MASTERS DEGREE:

BREAST CANCER DIAGNOSED IN WOMEN WITH THE HUMAN IMMUNE-DEFICIENCY VIRUS (HIV)

A Retrospective, Single-Institution Survey to Assess the Phenotype of Breast Cancer In Women Infected With the Human Immune-Deficiency Virus.

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M.MED RADIATION AND CLINICAL ONCOLOGY
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**Declaration**

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December 2014
DECLARATION IN TERMS OF INTELLECTUAL PROPERTY.

I, Dr. Lizanne Langenhoven, hereby certify that I have not used the words or ideas of another and presented them as my own.

The intellectual input of Dr. P. Barnardt, my mentor at Tygerberg Hospital is acknowledged, as well as that of Prof’s. MB Terry and J.S Jacobson from Columbia University.

DECLARATION IN TERM OF ETHICS COMMITTEE APPROVAL

I hereby declare that no data collection was carried out prior to approval by the HREC 1 Committee at Stellenbosch University (Addendum 2) on 13 March 2012.

Protocol Number: S12/02/048


DECLARATION IN TERMS OF INSTITUTIONAL PERMISSION TO CONDUCT RESEARCH AT TYGERBERG HOSPITAL

In accordance with the Provincial Research Policy and Tygerberg Hospital Notice No. 40/09, permission to conduct research as per Protocol S12/02/048, was obtained on 23 May 2012. (Addendum 3)

DECLARATION IN TERMS OF FINANCIAL INCENTIVES

Research conducted with regards to this project was made possible through the D43 Grant, Columbia University – South Africa Training Program for Research on AIDS-Related Malignancies through the National Cancer Institute, NIH Grant #1D43CA153715-02.

Funds were made available to acquire the services of a data capturer as approved by the HREC-1 committee.

No personal financial gain is anticipated from this thesis or any related article.

CONFLICT OF INTEREST

No conflict of interest is known.

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To my mentor Dr. Pieter Barnardt, thank you for directing these efforts, for your valued time and expertise, and most of all, your patience.

I would like to acknowledge the contribution made by each woman in this cohort as we attempt to understand the phenotype of breast cancer in the HIV-positive patient.

Signed at Tygerberg Hospital November 2012.

Dr. L. Langenhoven
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ABSTRACT

A Retrospective, Single-Institution Survey to Assess the Phenotype of Breast Cancer In Women Infected With the Human Immune-Deficiency Virus.

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Background: HIV is the defining pandemic of our era, with an estimated 5.9 million people infected in South Africa according to World Health Organization (WHO) estimates for 2011. The expression and treatment of non-AIDS defining cancers has become an important consideration in this cohort, as antiretroviral therapy (ART) has prolonged survival in a subgroup previously at risk of early mortality.

Study Design: A retrospective cohort study of all women seen at the Combined Breast Cancer Clinic at Tygerberg Hospital between January 2010 and December 2011, stratified into three subgroups based on HIV status.

Methods: Of the 816 patients screened, 586 met inclusion criteria; of which 31 (5.3%) patients were HIV positive, 420 (71.7%) HIV negative, and 135 (23%) had an unknown HIV status. The disease phenotype was described in each subgroup, as well as toxicities associated with standard chemotherapy regimens, with an emphasis on completion rates of systemic cytotoxic treatment. The insult of cytotoxic therapy to the CD4 count was described for this cohort.

Results: Women with HIV had a statistically significant (p<0.001) younger age at presentation of breast cancer with a median age of 42 years (range 39 – 45 years) in comparison with the HIV-negative cohort with a median age of 54 years (range 53 – 55 years).

No difference was detected in disease phenotype when stage at presentation (p=0.7874), histological subtype (p=0.3375), grade of differentiation (p=0.8297), nodal involvement (p=0.0998) or hormone-receptor positivity (p=0.6285) was considered. Completion rates for systemic cytotoxic treatment were excellent (>90%) regardless of HIV-status and no statistically significant toxicity was observed. The median CD4 count at diagnosis was 477 cells/µL (range 234 – 807 cells/µL), with a nadir value of 333 cells/µL (range 62 – 713 cells/µL), representing a decrease of 30.2% during treatment. One case of suspected treatment-related mortality was recorded.

Conclusion: This retrospective study confirmed that women infected with HIV had a younger age at breast cancer diagnosis when compared to women with a negative HIV-status. No difference in disease phenotype could be demonstrated for women with HIV, denoting the co-existence of two common chronic diseases. Chemotherapy was tolerated well, but caused a median decline in CD4-count of 30% during treatment.
OPSOMMING

‘n Retrospektiewe, Enkel-Instansie Oudit van die Borskanker-fenotipe in Vroue Geïnfekteerd met die Menslike Immungebreek-Virus (MIV).

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Achtergrond: Infeksie met die Menslike Immungebreek-Virus (MIV) is die pandemie van ons era, met ‘n geraamde 5.9 miljoen mense geïnfekteerd in Suid-Afrika volgens die Wêreld Gesondheids-Organisasie (WGO) in 2011. Kankers wat nie met MIV geassosieer word nie, het n belangrike oorweging in hierdie populasi-groep geword as gevolg van die gebruik van anti-retrovirale terapie wat die lewensverwagting van mense met MIV verleng.

Navorsingsontwerp: ‘n Retrospektiewe kohort studie van alle vroue wat tydens die tydperk Januarie 2010 en Desember 2011 by die Gekombineerde Borskanker Kliniek te Tygerberg Hospitaal behandel was, verdeel in 3 groepe volgens hul retrovirale status.

Metodes: ‘n Totaal van 819 vroue was oorweeg vir insluiting in die studie, waarvan 586 aan die insluitingskriteria voldoen het. Daar was 31 vroue met MIV (5.3%), 420 vroue het MIV-negatief getoets (71.7%), en 135 (23%) vroue waarvan die MIV-status onbekend was. Die fenotipe van borskanker was beskryf vir elke sub-groep, sowel as die toksiteite wat geassocieer word met die gebruik van standaard chemoterapie-skedules, insluitend die effek van chemoterapie op die CD4-telling.

Bevindinge: Vroue met MIV het op ‘n statisties noemenswaardige (p<0.001) jonger ouderdom gepresenteer met borskanker (gemiddelde ouderdom 42 jaar, reikwydte 39 – 45 jaar) in vergelyking met vroue wat MIV-negatief was (gemiddelde ouderdom 54 jaar, reikwydte 53 – 55 jaar). Geen verskil was waargeneem in die fenotipe van borskanker in vroue met MIV vir die stadium by diagnose (p = 0.7874), histologiese tipe (p=0.3375), graad van differensiasie (p = 0.8297), nodale betrekking (p = 0.0998) of hormoon-reseptor status (p=0.6285) nie. Voltooiing van sistemiese chemoterapie is bereik in meer as negentig persent van gevalle onafhanklik van MIV-status. ‘n Gemiddelde CD4-telling van 477 selle/µL (reikwydte 234 – 807 selle/ µL) met diagnose het ‘n gemiddelde afname van 30.2% tydens behandeling getoon na ‘n gemiddelde waarde van 333 selle/ µL (reikwydte 62 – 713 selle/ µL).

Gevolgtrekkings: Hierdie retrospektiewe studie het bevind dat vroue met MIV op ‘n jonger ouderdom met borskanker presentere as vroue wat MIV-negatief is. Geen noemenswaardige verskil was waargeneem in die fenotipe van borskanker in vroue met MIV nie, en dui daarop dat borskanker en MIV twee algemene, maar onafhanklike entiteite is. Chemoterapie was goed getolereer met ‘n gemiddelde afname in die CD4-telling van 30.2% tydens chemoterapie.
CHAPTER 1: INTRODUCTION

“It is estimated that 30 million lives have been lost globally to the human immune-deficiency virus (HIV) pandemic since 1981.”

Infection with the human immune-deficiency virus (HIV) is undoubtedly the defining pandemic of our era. According to the World Health Organization (WHO) Global HIV/AIDS Response Report 2011 an estimated 34 million people worldwide were living with HIV/AIDS in 2010. Sub-Saharan Africa remained disproportionately affected with an estimated 22.5 million cases, representing 67% of the global HIV burden and of these, 5.9 million cases were from South Africa, the country with the largest population of people living with HIV/AIDS in the world.

