic acid, in children under 1 year of age, or normetanephrine with homovanillic acid, in children older than 1 year of age, the diagnostic sensitivity and specificity of age group depend testing is 100% [48]. In another studies it has been suggested that dopamine (DA) is the preferred marker to determine diagnosis with VMA and HVA [49] or Noradrenaline [50]. Zambrano et al described an age correlation similar to previous studies but included DA and NA in the 1 year of age watershed [50].

Strenger et al suggested that urine catecholamines have prognostic capabilities. In the study sensitivity of VMA, HVA, and DA was 80.7, 71.9, and 61.3%, respectively [49]. In 114 patients with NB high VMA levels were associated with favourable biological features and high DA levels were associated with unfavourable biology [49, 50]. Patients with normal HVA levels had a significant better outcome [49]. For disseminated Neuroblastoma of infancy (stage 4 and stage 4s) the DA:VMA ratio proved to have value in the discrimination of stage 4 versus stage 4s [49]. Laug et al concluded that the HVA:VMA ratio had prognostic predictive value in disseminated disease [51]. It was further concluded that the presence of the DA, VMA, as well as increased amounts of cystathionine and VMA or low levels of VMA indicated poor prognosis [51].

Previous studies found that increased urinary DA:NA ratios have been associated with poor prognosis and low DA:NA ratios have been associated with longer disease-free survival [50].

Zambrano et al further described that unfavourable biology like MYCN and unfavourable histology. The study suggested that stage 3 and 4 disease had increased ratios of cathecholamines as opposed to stage 1 and 2 disease [50]. Their study results suggested that stage 3 and 4 cases had DA levels >2 times the upper limit of normal, where only 8% of stage 1-2 cases had DA levels twice the upper limit of normal [50]. In stage 3 and 4 cases the VMA level was >10 times the upper limit of normal as opposed to stage 1 and 2 cases where only one patient had VMA levels >10 times the upper limit of normal [50].

Even though tumour markers in the management of NB can predict outcome they are poor predictors for relapse or progression [52]. Simon et al proved the difference in diagnostic and relapse or progression prediction of tumour markers. At diagnosis the study found abnormal results in 75% for serum VMA and/or HVA, 92% for urine VMA and/or HVA, 90% for neuron specific enolase (NSE), and 81% for LDH. Yet the study found a lower incidence of abnormal results at relapse or progression with

Stellenbosch University https://scholar.sun.ac.za

40% for serum VMA and/or HVA, 54% for urine HVA and/or VMA, 61% for NSE, and 48% for LDH [52]. Hsiao et al described that serum Chromogranin A had a sensitivity of 91% and specificity of 100% [53]. The level of Chromogranin A at diagnosis correlated with outcomes. Levels <190ng/ml correlated with a good prognosis and levels >190ng/ml with a poor outcome [53].

In the study of Simon et al NSE was found to be the most sensitive marker for localised relapses (42%), combined local and metastatic relapses (77%) and of metastatic recurrences (69%) of NB [52]. Sensitivity of all markers was higher for metastatic disease compared with local recurrence [52]. In all these studies relapse and progression could not be predicted by tumour markers alone. Imaging studies must be done in con junction with tumour markers for accurate prediction [49].

Many clinical aspects, histology and imaging techniques are applied to prognosticate the outcomes in NB [54]. Yet identifying novel techniques refine earlier prognostic determinants and treatment responses could change the application of treatment [54]. Furthermore, it could aid in the risk classification of NB especially in the high-risk tumours that would benefit from novel first line treatments like immunotherapy with Anti-GD2 antibodies and IL-1 [54].

Although much has been published regarding the prognostication of each of these diagnostic modalities very little is known how they relate to each other in prognostication. Recent studies suggest that the levels of mRNA in the blood from NB patients could distinguish an ultra-high-risk group from a high-risk group [55]. The same risk distinction was concluded by a study of the pattern of ^{123}I-MIBG scans [34]. What is not known is whether there is a correlation between high mRNA levels and the patterns of ^{123}I-MIBG scans. Yet other tumour markers have not been correlated to imaging studies to assess the relation with regards to prognostication.

If the knowledge we gain from specific tumour markers in the laboratory is subjected to the principles of translational oncology in relation to sensitive and specific diagnostic techniques in nuclear medicine, the question can be asked if these specific tumour markers and diagnostic techniques can indicate various levels of risk in non-specific tumour markers.

As these non-specific tumour markers are more affordable and available in LMIC as risk classification for treatment, it can be utilised in more evidence-based treatment decisions.

AIMS AND OBJECTIVES

Aim:

The aim of the study is to determine the association between prognostic clinical characteristics and special investigations of children diagnosed with neuroblastoma by using treatment response and OS as measurement for outcome.

Objectives:

a) To determine the association between the prognostic value of non-specific tumours and specific tumour markers with the prognostic value of ^{123}I-MIBG-scan.
b) To evaluate how the pattern of metastatic distribution in ^{123}I-MIBG scan relates to treatment response and outcomes.

Stellenbosch University https://scholar.sun.ac.za

METHODOLOGY:

Study design:

The study will be a national multicentre, retrospective chart review and part of a prospective national treatment protocol. Data will be sourced from a completed retrospective study: A retrospective analysis of the management and outcome of Neuroblastoma (NB) in South African Children 2000-2014 (approved by UCT HREC 308/2017) and from a standard of care prospective treatment protocol for all children diagnosed with neuroblastoma in South Africa.

Study setting:

All Paediatric Haematology and Oncology Units in South Africa and associated Nuclear Medicine departments will be invited to participate in the study.