The WHO estimated that 6.65 million people received anti-retroviral therapy (ART) in 2010, representing 47% of people eligible to treatment. In a recent article by May et al. it was shown that the life expectancy for people receiving ART increased by an average of 15 years between 1996 and 2008. When compared to the general population of the United Kingdom for this period, the decrease in average life expectancy amounted to only 13 years.

Although no concrete data is available to reflect the impact of ART in the South African setting, it is accepted that persons with HIV infection are living longer as a result of ART, and are hence at risk of developing illnesses associated with ageing, including chronic illnesses and malignancies.

Data from the Swiss HIV Cohort Study showed that 19% of all cohort deaths in the ART-area were attributable to non-AIDS defining cancers (NADC). Much global effort is currently directed to describing the association between HIV and the NADC’s. An increased incidence was observed for anal cancer, lung cancer, certain head-and-neck cancers, hepatocellular carcinoma, as well as Hodgkin’s Lymphoma, while for breast cancer, prostate cancer and colorectal cancer no increased risk was observed in the HIV-positive cohort. Very little is known on the phenotype of these malignancies in the context of HIV, as well as the outcome of patients receiving standard oncological treatment.

Given that breast cancer is the most common NADC to affect the global female population, affecting 1 in every 8 women in their lifetime, the question as to whether HIV-infection will alter the incidence, phenotype, response to treatment, or outcomes of breast cancer remains unanswered. It is expected that the complex interactions between a host with an altered immune-regulation, receiving ART, while being exposed to all the effects of ART will alter the course of breast cancer expression in the HIV-positive host.

It is the primary objective of this retrospective cohort study to describe the phenotype of breast cancer in women diagnosed with HIV in comparison to the HIV-negative control group.

LITERATURE REVIEW

A PubMed literature review restricted to the English language was performed using the keywords ‘HIV’ and ‘breast cancer’ for the period 1981 to present. It is of interest that our current understanding of breast cancer in the HIV-positive patient is based on case series detailing single institution experience.

Amir et al. published data from the Tanzanian Cancer Registry and concluded that a statistically significant decreased incidence of breast cancer was observed for both men and women before and during the AIDS-pandemic. These results have been replicated by large
cancer registry based studies in Italy, France and the USA and possible explanations include:  

a) The competing mortality of opportunistic infections – patients do not live long enough to develop breast cancer, and succumb to HIV-related complications.

b) The possible underreporting of cases in the third world reflecting decreased access to health care and socio-economic status, and

c) The overall reduced risk in the developing world for breast cancer when the following known protective factors for breast cancer are considered:

   i. Early age of first childbirth  
   ii. High parity  
   iii. Low alcohol intake  
   iv. Lower social status.

According to the heterotypic model of carcinogenesis, it is postulated that decreased immunity associated with HIV-infection might be protective in developing breast cancer. A recent report on HIV-1 and glycoprotein-120 (gp120) induction of breast cancer cell apoptosis through gp120-CXCR4 receptor supports this theory. Further reports have shown ritonavir to inhibit breast cancer growth through the inhibition of Akt-regulated cell proliferation.

Currently any assumption made as to the protective/permissive role of HIV in the development of breast cancer would be premature. In clinical practice it is accepted that HIV is not permissive to the development of breast cancer and that the incidence of breast cancer in the HIV-positive person is the same, or slightly less than their congruent background populations.

Evidence to support a more aggressive breast cancer phenotype with poor outcome was presented by Guth in a case series of 17 patients published in 1999 in which 14 patients had metastatic disease at presentation and an associated poor outcome. Yet, this uniformly poor outcome could be greatly attributed to the following confounders:

Most cases were managed before the ART-era and represent a cohort of patients with progressive immune-impairment, prone to the development of opportunistic infections, and a poor tolerance of systemic chemotherapy due to the associated rise in viral load during treatment. Patients in the cohort were mostly young African-American women, a subgroup known to have a uniformly poor outcome, younger age at presentation and more advanced disease stage at diagnosis. Furthermore, poor socio-economic status is an independent risk factor for both HIV-infection and lack of access to mammography screening, and may represent a confounding effect in advanced stage at diagnosis.

Evidence to support a similar phenotype to that seen in the HIV-negative patients comes from a series published in 2000 describing the outcome of 20 consecutive patients treated at the Jackson Memorial Hospital in Miami. Of interest was that 12 of the 20 patients presented with early stage disease, and the stage distribution was similar to the HIV-negative cohort. The most important contribution made by this study was the inclusion of toxicity data in relation to the tolerance of chemotherapy. Hurley cautioned that doxorubicin-based chemotherapy was poorly tolerated in the HIV-positive patient, with a higher incidence of grade 3 and 4 toxicity observed including neutropenic sepsis, Candida esophagitis and Adult Respiratory Distress Syndrome (ARDS).

The overall survival in patients treated with chemotherapy was inferior to patients only offered hormonal therapy in both the adjuvant and metastatic setting. Hurley postulated that
chemotherapy dealt a blow to the immune-system that could not recover after the completion of chemotherapy and was permissive to the progression of the HIV-infection and early HIV/AIDS related mortality.\textsuperscript{21}

These findings were supported by another series of five HIV-positive women with breast cancer published in 2002.\textsuperscript{22} Eighty percent of patients developed grade 3 or 4 neutropenia, with one mortality due to neutropenic sepsis while two patients developed progression of their HIV-infection as confirmed by a rise in the HIV-1 RNA viral load. Both doxorubicin and taxane-based chemotherapy were poorly tolerated. The recommendation was to introduce the routine use of supportive prophylactic granulocyte-colony stimulating factor (G-CSF).

More recent data published by Sarhan et al. details the experience of the Harlem Medical Centre for the period 2002 to 2008.\textsuperscript{23} The phenotype of six HIV-positive women was compared to that of 57 negative women from the same cohort (n=63). No difference in stage at presentation, age, tumour morphology or outcome was observed between the two sub-groups. Chemotherapy was tolerated without significant toxicity, and the 5-year overall survival for the HIV-positive cohort was 75%. A major confounder became evident when the stage distribution was considered: only 34% of patients presented with stage 3 or 4 disease. This phenomenon was attributed to increased access to health care associated with the routine follow-up of HIV infected patients.

**STUDY OBJECTIVES**

Primary objective: It was the aim of this study to describe the phenotype of breast cancer in the HIV-positive population in relation to the HIV-negative control group. Attention was paid to the pattern of disease expression, and in particular the age at diagnosis, stage of presentation, hormone-sensitivity of the tumour, as well as markers of biological aggressiveness including the grade of differentiation, presence of nodal metastases and histological subtype.

Secondary objectives:

1. The ability of HIV positive patients to complete standard chemotherapy regimens. If planned treatment could not be completed, the reasons for cessation and the timing thereof were documented.
2. Acute toxicities were noted.
3. The trend of CD-4 count changes during chemotherapy was described.

**STUDY DESIGN**

This was a retrospective cohort study of all female patients seen at the Combined Breast Clinic at Tygerberg Hospital for the period January 2010 to December 2011. Consented HIV-testing was introduced in 2009, and despite attempts to attain 100% testing, the status of some patients were unknown. The cohort was divided into three sub-groups reflecting the HIV status of the patient: HIV-negative, HIV-positive, and HIV-unknown. All variables were compared between the three subgroups to determine whether a statistically significant difference could be detected when considering the phenotype of disease or outcome of these sub-groups.

**INCLUSION CRITERIA**

1. All patients newly diagnosed with breast cancer at Tygerberg Hospital during the period January 2010 to December 2011 were eligible for inclusion.
2. Patients were female and aged between 18-100 years.