The Nuclear Medicine Departments are:

University of Cape Town

- Groote Schuur Hospital, Cape Town
- Red Cross War Memorial Children's Hospital, Cape Town

University of the Freestate

- Universitas Hospital, Bloemfontein

University of KwaZulu-Natal

- Inkosi Albert Luthuli Central Hospital, Durban

University of Pretoria

-Steve Biko Academic Hospital, Pretoria

University of Limpopo

- Polokwane Provincial Hospital, Polokwane

University of Stellenbosch

- Tygerberg Hospital, Stellenbosch

University of Witwatersrand

-Baragwanath Hospital, Johannesburg

-Charlotte Maxeke Academic Hospital, Johannesburg

Paediatric Oncology Units are:

University of Cape Town

- Groote Schuur Hospital, Cape Town
- Red Cross War Memorial Children's Hospital, Cape Town
- Rondebosch Medical Centre, Cape Town

University of the Free State

- Universitas Hospital, Bloemfontein
- Kimberley Provincial Hospital

University of KwaZulu-Natal

- Inkosi Albert Luthuli Central Hospital, Durban
- Pietermaritzburg Metro Complex, Pietermaritzburg

University of Pretoria

-Steve Biko Academic Hospital, Pretoria

- Unitas Hospital, Pretoria

University of Limpopo

Stellenbosch University https://scholar.sun.ac.za

- Polokwane Provincial Hospital, Polokwane

Sefako Makgatho Health Sciences University

- George Mukhari Hospital, Ga-Rankuwa

University of Stellenbosch

- Tygerberg Hospital, Stellenbosch

Walter Sisulu University

-Frere Hospital, East London

-Port Elizabeth Provincial Hospital, Port Elizabeth

University of Witwatersrand

-Baragwanath Hospital, Johannesburg

-Charlotte Maxeke Academic Hospital, Johannesburg

-Donnie Gordon Private Hospital, Johannesburg

Private units

-Parklands Netcare Hospital, Durban

Study population and sampling:

Patients will be recruited both retrospectively and prospectively. Prospective patients will be recruited as part of a national NB treatment protocol.

Inclusion criteria:

All patients ≤ 18 years at diagnosis from 2000 – 2016 and completed treatment by December 2016 as well as patients diagnosed from August 2018 with:

a) Biopsy proven NB or

b) Radiological reported neuroblastoma with positive u-catecholamines or

c) BMAT with involvement of NB and a positive u-catecholamines (in cases where a biopsy is not possible or radiological evidence in inconclusive)

Exclusion criteria:

1) Patients with indeterminate or inconclusive histology results and/or BMAT and u-catecholamines to diagnose NB.
2) Poor quality investigations or investigations that provide incomplete information for study purposes.
3) If consent and/or assent for treatment has not be granted.

Methodology:

Data collection:

Stellenbosch University https://scholar.sun.ac.za

All charts of participants will be reviewed for information and collected according to a data collection sheet (see Appendix A). The dataset is standardised for both the retrospective and prospective participants.

MIBG-scan evaluation:

Each patient's pre-treatment and reassessment ^{123}I-MIBG scan (if available) during induction, regardless of treatment protocols, will be:

a) Scored according to the modified Curie score (see Appendix B) and

b) The investigation of metastatic patterns on MIBG-avid skeletal lesions will be categorized as "focal" or "diffuse".

Definitions:

Focal metastases are MIBG-avid spots with clear margins distinguishable from the background and/or located to a single region.

Diffuse metastases will be view as MIBG-avid regions with no clear margins and/or spread throughout body segment(s).

Reporter bias:

Two independent observations will be documented to exclude reporter bias. The first will be documented from the report of the treating POU and the second will be documented by a review Nuclear physician.

Correlation studies:

a) The prognostic value of the diagnostic LDH and Ferritin values will individually be correlated with the prognostic value of the diagnostic modified-Curie scores.

b) The clinical presentation, age, LDH, Ferritin and MYCN will be correlated with the findings in the bone marrow, CT-scan, bone scan and ^{123}I-MIBG scan (pattern of distribution and modified Curie-score).

STATISTICAL CONSIDERATIONS

Study sample:

In South Africa between 30 – 40 new cases of NB are diagnosed per year [56]. Correlating the Paediatric oncology units (POU) where patients can access ^{123}I-MIBG scan with the incidence from 2000 – 2016, it is expected to identify 80 – 90 patients eligible from retrospective data.

Stellenbosch University https://scholar.sun.ac.za

Prospectively over a 2-year period another 40 - 50 may be eligible for the study. Thus, an expected 120 – 140 patients can potentially be included into the study.

Data collection:

- Data will be collected by each individual participating unit and collated in a central anonymous database, managed by the principle investigator.
- Anonymity will be assured by awarding a unit specific number to each patient. The list of patients with their unique number will be kept in a password protected computer in a locked location. This information will only be known by the participating health care provider in each unit.
- The PI will only receive data with a site-specific identifier without knowledge of the patient's identity.
- Data will be stored in a password protected computer only accessible by the PIs in a locked office accessible by only the PIs.

Data analysis:

4) The data will be analysed using Stata 15.0 and R statistical package 3.5. (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP.).
5) Sample mean, median and standard deviations will be described. Normally distributed data will be presented as mean ± standard deviation (SD) and skewed data as median and range. Different degrees of OSA will compared by using Mann-Whitney test/Jonckheere-Terpstra Test or X^2 test. Spearman correlations will be performed between LDH, Ferritin and MYCN with the modified Curie score and pattern of distribution of diagnostic ^{123}I-MIBG scans. All analyses will be analysed with a significance was set up at p <0.05. In view of prediction of treatment outcome, multiple logistic regression analysis will be used.
6) We will attempt to analyse and compare those subjects with missing values to those without in terms of demographics and the outcome measures in an attempt to assess whether any potential selection biases are present in the data and to check the missing-at-random assumption. Sequential regression multivariate imputation will also be employed to constitute key markers with high missingness. The Youden index will be used to identify the optimal breakpoints for non-specific tumour markers and prognosis/outcome. The discriminatory power for each marker will be evaluated using the area under the ROC curve (AUC), and a comparison between the curves will be performed for significant differences. An

Stellenbosch University https://scholar.sun.ac.za

AUC value of value ≥0.8 will be targeted as an accepted cut-off for good to excellent predictive capability. Sensitivity, specificity, predictive positive value (PPV) and negative predictive value (NPV) based on the optimal cut-points will be estimated with associated 95% confidence intervals (CI's). To account for multiple comparison, we will also adjust p-values for multiple testing using a Bonferroni or related correction factor. Once highly predictive factors are identified we will extend to a multivariable logistic regression to create weighted risk scores and compare to unweighted scores of tumour markers for predictive capability.

7) If expected cell count in the cross tabulation contains fewer than 5 observations (sparse numbers) then the Fisher's exact test will be employed. The standard t-test will be used to compare the mean of continuous explanatory variables by dichotomous outcome classification (e.g. mortality or remission). If the normality assumption is not upheld, then the non-parametric equivalent Wilcoxon rank-sum test (Mann Whitney U test) will be used instead.
8) Kaplan-Meier survival curves will be developed and differences by groups assessed using a log rank test. This will be extended to a multivariable proportional hazards (Cox) regression model to adjust for confounding factors and test for potential interactions. The proportional hazards assumption of the model will be assessed to confirm adequacy. If this assumption is not upheld then a semi-parametric and/or parametric survival model will be used instead. An adjusted p-value of <0.05 will be considered statistically significant.

RELEVANCE

With a growing number of children in LMIC settings the burden of cancer in children will rise. This will be the first comprehensive multi-institutional, interdepartmental, retrospective and prospective study describing the role of ^{123}I-MIBG scans in NB in South Africa and Sub-Saharan Africa. The study will improve the understanding of the pathophysiology of NB in the South African context.

ETHICAL CONSIDERATIONS

All research conducted during this study will be done according to the benchmarks for research in LMIC [57].

Stellenbosch University https://scholar.sun.ac.za

Collaborative partnership:

The research is a national, multi departmental study which includes multiple hospital sites. The study is a joint partnership between pediatric oncologists, radiologists, surgeons, nuclear medicine physicians as well as staff active in the care of patients and diagnostics such as laboratories.

Social value:

The proposed management protocols will be sustainable treatment interventions. Future suggestions for management adaptations will benefit the South African population and needs.

Scientific validity:

The risk of intervention and scientific validity is according to acceptable standard medical care for all children diagnosed with NB. All treatments and palliation will be standard of international care with practical application to South African health care guidelines and resource restraints.

Study results will be given to participating parties and the data will be discussed in academic meetings and congresses, thesis purposes and published in peer reviewed journals.

Fair selection of study population:

All patients diagnosed with NB will be invited to participate in the study as part of a fair selection process.

Favourable risk benefit ratio:

Untreated NB is a terminal disease. The aim of the study is to improve outcomes as well as improve palliative care for patients without further treatment options. All interventions will only be done to benefit the patient and treatment above the negative effects of the treatment or interventions on the patient.

Independent review:

Applications for ethical approval will be submitted at the Health Research Evaluation Committee of respective universities attached to participating POUs. The ethical approval from respective Departments of Health will be obtained. The study will be open for regular review or audits.

Informed consent:

Informed consent will be waived on the retrospective data (as per HREC 308/2017 approval). The prospective data forms part of a standard of care prospective management protocol for which written consent will be obtained for the complete management from participants' caretakers as well as assent in children older than 7 years old. Assent from children below 7 years of age will not be

Stellenbosch University https://scholar.sun.ac.za

taken, however the willingness to participate in the management will be taken into consideration. Participation in the study is voluntary. Participants or their parents may withdraw at any stage of the study. All consent and assent will be obtained according to age, education and language sensitive requirements.

Respect for the recruited participants and study communities:

Where there is the possibility of discomfort or emotional distress all efforts will be made to minimize the discomfort without hazardous effects.

Each patient will receive a unique study number. A list linking the name and the unique study number will be kept separate in the individual POU and not be used in the analysis. The data will be collected prospectively and analysed without the name or any identifiable data of the individual patient to ensure confidentiality.

The electronic information will be kept in each POU on a password protected computer that will only be accessible by the staff participating in the study. The main data base will be kept by the principle investigator at Grey's Hospital on a password protected computer.

Human tissue and biological samples will be collected and treated according to the ethical guidelines of the ethical committees of participating Universities and guidelines of the Health Professions Council of South Africa.

All interventions and management recommendations will be done with consideration to social and cultural aspects of each patient.

SIGNIFICANCE OF THE STUDY

National resources should be utilised efficiently especially in regards to rare diseases. More specialised tests are developed to improve diagnostics and evaluation of treatment and outcomes. Yet less specialised tests with proven prognostic values have not been correlated with these specialised tests to assess the comparative use in the clinical setting.

By understanding how easily accessible tests in lower resourced settings relate to advanced tests more affordable management of patients is possible without compromising on patient care.

The importance of this study is to attempt to determine which investigations should be done in a setting with limited resources.

Stellenbosch University https://scholar.sun.ac.za

LIMITATIONS OF THE STUDY

Neuroblastoma is a rare disease and the sample size is small. As the study is retrospective the quality of the data might be poor or lacking in regards to the objectives. The retrospective nature of the study implies limitations associated with this study design. This data can be compared to the data gathered in the prospective management protocol.

As many of the investigations and interpretation is user dependent a degree of intra and inter reporter variability might exist and introduce bias into the information set of the study.