3. Patients of all ethnic groups were eligible for inclusion.

EXCLUSION CRITERIA

1. Benign or non-invasive disease.

2. Patients with insufficient data.


RESOURCES

Funding to the monetary value of R16 500 was made available through the D43 grant in lieu of the salary for the data capturer.
CHAPTER 2: METHODS

STUDY DESIGN:

A retrospective analysis was performed of all women registered at the Combined Breast Clinic at Tygerberg Hospital for the period January 2010 to December 2011. A total of 819 patients were seen during the trial period, of which 586 met the inclusion criteria. Exclusions were for: insufficient data (n=154), prior diagnosis of breast cancer (n=47), male gender (n=11), ductal carcinoma in situ (n=16) and benign breast disease (n=5).

RESEARCH PROCEDURES:

The demographic profile was considered in each subgroup as primary endpoint, and reported in terms of median age at presentation, HIV-status by ethnic group, menopausal status and Breast Cancer Gene (BRCA) status.

The disease phenotype was described in terms of stage at presentation (Breast cancer was staged according to the American Joint Committee on Cancer (AJCC) staging system 2007), histological subtype, grade of differentiation, nodal status and hormone-receptor positivity for the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (Her-2) receptor respectively.

Secondary objectives included the ability of patients to complete the number of intended chemotherapy cycles scheduled at the start of treatment. Allowance was made for interruptions or postponements due to any cause, as well as dose reductions due to toxicities. A patient was considered to have completed chemotherapy if the total number of cycles completed were in agreement with the number of cycles planned at the onset of treatment. A comparison was made between the completion rates of the respective subgroups.

The HIV-positive subgroup was considered separately in so far chemotherapy toxicity data was concerned, and scoring of toxicities were done according to the European Organization for Research and Treatment of Cancer (EORTC) Common Toxicity Criteria (CTC) Scoring system, version 4.0 A toxicity grading was assigned for each patient at each cycle for the following hematological parameters: hemoglobin (Hb), white cell count (WCC), platelets, neutrophils, lymphocytes and CD4 count. Clinical toxicities considered were for nausea and vomiting, alopecia, thrombophlebitis and loss of weight. A concise case report was offered for each patient who could not complete chemotherapy as planned, detailing the specific reasons for cessation of therapy.

The CD4-nadir was defined as the lowest value recorded during or at any stage after treatment, and reported as a mean value with a standard deviation, minimum and maximum values. It is practice at Tygerberg Hospital to repeat the CD4 value at cycle 3 and on completion of chemotherapy. The timing of the nadir values reflected this practice.

Data collection commenced on 13 March 2012 when ethical approval was obtained and included all potential patients from 14 January 2010 up to 31 December 2011 when data lock occurred.
DEFINITIONS:

**Breast Cancer Gene (BRCA):** Indications for Breast Cancer susceptibility gene 1 and 2 (BRCA) testing include:

- Breast cancer in women younger than 35 years old.
- A family history of breast cancer involving a first-degree relative.
- Male breast cancer.
- Bilateral breast cancer.

**Completion of chemotherapy regimen as planned:** Completion of the total number of chemotherapy cycles as planned despite postponements and/or dose-reductions made.

**Early-stage breast cancer:** Defined as all patients with disease that has not spread beyond the breast or axillary lymph nodes, and include patients presenting with Stage I and II disease according to the American Joint Committee on Cancer (AJCC) Staging system.  

**HIV-positive:** The serum HIV Enzyme Linked Immunosorbent Assay (ELISA) Antigen/Antibody Combination Assay test for the presence of both HIV antibody and antigen (p24) was used. Positive serologic results were repeated and patients were deemed HIV-positive if serum positivity was confirmed with 2 separate ELISA tests.

**Locally advanced disease:** Defined as all patients presenting with stage III breast cancer according to the AJCC Staging system.  

**Menopausal status:**

**Menopause:** A physiological occurrence marking the permanent cessation of ovarian function in women with an intact uterus (medical menopause) or without (surgical menopause), usually occurring during the late 40’s or early 50’s. Post-menopausal status is reached when no menstrual cycles are recorded for 12 consecutive months in the absence of pregnancy, lactation, or other pathological causes of amenorrhea.

**Peri-menopause** is descriptive of the period around menopause, where menstrual cycles are less frequent and less predictable in women in midlife, but not meeting the above criteria.

**Pre-menopause:** Intact ovarian function in women of reproductive age.

**Nodal sampling:** The surgical evaluation of axillary lymph nodes as appropriate in non-metastatic patients, including sentinel lymph node biopsy and axillary clearance.

**Performance status:** All patients were scored according to the Eastern Cooperative Oncology Group (ECOG) performance status scoring system.  

**Range:** The median age range was defined as the age that marks the -95% and +95% centiles respectively.

**Stage:** Breast cancer was staged according to the AJCC staging system 2007.  

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DATA CAPTURING AND SAFETY:

Data acquisition was performed at the Gene Louw Building at Tygerberg Hospital by the principal investigator and data capturer following protocol approval by the Health Research Ethics Committee 1 (HREC-1). Approval to conduct research at Tygerberg Hospital in accordance with the Provincial Research Policy and Tygerberg Hospital was granted.

Data was captured onto a password-protected database, with access restricted to the principal investigator, data capturer and supervisor. The format of the database was designed by Prof. Martin Kidd to facilitate statistical analysis subsequent to data acquisition. Patient confidentiality was protected through the use of registry numbers to which only the principal investigator has access. The datasheet is attached as Addendum 7.

DATA ANALYSIS:

Statistical analysis was performed by Prof Martin Kidd at the Centre for Statistical Consultation, Stellenbosch University. The completed database was imported into the Statistica software program, which was used for data analysis. Prior to data analysis, all variables were reviewed for incomplete data. Where data was missing, an ‘unknown’ variable was created.

Descriptive data analysis was utilized in nominal variables such as age, as well as ordinal variables for race, menopausal status, stage at presentation and histological subtype. Histograms, graphs and tables were utilized in data-representation. Hypothesis testing was done through linear regression models, Chi-square and ANOVA analysis, and expressed in terms of a p-value for which a p<0.05 was considered to be statistically significant.
CHAPTER 3: RESULTS

A total of 586 patients were included in the study from January 2010 to December 2011, of which 31 (5.3%) were HIV-positive, 420 (71.7%) were HIV-negative, and 135 (23%) had an unknown HIV status. The median age at presentation was 56 years, with a standard deviation (SD) of 14.1 years and a range of 20 to 97 years. The majority of women were from a Mixed Race, representing 61% of the cohort. Approximately a third of women (27%) were of European descent, while 12% was of African descent, reflecting the referral pattern and drainage areas for our institute. Bilateral breast cancer was observed in 20 patients (3%), with the remainder having an equal chance of having a left- (48%) or right-sided (49%) tumor.

A statistically significant age difference (p<0.001) was detected between the three subgroups, favoring a younger age at presentation for women infected with HIV. The median age at diagnosis for the HIV-positive subgroup was 42 years, a median age difference of 12 years when compared to the HIV-negative subgroup, and 19.5 years when compared to the subgroup with an unknown HIV-status (Graph 1). The age range between the 95th centiles was much smaller for the HIV-positive subgroup (7.5 vs. 13.1 years) when compared to the HIV-negative subgroup, denoting a more homogenous age distribution (Table 1).

<table>
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<th>Negative</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
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<td>42</td>
<td>54,3%</td>
<td>61,8%</td>
</tr>
<tr>
<td>Diagnosis (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (95th centiles)</td>
<td>39-45</td>
<td>53-56</td>
<td>59-64</td>
</tr>
<tr>
<td>African</td>
<td>40</td>
<td>48,7%</td>
<td>63,4%</td>
</tr>
<tr>
<td>Mixed race</td>
<td>45</td>
<td>54%</td>
<td>59,3%</td>
</tr>
<tr>
<td>European</td>
<td>-</td>
<td>57,6%</td>
<td>64,9%</td>
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Ethnic group

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<td>13</td>
<td>3,6%</td>
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<tr>
<td>African</td>
<td>18</td>
<td>26,1%</td>
<td>8</td>
</tr>
<tr>
<td>European</td>
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Menopausal status

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<tr>
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<td>20</td>
<td>64,5%</td>
<td>37</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>7</td>
<td>22,6%</td>
<td>97</td>
</tr>
<tr>
<td>Peri-menopausal</td>
<td>4</td>
<td>12,9%</td>
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BRCA-receptor status

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<th>Negative</th>
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<td>Not done</td>
<td>28</td>
<td>90,3%</td>
<td>125</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>0%</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>9,7%</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 1: Demographic data by HIV-status

In the subgroup stratification the HIV-status was known in 77% (n=451) of the cohort, of which 6.9% (n=31) of women tested positive for infection with HIV. The HIV-status was not determined in 23% of patients (n=135). A subgroup analysis was performed based on ethnic
group to address a possible confounding effect inherent to the association between HIV-positivity and ethnic group (Table 1). A statistically significant (p<0.01) younger age at presentation was seen for women infected with HIV after correcting for race. African women infected with HIV presented on average 8.2 years earlier when compared to the HIV-negative subgroup, and 22.9 years when compared to the HIV-unknown subgroup.