DISSEMINATION PLAN

The information will be distributed in four ways:

- Aim to publish the data in a medical journal with an impact factor.
- Present the findings at the SACCSG National meeting.
- Present the data in a dissertation in fulfilment of a PhD degree of the PI.
- Present the findings at International congresses such as SIOP-Africa or SIOP-International

BUDGET

The study will not generate any additional cost in terms of patient care as data will be gathered from files. There will be no additional administration costs as all data will be electronic. The study is based on the standard of care in public hospitals in South Africa and no added resources utilised in the management of patients.

CONFLICT OF INTEREST

The investigators wish to declare no conflict of interest in terms of this study.

REFERENCES

1. Smith, A; Altekruse, S; Adamson, P; et al. Declining childhood and adolescent cancer mortality. Cancer 2014;120 (16): 2497-506.
2. National Cancer Institute: Surveillance, Epidemiology and End Results Database http://seer.cancer.gov (Accessed Nov 2015)
3. National Cancer Institute: Neuroblastoma http://www.cancer.gov/types/neuroblastoma (Accessed Nov 2015)
4. Bhatnagar, S; Sarin, Y. Neuroblastoma: A Review of Management and Outcome. Indian J Pediatr (June 2012) 79 (6):787-792
5. Parikh, N; Howard, S; Chantada, G; Israels, T; Khattab, M; Alcasabas, P; Lam, C; Faulkner, L; Park, J; London, W; Matthay, K: SIOP-PODC adapted risk stratification and treatment

Stellenbosch University https://scholar.sun.ac.za

guidelines: Recommendations for neuroblastoma in low- and middle-income settings. Pediatr Blood Cancer 2015; 62:1305-1316

6. Kushner, B. Neuroblastoma: A disease requiring a multitude of imaging studies. J Nucl Med. 2004 Jul;45(7):1172-88
7. Monsaingeon, M; Perel, Y; Simonnet, G; Corcuff, J. Comparative values of catecholamines and metabolites for the diagnosis of neuroblastoma. European Journal of Pediatrics. 2003: 162(6), 397-403.
8. Asgharzadeh, S; Salo, J; Ji, L; Oberthuer, A; et al. Clinical significance of tumor-associated inflammatory cells in metastatic Neuroblastoma. J Clin Oncol 2012 Oct;30(28):3525-32.
9. www.cancer.gov. National Cancer Institute: Definition of Cancer Terms. (Accessed June 2016)
10. Maris, J; Hogarty, M; Bagatell, R; Cohn, S.: Neuroblastoma. Lancet 2007; 369: 2106-20
11. Sanai, N; Alvarez-Buylla, A; Berger, M. Mechanisms of disease: neural stem cells and the origin of gliomas. The New England Journal of Medicine 2005. 353(8): 811–822.
12. De Weille, J. On the genesis of Neuroblastoma and Glioma. International Journal of Brain Science. Volume 2014. 1-4
13. Melotte, V; Qu, X; Ongenaert, M et al. The N-myc downstream regulated gene (NDRG) family: diverse functions, multiple applications. The FASEB Journal 2010, 24(11) 4153–66
14. Jiang, M; Stanke, J and Lahti, J. The connections between neural crest development and Neuroblastoma. Current Topics in Developmental Biology vol. 94, chapter 4, page 77–127, Elsevier, New York, NY, USA, 2011.
15. Mohlin, S; Wigerup, C; P˚ahlman, S. Neuroblastoma aggressiveness in relation to sympathetic neuronal differentiation stage. Seminars in Cancer Biology 2011, 21(4): 276–282.
16. Patricia A. Aronica The Detection of Neuroblastoma in the Bone Marrow: Biopsy versus Aspiration Journal of Paediatric Haematology/Oncology 20(4): 330-334, July/August 1998
17. Maria Valeria Corrias Detection of Neuroblastoma Cells in Bone Marrow and Peripheral Blood by Different Techniques: Accuracy and Relationship with Clinical Features of Patients Clin Cancer Res 2004; 10: 7978-7985
18. Cohn, S; Diagnosis and classification of Small round-cell tumors of childhood. American Journal of Pathology, Vol. 155, No. 1, July 1999
19. Brodeur, G; Seeger, R; Barrett, A; et al.: International criteria for diagnosis, staging, and response to treatment in patients with neuroblastoma. J Clin Oncol 1988; 6 (12): 1874-81.
20. Chandra Krishnan Detection of Isolated Tumor Cells in Neuroblastoma by Immunohistiochemical Analysis in Bone Marrow Biopsy Specimens –Improved Detection with use of β-Catenin Am J Clin Pathol 2009; 131:49-57
21. Farzana D Pashankar MIBG and Somatostatin Receptor Analogs in Children: Current Concepts on Diagnostic and Therapeutic Use J Nucl Medicine 2005; 46: 55s-61s
22. Thomas Pfluger Integrated Imaging using MRI and MIBG scan to improve sensitivity and specificity in the diagnosis of Paediatric Neuroblastoma AJR: 181, October 2003
23. Brodeur GM, Pritchard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol.1993; 11(8):1466–1477