![Median age at diagnosis](image)

**Figure 1: Median age at presentation by HIV-status**

The proportion of HIV positive patients in the cohort tested, was 6.9%, but on ethnic subgroup analysis it was found that (Table 1):

1. No case of HIV-positivity was detected in the European subgroup, with the highest proportion of unknown status (33.6%).
2. The proportion of Mixed race patients testing positive for HIV was 3.6%, with 20.6% of patients not tested for HIV, while
3. The proportion of patients with HIV in the African subgroup was 26.1%. Only 11.6% of African patients were not tested for HIV.

In accordance with the pathophysiology and risk factors of breast cancer, 64% of women were post-menopausal, 2% were peri-menopausal, with the remaining 34% being pre-menopausal. A statistically significant (p=0.00004) difference in menopausal status was detected with the majority of HIV-positive patients being pre-menopausal (64.5%), a finding in contrast to the 34.1% recorded for the HIV-negative cohort.
BRCA testing was performed in 11% of the cohort, with a 2% BRCA-positive result recorded. No statistically significant difference \( (p=0.36294) \) was observed in the frequency with which BRCA status was determined or tested positive in.

No statistically significant difference in stage at presentation was observed between the subgroups \( (p=0.7874) \), despite a trend towards more advanced disease at presentation for the HIV-positive subgroup. The majority of the HIV-negative cohort (46.4%) presented with early-stage breast cancer, with locally advanced and metastatic disease representing 39% and 14.6% of cases respectively. A trend towards advanced disease at presentation became evident for the HIV-positive subgroup, 35.4% presented with locally advanced disease. An equal proportion of patients presented with early and metastatic disease. When the HIV-unknown subgroup was considered the majority of patients (43.4%) presented with early breast cancer.

Infiltrating duct carcinoma was the predominant histological subtype in all subgroups regardless of HIV-status, and represented 71% (HIV-positive), 84.9% (HIV-negative) and 82.1% (HIV-unknown) of all cases. Infiltrating lobular carcinoma was the second most recorded histological type. The remaining histological subtypes were grouped together as ‘other’ due to the small number of events and unsure statistical significance when considered in isolation.

A significant percentage of HIV-positive patients (22.5%) had a histological subtype classified as ‘other’ at presentation in contrast to the HIV-negative (8.4%) and HIV-unknown (11.9%) subgroups, but due to small numbers, this finding did not reach statistical significance. The subtypes classified as ‘other’ were reviewed, with the 22.5% \( (n=7) \) of cases represented by the following histologies:

A cytological diagnosis had not been made in one patient due to a poor performance status at disease presentation, while another patient defaulted management prior to confirmatory cytological testing. The remaining five cases were represented by carcinoma NOS \( (n=4) \) and mucinous adenocarcinoma \( (n=1) \). It is of interest that these subtypes classified as ‘other’ were still of epithelial origin, and not the less frequently-observed subtypes of lymphoma, sarcoma or inflammatory breast cancer.
No statistically significant (p=0.8297) difference in tumor differentiation was observed between the subgroups and ≈30% of patients presented with moderately differentiated tumors (grade 2).

In patients offered axillary sampling (58.1%), the majority of patients (78%) had less than four nodes involved, with no statistically significant difference (p=0.0998) detected between the subgroups. Her-2 receptor positivity to the human epidermal growth factor receptor-2 (HER-2) neu-oncogene was equally represented between the subgroups, being positive in a third of the patients in line with internationally expected 20-30% incidence of all breast cancer cases.

<table>
<thead>
<tr>
<th>HIV-status</th>
<th>Positive</th>
<th>Negative</th>
<th>p-value</th>
</tr>
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<tr>
<td></td>
<td>n = 31</td>
<td>n = 420</td>
<td></td>
</tr>
<tr>
<td>Stage at presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>2 6,5</td>
<td>21 4,9</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>8 25,8</td>
<td>174 41,5</td>
<td>p=0.7874</td>
</tr>
<tr>
<td>Stage 3</td>
<td>11 35,4</td>
<td>164 39</td>
<td></td>
</tr>
<tr>
<td>Stage 4</td>
<td>10 32,3</td>
<td>61 14,6</td>
<td></td>
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<td>Histological subtype</td>
<td></td>
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<tr>
<td>Infiltrating ductal carcinoma</td>
<td>22 71</td>
<td>355 84,9</td>
<td></td>
</tr>
<tr>
<td>Lobular carcinoma</td>
<td>2 6,5</td>
<td>28 6,7</td>
<td>p=0.3375</td>
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<tr>
<td>Other</td>
<td>7 22,5</td>
<td>35 8,4</td>
<td></td>
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<tr>
<td>Grade of differentiation</td>
<td></td>
<td></td>
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<tr>
<td>Well differentiated</td>
<td>3 9,7</td>
<td>38 9,1</td>
<td></td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>9 29</td>
<td>119 28,3</td>
<td>p=0.8297</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>5 16,1</td>
<td>89 21,2</td>
<td></td>
</tr>
<tr>
<td>Unknown differentiation</td>
<td>14 45,2</td>
<td>174 41,4</td>
<td></td>
</tr>
<tr>
<td>Nodal Status</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total sampled</td>
<td>15 265</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4 nodes involved</td>
<td>13 86,7</td>
<td>182 68,7</td>
<td>p=0.0998</td>
</tr>
<tr>
<td>≥ 4 nodes involved</td>
<td>2 13,3</td>
<td>83 31,3</td>
<td></td>
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<tr>
<td>Hormone Receptor Status</td>
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<td></td>
<td></td>
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<td>16 51,6</td>
<td>268 63,8</td>
<td></td>
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<td>Progesterone Receptor positivity</td>
<td>9 29</td>
<td>215 51,2</td>
<td>p=0.6285</td>
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<tr>
<td>HER-2 Receptor positivity</td>
<td>10 32,3</td>
<td>149 35,5</td>
<td></td>
</tr>
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Table 2: Disease and tumour characteristics by HIV status
No statistically significant difference (p=0.62585) in hormone receptor sensitivity was observed between the subgroups, but slightly less hormone-sensitive disease was observed for the HIV-positive subgroup. Estrogen receptor (ER) positive disease was observed in 51.6% of patients in contrast to the 63.8% in the HIV-negative subgroup. An even greater difference between the subgroups was observed when sensitivity to the Progesterone receptor (PR) was considered. Only 29% of the HIV-positive subgroup presented with PR-positive disease, in contrast to the 51.2% of the HIV-negative subgroup.

TREATMENT DEMOGRAPHICS

The administration of systemic chemotherapy was predominantly in the neo-adjuvant setting regardless of HIV-status at presentation (Table 3), with 42% of the HIV-positive, 37% of the HIV-negative, and 23% of the HIV-unknown subgroups receiving neo-adjuvant chemotherapy respectively. No statistically significant difference was observed when the administration of adjuvant chemotherapy was considered for the HIV-positive (10%), HIV-negative (14%) and HIV-unknown (11%) cohorts (p=0.1662).

Reflective of standard chemotherapy regimens employed in the treatment of breast cancer, the use of an anthracycline-based regimen in combination with cyclophosphamide and/or 5-flourouracil (FEC/EC) was administered in 85% of patients regardless of HIV-status (Table 3). The omission of anthracycline-based therapy in the cyclophosphamide/methotrexate/5-flourouracil (CMF) regimen was reflective of individual patient characteristics, especially cardiac compromise, a known contra-indication to the use of systemic anthracyclines. When the completion rate of standard chemotherapy regimens was considered, no statistically significant difference (p=0.7076) was observed between the subgroups. It is of interest that 85% of HIV-positive patients managed to complete the scheduled number of planned chemotherapy cycles as assigned at the initiation of their treatment.
HIV-status | Positive | Negative
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 20</td>
<td>%</td>
<td>n = 228</td>
</tr>
<tr>
<td>Regimen completed</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td>Regimen not completed</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

**Table 3: Chemotherapy completion rates**

**THE HIV-POSITIVE SUBGROUP:**

**TREATMENT DEMOGRAPHICS**

The HIV-positive cohort constituted 31 female patients, of which 20 patients were offered systemic chemotherapy. The majority of patients (78.1%) had a performance status graded as ECOG 0 or 1 at presentation, with performance status 2 and 3 observed in 6.3% and 9.4% of cases respectively. Five patients absconded after staging examinations were completed and prior to any treatment. Medical contra-indications precluded the use of chemotherapy in five patients, with the remaining patient not offered systemic cytotoxic therapy on the basis of early breast cancer.

The majority of patients (n=22) were known to be HIV-positive at diagnosis, with the remainder (n=9) being diagnosed at their first clinic visit. The mean CD4 count at diagnosis was 402 cells/µL, with a standard deviation of 244 cells/µL and a range of 66 to 1075 cells/µL. The CD4 nadir was not recorded in five patients and were subsequently excluded from the nadir value analysis. A CD4-value greater than or equal to the value at initiation of chemotherapy was observed for two patients, one patient who had started ART at diagnosis of her breast cancer, while the ART regimen of the second patient was changed to second-line therapy. When the use of ART was considered, only 12 patients (37.5%) had been on ART for three months or longer. The remainder of patients was referred for initiation of ART at the start of chemotherapy.

Co-morbid infections were present in 12.9% of patients at diagnosis, represented by Pulmonary Tuberculosis (PTB) (n=2), Aspergillosis (n=1) and *Staphylococcus aureus* pneumonia (n=1).

**CHEMOTHERAPY TOXICITY DATA**

Chemotherapy was administered in the neo-adjuvant (n=15), adjuvant (n=3), and palliative (n=2) settings and was generally well tolerated in the majority of patients. The most common toxicities observed were alopecia (n=16), nausea and vomiting (n=15), thrombophlebitis (n=3) and asthenia (n=3).

Hematological toxicities were predominantly limited to grade 0 and 1, with grade 3 or 4 toxicities commonly witnessed in the lymphocyte count (Table 4). The grade 4 hemoglobin toxicity observed in one patient (5.3%) was contributed to the additive suppressive bone marrow effect of Cyclophosphamide in combination with ART. No grade 3 or 4 toxicity was observed for the white cell- or platelet count. The depletion of the lymphocyte count innate to HIV infection was exacerbated by systemic cytotoxic therapy, with grade 3 and 4 toxicities witnessed in 26.4% of patients, representing the most frequently observed asymptomatic...
hematological toxicity. Only two patients (10.5%) had grade 3 toxicity of the neutrophil count, which did not preclude the successful completion of chemotherapy.

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 0</th>
<th>Grade 1/2</th>
<th>Grade 3/4</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1</td>
<td>5.3</td>
<td>17</td>
</tr>
<tr>
<td>White cell count</td>
<td>16</td>
<td>84.2</td>
<td>3</td>
</tr>
<tr>
<td>Platelets</td>
<td>19</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>12</td>
<td>63.2</td>
<td>5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2</td>
<td>10.5</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 4: Hematological toxicities scored by EORTC grading system

The administration of planned chemotherapy was not accomplished in three patients. The first patient defaulted mid-chemotherapy and returned 7 months later when she presented with local progression of her breast cancer (ulceration, fixation to chest wall and uncontrolled pain) and was offered palliative neutron external beam radiotherapy. The second patient was offered palliative systemic therapy for stage IV disease and completed 4 cycles prior to defaulting treatment. Collateral history suggested she died due to possible chemotherapy-related toxicity. The third patient was offered neo-adjuvant chemotherapy but developed progression of her disease (contra-lateral breast involvement) after four cycles of chemotherapy and was offered palliative radiotherapy.

Although not affecting the completion of planned chemotherapy, two patients required an adjustment to their ART-regimen during chemotherapy. Grade 4 anaemia was witnessed in one patient, with a second patient requiring an adjustment in ART due to decreased renal function as reflected by a deterioration in her glomerular filtration rate (GFR). One patient necessitated the initiation of bactrim® (trimetoprim-sulphametoxazole) prophylaxis when her initial CD4 count (466 cells/µL) dropped below 200 cells/µL. She received ART for more than three months at time of her diagnosis. Transport problems delayed treatment in two patients but both managed to complete planned chemotherapy.

Only patients with completed CD4 values (n=16) were included in data analysis utilizing the t-Test for dependent means (Figure 4). A statistically significant difference (p=0.0002) was detected in the mean CD4-count when measured at the start of treatment (477 cells/µL), and the lowest recorded value (333 cells/µL).
The observed decrease in median CD4 count amounted to 30.2% of the pre-treatment value. The Standard deviation (SD) remained consistent when pre-treatment (SD=160 cells/µL) and nadir (SD=166 cells/µL) values were considered, denoting a homogeneous insult to the CD4 value during chemotherapy treatment.

**Figure 4:** *Mean change in CD4-count.*
CHAPTER 4: DISCUSSION

A statistically significant younger age at presentation (p<0.001) was evident in our cohort for women infected with HIV after correcting for ethnic group, supporting the notion that these women were at least a decade younger (42 vs. 54 years) than their HIV-negative counterparts at the time of their breast cancer diagnosis. Yet, the interpretation of this finding was one of the most contentious aspects in the literature. Many authors put forth arguments to explain this phenomenon per se, but it was unlikely that any one explanation would suffice in explaining this phenomenon, rather it was expected that the symbiotic interaction of different factors were responsible for the observed younger age at presentation.

The majority of published case reports detailed the outcome of African American women, a cohort known to have a younger age at diagnosis, more aggressive disease and a universally poor outcome in comparison to other ethnic groups. The fact that the majority (59.4%) of HIV-positive patients in our cohort were of African descent was the first factor contributing to the younger age at diagnosis as witnessed in this retrospective study. A younger age at presentation was described by Voutsadakis, arguing that age at presentation reflected the younger age of women infected with HIV. When considering the South African HIV prevalence data, the highest prevalence was recorded in African women of reproductive age and supported this theory, the second factor to underlie the anticipation of age at diagnosis witnessed in our cohort. HIV remains a highly stigmatized entity in the South African context, with a resultant negative impact on the accurate reporting of prevalence data. Current HIV infection estimates are based on indirect measures such as ante-natal incidence data, shifts in mortality patterns, as well as the analysis of annual surveys. The reported national prevalence of HIV in women aged 55-59 years, was 7.7%, a figure not too dissimilar to the observed 5.4% in our cohort, especially when considering that 22% of the cohort was not tested for HIV. Only when prevalence data was considered by age group, did the dilutional effect of the predominantly older patient with breast cancer become evident.

Nearly a third of European women were not screened for HIV, while 11.6% of African women had an unknown HIV status. Possible explanations included the fact that African women have a younger median age at presentation and were screened more vigorously due to their reproductive status. Women of African descent are known to have the highest prevalence of HIV, hence influencing screening bias at first consultation. Of the African subgroup, 26.1% of women tested positive for HIV, with a median age of 40 years (range 39 - 45 years). The background HIV prevalence in Western Cape for this cohort was 18.5% in 2010. Whether this observed increased prevalence represented a true excess of women with HIV and breast cancer was uncertain. The prevalence of HIV in the Mixed Race and European subgroups was less than the expected national average, but still congruent to the background age per race stratification. The disproportionate burden of HIV infection in the African subgroup reflected the epidemiology of HIV infection in Southern Africa, currently the target of interventions aimed at understanding and controlling the pandemic.