Stellenbosch University https://scholar.sun.ac.za

24. Hero, B; Simon, T; Spitz, R; et al.: Localized infant neuroblastomas often show spontaneous regression: results of the prospective trials NB95-S and NB97. J Clin Oncol 2008; 26 (9): 1504-10.
25. Perel Y, Conway J, Kletzel M, Goldman J, Weiss S, Feyler A, Cohn SL. Clinical impact and prognostic value of metaiodobenzylguanidine imaging in children with metastatic neuroblastoma. J Pediatr Hematol Oncol 1999; 21(1): 13–18
26. Matthay KK, Shulkin B, Ladenstein R, Michon J, Giammarile F, Lewington V, Pearson ADJ, Cohn SL. Criteria for evaluation of disease extent by 123I-metaiodobenzylguanidine scans in neuroblastoma: a report for the International Neuroblastoma Risk Group (INRG) Task Force. British Journal of Cancer. 2010; 102: 1319-1326
27. Matthay KK, Edeline V, Lumbroso J, Tanguy ML, Asselain B, Zucker JM, Valteau-Couanet D, Hartmann O, Michon J. Correlation of early metastatic response by 123I-metaiodobenzylguanidine scintigraphy with overall response and event-free survival in stage IV neuroblastoma. J Clin Oncol. 2003; 21: 2486–2491
28. Katzenstein, H; Cohn, S; Shore, R et al. Scintigraphic Response by 123I-Metaiodobenzylguanidine Scan Correlates with Event-Free Survival in High-Risk Neuroblastoma. J Clin Oncol 2004; 22:3909-3915.
29. Bleeker, G; Tytgat, G; Adam, J; Caron, H; Kremer, L: Hooft, L; van Dalen, E. 123I-MIBG scintigraphy and 18F-FDG-PET imaging for diagnosing neuroblastoma (Review). The Cochrane Database of Syst Rev 2015;9.
30. Bombardieri E, Giammarile F, Aktolun C, Baum RP, Delaloye BA, Maffioli L, Moncayo R, Mortelmans L, Pepe G, Reske SN, Castellani MR, Chiti A. 131I/123I-Metaiodobenzylguanidine (mIBG) scintigraphy: procedure guidelines for tumour imaging. Eur J Nucl Med Mol Imaging. 2010; 37:2436–2446
31. Carlin S, Mairs RJ, McCluskey AG, Tweddle DA, Sprigg A, Estlin C, Board J, George RE, Ellershaw C, Pearson AD, Lunec J, Montaldo PG, Ponzoni M, van Eck-Smit BL, Hoefnagel CA, van den Brug MD, Tytgat GA, Caron HN. Development of a real-time polymerase chain reaction assay for prediction of the uptake of meta-[(131)I]iodobenzylguanidine by neuroblastoma tumors. Clin Cancer Res. 2003; 9:3338–3344.
32. Pfluger T, Schmied C, Porn U, Leinsinger G, Vollmar C, Dresel S, Schmid I, Hahn K. Integrated imaging using MRI and 123I metaiodobenzylguanidine scintigraphy to improve sensitivity and specificity in the diagnosis of pediatric neuroblastoma. Am J Roentgenol 2003; 181:1115 – 1124
33. Simon, T; Hero, B; Benz-Bohm, G; von Schweinitz, D; Berthold, F.
Review of image defined risk factors in localized neuroblastoma patients: Results of the GPOH NB97 trial. Pediatr Blood Cancer. May 2008; 50(5):965-9.
34. Bleeker, G; van Eck-Smit, B; Zwindermann, K; et al. MIBG scans in patients with stage 4 neuroblastoma reveal two metastatic patterns, one is associated with MYCN amplification and in MYCN-amplified tumours correlates with a better prognosis. Eur J Nucl Med Mol Imaging (2015) 42:222–230.
35. Yanik, G; Parisi, M; Shulkin, B; et al.: Semiquantitative MIBG scoring as a prognostic indicator in patients with stage 4 neuroblastoma: a report from the Children's oncology group. J Nucl Med 54 (4): 541-8, 2013.

Stellenbosch University https://scholar.sun.ac.za

36. Decarolis, B; Schneider, C; Hero, B; et al.: Iodine-123 metaiodobenzylguanidine scintigraphy scoring allows prediction of outcome in patients with stage 4 neuroblastoma: results of the Cologne interscore comparison study. J Clin Oncol 31 (7): 944-51, 2013
37. Andrich MP, Shalaby-Rana E, Movassaghi N, Majd M. The role of 131 Iodine-Metaiodobenzylguanidine scanning in the correlative imaging of patients with neuroblastoma. Paediatrics. 1996;97(2):246-250.
38. Fawzy, M; Moussa, E; Hamoda, A; Zaher, A; Hassanain, O; Azmy, S. PET/CT and MIBG scans in diagnosis and management of Neuroblastoma. Journal of Solid Tumors, 2015, 5(2): 32-40
39. Shulkin BL, Hutchinson RJ, Castle VP, et al. Neuroblastoma: positron emission tomography with 2-[fluorine-18]-fluoro-2-deoxyD-glucose compared with meta-iodobenzyl guanidine scintigraphy. Radiology. 1996; 199: 743-750.
40. Sharp SE, Shulkin BL, Gelfand MJ, et al. 123I-MIBG scintigraphy and 18F-FDG PET in neuroblastoma. J Nucl Med. 2009 Aug; 50(8): 1237-43.
41. Bleeker,G; Tytgat, G; Adam, J; Caron, H; Kremer,L; Hooft, L; van Dalen, E. 123I-MIBG scintigraphy and 18F-FDG-PET imaging for diagnosing neuroblastoma. Cochrane Database Syst Rev. 2015 Sep 29;(9):CD009263. doi: 10.1002/14651858.CD009263.pub2.
42. Hesseling PB, Ankone K, Wessels G, Schneider JW, Du Plessis, Moore L. Neuroblastoma in Southern Africa: Epidemiological features, prognostic factors and outcome. Ann Trop Paediatr 1999;19: 357–363.
43. Wang LL, Teshiba R, Ikegaki N, et al.: Augmented expression of MYC and/or MYCN protein defines highly aggressive MYC-driven neuroblastoma: a Children's Oncology Group study. Br J Cancer 113 (1): 57-63, 2015.
44. Bagatelle, R; Beck-Popovic, M; London, W; Zhang, Y; et al. Significance of MYCN Amplification in International Neuroblastoma Staging System Stage 1 and 2 Neuroblastoma: A Report from the International Neuroblastoma Risk Group Database. Journal of Clinical Oncology 2009; 27 (3): 365-370.
45. Brodeur, G. Neuroblastoma: Biological insights into a clinical enigma. Nature Reviews: Cancer March 2003;3: 203-2016.
46. Cangemi, G; Reggiardo, G; Barco, S; Barbagallo, L; Conte, M; D'Angelo, P; Bianchi, M; Favre, C; Galleni, B; Melioli, G; Haupt, R; Garaventa, A; Corrias, M. Prognostic value of ferritin, neuron-specific enolase, lactate dehydrogenase, and urinary and plasmatic catecholamine metabolites in children with neuroblastoma. Onco Targets Ther. 2012; 5:417–423.
47. Hsiao, R; Seeger, R; Yu, A; O'Connor, D. Chromogranin A in children with Neuroblastoma: Serum Concentration Parallels Disease Stage and Predicts Survival. J. Clin. Invest. 1990; 85:1555-1559.
48. Monsaingeon, M; Perel, Y; Simonnet, G; Corcuff, J. Comparative values of catecholamines and metabolites for the diagnosis of neuroblastoma. European Journal of Pediatrics. 2003: 162(6), 397-403.
49. Volker Strenger, V; Kerbl, R; Dornbusch, H; Ladenstein, R; Ambros, P; Ambros, I; Urban, C. Diagnostic and prognostic impact of urinary catecholamines in neuroblastoma patients. Pediatric Blood and Cancer May 2007. 48(5): 504-509
50. Zambrano, E; Reyes-Múgica, M. Hormonal activity may predict aggressive behavior in neuroblastoma. Pediatr Dev Pathol. Mar 2002.5(2):190-9.
51. Laug, W; Siegel, S; Shaw, K; Landing, B; Baptista, J; Gutenstein, M. Initial Urinary Catecholamine Metabolite Concentrations and Prognosis in Neuroblastoma. Pediatrics July 1978. 62(1): 77-83.

Stellenbosch University https://scholar.sun.ac.za

52. Simon, T; Hero, B; Hunneman, D; Berthold, F. Tumour markers are poor predictors for relapse or progression in Neuroblastoma. Eur Jour of Cancer. Sept 2003; (39) 13:1899–1903.
53. Hsiao, R; Seeger, R; Yu, A; O'Connor, D. Chromogranin A in children with Neuroblastoma: Serum Concentration Parallels Disease Stage and Predicts Survival. J. Clin. Invest. 1990; 85:1555-1559.
54. Marabelle, A; Gray, J. Tumour-Targeted and Immune-Targeted monoclonal antibodies: Going from passive to active immune therapy. Pediatr Blood and Cancer 2015; 62:1317-1325.
55. Viprey, V; Gregory, W; Corrias, M; et al. Neuroblastoma mRNAs predict outcome in children with Stage 4 Neuroblastoma: A European HR-NBL1/SIOPEN Study. J Clin Oncol. 2014 Apr; 32(10): 1074-1084.
56. Stefan, D; Stones, D; Wainright, R; Kruger, M; Davidson, A; Poole, J; Hadley, G; Forman, D; Colombet, M; Steliarova-Foucher, E. Childhood cancer incidence in South Africa, 1987–2007. S Afr Med J 2015; 105(11): 939-947.
57. Emmanuel, E; et al. What Makes Clinical Research in Developing Countries Ethical? The Benchmarks of Ethical Research. Perspective 2004; 189: 930-937.

Stellenbosch University https://scholar.sun.ac.za

APPENDICES

APPENDIX A: Data collection sheet

Data sheets codes:

Information	Classification	Code
Source ID code:		
Sex:	Female Male	F M
Race: (Statistical purposes)	African Indian Mixed race (Coloured) White Other (Specify)	A I M W O
Date of birth (DOB): yy/mm/dd		
Age (months):		
Date of diagnosis (DOD): yy/mm/dd		
Anthropometry (values): Weight Height/length BMI (Will be determined by the PI) MUAC TSF (Triceps skin fold)	(Will be determined by the PI)	
Presenting symptoms:		
Bone pain:	No Yes	N Y
Clinical skull metastasis:	No	N

Stellenbosch University https://scholar.sun.ac.za

	Yes	Y
Racoon eyes:	No Yes	N Y
Duration of symptoms: (From onset of disease related symptoms till diagnosis)	Days (State value i.e. 1 week = 7 days)	
Hypertension	No Yes	N Y
Paraneoplastic syndromes (ParaNS):	OMAS (Opsoclonus Myoclonus Syndrome) VIP (Vaso Intestinal Peptide Syndrome) Other (Specify)	O V
Emergency	Spinal compression Hepatic pain Respiratory distress Horner Syndrome/ Airway compromise Intestinal obstruction Other (Specify)	Sp L R HS IO
HIV status	Positive Negative Unknown	P N U
TB status	Positive – New Positive – on treatment Negative Unknown	PN PT N U
Other infectious diseases:		
Co-morbid disease or Syndromes (Sxd) (Specify):		

Stellenbosch University https://scholar.sun.ac.za

Site of primary	Adrenal Abdominal (other than adrenal) Chest Neck Primary not found Other (Specify)	Ad Am C N PNF
Stage (INSS):	Please see appendix A	
Stage (INRG):	Please see appendix B	
Sites of metastasis:	Bone (Specify) Bone marrow Intra abdominal Liver Local invasion Lungs Lymphnodes (Specify) Paraspinal Skull Skin	BT BM Am L I R N Sp Sl Sn
Cytopaenia:	Anaemia Leucopaenia Trombocytopaenia Bicytopaenia Pancytopaenia	A L T e.g. AT / AL/ LT P
LDH (value):		
Ferritin (value):		
ESR (value):		
Haptoglobin (value):		

Stellenbosch University https://scholar.sun.ac.za

ALP/ ALT (value):		
Albumin (value):		
Bloods at diagnosis (value): HCO3 Potassium Magnesium		
U-catecholamine State raised: R Not raised: N Example: HVA R or HVA N	HMA VMA Other	
CXR:	Lung parenchyma Paraspinal Other (Specify)	LP PS
Skull XR involvement:	Coin lesions Oribital lesions Other (Specify)	CL OR
Other X-rays: bone involvement	Describe main areas	
ABD U/S:	Adrenal Other (Specify)	Adr
CT scan/MRI: (Example for adrenal primary with liver and node involvement: Adr, L, M Or Paraspinal primary with local infiltration Non, I)	Primary: Adrenal Non adrenal Unkown Lymphnodes: Local infiltration: Metastasis liver	 Adr Non U LN I M
CT scan bone involvement:	Specify sites	

Stellenbosch University https://scholar.sun.ac.za

Pre-treatment MIBG scan: Modified Curie score (value)		
Pre-treatment MIBG scan: Pattern of metastases	Diffuse Focal	D F
Reassessment MIBG-scan: Modified Curie score (value)		
Reassessment MIBG-scan: Pattern of metastases: Diffuse or focal	Diffuse Focal	D F
Bone scan: Pre-treatment Reassessment	Describe main areas	
BMAT involved pre-treatment:	No Yes	N Y
BMAT involved reassessment:	No Yes Not applicable (non-stage 4)	N Y A
BM aspirate pre-treatment:	No Yes	N Y
% involvement pre-treatment	Packed Moderate	P M
BM aspirate reassessment:	No Yes Not applicable (non-stage 4)	N Y A
% involvement reassessment	Packed Improved Clear	P I C

Stellenbosch University https://scholar.sun.ac.za

Trephine pre-treatment:	No Yes	N Y
Trephine reassessment:	No Yes Not applicable (non-stage 4)	N Y A
Histology (INPC):	Favourable: Unfavourable:	F U
MYCN amplification:	Negative Extra copies Positive	N EC P
Ploidy (DNA index):	Diploid Hyperdiploid	D H
Genetics markers:	1p 11q	1p 11q
Risk classification: (INSS or INRG)	Very low risk Low risk Intermediate risk High risk	VLR LR IR HR
Treatment intent from onset:	Cure Palliative	C P
Chemotherapy (Regimes):		
Chemotherapy adapted or interrupted	No Yes	N Y
Reason:	Death Abandonment of treatment Tumour related complications Medical complication	D A T M

Stellenbosch University https://scholar.sun.ac.za

	Other (Specify)	
Surgery:	Complete resection: Incomplete resection: No surgery:	C I N
Radiotherapy:	Describe areas	
Remission:	No Yes	N Y
Autologous transplant (Auto Tx):	No Yes	N Y
Outcomes:	Remission (<5 years post last treatment) Relapse Primary site Metastatic site Alive (>5 years post last treatment) Dead	R PR MR A D
Lost to follow up (LTF):	No Yes	N Y
Date last seen (DLS): yy/mm/dd		
Date of Death: (DODx): yy/mm/dd		
Reason for death	Disease Toxicity (Specify) Disease related mortality (Specify) Other (Specify)	D T

Stellenbosch University https://scholar.sun.ac.za

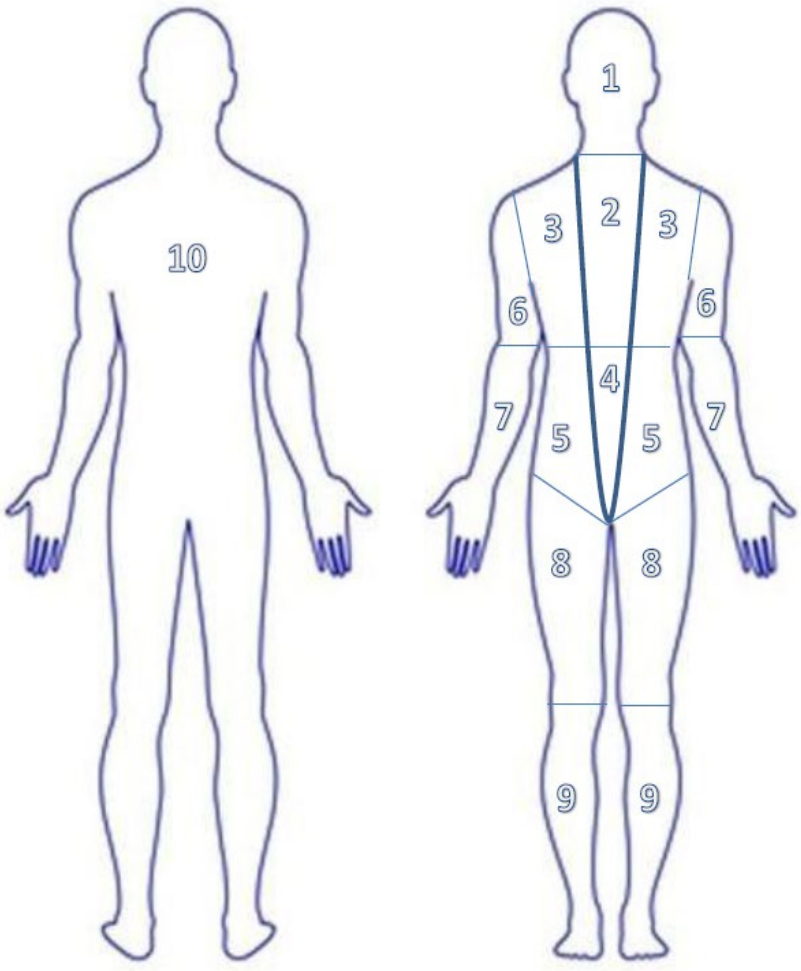
APPENDIX B:

^{123}I-MIBG SCORING SHEET

Data	Options	Code
Source code		
Induction regime	Rapid COJEC OJEC/OPEC Other (please specify)	rCJ JP
Type of scan	Pre-treatment scan Post induction treatment scan Post cycle 4 scan Unknown	Pre Post Fr U

MODIFIED CURIE-SCORE

The scoring system divides the skeleton into 9 compartments.
A 10th compartment represents the soft tissue lesions.



Scoring of MIBG scan
Level of lesion certainty:

0 = unknown
1 = possible
2 = probable
3 = definite

Extent of uptake:

0 = no uptake/ no foci per segment
1 = one focal lesion per segment
2 = more than one focal lesion per segment
3 = diffuse involvement (≥50% of segment involved)

Intensity of uptake:

0 = no sites of uptake
1 = doubtful uptake
2 = obvious but mild uptake
3 = obvious and intense uptake

Soft-tissue lesions scoring system:

0 = no MIBG involvement
1 = one MIBG-avid soft-tissue lesion present
2 = more than one MIBG-avid soft-tissue lesion present
3 = MIBG avidity in a soft-tissue lesion that occupies 50% of the chest or abdomen

*Maximum attainable score is 30
*Focal uptake: uptake with clearly defined margins
* Diffuse uptake: is indistinguishable from background

Stellenbosch University https://scholar.sun.ac.za

Location/ segment involved	**Level of certainty**	**Number of lesions in the segment**	**Extent of involvement and score**	**Level of certainty**	**Intensity of uptake**
Head					
Cervico-thorasic spine					
Ribs/sternum/ scapula					
Lumbosacfral spine					
Pelvis					
Upper arms					
Forearm and hands					
Upper legs/ Thighs					
Lower legs/ feet					
Soft tissue involvement					
				Score	

PATTERN OF UPTAKE

Focal metastases
(Clear margins distinguishable from the background and/or located to a single region) ☐

or

Diffuse metastases
(No clear margins and/or spread throughout body segments) ☐

Stellenbosch University https://scholar.sun.ac.za

Appendix G: Research and congress abstracts

Congress abstracts

1. Age at diagnosis as prognostic factor in South African children with neuroblastoma – SIOP International, Toronto, Canada 2020
2. Favourable outcomes in children and adolescents with sex cord stromal tumours (1990-2015): First report by the South African Children's Cancer Study Group - SIOP International Lyon, France 2019
3. Favourable Outcomes for South African Children and Adolescents with Mature and Immature Teratomas (1990-2015): First Report by the South African Children Cancer Study Group - SIOP International Lyon, France 2019
4. The prognostic significance of surgery and radiotherapy for cure in high risk neuroblatoma in South Africa – SIOP International, Lyon, France 2019
5. The management of neuroblastoma in South Africa between 2000 and 2014 – SIOP International Lyon, France 2019
6. Collaborative National Protocol Design – SACO, Cape Town, South Africa 2019
7. Favourable outcomes in children and adolescents with sex cord stromal tumours (1990-2015): First report by the South African Children's Cancer Study Group – SACO, Cape Town, South Africa 2019
8. Favourable outcomes for South African children and adolescents with mature and immature teratomas (1990 -2015): First report by the South African Children Cancer Study Group – SACO, Cape Town, South Africa 2019
9. Carboplatin-based chemotherapy delivers superior results in children and adolescents with extracranial germ cell tumours: First report by the South African Children's Cancer Study Group, 1990-2015 – SACO, Cape Town, South Africa 2019
10. The role of surgery and radiotherapy in the management of Neuroblastoma in South Africa – SACO, Cape Town, South Africa 2019
11. The management of neuroblastoma in South Africa between 2000 and 2014 – SACO, Cape Town, South Africa 2019
12. Iron deficiency anaemia: the forgotten culprit of thrombocytopenia in female adolescents - Annual Congress of the Belgium Society of Paediatrics 2019

Publications

1. Van Heerden J, Zaghloul M, Neven A, de Rojas T, Geel J, Patte C; et al.: Pediatric Oncology Clinical Trials and Collaborative Research in Africa: Current Landscape and Future Perspectives. JCO Global Oncology. 2020; 6:1264-1275.
2. Eelen Y, Norga K, Verlooy J, Tousseyn T, Van Heerden J. Primary Thyroid Lymphoma in an Adolescent with Hashimoto's Thyroiditis and Congenital Deafness. Journal of Clinical Oncology and Research. 2020; 3(5): 2-5.
3. Lopes L, Verlooy J, Norga K, van Heerden J. The multifactorial etiology and approach to iron deficiency anemia in adolescent girls. International Journal of Case Reports. 2020; 4:130.
4. J van Heerden, R Delport, M Kruger. The ability of children to consent to medical procedures. South African Journal of Child Health. 2020;14(1):25-29.
5. T. Van Genechten, J. van Heerden, T. Bauters, C. Dhooge. Successful treatment of adenovirus infection with Brincidofovir in an immunocompromised patient after hematological stem cell transplantation. Case Reports of Infectious diseases. 2020;2020.

Stellenbosch University https://scholar.sun.ac.za

6. Van Heerden J, Tjelma W. The multidisciplinary approach to ovarian masses in children and adolescents. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2019; 243(10): 103-110.
7. Claes L, Dendooven A, van Heerden J. The Challenges of diagnosing ectopic thymic tissue in children. BMJ Case Reports CP 2019;12: e228807.
8. Jaques van Heerden, Hansraj Mangray, Fernando Ghimenton, Deneys Reitz. Significant haematuria caused by a pseudo-aneurysm in nephroblastoma. Pediatric Surgery Case Reports. 2019; 41(2): 30-32.