No statistically significant difference (p=0.36294) was observed in the frequency with which BRCA status was determined. In line with recorded experience, 19.6% of the HIV-negative subgroup tested positive for an alteration in the BRCA gene. When considering the advanced median age at presentation of the HIV-unknown cohort, 10% of patients tested positive for the BRCA-gene. Due to the younger age at presentation of HIV-positive patients, an expected increase incidence of BRCA-1 and BRCA-2 gene mutations would be observed in this subgroup. Yet, no statistically significant difference was detected, and no HIV-positive
patient tested positive for the germline mutation for BRCA1 or BRCA2 genes. The notion that younger age at presentation might be due to a hereditary trait was thus excluded.

No statistically significant difference ($p=0.62585$) in hormone receptor sensitivity was observed between the subgroups, but a trend towards less hormone-sensitive disease was observed for the HIV-positive subgroup. This finding was thought to be representative of the fact that hormone-sensitive tumours are generally associated with women of post-menopausal status, in this case, women with HIV-negative and HIV-unknown status.

No statistically significant difference was detected in stage at presentation, histological subtype and grade of tumour differentiation, presence of nodal metastases or lymphovascular invasion when disease presentation in women with HIV was contrasted to women who were HIV negative or had an unknown HIV status. The notion that HIV altered the phenotype of disease expression was thus rejected, and in accordance with the phenotype described by both Sarhan and Hurley.\textsuperscript{21, 23}

Chemotherapy was tolerated well in all subgroups regardless of HIV status, with completion rates in excess of 90%. Five patients absconded after staging examinations were completed and prior to any treatment. The high rate of patients absconded prior to treatment was contributed to the multiple socio-economic confounders associated with HIV infection and ethnicity. Our finding of high chemotherapy completion rates was in contrast to earlier case reports where adverse outcomes were reported for systemic anthracycline-based cytotoxic therapy, including neutropenic sepsis, ARDS, Candida Oesophagitis and death.\textsuperscript{21, 22, 33} It was thought that the careful selection of HIV-positive patients receiving chemotherapy contributed to excellent completion rates, as well as the fact that patients were started on ART at initiation of chemotherapy regardless of CD4 count as per national ART treatment guidelines.

Grade 3 and 4 myelosuppression as reported in the literature was replicated only in so far lymphopenia was concerned, where 26.4% of the HIV positive cohort presented with grade 3 and 4 lymphopenia. This finding represented the synergistic effect of HIV and chemotherapy on the lymphocyte population. No dose adjustments were made for this toxicity and no resultant impact on treatment completion ensued. No dose-limiting toxicity was observed in the neutrophil count, with only 10.5% of patients developed grade 3 neutropenia. A single case of grade-4 toxicity was observed in the Hemoglobin levels of a patient, and contributed to the additive effect of chemotherapy and ART on bone marrow. No case of platelet toxicity was reported, with 100% of values graded as grade 0.

A single case of suspected treatment related mortality was observed in this cohort, but could not be confirmed by special investigations as the patient did not present to a medical institution prior to her demise. This incident was thought to elude to a much greater problem underlying the administration of cytotoxic therapy in patients with HIV – the fact that infection with HIV serves as a proxy for poor socio-economic means, a problem further compounded by language barriers and cultural beliefs.

No case of breast cancer was diagnosed at a CD4 value below 200 cells/µL and supported the notion that infection with HIV was neither permissive nor protective to the development of breast cancer, but rather represented the co-existence of two common chronic diseases. To date, the only association between CD4 count and the relative risk of developing a malignancy was shown in cancers with a viral pathogenesis, the so-called AIDS-defining cancers. No association between progressive immune-suppression as witnessed by a decreased CD4 count was shown for the NADC’s, a finding echoed in our study population.

Lymphoma studies published during the pre-ART era established the perception that chemotherapy administered in the HIV setting led to persistent, severe compromised
immunologic function and poor outcomes.\textsuperscript{32} The results of the AIDS malignancies consortium trial 010 confirmed an excess of deaths attributable to opportunistic infections, post-chemotherapy in patients with CD4 counts <50 cells/\mu L.\textsuperscript{33} However, a declined mean CD4 count was reported in trials but recent studies confirmed that chemotherapy was tolerated without significant opportunistic toxicity in the ART setting despite initial low CD4 counts.\textsuperscript{34, 35, 36} In our study the observed decrease in CD4 count amounted to a third of pre-treatment values, with a mean CD4 count at diagnosis of 477 cells/\mu L (234-807 cells/\mu L) in comparison with a mean nadir CD4 count of 333 cells/\mu L (62-713 cells/\mu L).

**STRENGTHS & LIMITATIONS**

Describing the phenotype of breast cancer in the HIV positive patient is ideally suited to the South-African academic environment, as South Africa currently has the highest HIV prevalence in the world. The excellent administrative system at the Combined Breast Clinic formed an ideal basis for retrospective data analysis, especially given the limitations inherent to retrospective data analysis.

The retrospective nature of data collection formed the greatest limitation of this study, with missing data and a non-uniform reporting of side effects evident at the time of data capturing. CD4 values were not determined at set intervals on completion of treatment, hence no recommendations could be made regarding the concern that systemic chemotherapy leads to progression of HIV itself and subsequent poor outcomes.

A prospective study evaluating the recovery of the CD4 nadir in terms of timing and absolute values should be undertaken to address this issue. The observed frequency of poor compliance, haphazard attendance and patient absconding during treatment were contributed to socio-economic and cultural issues and was a major limitation in patient access to health care. The triad of poor socio-economic means, strong cultural beliefs and the effects of a language barrier became evident as confounding factors, as witnessed in the unacceptably high rates of recorded defaulting for the HIV-positive subgroup.

A shortcoming in the reporting of CD-4 toxicity values became evident when utilizing the EORCT-CTC system, as CD4-values are reported in terms of absolute values, and no allowance is made to capture the percentage decline in CD-4 count during therapy. The EORCT-CTC version 2 was used to score toxicity in this study, but on evaluating the differences between the earlier and later versions, the subtle changes in toxicity definitions did not translate to a different statistical result, mainly as a result of grouping low grade (grade 1&2) and high grade (grade 3&4) toxicities together.
CHAPTER 5: CONCLUSION

This retrospective study confirmed that women infected with HIV presented at a younger age with breast cancer (42 years; range 39–45 years) when compared to women with a negative-HIV status (54 years, range 53 – 55 years). This finding was attributed to the younger age of women infected with HIV and the predominant African ethnicity of the HIV-positive subgroup, a known risk factor for a younger age at presentation.

No difference in disease phenotype could be demonstrated for women with HIV, denoting the co-existence of two common chronic diseases. Systemic chemotherapy was tolerated well in women infected with HIV, attributable to the careful selection of patients offered chemotherapy and the fact that all newly diagnosed HIV-patients were offered ART at their breast cancer diagnosis.

No statistically significant chemotherapy toxicity was observed and no patient developed opportunistic infections during treatment. The mean decline in CD4-count amounted to 30% during chemotherapy, and this value should be kept in mind by the clinician when selecting HIV-positive patients for chemotherapy.

In conclusion, data from this retrospective review confirmed that in a resource limited environment a service comparable to developed countries was provided. Data and observations from this study can be used for future treatment recommendations when the phenotype of breast cancer and the ability to complete systemic chemotherapy is offered to women infected with HIV.
REFERENCES.

## ABBREVIATIONS

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<tr>
<th>Abbreviation</th>
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<td>AIDS:</td>
<td>Acquired Immune-Deficiency Virus</td>
</tr>
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<td>AJCC:</td>
<td>American Joint Committee on Cancer</td>
</tr>
<tr>
<td>ARDS:</td>
<td>Adult Respiratory Distress Syndrome</td>
</tr>
<tr>
<td>ART:</td>
<td>Anti-Retroviral Therapy</td>
</tr>
<tr>
<td>BRCA:</td>
<td>Breast Cancer susceptibility gene 1 and 2 (tumour suppressor genes)</td>
</tr>
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<td>CD4:</td>
<td>Cluster of differentiation 4, a glycoprotein predominantly found on the surface of T-helper cells.</td>
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<td>CMF:</td>
<td>Cyclophosphamide/Methotrexate/5-Flourouracil</td>
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<td>Common Toxicity Criteria</td>
</tr>
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<td>EC:</td>
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<td>Eastern Cooperative Oncology Group</td>
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<td>ELISA:</td>
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<td>EORTC:</td>
<td>European Organization for Research and Treatment of Cancer</td>
</tr>
<tr>
<td>ER:</td>
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<tr>
<td>SD:</td>
<td>Standard Deviation</td>
</tr>
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<td>SEER:</td>
<td>Surveillance Epidemiology End Results</td>
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</table>
USA: United States of America
WCC: White Cell Count
WHO: World Health Organization
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Table 1: Demographic data by HIV-status
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Figure 2: Menopausal status by HIV status
Figure 3: Nodal involvement by HIV-status
Figure 4: CD4 count trend during treatment
Breast cancer diagnosed in women with the Human Immune-deficiency Virus.

Principal Investigator: Dr. L. Langenhoven. Tygerberg Academic Hospital, Cape Town, SA.

Supervisor/Co-Investigator: Dr. P. Barnardt. Tygerberg Academic Hospital, Cape Town, SA.

Introduction and literature review:

HIV/AIDS is the defining pandemic of our era. The widespread use of HAART has led to improved survival in a population previously at risk for early mortality. Given that breast cancer is the biggest global malignancy affecting the female population, it is expected that the number of immune-compromised survivors developing breast cancer will increase with time. To date, only 48 case reports have been published on the HIV-positive patient with breast cancer. Initial reports suggested a more aggressive tumour, triple negative receptor status, a younger age at presentation, a poorer prognosis, and an inability to complete chemotherapy.

Study Objectives

1. The primary objective of this retrospective analysis is to describe the phenotype of breast cancer in the HIV positive population in relation to its HIV negative control group.

2. Secondary objectives include the following:
   2.1. The ability of HIV positive patients to complete standard chemotherapy regimes. If treatment could not be completed, the reasons for cessation and the timing thereof will be documented.
   2.2. The trend of the CD4 count changes during chemotherapy.
   2.3. Disease Free Survival and Time to Progression.

Study design: The study will take the form of a retrospective descriptive, clinical audit and will be submitted as the thesis for my M.Med Rad. Onc degree.

Inclusion Criteria: Female patients of all ethnic groups newly diagnosed with breast cancer at Tygerberg Hospital during the period Jan 2010 – Dec 2011 will be eligible for inclusion.

Exclusion Criteria: Any patient managed only with surgery, palliative radiotherapy or patients included in the neo-adjuvant Paclitaxel trial will be excluded from participation in the study.

Methods: The cohort will be stratified into 2 subgroups: The HIV positive patient with breast cancer and the HIV negative patient with breast cancer. The age and stage of presentation will be compared between these 2 subgroups, as well as tumor characteristics such as: Histological subtype, grade of differentiation, receptor status, nodal status and tumour size. In the HIV positive group, the CD4 count, use of ART and the ability to complete planned treatment will be noted. The data collection sheet is attached as an addendum.

Data Management and Statistical analysis: All the patient and treatment information will be collected from the patients’ hospital folders, and will be conducted at the radiotherapy department of Tygerberg hospital. A database using Microsoft Excel 2003 will be created for
the purpose of the review (Addendum). Once the data is collected it will be analyzed with the help of US statistician, Prof Martin Kidd.

**Time Plan and logistics:** The study will commence as soon as ethics committee approval is granted. Data collection is estimated to take 3 months.

**Ethical Aspects:** This project will be an internal, departmental review, primarily based on folder reviews. Information will be collected to protect the patient confidentiality.

- No name, date of birth or hospital folder number will be mentioned.
- The data capture sheet will only have a study code attached
- Identifying information linked to the study code, will be kept in a password protected database.
- Access to this information will be restricted to the principal and co-investigators.
- Access to the database as a whole will be password protected.

Waiver of informed consent is requested from the ethics committee on the following grounds:

1. Each patient in the cohort gave informed consent to the testing of their HIV status prior to the test being done.
2. Patient information will be collected in such a way as to protect patient confidentiality as discussed.
3. The research will produce valuable information that would benefit the HIV positive patient receiving treatment for breast cancer. Recommendations regarding the tolerance of chemotherapy and possible dose-reductions will be made.
4. It would be impossible to trace individuals because of the retrospective nature of the audit.

Publication of the results will answer many of the current controversies in terms of the phenotype of disease, and the tolerance of the HIV-positive patient to current treatment regimens. Recommendations on dose-alterations in the HIV positive patient will be made based on the witnessed CD4-count trend.

**Resources:** No financial resources have been made available for this project. It is an M.Med thesis without financial incentive or interest other than those academic in nature.

**Strengths and Limitations:** Describing the phenotype of breast cancer in the HIV positive patient is ideally suited to the South-African academic environment as SA has the highest HIV prevalence in the world. Ideally prospective data should be collected, in this case a limitation in the study design.

**References**

Addendum 2: Protocol.

**Breast cancer diagnosed in women with the Human Immune-deficiency Virus.**

**Principal Investigator:** Dr. L. Langenhoven. Tygerberg Academic Hospital, Cape Town, SA.

**Supervisor/Co-Investigator:** Dr. P. Barnardt. Tygerberg Academic Hospital, Cape Town, SA.

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Introduction and Literature review

HIV is undeniably the defining pandemic of our era. According to the 2008 WHO statistics, it is estimated that more than 33.4 million people globally are infected with HIV, approximately 70% of which reside in sub-Saharan Africa.\(^1\) With the widespread introduction of ART, the life expectancy of patients living with HIV/AIDS has improved dramatically. Considering that breast cancer is the most common cancer in women, it is projected that the number of HIV-positive patients with breast cancer will increase in the foreseeable future.\(^2\)

The impact of HIV on breast cancer incidence, stage at presentation, response to treatment and treatment toxicity remains largely contentious at present. Case reports dominate the literature, of which 48 in total have been published. Hurley et al contributed the largest case series describing the treatment and outcome of 20 HIV positive patients with breast cancer.\(^3\) A more aggressive phenotype was described in the HIV positive patient, dominated by a triple negative receptor status, younger age at presentation, inability to complete chemotherapy and greater treatment related toxicity.

Palan et al published a report on 11 patients in 2011, all of which had a similar outcome to that witnessed in the HIV negative patient.\(^4\) Unfortunately these series were by American and European investigators, evaluating a disease relatively uncommon in their society. The South African experience of breast cancer in the HIV positive patient is much greater, yet no publication on the data has been presented for publication.

It is the aim of this study to describe the phenotype of breast cancer in the HIV positive patient in terms of age and stage at presentation, tumor grade and receptor status, as well as tolerance to standard treatment regimens.

Study Objectives

The primary objective of this retrospective analysis is to describe the phenotype of breast cancer in the HIV positive population in relation to its HIV negative control group.

Secondary objectives include the following:

1. The ability of HIV positive patients to complete standard chemotherapy and radiotherapy regimes. If planned treatment could not be completed, the reasons for cessation and the timing thereof will be documented.
2. The trend of the CD4 count changes during chemotherapy.
3. Disease Free Survival and Time to Progression.

Study design

The study will take the form of a retrospective descriptive, clinical audit and will be submitted as the thesis for my M.Med Rad. Onc degree.

All newly diagnosed breast cancer patients seen at Tygerberg Hospital between January 2010 and December 2011 will be included. Consented HIV testing was introduced in 2009, and with the exception of a few patients, the HIV status of every new patient is known. The study cohort is estimated to be 800 patients over the 2 year study period, of which 75 is estimated to be HIV-positive.
**Inclusion Criteria**

All newly diagnosed patients with breast cancer at Tygerberg Hospital during the period Jan 2010 – Dec 2011 will be eligible for inclusion

Female patients between the ages of 18-75 years

Patients of all ethnic groups will be included.

**Exclusion Criteria**

Any patient managed with only surgery or

Patients managed with only palliative radiotherapy.

Patients enrolled in the neo-adjuvant Paclitaxel trial.

**Objectives**

The primary objective of this retrospective analysis it to describe the phenotype of breast cancer in the HIV positive population in relation to its HIV negative control group.

Secondary objectives include the following:

1. The ability of HIV positive patients to complete standard chemotherapy and radiotherapy regimes. If planned treatment could not be completed, the reasons for cessation and the timing thereof will be documented.
2. The trend of the CD4 count changes during chemotherapy.
3. Disease Free Survival and Time to Progression.

**Methods**

The cohort will be stratified into 2 subgroups:

1. The HIV-negative patient with breast cancer.
2. The HIV-infected patient with breast cancer.

The age and stage of presentation will be compared between these 2 subgroups, as well as tumor characteristics such as:

- Histological subtype
- Grade of differentiation
- Receptor status
- Her2neu expression status
- Nodal status
- Tumour size

In the HIV positive group, the CD4 count, use of ART and the ability to complete standard treatment regimens will be documented.

All the patient and treatment information will be collected from their oncology therapy folders, radiotherapy treatment files, TBH folders, the NHLS results database and the Clinicom patient administration system used at Tygerberg Hospital. It will be conducted at the radiotherapy department (X Block) of Tygerberg hospital.
If a patient is found to be “lost to follow up” according to their hospital records, follow up information will be updated by telephonic contact with the patient or their immediate relatives to assess the patients status (alive /deceased).

**Data Management and Statistical analysis**

A database using Microsoft Excel 2003 will be created for the purpose of the review. (Appendix A). Once the data is collected on the database, it will be analyzed with the help of US statistician, Prof Martin Kidd. For descriptive purposes, frequency tables, histograms, means, standard deviation etc. will be used. For comparison between groups, the Chi- square test or ANOVA will be used depending on the types of data (continuous or categorical). Final decisions on which statistical techniques are most appropriate, will be made once the data have become available.

**Time Plan and logistics**

The study will commence as soon as ethics committee approval is granted.

Data collection is estimated to take 3 months.

The aim will be to finalize the analysis and study to be presented at the University Stellenbosch Academic Year Day in August 2012.

**Ethical Aspects**

This project will be an internal, departmental review, primarily based on folder reviews, with where required, a telephonic update of patient’s health status. Information will be collected in such away to protect the patient confidentiality.

- No name, date of birth or hospital folder number will be mentioned.
- The data capture sheet will only have a study code attached
- Identifying information linked to the study code, will be kept in a password protected database.
- Access to this information will be restricted to the principal and co-investigators.
- Access to the database as a whole will be password protected.

Waiver of informed consent is requested from the ethics committee on the following grounds:

1. Each patient in the cohort gave informed consent to the testing of their HIV status prior to the test being done.
2. Patient information will be collected in such a way as to protect patient confidentiality as discussed.
3. The research will produce valuable information that would benefit the HIV positive patient receiving treatment for breast cancer. Recommendations regarding the tolerance of chemotherapy and possible dose-reductions will be made.
4. It would be impossible to trace individuals because of the retrospective nature of the audit.
5. Publication of the results will answer many of the controversies in terms of the phenotype of disease, and the tolerance of the HIV-positive patient to current treatment regimens. Recommendations on dose-alterations in the HIV positive patient will be made based on the witnessed CD4-count trend.
The database could be used in the department in the future to serve as an audit for both HIV positive and negative patients receiving treatment for breast cancer at Tygerberg Hospital. Comparison with international results could be made to ensure that the standard of care is acceptable and in line with what is predicted.

**Resources**

No financial resources have been made available for this project. It is a M.Med thesis and data collection will be done by the principal investigator.

**Strengths and Limitations**

Describing the phenotype of breast cancer in the HIV positive patient is ideally suited to the South-African academic environment as SA has the highest HIV prevalence in the world.

Ideally prospective data should be collected, in this case a limitation in the study design.

**Bibliography**

Addendum 3: Approval notice by HREC-1 Committee.

22-Mar-2012

LANGENHOVEN, Lizanne

Approval Notice New Application

Protocol #: S12/02/048 Title: Breast cancer diagnosed in women with the Human Immune-deficiency Virus (HIV)

Dear Dr Lizanne LANGENHOVEN,

The New Application received on 17-Feb-2012, was reviewed by members of Health Research Ethics Committee 1 via Expedited review procedures on 13-Mar-2012 and was approved. Please note the following information about your approved research protocol:

Protocol Approval Period: 13-Mar-2012 -13-Mar-2013 Please remember to use your protocol number (S12/02/048) on any documents or correspondence with the REC concerning your research protocol.

Please note that the REC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review:

Please note a template of the progress report is obtainable on www.sun.ac.za/rds 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 projects may be selected randomly for an external audit. Translation of the consent document in the language applicable to the study participants should be submitted.

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 2004 (Department of Health).

**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 Hlne 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 REC forms and documents please visit: [www.sun.ac.za/rds](http://www.sun.ac.za/rds)

If you have any questions or need further help, please contact the REC office at 0219389657.

**Included Documents:**

- Synopsis
- Declaration
- Protocol
- Checklist
- Application
- CV

Sincerely,

Franklin Weber, REC Coordinator, Health Research Ethics Committee 1
Addendum 4: HREC-1 Application for amendments to protocol.

No 20, 17th Avenue
Boston
Bellville
7530

13/04/2012

The Health Research Ethics Committee 1,

Re: Addendum to Protocol S12/02/048

Breast cancer diagnosed in women with the Human Immune-deficiency Virus

1. Request to extend the research period as per protocol (2010-2011) to include the years 2008-2009 due to an unexpected lack of HIV-positive patients.

2. The principal investigator was offered an educational grant (The D43 Grant) by the Mailman School of Epidemiology, Columbia University, New York City. Dr. Lizanne Langenhoven will attend summer school in NYC during June 2012, followed by a month period of hands-on guidance in research ethics, epidemiology and biostatistics. The chosen project for assistance is the project of breast cancer diagnosed in women with the Human Immune-deficiency Virus.

The contractual agreement between Dr. Langenhoven and Columbia University states that any publication arising from the educational grant has to be published with the following paragraph:

- Appropriate acknowledgement of the funding source of your Traineeship on any scientific publications or presentations emanating from this Traineeship as follows: [your name] was supported by the Columbia University – South Africa Training Program for Research on AIDS-related Malignancies through the National Cancer Institute, NIH (grant # 1D43CA153715-02).

3. Through the funding of Columbia University, a research assistant will be employed to assist with data collection onto a password protected database. The assistant is contractually bound to patient privacy, and the principal investigator accepts the responsibility of ensuring that her actions are professional, ethically correct, that she respects patient privacy and serves the medical community at a large.

Regards,

Dr. Lizanne Langenhoven
079 5244 754
kibouter@yahoo.com

Addendum 5: HREC-2 approval of amendments to protocol.

10-May-2012
Barnardt, Pieter P

Approved with Stipulations Amendment

Ethics Reference #: S12/02/048 Title: Breast cancer diagnosed in women with the Human Immune-deficiency Virus (HIV)

Dear Doctor Pieter Barnardt,

The Amendment received on 13-Apr-2012, was reviewed by members of Health Research Ethics Committee 2 via Expedited review procedures on 18-Apr-2012.

Sincerely,

Mertrude Davids
REC Coordinator
Health Research Ethics Committee 2
Addendum 6: Approval to conduct research at Tygerberg Hospital in accordance with the Provincial Research Policy and Tygerberg Hospital Notice No. 40/09.

ETHICS NO: S12/02/048

Title: Breast Cancer diagnosed in women with the Human Immune-deficiency Virus (HIV).

Dear Dr Langenhoven

PERMISSION TO CONDUCT YOUR RESEARCH AT TYGERBERG HOSPITAL

In accordance with the Provincial Research Policy and Tygerberg Hospital Notice No 40/2009, permission is hereby granted for you to conduct the above-mentioned research here at Tygerberg Hospital.

Permission is hereby granted for you to access patient data for this retrospective study.

[Signature]

DR MUKOSI
MANAGER: MEDICAL SERVICES
RESEARCH
Date: