Reference intervals for common chemistry and haematology laboratory tests in a healthy Kenyan population: variation with age, sex, BMI and comparison with a South African population

By

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DECLARATION

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This dissertation includes four original papers published in peer-reviewed journals and one unpublished publication. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and, for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contributions of co-authors.

December 2020

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With regard to Chapters 3-7, the nature and scope of my contribution were as follows:

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2. No other authors contributed to Chapters 3-7 besides those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapters 3-7 of this dissertation.

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ABSTRACT

Introduction

Laboratory results play an important role in assessment of one's health status including detection of sub-clinical disease. Reference intervals (RIs) have been shown to vary across different populations due to various reasons including ethnic and racial differences, differences in reference populations and statistical approaches used in deriving the RIs. The International Federation of Clinical Chemistry committee on reference intervals and decision limits has been carrying out a global study aimed at harmonizing RIs. Kenya is the only country in East Africa that participated in this study and therefore the derived RIs will serve as a reference point for laboratories in the region. It is also important to identify what factors cause variations in RIs and whether partitioning based on age or sex will make them more specific. We subsequently compared our RIs with those from South Africa also derived as part of the global RI study.

Methods

Recruitment of study participants in Kenya was carried out between January and October 2015 in several counties after obtaining informed consent. Inclusion of participants was limited to healthy adults aged 18-65 years stratified into 4 age groups: 18-29, 30-39, 40-49 and 50-65 years. Haematology tests were performed at the PathCare laboratories in Nairobi, Kenya while all other analysis was done at the PathCare reference laboratory in Cape Town, South Africa. For purposes of the global RI study, all participating laboratories received a panel of sera that had assigned values to enable recalibration of reference values (RVs) and alignment across different countries. RIs were determined using both parametric and non-parametric methods before and after applying the latent abnormal values exclusion (LAVE) method.

Results

Out of 596 volunteers, 533 met the inclusion criteria: 260 (48.8%) males and 273 (51.2%) females. The prevalence of metabolic syndrome (MetS) was 25.6% and less than 1% of participants had reduced estimated glomerular filtration rate (eGFR). Sex-specific RIs were required for uric acid, creatinine, total bilirubin (TBil), total cholesterol (TC), transaminases, transferrin, transferrin saturation and immunoglobulin-M. Age-specific RIs were required for glucose and triglyceride for both sexes, and for urea, magnesium (Mg), TC, HDL-cholesterol ratio, alkaline phosphatase

(ALP), and ferritin for females. Kenyan RIs were comparable to those of other countries participating in the global study with a few exceptions such as higher ULs for TBil and c-reactive protein (CRP). South African RIs for uric acid, TC, low density lipoptotein cholesterol, alanine transaminase, lactate dehydrogenase, ALP, albumin, Mg, thyroid stimulating hormone and prostate specific antigen were lower than the Kenyan RIs.

Conclusion

Kenyan RIs for several analytes were established using a harmonized protocol from well-defined reference individuals. Given the rigour with which the study was conducted, the derived RIs will provide a useful reference for laboratories in sub-Saharan Africa that are looking for RIs for common haematology and biochemistry tests. For most analytes, harmonization of RIs between Kenyans and South Africans of African ancestry was not possible as they could result in misclassification of individuals as either diseased or healthy given the differences seen.

OPSOMMING

Inleiding

Laboratorium uitslae speel ‘n belangrike rol in die behandeling en diagnose van onderliggende siektes in pasiente. Normale reikwydtes (NW) vertoon verskillend oor bevolkingsgroepe om verskeie redes: etniese- en verwysings polulasie verskille asook verskillende statistiese metodes wat gebruik word. Die Internationale Federasie van Kliniese Chemie se kommitee vir reikwydtes en bepalings limiete koördineer ‘n globale projek om te bepaal of NW geharmoniseer kan word of nie. Kenya was die enigste land in Oos Afrika wat aan die globale projek deegeneem het. Kenya NW’s kan dien as verwysingsraamwerk vir ander laboratoria in die streek. Dit is belangrik in studies van hierdie aard om te bepaal watter faktore variasies in NW sal veroorsaak en die noodsaklikheid om ‘n skeiding te maak in NW om dit spesifiek te maak vir ouderdom en geslag. Hierdie studie het die NW van Kenya met die van Suid Afrika (RSA) vergelyk wat ook deegeneem het aan die globale projek.

Metode

Werwing van deelnemers in diè studie was gedoen in verskeie graafskappe in Kenya tussen Januarie en Oktober 2015 met ingeligte toestemming. Gesonde deelnemers was toegelaat om deel te neem tussen 18 – 65 jarige ouderdom, met verdere subverdeel in 4 verskillende ouderdomsgroepe: 18 – 29, 30 – 39, 40 – 49 en 50 – 65. Haematologiese analise van bloedmonsters was gedoen by PathCare Laboratorium in Nairobi, Kenya. Alle ander analise was gedoen was by PathCare se Verwysingslaboratorium in Kaapstad, RSA. Vir die doeleindes van die globale studie het alle deelnemende lande ‘n paneel sera ontvang met voorafbepaalde waardes wat hulle moes analiseer. Diè waardes was gebruik om verwysingswaardes te herkalibeer asook om waardes van die verskillende lande te kombineer en harmoniseer. Parametiese – asook nie-parametiese statisiek metodes was gebruik om NW te bepaal voor en na latente abnormale waarde uitskakelings (LAVE) toegepas was.

Resultate

596 deelenemers het deelgeneem; daar slegs 533 oor na toepassing van streng kriteria waarvan 260 (48.8%) mans en 273 (51.2%) vrouens was. Metaboliese sindroom (MetS) voorkoms onder deelnemers was 25.6%. < 1% van deelnemers het ‘n verminderde geskatte glomulêre filtrasie

tempo (eGFR) gehad. Geslag spesifieke NW was nodig vir uriensuur, kreatinien, totale bilirubin (TBil), totale cholesterol (TC), transaminases, transferrien, transferrien saturasie en immunoglobulien – M. Ouderdom spesifieke NW was nodig vir glukose en trigliseriede vir beide geslagte. NW vir vroulike deelnemers vir ureum, magnesium, TC, HDL-cholesterol verhouding, alkaliese phosfatase (ALP) en ferritien. NW vir Kenya kon vergelyk word met ander lande wat deelgeneem het met etlike uitsonderings vir boonste limiet waardes vir TBil en c-reaktiewe protein (CRP). Die NW van Suid Afrika vir uriensuur, lae densiteit lipoprotein cholesterol, alanine transaminase, laktaat dehidrogenase, ALP, albumien, magnesium, tiroïd stumulerings hormoon en prostaat spesifieke antigeen was laer as die NW van Kenya.

Konklusie

NW vir Keyna was bepaal deur ‘n geharmoniseerde protokol met goed gedefiniheerde verwysingsraamwerk en kan dien as verwysingsraamwerk vir ander laboratoria in die streek asook vir sub-Sahara Afrika lande vir algemene haematologiese – en kliniese chemie toetse. Kenya en RSA se NW’s kon nie geharmoniseer word nie; dit kan lei tot misklassifikasie van pasiente as óf gesond óf met onderliggende siektetoestande.

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LIST OF ABBREVIATIONS

4v-MDRD-Re-expressed 4 variable modified diet in renal disease

Abd.Circ. or AC-Abdominal circumference

Abs-Absolute count

ADA-American Diabetes Association

AE-Adverse event

ALB-Albumin

ALP-Alkaline phosphatase

ALT-Alanine aminotransferase

AMY-Amylase

AST-Aspartate aminotransferase

ATP III-Adult treatment panel III

AUC-Area under the curve

Bas-Basophil

BMI-Body mass index

BP-Blood pressure

BSA-Body surface area

C3-complement 3

C4-complement 4

C-RIDL-Committee of reference intervals and decision limits

CBC-Complete blood count

CDC-Center for Disease control

CDL-Clinical decision limit

CholRem-Cholesterol remnants

CG-Cockcroft-Gault

CK-Creatinine kinase

CKD-Chronic kidney disease

CKD-EPI- Chronic kidney disease epidemiology collaboration

CI-Confidence interval

cLDL-C-calculated low density lipoprotein cholesterol

CLSI-Clinical Laboratory Standards Institute

CrCl-Creatinine clearance

CRP-High sensitive c-reactive protein

CT-Computed tomography

CVD-Cardiovascular disease

DAIDS-Division of AIDS, National Institute of Allergy and Infectious Diseases

DARC-Duffy antigen receptor complex

eGFR-Estimated glomerular filtration rate

Eos-Eosinophil

FAS-Full age spectrum

Fe-Iron

Fer-Ferritin

FPG-Fasting plasma glucose

FT3-Free tri-iodothyronine

FT4-Free thyroxine

GFR-Glomerular filtration rate

GGT-Gamma glutamyl transferase

HB or Hb-Haemoglobin

HCT-Haematocrit

HDL-C-High density lipoprotein cholesterol

HIV-Human immunodeficiency virus

hsCRP-Highly sensitive c-reactive protein

IAVI-International Aids and Vaccine Initiative

IDF-International diabetes federation

IDL-Intermediate density lipoprotein

IFCC-International Federation of Clinical Chemistry

IFG-Impaired fasting glucose

IgA-Immunoglobulin A

IgG-Immunoglobulin G

IgM-Immunoglobulin M

IGT-Impaired glucose tolerance

IQR-Interquartile range

IQR-Interquartile range

ISO-International Organization of Standards

LAP-Lipid accumulation product

LAVE-latent abnormal value exclusion

LDH-lactate dehydrogenase

LDL-C-Low density lipoprotein cholesterol

LL-Lower limit

Lym-Lymphocyte

KDIGO-Kidney Disease: Improving Global Outcomes

Max.-Maximum

MCH-Mean corpuscular haemoglobin

MCHC-Mean corpuscular haemoglobin concentration

MCV-Mean corpuscular volume

MDRD- Modified diet in renal disease

MetS-Metabolic syndrome

mGFR- Measured glomerular filtration rate

Min.-Minimum

mLDL-C-Measured low density lipoprotein cholesterol

Mon-Monocyte

MRI-Magnetic resonance imaging

NCD-Non communicable disease

Neu-Neutrophil

NHIS-National Health Interview Survey

NORIP-Nordic Reference Interval Project

OR-Odds ratio

PLT-Platelets

PSA-Prostate specific antigen

RBC-Red blood cell

RDW-Red cell distribution width

RI-Reference interval

ROC-Receiver operator curves

RV-Reference value

SAT-Subcutaneous adipose tissue

SD-Standard deviation

SDR-Standard deviation ratio

SDR_{age}-between-age standard deviation ratio

SD_{RI}-Standard deviation comprising the reference interval

SDR_{sex}-between-sex standard deviation ratio

SSA-sub-Saharan Africa

TBIL or TBil-Total bilirubin

TC-Total cholesterol

Tf-Transferrin

TfSat-Transferrin saturation

TG-Triglycerides

TgAb-Anti-thyroglobulin

TIBC-Total iron binding capacity

TPOAb-Anti-thyroid peroxidase

TSH-Thyroid stimulating hormone

UA-Uric acid

UIBC-Unsaturated iron binding capacity

UL-Upper limit

UN-urea nitrogen

USA or US-United States of America

VAI-Visceral adiposity index

VAT-Visceral adipose tissue

VLDL-Very low density lipoprotein

WBC-White blood cell

WC-Waist circumference

YI-Youden index

OUTLINE OF THE DISSERTATION

The dissertation is organized into eight chapters. **Chapter 1** is a general introduction that surmises the importance of deriving population specific reference intervals for laboratory tests, study justification, hypotheses, research questions and objectives. **Chapter 2** is the literature review that highlights the variation of reference intervals across populations, various statistical methods used in deriving RIs, review of clinical decision limits, biochemical abnormalities in a reference population and assessment of renal dysfunction in asymptomatic individuals. **Chapter 3** is a published manuscript on the prevalence of metabolic syndrome in adult reference individuals recruited from Kenya as part of the global study on reference intervals. This study also proposes optimal cut-offs for the different components of metabolic syndrome for the Kenyan population. **Chapter 4** is a published manuscript on comparison of equations for estimating glomerular filtration rate in screening for chronic kidney disease in healthy Kenyan adults recruited as part of the global study on reference intervals. **Chapter 5** is a published manuscript on reference intervals for complete blood count parameters for the Kenyan population and comparison with similar studies from Africa and North America highlighting similarities and differences. **Chapter 6** is a published manuscript on reference intervals for clinical chemistry and immunoassay tests for the Kenyan adult population and comparison with similar studies carried out by the committee of reference intervals and decision limits (C-RIDL) of the International Federation of Clinical Chemistry (IFCC). **Chapter 7** is a yet to be published manuscript that compares reference intervals between Kenya and South Africa. It also explores sources of variation in reference values including age, sex and BMI. **Chapter 8** is an integrated summary of the key findings and recommendations on what needs to be done with regards to reference interval determination. Each chapter has its own references which have all been formatted in a common style across all chapters to provide consistency. However, some chapters retain some formatting aspects unique to the journal requirements. The last part of the dissertation is the **appendix** which has copies of study approvals, consent form and questionnaire.

CHAPTER 1: GENERAL INTRODUCTION

1.1. Introduction

Laboratory results play an important role in assessment of one's health status including detection of sub-clinical disease and ruling in or ruling out clinical diagnosis. In order to make laboratory test results more reliable, harmonization of all levels of the testing cycle is required (Miller, Tate, Barth, & Jones, 2014). Harmonization in laboratory testing goes beyond standardization of the analytical process, it includes standardizing pre-analytical processes such as sample collection and processing as well as post analytical processes such as use of appropriate reference intervals (RIs) and interpretive comments. It has been argued that RIs are the most common decision support tool used for interpretation of quantitative laboratory results and that the quality of the RIs may be as important as the quality of the result itself (G. Jones & Barker, 2008). It has been shown that the variation in RIs across laboratories for some tests may be much greater than the analytical accuracy of their measurements (G. R. D. Jones, Barker, Tate, Lim, & Robertson, 2004). This implies that the same patient result obtained by two laboratories that use the same assay but different RIs can result in different clinical interpretation and possibly initiate a cascade of testing that is unnecessary.

In Europe, in-vitro diagnostic companies are required by law to provide RIs for healthy and diseased individuals where appropriate for the test values determined using their equipment as this aids in better interpretation of the generated values (EU, 2017). The International Organization for Standardization (ISO) 15189 standard for medical laboratories states that "a laboratory shall define the biological reference intervals or clinical decision values, document the basis for the reference intervals or decision values and communicate this information to users" (ISO, 2012). The Clinical Laboratory Standards Institute (CLSI) has also published guidelines that guide the establishment of reference intervals in clinical laboratories in order to standardize the process of determining RIs. These guidelines are modelled around discussions of an expert panel of the International Federation of Clinical Chemistry (IFCC) on reference intervals that were published as 6 papers from as far back as 1986 (Dybkœr & Solberg, 1987; PetitClerc & Solberg, 1987; Solberg, 1984, 1987; Solberg & PetitClerc, 1988; Solberg & Stamm, 1991). The best method for deriving RIs is a formal study where samples are collected from a reference

group comprising a minimum of 120 individuals identified from a reference population through probability sampling (CLSI, 2010). CLSI recommends verification of RIs as an option in the event that establishing population specific RIs is not possible. In-vitro diagnostic companies also recommend local validation of the RIs reported in their reagent inserts. Even though verification is an affordable alternative, formal derivation of RIs should be attempted where possible.

RIs have been shown to vary across different populations due to various reasons including ethnic and racial differences, differences in reference populations including differences in body mass index (BMI), and differences in statistical approaches used in deriving RIs (Ichihara et al., 2008; Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017). Despite these known sources of variation in RIs, many countries in sub-Saharan Africa (SSA) adopt manufacturer proposed RIs which are largely derived from a Caucasian population and some of these RIs may not be appropriate. For instance, it is known that Africans in SSA have low absolute neutrophil counts compared to individuals of European ancestry (Karita et al., 2009). This lower neutrophil count is thought to be an adaptation that is related to the presence of a null genotype of the duffy antigen receptor complex (DARC) which confers resistance to infection by *Plasmodium vivax* (Haddy, Rana, & Castro, 1999; Thobakgale & Ndung'u, 2014). In Tanzania, RI upper limits for lactate dehydrogenase (LDH), creatine kinase (CK) and total bilirubin (TBil) were found to be double that of similar RIs from the United States of America (USA) (Saathoff et al., 2008). Despite the well-established evidence of such differences, there is reluctance by many laboratories in SSA to adopt RIs derived from their own populations possibly due to a paucity of high quality studies carried out using universally approved and accepted guidelines for RI derivation.

The International Federation of Clinical Chemistry (IFCC) committee on reference intervals and decision limits (C-RIDL) has been carrying out a global study aimed at harmonizing RIs. A key strategy for this study is the use of harmonized protocols for the pre-analytical, analytical and post-analytical processes (Ozarda, Ichihara, Barth, & Klee, 2013). This will enable comparison of reference values and RIs across different geographical jurisdictions and exploration of sources of variation such as age, sex and BMI. A critical component of this process is ensuring that reference individuals who are recruited are reflective of a healthy population enabling the extrapolation of results to the community. Unfortunately, given the subjectivity associated with

defining health (Petitclerc, 2004), it is not always a guarantee that people who define themselves as healthy are free of sub-clinical disease given that some medical conditions are preceded by a prolonged asymptomatic period. This has been a major confounder in the derivation of RIs.

It has been demonstrated that the association between BMI and health risks varies across races and ethnicities and that the prevalence of conditions such diabetes and hypertension vary across races even for the same BMI categories. Across races, increasing BMI is associated with increased health risks (Stommel & Schoenborn, 2010). The presence of conditions such as metabolic syndrome (MetS) and elevated BMI have been associated with variation in reference values necessitating their consideration when deriving RIs (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017). Therefore, it is important to know the BMI of reference individuals and the prevalence of conditions such as MetS in your reference population in order to better understand their association with reference values.

Early chronic kidney disease (CKD) is often times asymptomatic and could be present in individuals who are subjectively healthy including those who would volunteer as reference individuals in a RI study. In such individuals, screening for CKD is dependent on determining their glomerular filtration rate (KDIGO, 2013). It is recommended that routine reporting of estimated glomerular filtration rate (eGFR) with every request for serum creatinine be adopted to enhance awareness of CKD, early detection of CKD and the institution of early interventions to slow its progression. There is value in determining the prevalence of reduced eGFR in reference individuals as this would affect RIs derived for renal function tests. However, there is controversy on which equation should be used in determining eGFR in an asymptomatic population given that serum creatinine based equations derived from Caucasian populations have been shown to be inappropriate for other races (Hans Pottel, Hoste, Delanaye, Cavalier, & Martens, 2012). Furthermore, the issue of whether to correct for race when screening asymptomatic Africans for CKD is yet to be settled.

1.2. Justification

The IFCC recommends that population specific RIs should be determined in recognition of variations that may arise due to various reasons including ethnic, racial, diet and environmental differences. There is a need for RIs for haematology and biochemistry laboratory tests derived using well standardized guidelines for the Kenyan and East African population. Most of the studies carried out in the East Africa region were carried out by the Center for Disease control (CDC) and International Aids and Vaccine Initiative (IAVI) either as part of disease surveillance or prior to the roll out of HIV related clinical trials. Many of these studies were not meticulous in identifying reference populations and often times recruited reference individuals who would not suffice as being representative of a healthy population. Kenya is the only country in East Africa that participated in the global RI study and therefore the derived RIs will serve as a reference point for laboratories in the region in the short term but can also provide leadership in this field so that intra-regional differences in RIs can be determined. The determination and use of RIs that are specific to Kenya and by extension the wider East African region will ensure that diagnosis, prognosis, treatment, follow up and disease surveillance is done based on appropriate laboratory cut-offs.

It is also important to identify what factors cause variations in RIs in the Kenyan population and whether partitioning of RIs based on age or sex will make them more specific. The magnitude of the effect of these variables on the distribution of reference values in different populations is not necessarily the same. We have no data from Kenya or the East African region on how RIs for certain analytes vary with age or sex and the effect of BMI. Furthermore, the use of clinical decision limits (CDLs) to guide diagnosis of diseases like diabetes or to influence treatment of dyslipidemia requires consideration of local RIs for the respective tests.

Few studies have been carried out in Kenya looking at the prevalence of MetS or renal dysfunction adjudged by reduced estimated glomerular filtration rate (eGFR) in the general population and in particular healthy adults. The prevalence of these conditions which often times are sub-clinical may impact derivation of RIs and more importantly can inform public health interventions aimed at reducing their prevalence given the associated morbidity and mortality if not addressed.

In order to investigate the potential influence of environmental factors such as geographical location, diet, physical activity, alcohol intake and smoking on RIs, the derived Kenyan RIs were compared to South African RIs derived from a similar study carried out using the same harmonized protocol. This will help determine whether different regions within Africa can use common RIs.

1.3. Hypotheses

1. Kenyan RIs for common haematology and biochemical tests differ from those proposed by in-vitro diagnostic companies and published studies
2. Kenyan RIs vary with age, sex and BMI
3. Kenyan RIs differ from South African RIs

1.4. Research questions

1. Are CLSI/IFCC protocol derived RIs for common haematology and biochemical analytes in healthy adult Kenyan subjects different from those stated in manufacturer reagent inserts or published studies?
2. Are such derived RIs affected by age, sex or BMI?
3. Will such RIs derived from a Kenyan population be different from those derived from a South African population?
4. Would such healthy populations identified through a questionnaire and physical examination have metabolic syndrome or renal dysfunction?
5. Which equation for estimating eGFR is most appropriate to use in assessing renal dysfunction in healthy Kenyan adults?

1.5. Objectives

1. To establish age and sex specific haematology and chemistry RIs for an adult Kenyan population
2. To determine the association between age, sex, BMI and RIs in the Kenyan population

3. To determine whether there is a difference in RIs between Kenya and South Africa
4. To determine the prevalence of metabolic syndrome and reduced estimated glomerular filtration rate in healthy Kenyan adults
5. To evaluate various equations for eGFR determination in subjectively healthy Kenyan adults

1.6 References

CLSI. (2010). *EP28-A3C: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline* (Third ed.): Clinical Laboratory Standards Institute.

Dybkœr, R., & Solberg, H. E. (1987). Approved recommendation (1987) on the theory of reference values. Part 6. Presentation of observed values related to reference values. *Clinica Chimica Acta, 170*(2-3), S33-S41. doi:10.1016/0009-8981(87)90152-5

REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU, 2017/746 C.F.R. (2017).

Haddy, T. B., Rana, S. R., & Castro, O. (1999). Benign ethnic neutropenia: what is a normal absolute neutrophil count? *The Journal of Laboratory and Clinical Medicine, 133*(1), 15-22. doi:10.1053/lc.1999.v133.a94931

Ichihara, K., Itoh, Y., Lam, C. W. K., Poon, P. M. K., Kim, J.-H., Kyono, H., . . . Muliaty, D. (2008). Sources of variation of commonly measured serum analytes in 6 Asian cities and consideration of common reference intervals. *Clinical Chemistry, 54*(2), 356-365. doi:10.1373/clinchem.2007.091843

Ichihara, K., Ozarda, Y., Barth, J. H., Klee, G., Qiu, L., Erasmus, R., . . . Yadav, B. (2017). A global multicenter study on reference values: 1. Assessment of methods for derivation and comparison of reference intervals. *Clinica Chimica Acta, 467*, 70-82. doi:10.1016/J.CCA.2016.09.016

ISO. (2012). *ISO 15189:2012(en), Medical laboratories — Requirements for quality and competence*.

Jones, G., & Barker, A. (2008). Reference intervals. *The Clinical Biochemist Reviews, 29 Suppl 1*, S93-97.

Jones, G. R. D., Barker, A., Tate, J., Lim, C., & Robertson, K. (2004). The case for common reference intervals. *The Clinical Biochemist Reviews, 25*(2), 99-104.

Karita, E., Ketter, N., Price, M. A., Kayitenkore, K., Kaleebu, P., Nanvubya, A., . . . Kamali, A. (2009). CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. *PLOS ONE, 4*(2), e4401. doi:10.1371/journal.pone.0004401

KDIGO. (2013). KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney International Supplements, 3*(1), 4-4. doi:10.1038/kisup.2012.76

Miller, W. G., Tate, J. R., Barth, J. H., & Jones, G. R. D. (2014). Harmonization: the sample, the measurement, and the report. *Annals of Laboratory Medicine, 34*(3), 187-197. doi:10.3343/alm.2014.34.3.187

Ozarda, Y., Ichihara, K., Barth, J. H., & Klee, G. (2013). Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. *Clinical Chemistry and Laboratory Medicine (CCLM), 51*(5), 1027-1040. doi:10.1515/cclm-2013-0249

Petitclerc, C. (2004). Normality: the unreachable star? *Clinical Chemistry and Laboratory Medicine, 42*(7), 698-701. doi:10.1515/CCLM.2004.119

PetitClerc, C., & Solberg, H. E. (1987). Approved recommendation (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values. *Clinica Chimica Acta, 170*(2-3), S1-S11. doi:10.1016/0009-8981(87)90150-1

Pottel, H., Hoste, L., Delanaye, P., Cavalier, E., & Martens, F. (2012). Demystifying ethnic/sex differences in kidney function: Is the difference in (estimating) glomerular filtration rate or in serum creatinine concentration? *Clinica Chimica Acta, 413*(19), 1612-1617. doi:10.1016/j.cca.2012.04.034

Saathoff, E., Schneider, P., Kleinfeldt, V., Geis, S., Haule, D., Maboko, L., . . . Hoelscher, M. (2008). Laboratory reference values for healthy adults from southern Tanzania. *Tropical Medicine & International Health, 13*(5), 612-625. doi:10.1111/j.1365-3156.2008.02047.x

Solberg, H. E. (1984). The theory of reference values Part 5. Statistical treatment of collected reference values. Determination of reference limits. *Clinica Chimica Acta, 137*(1), 95-114. doi:10.1016/0009-8981(84)90319-X

Solberg, H. E. (1987). Approved recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. *Clinica Chimica Acta, 165*(1), 111-118. doi:10.1016/0009-8981(87)90224-5

Solberg, H. E., & PetitClerc, C. (1988). Approved recommendation (1988) on the theory of reference values. Part 3. Preparation of individuals and collection of specimens for the production of reference values. *Clinica Chimica Acta, 177*(3), S3-S11. doi:10.1016/0009-8981(88)90074-5

Solberg, H. E., & Stamm, D. (1991). IFCC recommendation: The theory of reference values. Part 4. Control of analytical variation in the production, transfer and application of reference values. *The Journal of Automatic Chemistry, 13*(5), 231-234. doi:10.1155/S146392469100038X

Stommel, M., & Schoenborn, C. A. (2010). Variations in BMI and Prevalence of Health Risks in Diverse Racial and Ethnic Populations. *Obesity, 18*(9), 1821-1826. doi:10.1038/oby.2009.472

Thobakgale, C. F., & Ndung'u, T. (2014). Neutrophil counts in persons of African origin. *Current Opinion in Hematology, 21*(1), 50-57. doi:10.1097/MOH.0000000000000007

CHAPTER 2: LITERATURE REVIEW

2.1 Variation of reference intervals in different populations

There have been several attempts to derive RIs in various continents. The Nordic Reference Interval Project (NORIP) was one of the landmark studies whose aim was to establish common adult Nordic RIs for 25 clinical biochemical tests frequently measured in serum and plasma. Recruitment of reference individuals above the age of 18 years, collection of samples and analyses was done in 102 medical laboratories in 5 Nordic countries using a common protocol. The results showed no significant differences for the included tests across the 5 countries and subsequently common RIs were adopted (Rustad et al., 2009). The upper reference limits for alanine transaminase (ALT), aspartate transaminase (AST), creatinine kinase (CK) and gamma-glutamyl transferase (GGT) were found to be higher compared to the ones recommended by the IFCC. The study also demonstrated increase in total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) with increase in age for both males and females. High density lipoprotein cholesterol (HDL-C) was found to be lower in males than in females which is in keeping with what has been published (Burtis, Ashwood, & Bruns, 2005). One interesting finding was the lack of a significant difference in the RI upper limit for potassium between serum and plasma samples. Potassium levels are usually higher in serum as a result of cellular leakage that occurs during clot formation. A reference clinical chemistry text book recommends a RI of 3.5-5.1 mmol/L for serum and 3.4-4.4 mmol/L for plasma (Burtis et al., 2005). For the NORIP study, RIs for serum and plasma were 3.6-4.6 mmol/L and 3.5-4.4 mmol/L respectively.

Several RI studies have been published from the Asian continent including one that derived RIs for selected serum proteins in 6 Asian countries from adults aged 20-60 years. Despite analysis being performed in a central laboratory to reduce chances of variation due to different measurement procedures and adjustment done for age, sex, and lifestyle variables, marked regional variations were still seen between the participating countries for some proteins like c-reactive protein (CRP), complement 3 (C3) and immunoglobulin G (IgG) (Ichihara et al., 2008). These differences were thought to be due to genetic or environmental differences. A more recent study reported on RIs for 31 standardized tests in both males and females aged 20-65 years from Japan and a few other countries in South East Asia. Despite all attempts to standardize recruitment so as to have a fairly homogeneous population, it was surprising that significant

regional variation was seen for HDL-C, IgG, C3, complement 4 (C4), and CRP. However, when data was analyzed for Japan only, there were no significant age and gender variations hence common RIs were calculated without age and sex stratification (Ichihara, Ceriotti, Tam, et al., 2013). The hypothesis suggested for the increase in inflammatory markers seen in these 2 Asian studies is increased exposure to infectious agents as you move closer to the equator.

In Turkey, a RI study enrolled 3066 healthy individuals in the age group 20-65 years from 7 regions in the country. This study found gender related differences in 10 analytes; uric acid (UA), urea nitrogen (UN), total bilirubin (TBIL), creatinine, triglycerides (TG), HDL-C, ALT, AST, CK, and GGT), and age-related differences in 7 analytes (albumin (ALB), UN, TG, Low Density Lipoprotein cholesterol (LDL-C), total cholesterol, glucose and alkaline phosphatase. The study showed no major differences between the selected regions within the country and therefore proposed common RIs for the selected tests. A secondary exclusion of individuals with a BMI >28 kg/m2 resulted in narrower RIs for UA, GLU, TG, HDL-C, LDL-C, ALT and GGT. This shows that BMI is an important covariate for specific RIs in particular those associated with metabolic syndrome (MetS). Of note, the upper limit for RIs for glucose, TC, HDL-C, LDL-C and TGs were higher than the well-recognized clinical decision limits (CDLs). For example, the upper limit for TC was determined to be 6.45 mmol/L which is significantly higher than the CDL of 5 mmol/L. The authors hypothesized that this could be due to the presence of sub-clinical disease such as MetS or pre-diabetes in the individuals enrolled for the study (Ozarda et al., 2014).

Regional variations in RIs even within the same country have been described especially for haematological parameters. Ozarda et al carried out a multicentre study in Turkey to establish RIs for haematological parameters and found regional differences for red blood cells (RBC), haemoglobin (HB), hematocrit (HCT), unsaturated iron binding capacity (UIBC), and total iron binding capacity (TIBC). Not surprisingly, there was an association between altitude and HB, HCT and ferritin in males, and RBC, HB, HCT, and TIBC in females with higher values found in areas with high altitude (Ozarda et al., 2017).

In Africa, there are several published studies on RIs from different countries (Karita et al., 2009; Miri-Dashe et al., 2014; Segolodi et al., 2014). Many of the studies have been driven by the need for robust population specific RIs required for clinical trials where patient follow up and laboratory monitoring of adverse events is essential. Using RIs derived from reference populations that are not similar to the local population where the clinical trial is being carried out may result in inappropriate exclusion or inclusion of potential trial volunteers and makes laboratory assessment of adverse events challenging. Karita et al carried out a cross sectional, observational study of healthy adults aged 18-60 years in multiple African research centers in Kenya, Uganda, Zambia and Rwanda from December 2004 to October 2006. Compared to RIs from the United States of America (USA), the study found lower HCT and HB levels which were more pronounced in women, lower total white blood cell (WBC) and neutrophil counts, and lower amylase levels (Karita et al., 2009). Genetic deletion of the Duffy antigen receptor for chemokines (DARC-null genotype) is thought to contribute to the neutropenia seen in Africans and African-Americans. DARC is thought to be a receptor for *Plasmodium vivax* malaria and the null genotype may have been positively selected among Africans (Thobakgale & Ndung'u, 2014). Both sexes showed elevated eosinophil counts, IgG, total and direct bilirubin, lactate dehydrogenase (LDH) and CK. Most volunteers with elevated LDH had normal liver function tests ruling out underlying hepatic disease as a possible cause. There was significant variability in some analytes between the different study sites. For example, the lower limit for HB from the coastal site in Kilifi, Kenya was less than 8 g/dL which would normally be considered anaemic. The lower limit of the consensus RI for the 5 countries was 9.5 g/dL. It was hypothesized that the low haemoglobin seen in women was most likely due to iron deficiency anaemia. This study further went on to evaluate how many of the healthy volunteers would have been classified as having a laboratory adverse event (AE) according to the 2004 grading criteria provided by Division of AIDS, National Institute of Allergy and Infectious Diseases, of the U.S. National Institutes of Health (DAIDS). Based on the US cut-offs used as the comparison RI in this study (Kratz, Ferraro, Sluss, & Lewandrowski, 2004), a total of 744 out of 2105 (35.3%) healthy volunteers would have been classified as having at least one laboratory-based AE at the time of recruitment. Three volunteers would have been classified as having a grade 4 (‘‘life threatening’’) AE (Karita et al., 2009). This would result in unnecessary exclusion of individuals from the vaccine trials due to misclassification.

The question that arises when examining RIs derived from people being screened as part of a HIV clinical trial is whether such volunteers are truly representative of the health status of the reference population and whether any RIs derived are generalizable. Participants who volunteer in such clinical trials have different motivations which can result in a selection bias hence compromising the external validity of the study results. Individual gain is a major motivator especially access to free medical care as reported by Townsend et al who interviewed 39 individuals who had previously participated in clinical trials in Canada (Townsend & Cox, 2013). There is reason to believe that individuals who volunteer to participate in vaccine trials may be more representative of the population who would benefit from the trial intervention rather than the general healthy population.

2.2 Statistical methods and reference intervals

The main source of post analytical variation in RIs is the adoption of different statistical methods when analyzing reference values. Computation of RIs can be done using parametric or non-parametric methods. Parametric methods assume that the distribution of data can be predicted by a standard statistical distribution. For non-parametric methods no such assumption is made. CLSI recommends the use of non-parametric methods without the exclusion of outliers as a simple way of deriving RIs (CLSI, 2010). It is recommended that confidence intervals should be determined around the upper and lower limits of the RI which is usually done using a bootstrapping method to give an estimate of error around each limit. Generally, the parametric method gives narrower confidence intervals for the lower and upper limits of the RI (Ichihara & Boyd, 2010). Parametric computations can be done on transformed or untransformed data. Data transformation is done to normalize data especially when the distribution of the reference values is skewed or has kurtosis. Several power transformation methods can be used but the one most commonly used in RI calculations is a modification of the original Box-Cox transformation (Box & Cox, 1964). Testing for normality can be done using graphical methods like box plots and histograms, numerical methods like kurtosis and skewness indices or formal normality tests. Graphical methods are good but they do not give conclusive evidence of normality. Razali et al evaluated the performance of four standard normality tests namely Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors and Anderson-Darling. Shapiro-Wilk was shown to have the highest power

to determine normality followed by Andrerson-Darling (Razali & Wah, 2011). However, the Anderson-Darling method is preferred for large data sets and is optimized to look for normality at both ends of the data distribution which is important when determining RIs. A study by Pavlov et al used 3 different methods to calculate RIs from the same set of data for 33 analytes and demonstrated that the larger your data set the lower the likelihood that it will be normally distributed. In his study, none of the data sets with more than 240 values achieved normality. This basically implies that the number of reference individuals recruited for a RI study will impact on the approach used to calculate RIs.

Partitioning of RIs is commonly done in order to make them more specific if statistically significant differences exist between different groups. There are several methods that can be used to determine whether there is a significant difference in RIs between sub-groups. For univariate analysis, the *t*-test or analysis of variance (ANOVA) can be used depending on whether the data is normally distributed and the number of groups being compared. Ichihara et al argues that being observational in nature, RI studies potentially have many confounders that may affect the distribution of reference values and as such multiple regression analysis (MRA) models may be more appropriate when assessing the contribution of different variables on the distribution of reference values (Ichihara, 2014). After identifying major sources of variation, it is worth determining whether there is a need to stratify the reference values depending on the magnitude of the contribution of each source of variation. Different methods can be used to determine this magnitude and include Harris-Boyd, Lahti, Fraser and Ichihara methods. After partitioning and normalizing data, parametric methods require that outlier reference values are removed. This can be done using various methods such as Tukey's or the Dixon's method which consider outliers in analytes independently as opposed to looking for outliers in groups of related analytes. The latent abnormal value exclusion (LAVE) method developed by Ichihara considers multiple related analytes when determining whether to exclude a reference individual's values from analysis. The rationale for this approach is that one may have a normal value for one analyte like glucose but out of range values for a different but related analyte such as TGs or HDL-C as seen in MetS. In a RI study, it is thought that this approach would help weed out individuals who may have subclinical disease (Ichihara, 2014). This approach has been used in several studies and has been shown to be quite efficient in reducing the impact of MetS, anaemia and inflammation on RIs for

analytes that are associated with these conditions (Borai et al., 2016; Evgina et al., 2020; Omuse et al., 2018; Ozarda et al., 2017). The CLSI guideline recommends that outliers should not be deleted as there isn't a generally applicable method of outlier detection (CLSI, 2010).

Selection of appropriate statistical methods when determining RIs is important as it contributes to the variability seen even after standardizing pre–analytic and analytic processes. In order to compare RIs across different regions, age and sex stratifications, the same statistical analysis method needs to be used as different statistical analysis on the same dataset can result in very different RIs (Ichihara, 2014).

2.3 Clinical decision limits and reference intervals

Certain biochemical tests have known CDLs that are used in diagnosis, risk stratification or follow up of patients. Fasting plasma glucose (FPG), TGs, TC, HDL-C and LDL-C are common analytes for which CDLs have been defined by expert panels and committees to guide practice (Alberti et al., 2009; American Diabetes Association, 2014; NCEP, 2001).

The third report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (ATP III) has published guidelines for cholesterol treatment targets. The optimal or desirable levels for TC, LDL-C and HDL-C levels are < 5.17 mmol/L, < 2.59 mmol/L and > 1.03 mmol/L respectively (NCEP, 2001). These CDLs have replaced RIs and have generally been adopted by laboratories in the USA to guide interpretation of lipid test results based on individual patient risk. The2013 American College of Cardiology (ACC)/American Heart Association (AHA) guidelines on the treatment of blood cholesterol in adults recommends that individuals with primary, severe elevations of LDL-C (> 4.91 mmol/L) should receive treatment even as early as 21 years of age. This is because of increased risk of atherosclerotic cardiovascular disease (Stone et al., 2014).

A recent RI study carried out in Turkey derived a RI for LDL-C that had upper limits that were significantly higher than the CDLs. For males and females aged 20-29 years, the RI was 1.47-3.92 mmol/L. For females above 50 years and above the RI was 1.78-4.91 mmol/L (Ozarda et al., 2014). The upper limit of 4.91 mmol/L is equivalent to the level that would prompt

immediate treatment in a young adult with a primary cause for elevation. In the NORIP study, LDL-C RIs for adults 18-29 years was 1.2-4.3 mmol/L, 1.4-4.7 mmol/L for those aged 30-49 years and 2.0-5.3 mmoI/L for those ≥ 50 years. In the wake of well accepted CDLs, it is unlikely that these RIs would be adopted despite the possibility that the predictive values of a common cut off could differ significantly across different populations thereby affecting the diagnostic accuracy of the CDLs. Population specific RIs can however serve as a baseline to inform local cholesterol treatment guidelines.

The American Diabetes Association (ADA) defines a FPG level of 5.6-6.9 to diagnose pre-diabetes and a value ≥ 7.0 mmol/L or a 2 hour plasma glucose value (2hPG) ≥ 11.1 mmol/L post a 75g glucose load to define diabetes (American Diabetes Association, 2014). Some studies have however shown that these cut offs are inappropriate in some populations. For example, in an Australian cohort, the levels that best correlated with increased risk of diabetic retinopathy were FPG ≥ 7.1 mmol/L, 2hPG ≥ 13.1 mmol/L and HbA1c 6.1% (Tapp et al., 2006). The appropriateness of the 2hPG of 11.1 mmol/L in this population is therefore questionable. Similarly, in a Chinese cohort, significant increase in retinopathy occurred among individuals with FPG ≥ 7.2 mmol/l, 2hPG ≥ 10.5 mmol/L and HbA1c ≥ 6.4% (Xin et al., 2012).

The ADA recommends testing adults to assess risk for future diabetes in asymptomatic people who are overweight or obese and who have one or more additional risk factors for diabetes. It also recommends testing of all patients, particularly those who are overweight or obese and this should begin at 45 years of age (American Diabetes Association, 2010). The idea of testing for pre-diabetes which includes impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) is due to its association with increased risk of developing diabetes. The FPG CDL for pre-diabetes is 5.6-6.9 mmol/L. Some of the studies done as part of the global RI study have derived FPG upper limits that are higher than the widely adopted CDLs. For example, in Turkey, the RI for FPG was 3.96-5.88 mmol/L suggesting a good number of individuals have higher values compared to the ADA criteria (Ozarda et al., 2014). The adoption of this higher upper limit to define normality would reduce the number of people classified as pre-diabetic in Turkey.

The Japanese Society of Gout and Nucleic Acid Metabolism defines hyperuricaemia as a UA level above 416 µmol/L (7 mg/dL). It has published guidelines for the management of hyperuricaemia and gout which recommends urate-lowering therapy for asymptomatic hyperuricaemia greater than 476 µmol/L (8 mg/dL) especially in individuals with complications such as hypertension, ischemic heart disease, diabetes mellitus, and metabolic syndrome, which are considered risk factors for renal disorders, including urinary calculus, and cardiovascular disorders (Yamanaka, Japanese Society of, & Nucleic Acid, 2011). The definition of hyperuricaemia varies across different populations and men usually have higher levels than women. The National Health and Nutrition Examination Survey (NHANES) uses cut offs of 416 µmol/L (7 mg/dl) and 339 µmol/L (5.7 mg/dl) to define hyperuricaemia in men and women respectively (Zhu, Pandya, & Choi, 2011). In Turkey, the RI for men was determined to be 226-458 µmol/L for men and 166-345 µmol/L for women (Ozarda et al., 2014). In the US, the RIs for men are 150-480 and 90-360 for women (Kratz et al., 2004). In Asia, the RIs were 223-471 µmol/L in men and 153-344 µmol/L in women (Ichihara, Ceriotti, Tam, et al., 2013). These differences in UA RIs suggest that the same cut-off to determine hyperuricaemia across different populations will not be ideal. There is paucity of data on UA RIs from Africa but it has been documented that UA levels are higher in urban populations compared to semi-urban and rural populations (Reimann, Schutte, Malan, Huisman, & Malan, 2008). A study in South Africa also showed lower levels of UA in black Africans compared to Caucasians with no obvious explanation for this difference (Palmer, Schutte, & Huisman, 2010; Palmer et al., 2007).

The differences seen in RIs for analytes whose interpretation is based on CDLs emphasizes the point that a single cut off for a CDL may perform differently across different populations and therefore their appropriateness should be evaluated before adaptation. RI studies can provide evidence for the need to validate CDLs especially if there are significant differences in RIs between a reference population and the population where the CDLs were derived.

2.4 Biochemical abnormalities in a reference population

Despite all attempts at ensuring that only healthy individuals are included in a RI study, this can never be achieved due to the presence of subclinical disease. Reference individuals are quite heterogeneous in their state of health and there isn't a perfect standard to define health or

normality (Petitclerc, 2004). A study in Turkey revealed that BMI levels are clearly associated with test results for UA, GLU, TG, HDL-C, LDL-C, ALT and GGT either in males and/ or females. It was found that RIs derived for these analytes were wider compared with RIs derived after excluding reference values from individuals with a BMI $\geq$ 28 kg/m^2. Despite this secondary exclusion, it was found that upper reference limits for TC, LDL-C, TG, ALT, GGT and GLU were higher than published CDLs. The authors concluded that this was due to the presence of latent diseases such as MetS in the reference population (Ozarda et al., 2014). The observed prevalence of the MetS in the National Health and Nutrition Examination Survey (NHANES) was 5% among the subjects of normal weight, 22% among the overweight, and 60% among the obese (Park et al., 2003). This raises the question whether BMI should be included as part of the exclusion criteria in a RI study.

In order to minimize the recruitment of individuals with possible latent diseases, IFCC have provided fairly strict inclusion and exclusion criteria to be used in the global RI study (Ozarda et al., 2013). Some RI studies have gone even further and carried out secondary exclusion based on BMI to further reduce the likelihood of including reference values from individuals with sub clinical disease like MetS. According to the USA "Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults" published in 1998, all overweight and obese adults are at an increased risk for developing diseases such as hypertension, elevated cholesterol, type 2 diabetes and coronary heart disease (NHLBI Obesity Education Initiative Expert Panel, 1998). Stommel et al used data from the National Health Interview Survey (NHIS) to determine the prevalence of hypertension, diabetes, CHD and asthma in 4 racial and ethnic groups, and the influence of BMI on the prevalence of these diseases. It was clearly demonstrated that increasing BMI levels are associated with higher levels of chronic disease burden in the four major racial and ethnic groups represented namely Hispanics, non-Hispanic whites, non-Hispanic blacks and East Asians. This association persisted even after adjusting for possible confounders such as smoking, physical activity and alcohol consumption. A notable observation was that even small 1 unit kg/m^2 increments in BMI values were associated with increased prevalence of the four chronic diseases (Stommel & Schoenborn, 2010). Initial results of RI studies carried out in Japan, China, Turkey, Saudi Arabia and USA as part of the global study being conducted by the IFCC have shown that there is an increase in the

levels of TG, ALT and GGT as BMI increases while HDL decreases. This association was also seen for glucose, UA, TC, and LDL-C. In order to compare RIs across the different regions and populations, there may be need to correct the RIs for BMI (Ichihara, 2014). The pattern of reduced HDL-C, increased TG and glucose with increasing BMI suggests the presence of MetS in some of the reference populations.

2.5 Assessment of renal dysfunction in asymptomatic individuals

The increasing prevalence of chronic kidney disease (CKD) and its associated morbidity and mortality has necessitated the development of interventions geared at reducing its prevalence as well as reducing the rate of progression from early to late stage disease (Levey, Atkins, et al., 2007). One of the interventions from a public health perspective is the routine reporting of estimated glomerular filtration rate (eGFR) for every serum creatinine request. The purpose of this is to create awareness of CKD and more importantly, identify early cases of CKD which are invariably asymptomatic. A key component of determining RIs for a general population is ensuring that recruited individuals are free from disease including sub-clinical conditions such as early CKD. Renal dysfunction will impact on RIs especially those used in assessing renal function. Determination of eGFR is a useful tool in identifying individuals who would pass off as healthy but have underlying CKD. Part of the challenge with eGFR determination is the lack of consensus on the issue of correction for race in black Africans. Studies carried out in the USA clearly demonstrated the need to correct for race when determining eGFR in black Americans due to greater muscle mass (Levey et al., 2009). Similar studies carried out in sub-Saharan Africa have suggested that correction for race is not necessary. A study carried out in South Africa compared eGFR with and without correction for race to measured GFR (mGFR) and concluded that the bias was less when the correction factor for race was excluded. Specifically, the correction factor of 1.212 as established for African Americans resulted in a median positive bias of 13.1 (95% CI 5.5 to 18.3) mL/min/1.73m^2 for eGFR determined using the 4-variable modified diet in renal disease formula (4-v MDRD). Without the ethnicity factor, the median bias was 1.9 (95% CI_0.8 to 4.5) mL/min/1.73m^2. However, one of the major criticisms of this study is that the population recruited comprised individuals with CKD or risk factors for CKD of whom 20% had HIV/AIDS (van Deventer, George, Paiker, Becker, & Katz, 2008). The findings from this study can therefore not be directly extrapolated to an asymptomatic or subjectively healthy black

African population. It has also been demonstrated that serum creatinine based eGFR equations derived from a Caucasian population may not be appropriate for other populations (Hans Pottel et al., 2012). This highlights the need for the evaluation of the various proposed equations in order to determine their appropriateness or derivation of population specific eGFR equations to enable screening for CKD in asymptomatic black Africans.

In order to evaluate which equation best estimates GFR in healthy individuals, the ideal methodology would be to compare eGFR to GFR measured using exogenous filtration markers such as inulin. Measurement of GFR is technically challenging, expensive and not practical for routine clinical practice. There are also ethical issues around subjecting healthy individuals with no known risk factors for CKD to such an invasive test. Alternative ways of evaluating equations for eGFR determination and their appropriateness for routine use in screening for CKD in asymptomatic individuals need to be developed and evaluated.

2.6 References

Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., . . . Smith, S. C. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International. *Circulation, 120*(16), 1640-1645. doi:10.1161/CIRCULATIONAHA.109.192644

American Diabetes Association. (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 33 Suppl 1*(Supplement_1), S62-69. doi:10.2337/dc10-S062

American Diabetes Association. (2014). Classification and Diagnosis of Diabetes. *Diabetes Care, 38*(Supplement_1), S8-S16. doi:10.2337/dc15-S005

Borai, A., Ichihara, K., Al Masaud, A., Tamimi, W., Bahijri, S., Armbuster, D., . . . Abdelaal, M. (2016). Establishment of reference intervals of clinical chemistry analytes for the adult population in Saudi Arabia: A study conducted as a part of the IFCC global study on reference values. *Clinical Chemistry and Laboratory Medicine, 54*(5), 843-855. doi:10.1515/cclm-2015-0490

Box, G. E. P., & Cox, R. D. (1964). An Analysis of Transformations. *Journal of the Royal Statistical Society. Series B (Methodological), 26*(2), 211-252.

Burtis, C., Ashwood, E., & Bruns, D. (2005). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*.

CLSI. (2010). *EP28-A3C: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline* (Third ed.): Clinical Laboratory Standards Institute.

Evgina, S., Ichihara, K., Ruzhanskaya, A., Skibo, I., Vybornova, N., Vasiliev, A., . . . Emanuel, V. (2020). Establishing reference intervals for major biochemical analytes for the Russian population: a research conducted as a part of the IFCC global study on reference values. *Clin Biochem, 81*, 47-58. doi:10.1016/j.clinbiochem.2020.04.001

Ichihara, K. (2014). Statistical considerations for harmonization of the global multicenter study on reference values. *Clinica Chimica Acta, 432*, 108-118. doi:10.1016/j.cca.2014.01.025

Ichihara, K., & Boyd, J. C. (2010). An appraisal of statistical procedures used in derivation of reference intervals. *Clinical Chemistry and Laboratory Medicine, 48*(11), 1537-1551. doi:10.1515/CCLM.2010.319

Ichihara, K., Ceriotti, F., Tam, T. H., Sueyoshi, S., Poon, P. M. K., Thong, M. L., . . . Panteghini, M. (2013). The Asian project for collaborative derivation of reference intervals: (1) strategy and major results of standardized analytes. *Clinical Chemistry and Laboratory Medicine, 51*(7), 1429-1442. doi:10.1515/cclm-2012-0421

Ichihara, K., Itoh, Y., Lam, C. W. K., Poon, P. M. K., Kim, J.-H., Kyono, H., . . . Muliaty, D. (2008). Sources of variation of commonly measured serum analytes in 6 Asian cities and consideration of common reference intervals. *Clinical Chemistry, 54*(2), 356-365. doi:10.1373/clinchem.2007.091843

Karita, E., Ketter, N., Price, M. A., Kayitenkore, K., Kaleebu, P., Nanvubya, A., . . . Kamali, A. (2009). CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. *PLOS ONE, 4*(2), e4401. doi:10.1371/journal.pone.0004401

Kratz, A., Ferraro, M., Sluss, P. M., & Lewandrowski, K. B. (2004). Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Laboratory

reference values. *The New England Journal of Medicine, 351*(15), 1548-1563. doi:10.1056/NEJMcpc049016

Levey, A. S., Atkins, R., Coresh, J., Cohen, E. P., Collins, A. J., Eckardt, K. U., . . . Eknoyan, G. (2007). Chronic kidney disease as a global public health problem: approaches and initiatives - a position statement from Kidney Disease Improving Global Outcomes. *Kidney Int, 72*(3), 247-259. doi:10.1038/sj.ki.5002343

Levey, A. S., Stevens, L. A., Schmid, C. H., Zhang, Y. L., Castro, A. F., Feldman, H. I., . . . Coresh, J. (2009). A new equation to estimate glomerular filtration rate. *Annals of Internal Medicine, 150*(9), 604-612. doi:10.7326/0003-4819-150-9-200905050-00006

Miri-Dashe, T., Osawe, S., Tokdung, M., Daniel, N., Choji, R. P., Mamman, I., . . . Morkve, O. (2014). Comprehensive Reference Ranges for Hematology and Clinical Chemistry Laboratory Parameters Derived from Normal Nigerian Adults. *PLOS ONE, 9*(5), e93919-e93919. doi:10.1371/journal.pone.0093919

NCEP. (2001). Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA, 285*(19), 2486-2497. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11368702

NHLBI Obesity Education Initiative Expert Panel. (1998). Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK2003/

Omuse, G., Maina, D., Mwangi, J., Wambua, C., Radia, K., Kanyua, A., . . . Erasmus, R. (2018). Complete blood count reference intervals from a healthy adult urban population in Kenya. *PLOS ONE*, 1-19.

Ozarda, Y., Ichihara, K., Aslan, D., Aybek, H., Ari, Z., Taneli, F., . . . Degirmen, E. (2014). A multicenter nationwide reference intervals study for common biochemical analytes in Turkey using Abbott analyzers. *Clinical Chemistry and Laboratory Medicine (CCLM), 52*(12). doi:10.1515/cclm-2014-0228

Ozarda, Y., Ichihara, K., Bakan, E., Polat, H., Ozturk, N., Baygutalp, N. K., . . . Eker, P. (2017). A nationwide multicentre study in Turkey for establishing reference intervals of haematological parameters with novel use of a panel of whole blood. *Biochemia Medica, 27*(2), 350-377. doi:10.11613/BM.2017.038

Ozarda, Y., Ichihara, K., Barth, J. H., & Klee, G. (2013). Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. *Clinical Chemistry and Laboratory Medicine (CCLM), 51*(5), 1027-1040. doi:10.1515/cclm-2013-0249

Palmer, I. M., Schutte, A. E., & Huisman, H. W. (2010). Uric acid and the cardiovascular profile of African and Caucasian men. *J Hum Hypertens, 24*(10), 639-645. doi:10.1038/jhh.2010.1

Palmer, I. M., Schutte, A. E., Huisman, H. W., Van Rooyen, J. M., Schutte, R., Malan, L., & Malan, N. T. (2007). A comparison of uric acid levels in Black African vs Caucasian women from South Africa: the POWIRS study. *Ethn Dis, 17*(4), 676-681. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18072378

Park, Y.-W., Zhu, S., Palaniappan, L., Heshka, S., Carnethon, M. R., & Heymsfield, S. B. (2003). The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. *Archives of Internal Medicine, 163*(4), 427-436. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3146257&tool=pmcentrez&rendertype=abstract

Petitclerc, C. (2004). Normality: the unreachable star? *Clinical Chemistry and Laboratory Medicine, 42*(7), 698-701. doi:10.1515/CCLM.2004.119

Pottel, H., Hoste, L., Delanaye, P., Cavalier, E., & Martens, F. (2012). Demystifying ethnic/sex differences in kidney function: Is the difference in (estimating) glomerular filtration rate or in serum creatinine concentration? *Clinica Chimica Acta, 413*(19), 1612-1617. doi:10.1016/j.cca.2012.04.034

Razali, N. M., & Wah, Y. B. (2011). Power comparisons of Shapiro-Wilk, Kolmogorov-Smirnov, Lilliefors, and Anderson-Darling test. *Journal of Statistical Modeling and Analytics, 2*(1), 21-33.

Reimann, M., Schutte, A. E., Malan, L., Huisman, H. W., & Malan, N. T. (2008). Hyperuricaemia is an independent factor for the metabolic syndrome in a sub-Saharan African population: a factor analysis. *Atherosclerosis, 197*(2), 638-645. doi:10.1016/j.atherosclerosis.2007.09.011

Rustad, P., Felding, P., Franzson, L., Kairisto, V., Lahti, A., Mårtensson, A., . . . Uldall, A. (2009). The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. *Scandinavian Journal of Clinical and Laboratory Investigation*. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/00365510410006324

Segolodi, T. M., Henderson, F. L., Rose, C. E., Turner, K. T., Zeh, C., Fonjungo, P. N., . . . Paxton, L. A. (2014). Normal laboratory reference intervals among healthy adults screened for a HIV pre-exposure prophylaxis clinical trial in Botswana. *PLOS ONE, 9*(4), e93034-e93034. doi:10.1371/journal.pone.0093034

Stommel, M., & Schoenborn, C. A. (2010). Variations in BMI and Prevalence of Health Risks in Diverse Racial and Ethnic Populations. *Obesity, 18*(9), 1821-1826. doi:10.1038/oby.2009.472

Stone, N. J., Robinson, J. G., Lichtenstein, A. H., Bairey Merz, C. N., Blum, C. B., Eckel, R. H., . . . Wilson, P. W. F. (2014). 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation, 129*(25 Suppl 2), S1-S45. doi:10.1161/01.cir.0000437738.63853.7a

Tapp, R. J., Zimmet, P. Z., Harper, C. A., de Courten, M. P., McCarty, D. J., Balkau, B., . . . Shaw, J. E. (2006). Diagnostic thresholds for diabetes: the association of retinopathy and albuminuria with glycaemia. *Diabetes Research and Clinical Practice, 73*(3), 315-321. doi:10.1016/j.diabres.2006.02.008

Thobakgale, C. F., & Ndung'u, T. (2014). Neutrophil counts in persons of African origin. *Current Opinion in Hematology, 21*(1), 50-57. doi:10.1097/MOH.0000000000000007

Townsend, A., & Cox, S. M. (2013). Accessing health services through the back door: a qualitative interview study investigating reasons why people participate in health research in Canada. *BMC Medical Ethics, 14*(1), 40-40. doi:10.1186/1472-6939-14-40

van Deventer, H. E., George, J. A., Paiker, J. E., Becker, P. J., & Katz, I. J. (2008). Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft-Gault equations. *Clinical Chemistry, 54*(7), 1197-1202. doi:10.1373/clinchem.2007.099085

Xin, Z., Yuan, M.-X., Li, H.-X., Hua, L., Feng, J.-P., Shi, J., . . . Yang, J.-K. (2012). Evaluation for fasting and 2-hour glucose and HbA1c for diagnosing diabetes based on prevalence of retinopathy in a Chinese population. *PLOS ONE, 7*(7), e40610-e40610. doi:10.1371/journal.pone.0040610

Yamanaka, H., Japanese Society of, G., & Nucleic Acid, M. (2011). Japanese guideline for the management of hyperuricemia and gout: second edition. *Nucleosides Nucleotides Nucleic Acids, 30*(12), 1018-1029. doi:10.1080/15257770.2011.596496

Zhu, Y., Pandya, B. J., & Choi, H. K. (2011). Prevalence of gout and hyperuricemia in the US general population: the National Health and Nutrition Examination Survey 2007-2008. *Arthritis Rheum, 63*(10), 3136-3141. doi:10.1002/art.30520

CHAPTER 3: METABOLIC SYNDROME AND ITS PREDICTORS IN AN URBAN POPULATION IN KENYA: A CROSS SECTIONAL STUDY

Omuse, G., Maina, D., Hoffman, M., Mwangi, J., Wambua, C., Kagotho, E., . . . Erasmus, R. (2017). Metabolic syndrome and its predictors in an urban population in Kenya: A cross sectional study. *BMC Endocrine Disorders, 17*(1).

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3.1 Abstract

Background

The metabolic syndrome (MetS) is a clustering of interrelated risk factors which doubles the risk of cardio-vascular disease (CVD) in 5-10 years and increases the risk of type 2 diabetes 5 fold. The identification of modifiable CVD risk factors and predictors of MetS in an otherwise healthy population is necessary in order to identify individuals who may benefit from early interventions. We sought to determine the prevalence of MetS as defined by the harmonized criteria and its predictors in subjectively healthy black Africans from various urban centres in Kenya.

Method

We used data collected from healthy black Africans in Kenya as part of a global study on establishing reference intervals for common laboratory tests. We determined the prevalence of MetS and its components using the 2009 harmonized criterion. Receiver operator characteristic (ROC) curve analysis was used to determine the area under the curves (AUC) for various predictors of MetS. Youden index was used to determine optimum cut-off for quantitative measurements such as waist circumference (WC).

Results

A total of 528 participants were included in the analysis. The prevalence of MetS was 25.6% (95% CI: 22.0%-29.5%). Among the surrogate markers of visceral adiposity, lipid accumulation product was the best predictor of MetS with an AUC of 0.880 while triglyceride was the best predictor among the lipid parameters with an AUC of 0.816 for all participants. The optimal WC cut-off for diagnosing MetS was 94cm and 86 cm respectively for males and females.

Conclusions

The prevalence of MetS was high for a healthy population highlighting the fact that one can be physically healthy but have metabolic derangements indicative of an increased CVD risk. This is likely to result in an increase in the cases of CVD and type 2 diabetes in Kenya if interventions are not put in place to reverse this trend. We have also demonstrated the inappropriateness of the

WC cut-off of 80 cm for black African women in Kenya when defining MetS and recommend adoption of 86 cm.

Key words

Metabolic syndrome, waist circumference, visceral adiposity, cardiovascular risk, Kenya, Africa

3.2 Background

The non-communicable disease (NCD) burden is expected to increase globally by 17% and by 27% in the African region in the next 10 years. NCDs are projected to overtake communicable diseases as the major cause of morbidity in sub Saharan Africa by the year 2030 (Mathers & Loncar, 2006). Smoking, hypertension, abdominal obesity, diabetes mellitus and elevated Apolipoprotein B/A-1 ratio have been shown to account for up to 90% of the risk for a first myocardial infarction in Africa according to the INTERHEART study (Steyn et al., 2005). Unfortunately, the prevalence of these risk factors continues to increase as urbanization takes root in the African continent (Vorster, 2002).

The metabolic syndrome (MetS) is a clustering of interrelated risk factors which doubles the risk of cardio-vascular disease (CVD) in 5-10 years and increases the risk of type 2 diabetes 5 fold (Alberti et al., 2009). The identification of modifiable CVD risk factors and predictors of MetS in an otherwise healthy population is necessary in order to identify individuals who may benefit from early interventions. It has been shown that subjectively healthy individuals may have biochemical abnormalities in keeping with the presence of MetS (Gami et al., 2007). A study carried out in the US showed that 23.5% of normal-weight adults were metabolically abnormal and conversely, 51.3% of overweight adults and 31.7% of obese adults were metabolically healthy (Wildman et al., 2008). Metabolic derangements may be an early indicator of increased CVD risk even in normal weight or subjectively healthy individuals. Initial results of reference interval (RI) studies carried out in Japan, China, Turkey, Saudi Arabia and USA have shown that the levels of fasting plasma glucose (FPG), triglycerides (TGs), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) increase while high density lipoprotein cholesterol (HDL-C) decreases as body mass index (BMI) increases. This pattern of reduced HDL-C,

increased TG and FPG suggests the presence of MetS in some of the reference individuals recruited despite adherence to a strict inclusion criteria designed to exclude unhealthy individuals. This has raised the question whether RIs should be further stratified according to BMI for those analytes where it is a major source of variation (Ichihara, 2014).

Whereas increasing levels of waist circumference (WC) and BMI have been associated with increased CVD risk, there is a continuous search for robust predictors of MetS. Both BMI and WC do not distinguish between visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). VAT plays a significant role in the pathogenesis of CVD due to its association with insulin resistance and increased levels of VAT has been associated with increased cardio-metabolic risk and coronary artery calcification regardless of BMI (Reilly, 2003; R. V. Shah et al., 2014). One of the emerging surrogate markers of visceral adiposity and predictor of MetS yet to be extensively studied in a black African population is the lipid accumulation product (LAP), a parameter whose calculation is based on WC and serum TG levels (Kahn, 2005). LAP has been shown to have a strong association with the presence of MetS in healthy adults, an accurate predictor of MetS in those aged 50 years and above, as well as an association with diabetes in studies carried out in Europe and Asia (Chiang & Koo, 2012; Taverna, Martinez-Larrad, Frechtel, & Serrano-Rios, 2011; Tellechea et al., 2009; Wakabayashi & Daimon, 2014).

Visceral adiposity index (VAI) is another emerging parameter that indirectly expresses visceral fat function and has been found to be independently associated with coronary heart disease, myocardial infarction, transient ischemic attack and ischemic stroke. The calculation of VAI is based on WC, BMI, triglycerides, and HDL cholesterol and hence includes both physical and metabolic parameters (Amato et al., 2010). In China, VAI was shown to be positively associated with type 2 diabetes and was a better predictor of diabetes than BMI, WC and waist to height ratio (Chen et al., 2014). VAI was derived from an Italian cohort largely comprising of Caucasians and hence needs validation in a black African population.

Hypertriglyceridaemia and low HDL-C are the only lipid parameters included in the harmonized MetS criterion (Alberti et al., 2009). There is controversy as to the specific role elevated TGs play in atherogenesis. Whereas increased TGs have been associated with increased risk of CVD,

it is thought that they are a surrogate marker for cholesterol remnants which play a direct role in atheromatous plaque formation (Jorgensen et al., 2013). Varbo *et al* demonstrated in a Danish population that non-fasting cholesterol remnants are a risk factor for ischaemic heart disease independent of HDL-C levels with an increase of 1 mmol/L associated with a 2.8 fold increase in risk (Varbo et al., 2013). The utility of cholesterol remnants in determination of CVD risk or MetS in healthy black Africans is unknown.

The prevalence of MetS in any population is influenced by many factors including the specific criteria adopted to define it. For example, in a rural population in Ghana, the overall prevalence of MetS defined by the International Diabetic Federation (IDF) and the National Cholesterol Education Programme Adult Treatment Panel III (ATP III) criteria was 35.9% and 15.0% respectively (Gyakobo, Amoah, Martey-Marbell, & Snow, 2012). In 2009, a harmonized criterion for diagnosing MetS was developed whose components included abdominal obesity, dyslipidemia, hyperglycemia, and hypertension (Alberti et al., 2009). Using the harmonized definition, the prevalence of MetS in an urban population in Kenya was reported as 34.6% with the prevalence being significantly higher in women at 40.2%. However, this study was only limited to one constituency in the capital city of Nairobi which limits the generalizability of the results despite the use of random sampling when identifying participants (Kaduka et al., 2012). Furthermore, this study didn't explore the utility of LAP and VAI as predictors of MetS in a black African population.

We sought to determine the prevalence of MetS as defined by the harmonized criteria in subjectively healthy black Africans from various urban centres in Kenya. We also determined the ability of various lipid parameters, LAP, VAI, WC and BMI to predict the presence of MetS.

3.3 Methods

We used data from 533 healthy black Africans participating in a global RI study in Kenya. This study is part of an initiative by the Committee of Reference Intervals and Decision Limits (C-RIDL) under the auspices of the International Federation of Clinical Chemistry (IFCC). Kenya is one of 3 participating countries in Africa, the other ones being Nigeria and South Africa.

Majority of recruited participants in Kenya were urban dwellers from the capital city Nairobi and its environs, Kisii County in the western part of Kenya and Nakuru town based in the Great Rift valley. Recruitment was mainly done by seeking adult volunteers aged 18-65 years of age through use of posters in public areas including churches, universities, colleges, hospitals and companies. Social media and word of mouth were also used. This was done between January and October 2015 and written informed consent was sought from each participant after giving a written and verbal explanation of the study.

Inclusion criteria

Inclusion was limited to healthy adults 18-65 years of age and was stratified into 4 age groups: 18-29, 30-39, 40-49 and 50-65 years with a similar distribution of males and females in each age strata.

Exclusion criteria

Exclusion criteria included participants with a BMI greater than 35 kg/m^2, consumption of ethanol greater than or equal to 70g per day [equivalent to 5 alcoholic drinks], smoking more than 20 tobacco cigarettes per day, taking regular medication for a chronic disease (diabetes mellitus, hypertension, hyperlipidemia, allergic disorders, depression), recent (less than 15 days) recovery from acute illness, injury or surgery requiring hospitalization, known carrier state of hepatitis B, hepatitis C or human Immunodeficiency virus, pregnant or within one year after childbirth. Individuals with any chronic disease were excluded except for the age group 50-65 years where those with well controlled hypertension were recruited. Towards the end of the study, a few individuals with a BMI greater than 35 kg/m^2 but less than 40 kg/m^2 were recruited due to difficulties in recruiting healthy participants in the older age groups. The exclusion criteria were defined in the questionnaire filled by the participants and those who were excluded didn't have blood samples taken.

Measurements

All participants had measurements of blood pressure (BP), WC and body mass index (BMI) done. A single measurement of BP was performed in a sitting position after at least 15 minutes of rest using a calibrated OMRON M3 automated BP monitor (Omron Healthcare, Kyoto, Japan)

that uses an upper arm cuff. A repeat BP measurement was done if the initial reading was consistent with hypertension. If the initial and second reading were in agreement then the first reading was adopted. If there was a discrepancy in terms of BP status then a third reading was done as a tie breaker. WC was measured using a tape measure over light clothing at the level of the umbilical cord to ensure a consistent reference point while BMI was automatically calculated after measuring the participants' height and weight using a Seca 703 weighing scale digital column with height meter scale (Seca, Hamburg, Germany). On average, measuring WC over light clothing increased the measurement by approximately 1cm which was subsequently deducted. Height was measured after removal of shoes to the nearest 0.5cm, WC to the nearest 1cm and weight to the nearest 0.1kg after removal of shoes and bulky clothing.

Sample handling

All participant samples were collected after obtaining informed consent and filling of a questionnaire. All participants had an overnight fast as per the study protocol. Samples were collected and centrifuged within 4 hours after collection and stored at -80^{o}c until shipment on dry ice to the reference laboratory in South Africa. All the sample analysis for the biochemistries and immunoassays were performed in the Pathcare reference laboratory in Cape Town, South Africa which is an International Organization for Standardization (ISO) 15189 accredited laboratory. Thawing was only done once before sample analysis. As part of the RI study, all participating laboratories used a common panel of sera with assigned values to ensure accuracy of reported results and alignment of values if any biases were identified. All the listed tests were carried out on a Beckman Coulter AU5800. The analytical methods for the various tests are shown in **Table 3.1**.

Table 3.1: List of tests, methodologies and coefficient of variation

Test	Method	Between run CV
UA	Enzymatic colour	0.1%
TC	Enzymatic colour calibrated to CDC Reference Method (Abell-Kendall)	0.3%
LDL-C	Enzymatic colour calibrated to US CDC	0.4%
TGs	Enzymatic colour	0.4%
HDL-C	Enzymatic colour calibrated to US CDC	0.3%
Glucose	Enzymatic UV test (hexokinase method)	0.1%
hsCRP	Immuno-turbidimetric	0.8%
ALP	Kinetic colour IFCC	0.1%
ALT	Kinetic UV IFCC	0.8%
AST	Kinetic UV IFCC	0.4%
GGT	Kinetic colour IFCC	0.4%

Key: ALP-Alkaline phosphatase, ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, CDC-Centre of Disease Control, CV-Coefficient of variation, GGT-Gama glutamyl transferase, HDL-C-High density lipoprotein cholesterol, hsCRP-Highly sensitive C-reactive protein, IFCC-International Federation of Clinical Chemistry, LDL-C-Low density lipoprotein cholesterol, TGs-Triglycerides, UA-Uric acid, US-United States

MetS diagnosis

The 2009 harmonized definition was used to diagnose MetS which requires the presence of any 3 of the following: increased WC (men: ≥ 94 cm, women: ≥ 80 cm), low HDL-C (men: <40 mg/dl (1 mmol/l), women: <50 mg/dl (1.3 mmol/l)), hypertriglyceredimia ≥150 mg/dl (1.7 mmol/l), elevated BP (systolic BP ≥ 130 mmHg and/or diastolic ≥ 85 mmHg or drug treatment for hypertension) and elevated blood sugar (FPG ≥ 100 mg/dl (5.6 mmol/l) or diabetes mellitus (Alberti et al., 2009).

CVD risk calculation

The 10 year CVD risk was calculated using the Framingham risk calculation (Blom, 2011). Family history of CVD was not included as this information wasn't captured in the questionnaire used for the study.

LAP calculation

LAP was calculated as (WC [cm] − 65) × (TG [mmol/L]) for males, and (WC [cm] − 58) × (TG [mmol/L]) for females (Kahn, 2005).

VAI calculation

VAI was calculated as (WC[cm] / (39.68 + (1.88 x BMI))) x (TG / 1.03) x (1.31 / HDL-C [mmol/L]) for males and (WC[cm] / (36.58 + (1.89 x BMI))) x (TG / 0.81) x (1.52 / HDL-C [mmol/L]) for females (Amato et al., 2010).

Non HDL-C

Non HDL-C was calculated as fasting total cholesterol minus HDL-C.

Cholesterol remnants

Cholesterol remnants were calculated as fasting total cholesterol minus HDL-C minus LDL-C.

Statistical analysis

The prevalence of MetS was presented as a percentage with 95% confidence intervals (CI). Descriptive statistics for continuous variables were presented as medians with interquartile ranges (IQRs). The Mann Whitney *U* test was used to compare the distribution pattern of continuous variables between males and females and those with and without. Where the distribution patterns were similar, medians were compared using the independent samples median test. Mean ranks were compared where the distribution patterns not similar. Binary logistic regression was used to determine the association between cardio-metabolic risk factors and MetS presence. Adjusted odds ratios (ORs) were subsequently determined in a model that excluded the MetS components. For hs-CRP, uric acid and GGT, the median value was used to as a cut-off to classify individuals as having increased or normal levels. Binary logistic regression was also used to determine the strength of association between MetS and CVD risk. Chi-square was used to compare cardio-metabolic factors between male and female participants as well as between individuals with a BMI < 25 kg/m^2 and those with BMIs ≥ 25 kg/m^2. A p-value < 0.05 was considered statistically significant. Receiver operator characteristic (ROC) curves were created and area under the curve (AUC) determined in order to compare lipid parameters, LAP, VAI, WC and BMI as predictors of MetS. An AUC ≥ 0.90 was considered excellent; 0.80 - 0.90, good; 0.70 - 0.80, fair; and 0.70 - 0.50, poor test performance. The Youden's index was calculated and used to determine the cut-offs that gave the best combination

of sensitivity and specificity. Statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp).

3.4 Results

A total of 533 participants met the inclusion criteria and subsequently had samples collected and analysed. However, 5 participants didn't have FPG results due to sample insufficiency and were excluded from this analysis leaving 528 participants, 255 (48.3%) males and 273 (51.7%) females. BMI was higher in female participants while WC and BP were higher in males. The difference in total cholesterol (TC) between male and female participants was not statistically significant but TGs were significantly higher in males and HDL-C lower in females. FPG levels and liver enzymes were higher in male participants as shown in **Table 3.2**. Only 2 (0.7%) women were smokers compared to 13 (5.1%) men, a difference that was statistically significant ($p = 0.003$).

Table 3.2: Descriptive characteristics of participants

	Male (n=254)		Female (n=274)		Total (n=528)		Male vs Female
	Median (IQR)	Min-Max	Median (IQR)	Min-Max	Median (IQR)	Min-Max	*p*-value
Age (years)	38 (19)	20-65	39 (21)	18-64	39 (20)	18-65	0.986*
BMI (kg/m^2)	24.87 (5.66)	16.29-34.94	26.10 (6.25)	17.10-38.05	25.46 (5.96)	16.29-38.05	0.000†
LAP	29.52 (40.95)	0.00-388.87	23.97 (29.69)	3.30-205.54	26.94 (35.96)	0.00-388.87	0.055*
VAI	1.51 (1.45)	0.26-21.66	1.35 (1.13)	0.42-20.42	1.43 (1.22)	0.26-21.66	0.163*
WC (cm)	90 (15)	65-124	86 (16)	64-115	89 (17)	64-124	0.000†
SBP (mmHg)	127 (18)	94-167	118 (20)	77-194	123.5 (20)	77-194	0.000†
DBP (mmHg)	81 (12)	56-101	79 (14)	57-112	80 (14)	56-112	0.003†
FPG (mmol/L)	4.9 (0.8)	3.0-15.6	4.8 (0.7)	3.3-19.5	4.9 (0.8)	3.0-19.5	0.022†
TC (mmol/L)	4.7 (1.2)	2.3-8.2	4.6 (1.2)	2.6-7.7	4.6 (1.1)	2.3-8.2	0.732*
HDL-C (mmol/L)	1.1 (0.3)	0.5-2.0	1.2 (0.3)	0.3-2.4	1.1 (0.3)	0.3-2.4	0.000†
cLDL-C (mmol/L)	2.9 (1.1)	1.0-5.8	2.9 (1.0)	1.1-5.4	2.9 (1.0)	1.0-5.8	0.650*
mLDL-C (mmol/L)	2.9 (1.1)	0.9-5.8	2.8 (1.0)	1.3-5.2	2.8 (1.1)	0.9-5.8	0.141*
TG (mmol/L)	1.2 (0.90)	0.32-10.51	0.9 (0.61)	0.33-4.78	1.05 (0.75)	0.32-10.51	0.000†
UA (mmol/L)	0.35 (0.10)	0.18-0.64	0.27 (0.08)	0.13-0.47	0.31 (0.11)	0.13-0.64	0.000†
ALP (U/L)	86 (35)	34-179	84 (38)	31-191	85 (37)	31-191	0.497*
ALT (U/L)	21 (14)	Aug-96	14 (9)	6-123	18 (11)	6-123	0.000†
AST (U/L	25 (8)	14-73	21 (6)	11-102	23 (7)	11-102	0.000†
GGT (U/L)	31 (24)	9-701	21 (12)	7-211	25 (19)	7-701	0.000†
hsCRP (mg/L)	0.99 (2.05)	0.20-36.25	1.65 (3.19)	0.20-60.75	1.31 (2.63)	0.20-60.80	0.000†

Key: ALP-Alkaline phosphatase, ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, BMI-Body Mass Index, BP-Blood pressure, CVD-Cardiovascular disease, DBP-Diastolic blood pressure, F-Female, FPG-Fasting plasma glucose, GGT-Gama glutamyl transferase, HDL-C-High density lipoprotein cholesterol, hsCRP-Highly sensitive C-reactive protein, LAP-Lipid accumulation product, cLDL-C-calculated low density lipoprotein cholesterol, mLDL-C-measured low density lipoprotein cholesterol, M-Male, SBP-Systolic blood pressure, TGs-Triglycerides, VAI-Visceral adiposity index, UA-Uric acid, WC-Waist circumference, *comparison of medians, †comparison of mean ranks

The overall prevalence of MetS was 25.6% (95% CI: 22.0%-29.5%). The prevalence increased with increase in age and BMI. The most prevalent component of the MetS was increased WC which was present in 294 (55.7%) participants while the least prevalent was elevated FPG which was found in 83 (15.7%) participants. Having GGT or UA values above the median more than doubled the odds of having MetS as shown in **Table 3.3**. Only 2 out of 135 (1.5%) participants with MetS were smokers compared to 13 out of 393 (3.3%) with no MetS (p = 0.375). Those who consumed alcohol were 26.7% (36/135) and 34.6% (136/393) for those with and without MetS respectively (p = 0.110). A comparison of ALT (U=17197.0, p=0.000), AST (U=22805.0, p=0.015) and ALP (U=21887.5, p=0.002) mean ranks in participants with and without Mets found that they were all higher in those with MetS.

Table 3.3: Association between cardio-metabolic risk factors and metabolic syndrome

Variable	Categories	Number	Metabolic syndrome status		Univariate		**Multivariate	
			Present-No. (%)	Absent-No. (%)	p-value	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)
Gender	Male*	255	64 (25.1%)	191 (74.9%)				
	Female	273	71 (26.0%)	202 (74.0%)	0.811	1.05 (0.71-1.55)	0.523	1.20 (0.68-2.13)
Age (years)	18-29*	134	6 (4.5%)	128 (95.5%)				
	30-39	136	23 (16.9%)	113 (83.1%)	0.002	4.34 (1.71-11.04)	0.051	2.64 (1.00-6.99)
	40-49	131	45 (34.4%)	86 (65.6%)	0.000	11.16 (4.56-27.31)	0.000	6.06 (2.35-15.60)
	50-65	127	61 (48.0%)	66 (52.0%)	0.000	19.72 (8.10-48.00)	0.000	11.74 (4.61-29.88)
BMI (kg/m^2)	< 25.00*	242	19 (7.9%)	223 (92.1%)				
	25.00-29.99	198	76 (38.4%)	122 (61.6%)	0.000	7.31 (4.22-12.66)	0.000	5.08 (2.80-9.22)
	30.00-39.99	88	40 (45.5%)	48 (54.5%)	0.000	9.78 (5.22-18.34)	0.000	5.23 (2.57-10.66)
WC (cm)	< 94 (M) OR < 80 (F)*	234	4 (1.7%)	230 (98.3%)				
	≥ 94 (M) OR ≥ 80 (F)	294	131 (44.6%)	163 (55.4%)	0.000	46.21 (16.75-127.50)		
TGs (mmol/L)	< 1.7*	437	63 (14.4%)	374 (85.6%)				
	≥ 1.7	91	72 (79.1%)	19 (20.9%)	0.000	22.50 (12.70-39.85)		
HDL-C (mmol/L)	≥ 1(M) OR ≥ 1.3 (F)*	304	38 (12.5%)	266 (87.5%)				
	< 1(M) OR < 1.3 (F)	224	97 (43.3%)	127 (56.7%)	0.000	5.35 (3.48-8.22)		
BP (mmHg)	SBP < 130 OR DBP < 85*	287	21 (7.3%)	266 (92.7%)				
	SBP ≥ 130 OR DBP ≥ 85	241	114 (47.3%)	127 (52.7%)	0.000	11.37 (6.82-18.96)		
FPG (mmol/L)	< 5.6*	445	65 (14.6%)	380 (85.4%)				
	≥ 5.6	83	70 (84.3%)	13 (15.7%)	0.000	31.48 (16.47-60.16)		
hsCRP (mg/L)	< 1.3*	262	44 (16.8%)	218 (83.2%)				
	≥ 1.3	266	91 (34.2%)	175 (65.8%)	0.000	2.58 (1.71-3.89)	0.620	1.14 (0.686-1.880)
GGT (IU/L)	< 25*	263	45 (17.1%)	218 (82.9%)				
	≥ 25	265	90 (34.0%)	175 (66.0%)	0.000	2.491 (1.654-3.752)	0.003	2.173 (1.295-3.648)
UA (mmol/L)	< 0.31*	252	47 (18.7%)	205 (18.3%)				
	≥ 0.31	276	88 (31.9%)	188 (68.1%)	0.000	2.042 (1.361-3.063)	0.000	2.042 (1.361-3.063)

Key: BMI-Body Mass Index, BP-Blood pressure, CI-Confidence interval, CVD-Cardiovascular disease, DBP-Diastolic BP, F-Female, FPG-Fasting plasma glucose, GGT-Gamma-glutamyl transferase, HDL-C-High density lipoprotein cholesterol, hsCRP-highly sensitive C-reactive protein, M-Male, OR-Odds Ratio, SBP-Systolic BP, TGs-Triglycerides, UA-uric acid, WC-Waist circumference, * Indicates reference category, **Components of MetS excluded in the multivariate analysis

The prevalence of MetS was higher in females though the difference was not statistically significant. There was a statistically significant difference in the prevalence of each component of the MetS when comparing male and female participants except for FPG. The commonest component of the MetS that was present in male participants was elevated BP (53.3%) while in females it was increased WC (71.8%). No female participant had a 10 year CVD risk > 10% based on their Framingham risk score compared to 10.6% of males as shown in **Table 3.4**.

Table 3.4: Gender comparison of metabolic syndrome component prevalence

Variable	Categories	Male (n=255) No. (%)	Female (n=273) No. (%)	*p*-value
BMI (kg/m^2)	< 25.00	132 (51.8%)	110 (40.3%)	0.001
	25.00-29.99	95 (37.3%)	103 (37.7%)	
	30.00-39.99	28 (11.0%)	60 (22.0%)	
WC (cm)	< 94 (M) OR < 80 (F)	157 (61.6%)	77 (28.2%)	0.000
	≥ 94 (M) OR ≥ 80 (F)	98 (38.4%)	196 (71.8%)	
TGs (mmol/L)	< 1.7	194 (76.1%)	243 (89.0%)	0.000
	≥ 1.7	61 (23.9%)	30 (11.0%)	
HDL-C (mmol/L)	≥ 1(M) OR ≥ 1.3 (F)	179 (70.2%)	125 (45.8%)	0.000
	< 1(M) OR < 1.3 (F)	76 (29.8%)	148 (54.2%)	
BP (mmHg)	SBP < 130 OR DBP < 85	119 (46.7%)	168 (61.5%)	0.001
	SBP ≥ 130 OR DBP ≥ 85	136 (53.3%)	105 (38.5%)	
FPG (mmol/L)	< 5.6	212 (83.1%)	233 (85.3%)	0.486
	≥ 5.6	43 (16.9%)	40 (14.7%)	
10 year CVD risk (%)	≤ 10%	228 (89.4%)	273 (100.0%)	0.000
	> 10%	27 (10.6%)	0 (0.0%)	

Key: BMI-Body Mass Index, BP-Blood pressure, CVD-Cardiovascular disease, DBP-Diastolic BP, FPG-Fasting plasma glucose, F-Female, HDL-C-High density lipoprotein cholesterol, M-Male, SBP-Systolic BP, TGs-Triglycerides, WC-Waist circumference

The prevalence of each component of the MetS was significantly higher in those with a BMI greater ≥ 25 kg/m^2 compared to those with a BMI < 25 kg/m^2 as shown in **Table 3.5**.

Table 3.5: Comparison of metabolic syndrome component prevalence in different BMI categories

Variable	Categories	BMI < 25.00 (n=242) No. (%)	BMI ≥ 25.00 (n=286) No. (%)	*p*-value
WC (cm)	< 94 (M) OR < 80 (F)	188 (77.7%)	46 (16.1%)	0.000
	≥ 94 (M) OR ≥ 80 (F)	54 (22.3%)	240 (83.9%)	
TGs (mmol/L)	< 1.7	223 (92.1%)	214 (74.8%)	0.000
	≥ 1.7	19 (7.9%)	72 (25.2%)	
HDL-C (mmol/L)	≥ 1(M) OR ≥ 1.3 (F)	157 (64.9%)	147 (51.4%)	0.002
	< 1(M) OR < 1.3 (F)	85 (35.1%)	139 (48.6%)	
BP (mmHg)	SBP < 130 OR DBP < 85	152 (62.8%)	135 (47.2%)	0.000
	SBP ≥ 130 OR DBP ≥ 85	90 (37.2%)	151 (52.8%)	
FPG (mmol/L)	< 5.6	230 (95.0%)	215 (75.2%)	0.000
	≥ 5.6	12 (5.0%)	71 (24.8%)	

Key: BMI-Body Mass Index, BP-Blood pressure, DBP-Diastolic BP, FPG-Fasting plasma glucose, F-Female, HDL-C-High density lipoprotein cholesterol, M-Male, SBP-Systolic BP, TGs-Triglycerides, WC-Waist circumference

ROC analysis of LAP, VAI, WC and BMI ability to predict the presence of MetS showed LAP as having the highest AUC at 0.880. When male and female data were analysed separately, the AUC for the 4 parameters were higher in males with WC, LAP and VAI having excellent AUCs. With the exception of VAI, all other optimum cut-offs were lower in females as shown in **Table 3.6**. Using a WC cut-off of 86 cm reduced the prevalence of MetS in female participants from 26.0% to 23.4%.

Table 3.6: Summary of ROC curve and Youden Index analysis for BMI, WC, VAI and LAP as predictors of MetS

Gender	Variable	AUC (95% CI)	YI	Cut-off	Sensitivity	Specificity
Males & Females	BMI	0.766 (0.725-0.808)	0.457	26.23	0.770	0.687
	WC	0.825 (0.789-0.862)	0.518	89.5	0.867	0.651
	VAI	0.858 (0.818-0.897)	0.599	2.057	0.726	0.873
	LAP	0.880 (0.846-0.914)	0.591	37.205	0.815	0.776
Males	BMI	0.841 (0.789-0.892)	0.587	25.71	0.859	0.728
	WC	0.913 (0.879-0.947)	0.759	93.5	0.957	0.806
	VAI	0.905 (0.859-0.951)	0.697	1.727	0.922	0.775
	LAP	0.949 (0.923-0.976)	0.749	42.895	0.843	0.749
Females	BMI	0.702 (0.638-0.765)	0.363	24.99	0.873	0.49
	WC	0.766 (0.710-0.821)	0.453	85.5	0.859	0.594
	VAI	0.814 (0.752-0.876)	0.529	2.065	0.648	0.881
	LAP	0.822 (0.764-0.879)	0.502	30.56	0.775	0.728

Key: AUC-Area under the curve, BMI-Body mass index, CI-Confidence intervals, LAP-Lipid accumulation product, WC-Waist circumference, VAI-Visceral adiposity index, YI-Youden Index

The lipid parameter that best predicted MetS was TG with AUCs of 0.816, 0.887 and 0.770 for all participants, male and female participants respectively. Measured LDL-C (mLDL-C) was consistently better than calculated LDL-C (cLDL-C) at predicting MetS. Non-HDL-C and cholesterol remnants performed better in males compared to females as shown in **Table 3.7**.

Table 3.7: ROC curve analysis for lipid parameters as predictors of MetS

Gender	Variable	AUC (95% CI)	YI	Cut-off	Sensitivity	Specificity
Males & Females	TG	0.816 (0.769-0.862)	0.517	1.27	0.726	0.791
	HDL-C	0.721 (0.669-0.774)	0.318	1.1	0.756	0.562
	mLDL-C	0.660 (0.605-0.714)	0.268	3.0	0.652	0.616
	cLDL-C	0.578 (0.520-0.636)	0.146	3.2	0.459	0.687
	TC	0.648 (0.592-0.704)	0.258	5.0	0.548	0.710
	non HDL-C	0.706 (0.654-0.759)	0.342	3.4	0.785	0.557
	CholRem	0.751 (0.701-0.801)	0.421	0.7	0.622	0.799
Males	TG	0.887 (0.841-0.933)	0.645	1.68	0.734	0.911
	HDL-C	0.722 (0.642-0.803)	0.395	0.9	0.594	0.801
	mLDL-C	0.719 (0.650-0.789)	0.346	2.8	0.828	0.518
	cLDL-C	0.613 (0.532-0.693)	0.190	2.8	0.703	0.487
	TC	0.731 (0.660-0.802)	0.358	4.9	0.688	0.670
	non HDL-C	0.778 (0.714-0.843)	0.456	3.5	0.891	0.565
	CholRem	0.814 (0.748-0.879)	0.551	0.7	0.813	0.738
Females	TG	0.770 (0.697-0.843)	0.471	1.25	0.620	0.851
	HDL-C	0.752 (0.684-0.820)	0.412	1.1	0.704	0.708
	mLDL-C	0.605 (0.525-0.685)	0.214	3.1	0.521	0.693
	cLDL-C	0.547 (0.464-0.629)	0.123	3.5	0.296	0.827
	TC	0.572 (0.490-0.654)	0.173	5.0	0.465	0.708
	non HDL-C	0.645 (0.567-0.722)	0.264	3.4	0.690	0.574
	CholRem	0.712 (0.642-0.782)	0.307	0.7	0.451	0.856

Key: AUC-Area under the curve, CholRem-Cholesterol remnants, cLDL-C-calculated LDL cholesterol, CI-Confidence intervals, mLDL-C-measured LDL cholesterol, non HCL-C-non HDL cholesterol, TC-Total cholesterol, TG-Triglycerides, YI-Youden Index

3.5 Discussion

The prevalence of MetS of 25.6% found in this study is quite high given the strict inclusion and exclusion criteria used for the RI study. This sample was meant to be representative of a healthy black African population in Kenya and as such the percentage of individuals with MetS was

expected to be much lower. Several studies have reported the prevalence of MetS in Africa. These studies are quite heterogeneous by way of individuals included and MetS definitions used hence limiting direct comparisons. Ogbera reported a MetS prevalence of 86% using the 2009 harmonized definition in diabetic patients in Lagos, Nigeria (Ogbera, 2010). This high prevalence in a diabetic population is expected given the known association between MetS and increased risk of developing type 2 diabetes (Marott, Nordestgaard, Tybjærg-Hansen, & Benn, 2016). In Ethiopia, Tran *et al* reported a prevalence of 12.5% and 17.9% using the ATP III and IDF definitions respectively in a presumably healthy population comprising working class individuals in Addis Ababa (Tran et al., 2011). This prevalence is much lower than what we have found in our study. Of, note is that the prevalence of overweight and obese individuals was lower in the Ethiopian population which we hypothesize may be attributed to genetic and/or environmental differences. Kaduka *et al* reported a prevalence of 34.6% in an urban population in Nairobi, Kenya. This study was however limited to one constituency in Nairobi hence limiting the generalizability of the findings. The inclusion criteria was also not as strict as what we used in our study and this could partly explain the higher reported prevalence (Kaduka et al., 2012).

The most prevalent component of the MetS was increased WC which was present in 294 (55.7%) participants followed by elevated BP found in 241 (45.6%) of study participants. This is consistent with what has been found in other studies carried out in sub-Saharan Africa (SSA) (Kaduka et al., 2012; Tran et al., 2011). However, the most common MetS component in males was hypertension (53.3%) and increased WC (71.8%) in females. In South Africa, HDL-C < 1.3 mmol/L was found to be the most prevalent of the MetS components in black African women at 70.1% closely followed by increased WC at 69.3% compared to 54.2% and 71.8% for low HDL-C and increased WC respectively in our study (Crowther & Norris, 2012). WC is a measure of both VAT and SAT. Increased visceral adiposity contributes to the insulin resistance that is central to the pathogenesis of MetS and is associated with the production of adipocytokines which contribute to the chronic low grade inflammation seen in MetS. WC was a better predictor of MetS than BMI in our study but had a lower AUC than both LAP and VAI. Chen *et al* found that VAI was better than BMI and WC in predicting the presence of diabetes in a Chinese cohort (Chen et al., 2014). Gender specific ROC curve analysis in our study showed that WC was a better predictor of MetS in males than in females and was superior to VAI. A WC greater than

93.5 cm in male participants had a sensitivity of 95.7%, specificity of 80.6% and AUC of 0.913 which is excellent for a simple measurement that can be carried out in any clinical setting. It is interesting that the optimum WC cut-off of 93.5 cm is similar to the proposed European cut-off for abdominal obesity of 94 cm that is used in the MetS 2009 harmonized criterion for men. The women cut-off of 85.5 cm is much higher than the European cut-off of 80 cm highlighting the need to adopt population specific cut-offs (Alberti et al., 2009). The use of the 80 cm cut-off in black African women may be misclassifying many of them as having abdominal obesity. A study carried out in Johannesburg, South Africa derived an optimal cut-off to diagnose MetS in urban black African women of 91.5 cm. The prevalence of MetS in this population was 42.1% and the WC was measured at the smallest girth above the umbilicus (Crowther & Norris, 2012). This suggests that the optimal cut-off would have been higher if measured at the level of the umbilicus like we did in our study. Further longitudinal studies are required to validate the WC cut-offs we have proposed and assess their ability to predict CVD risk in a black African population.

Elevated GGT and UA levels independently more than doubled the odds of having MetS. Surprisingly, elevated hsCRP did not independently increase the odds of one having MetS. Elevated GGT is known to occur in non-alcoholic fatty liver disease (NAFLD) which represents the hepatic component of MetS and its increase may be a physiological response to the increased oxidation stress seen in MetS as GGT plays a role in the metabolism of glutathione which is an important anti-oxidant (Lee, Blomhoff, & Jacobs, 2004). Matsha *et al* demonstrated that increasing levels of GGT were associated with increasing levels of insulin resistance as well as an increase in the number of components of MetS present in a mixed ancestry population in South Africa. This was so even in GGT levels within the reference interval. However, additional tests of liver enzymes were not performed in this study to enable inferences on the pathophysiology behind the increase of GGT in MetS (Tandi Edith Matsha et al., 2014). In our study, elevated GGT was associated with an increase in ALT, AST and ALP suggesting that MetS may be associated with mild hepatitis and cholestasis. It is known that hepatocyte injury does occur in individuals with NAFLD, however, canalicular cholestasis is more common in alcoholic liver disease (Takahashi & Fukusato, 2014).

Despite the difference in prevalence of Mets in male and female participants not being statistically significant, the 10 year CVD risk was significantly higher in male participants with 10.6% having a > 10% risk compared to 0% among females. This suggests that the presence of MetS confers a different CVD risk depending on gender with the risk being lower in females. We explored the possibility of over diagnosis of MetS in female participants given the disparity in WC between the Caucasian cut-off and the higher values for females reported in studies carried out in Africa including our study. However, whereas using a WC cut-off of 86 cm reduced the MetS prevalence in females from 26.0% to 23.4%, the difference compared to males was still not statistically significant hence this doesn't explain the observed disparity in CVD risk. This is even more surprising when you consider that women had a statistically significant higher level of hsCRP than men. This finding further adds to the uncertainty surrounding the use of hsCRP in CVD risk prediction (Yousuf et al., 2013). The optimal cut-off for TGs that predicted the presence of MetS was 1.27 mmol/L for combined males and females, 1.68 mmol/L for males and 1.25 mmol/L for females. Given these findings, we do advocate the adoption of separate TG cut-offs for males and females for the diagnosis of MetS in a black African population. The higher TG levels and WC in men compared to women is in keeping with the known positive correlation between TG levels and visceral adiposity (Taniguchi et al., 2002). The MetS 2009 harmonized criterion uses a cut-off of 1.7 mmol/L for both males and females which could under estimate the prevalence of hypertriglyceridaemia in female black Africans and by extension MetS. Using gender specific TG cut-offs in addition to adjusting the WC cut-off to 86 cm increased the MetS prevalence in females to 29.7 %. The prevalence in males remained at 25.1% which is not surprising given the similarities in our male TG and WC cut-offs to those used in the harmonized criterion (Alberti et al., 2009).

Of the lipid parameters that are not included in the MetS definition, cholesterol remnants outperformed non-HDL-C, mLDL-C and cLDL-C in predicting the presence of MetS. Calculated non-fasting cholesterol remnant has been shown to be an independent risk factor for ischaemic heart disease and myocardial infarction in a Caucasian population (Jorgensen et al., 2013; Varbo et al., 2013). There is a scarcity of data on the utility of cholesterol remnants and non HDL-C in predicting the presence of MetS in black Africans. In our study, the lipid parameters were better

predictors in males than in females. Prospective cohort studies are needed to determine whether cholesterol remnants are an independent risk factor for CVD in black Africans.

This study has several limitations. It is a cross sectional study hence causal inferences cannot be made. The predictive ability of the factors analysed would best be studied in a longitudinal study and as such, our findings would need to be validated in a prospective cohort study. However, with a sample size of over 528, the study has sufficient power to draw conclusions on optimal cut-offs for MetS prediction. We also didn't collect data on family history of CVD and therefore our estimation of CVD risk based on Framingham calculations may have underestimated the 10 year risk. However, it is unlikely that this omission significantly affected the comparison between CVD risk in males and females. Our study was carried out in an urban population hence the findings cannot be directly extrapolated to a rural population who are known to have a lower prevalence of obesity and MetS.

Conclusion

We propose 94 cm and 86 cm as WC cut-offs for males and females respectively for black Africans in Kenya when diagnosing MetS. These however need to be validated prospectively in other countries in SSA. There is also a need to adopt gender specific MetS TG cut-offs to avoid over diagnosing hypertriglyceridaemia in black African women who have a lower TG level compared to the 1.7 mmol/L cut-off. The high prevalence of abdominal obesity, hypertension and MetS in our subjectively healthy population emphasizes the need for primary healthcare interventions to control the epidemic of CVD in Kenya. Urbanization in SSA will continue to accelerate the incidence of obesity, diabetes and hypertension all of which can be avoided or at least reduced by adopting lifestyle changes such as reduced intake of processed food that is high in calories and regular exercise to ensure a reasonable balance between what is consumed and what is expended. Unless urgent interventions are put in place to prevent this epidemic, the additional morbidity due to CVD will over burden the already stretched healthcare services in SSA leading to increased mortality.

Ethics approval and consent to participate

Ethical approval was obtained from the Aga Khan University, Nairobi Health Research Ethics Committee (2014/REC-46).

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to study participant privacy.

Competing interests

The author(s) declare that they have no competing interests.

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Author contributions

GO designed the study, collected data, performed statistical analysis and wrote the manuscript. DM designed and coordinated the study, collected data and critiqued the manuscript, JM and CW coordinated the study and critiqued the manuscript, EK collected data and critiqued the manuscript, MH, KI and RE designed the study, facilitated sample analysis and critiqued the manuscript, AA, PJ and ZP critiqued the manuscript.

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3.6 References

Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., . . . Smith, S. C. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International. *Circulation, 120*(16), 1640-1645. doi:10.1161/CIRCULATIONAHA.109.192644

Amato, M. C., Giordano, C., Galia, M., Criscimanna, A., Vitabile, S., Midiri, M., . . . AlkaMeSy Study, G. (2010). Visceral Adiposity Index: A reliable indicator of visceral fat function associated with cardiometabolic risk. *Diabetes Care, 33*(4), 920-922. doi:10.2337/dc09-1825

Blom, D. (2011). Cardiovascular risk assessment. © *Medpharm S Afr Fam Pract, 53*(2), 2010-2019.

Chen, C., Xu, Y., Guo, Z.-r., Yang, J., Wu, M., & Hu, X.-s. (2014). The application of visceral adiposity index in identifying type 2 diabetes risks based on a prospective cohort in China. *Lipids in Health and Disease, 13*, 108-108. doi:10.1186/1476-511X-13-108

Chiang, J.-K., & Koo, M. (2012). Lipid accumulation product: a simple and accurate index for predicting metabolic syndrome in Taiwanese people aged 50 and over. *BMC Cardiovascular Disorders, 12*(1), 78-78. doi:10.1186/1471-2261-12-78

Crowther, N. J., & Norris, S. A. (2012). The current waist circumference cut point used for the diagnosis of metabolic syndrome in sub-Saharan African women is not appropriate. *PLOS ONE, 7*(11), e48883-e48883. doi:10.1371/journal.pone.0048883

Gami, A. S., Witt, B. J., Howard, D. E., Erwin, P. J., Gami, L. A., Somers, V. K., & Montori, V. M. (2007). Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *Journal of the American College of Cardiology, 49*(4), 403-414. doi:10.1016/j.jacc.2006.09.032

Gyakobo, M., Amoah, A. G. B., Martey-Marbell, D.-A., & Snow, R. C. (2012). Prevalence of the metabolic syndrome in a rural population in Ghana. *BMC Endocrine Disorders, 12*(1), 25-25. doi:10.1186/1472-6823-12-25

Ichihara, K. (2014). Statistical considerations for harmonization of the global multicenter study on reference values. *Clinica Chimica Acta, 432*, 108-118. doi:10.1016/j.cca.2014.01.025

Jorgensen, A. B., Frikke-Schmidt, R., West, A. S., Grande, P., Nordestgaard, B. G., & Tybjaerg-Hansen, A. (2013). Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *European Heart Journal, 34*(24), 1826-1833. doi:10.1093/eurheartj/ehs431

Kaduka, L. U., Kombe, Y., Kenya, E., Kuria, E., Bore, J. K., Bukania, Z. N., & Mwangi, M. (2012). Prevalence of metabolic syndrome among an urban population in Kenya. *Diabetes Care, 35*(4), 887-893. doi:10.2337/dc11-0537

Kahn, H. S. (2005). The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison. *BMC Cardiovascular Disorders, 5*, 26-26. doi:10.1186/1471-2261-5-26

Lee, D., Blomhoff, R., & Jacobs, D. R. (2004). Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Radical Research, 38*(6), 535-539. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15346644

Marott, S. C. W., Nordestgaard, B. G., Tybjærg-Hansen, A., & Benn, M. (2016). Components of the Metabolic Syndrome and Risk of Type 2 Diabetes. *The Journal of Clinical Endocrinology & Metabolism, 101*(8), 3212-3221. doi:10.1210/jc.2015-3777

Mathers, C. D., & Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Medicine, 3*(11), e442-e442. doi:10.1371/journal.pmed.0030442

Matsha, T. E., Macharia, M., Yako, Y. Y., Erasmus, R. T., Hassan, M. S., & Kengne, A. P. (2014). Gamma-glutamyltransferase, insulin resistance and cardiometabolic risk profile in a middle-aged African population. *European Journal of Preventive Cardiology, 21*(12), 1541-1548. doi:10.1177/2047487313501967

Ogbera, A. O. (2010). Prevalence and gender distribution of the metabolic syndrome. *Diabetology & Metabolic syndrome, 2*, 1-1. doi:10.1186/1758-5996-2-1

Reilly, M. P. (2003). The Metabolic Syndrome: More Than the Sum of Its Parts? *Circulation, 108*(13), 1546-1551. doi:10.1161/01.CIR.0000088846.10655.E0

Shah, R. V., Murthy, V. L., Abbasi, S. A., Blankstein, R., Kwong, R. Y., Goldfine, A. B., . . . Allison, M. A. (2014). Visceral Adiposity and the Risk of Metabolic Syndrome Across Body Mass Index: The MESA Study. *JACC: Cardiovascular Imaging, 7*(12), 1221-1235. doi:10.1016/j.jcmg.2014.07.017

Steyn, K., Sliwa, K., Hawken, S., Commerford, P., Onen, C., Damasceno, A., . . . Africa, I. I. i. (2005). Risk factors associated with myocardial infarction in Africa: the INTERHEART Africa study. *Circulation, 112*(23), 3554-3561. doi:10.1161/CIRCULATIONAHA.105.563452

Takahashi, Y., & Fukusato, T. (2014). Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World Journal of Gastroenterology, 20*(42), 15539-15548. doi:10.3748/wjg.v20.i42.15539

Taniguchi, A., Nakai, Y., Sakai, M., Yoshii, S., Hamanaka, D., Hatae, Y., . . . Fukushima, M. (2002). Relationship of regional adiposity to insulin resistance and serum triglyceride levels in nonobese Japanese type 2 diabetic patients. *Metabolism: Clinical and Experimental, 51*(5), 544-548. doi:10.1053/META.2002.31984

Taverna, M. J., Martinez-Larrad, M. T., Frechtel, G. D., & Serrano-Rios, M. (2011). Lipid accumulation product: a powerful marker of metabolic syndrome in healthy population. *European Journal of Endocrinology, 164*(4), 559-567. doi:10.1530/EJE-10-1039

Tellechea, M. L., Aranguren, F., Martínez-Larrad, M. T., Serrano-Ríos, M., Taverna, M. J., & Frechtel, G. D. (2009). Ability of Lipid Accumulation Product to Identify Metabolic Syndrome in Healthy Men From Buenos Aires. *Diabetes Care, 32*(7), e85-e85.

Tran, A., Gelaye, B., Girma, B., Lemma, S., Berhane, Y., Bekele, T., . . . Williams, M. A. (2011). Prevalence of Metabolic Syndrome among Working Adults in Ethiopia. *International Journal of Hypertension, 2011*, 193719-193719. doi:10.4061/2011/193719

Varbo, A., Benn, M., Tybjærg-Hansen, A., Jørgensen, A. B., Frikke-Schmidt, R., & Nordestgaard, B. G. (2013). Remnant Cholesterol as a Causal Risk Factor for Ischemic Heart Disease. *Journal of the American College of Cardiology, 61*(4), 427-436. doi:10.1016/j.jacc.2012.08.1026

Vorster, H. H. (2002). The emergence of cardiovascular disease during urbanisation of Africans. *Public Health Nutrition, 5*(1A), 239-243. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12027290

Wakabayashi, I., & Daimon, T. (2014). A strong association between lipid accumulation product and diabetes mellitus in japanese women and men. *Journal of Atherosclerosis and Thrombosis, 21*(3), 282-288.

Wildman, R. P., Muntner, P., Reynolds, K., McGinn, A. P., Rajpathak, S., Wylie-Rosett, J., & Sowers, M. R. (2008). The Obese Without Cardiometabolic Risk Factor Clustering and the Normal Weight With Cardiometabolic Risk Factor Clustering. *Archives of Internal Medicine, 168*(15), 1617-1617. doi:10.1001/archinte.168.15.1617

Yousuf, O., Mohanty, B. D., Martin, S. S., Joshi, P. H., Blaha, M. J., Nasir, K., . . . Budoff, M. J. (2013). High-Sensitivity C-Reactive Protein and Cardiovascular Disease: A Resolute Belief or an Elusive Link? *Journal of the American College of Cardiology, 62*(5), 397-408. doi:10.1016/j.jacc.2013.05.016

CHAPTER 4: COMPARISON OF EQUATIONS FOR ESTIMATING GLOMERULAR FILTRATION RATE IN SCREENING FOR CHRONIC KIDNEY DISEASE IN ASYMPTOMATIC BLACK AFRICANS: A CROSS SECTIONAL STUDY

Omuse, G., Maina, D., Mwangi, J., Wambua, C., Kanyua, A., Kagotho, E., . . . Erasmus, R. (2017). Comparison of equations for estimating glomerular filtration rate in screening for chronic kidney disease in asymptomatic black Africans: a cross sectional study. *BMC Nephrology*, *18*(1), 369-369.

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4.1 Abstract

Background

Several equations have been developed to estimate glomerular filtration rate (eGFR). The common equations used were derived from populations predominantly comprised of Caucasians with chronic kidney disease (CKD). Some of the equations provide a correction factor for African-Americans due to their relatively increased muscle mass and this has been extrapolated to black Africans. Studies carried out in Africa in patients with CKD suggest that using this correction factor for the black African race may not be appropriate. However, these studies were not carried out in healthy individuals and as such the extrapolation of the findings to an asymptomatic black African population is questionable. We sought to compare the proportion of asymptomatic black Africans reported as having reduced eGFR using various eGFR equations. We further compared the association between known risk factors for CKD with eGFR determined using the different equations.

Methods

We used participant and laboratory data collected as part of a global reference interval study conducted by the Committee of Reference Intervals and Decision Limits (C-RIDL) under the International Federation of Clinical Chemistry (IFCC). Serum creatinine values were used to calculate eGFR using the Cockcroft-Gault (CG), re-expressed 4 variable modified diet in renal disease (4v-MDRD), full age spectrum (FAS) and chronic kidney disease epidemiology collaboration equations (CKD-EPI). CKD classification based on eGFR was determined for every participant.

Results

A total of 533 participants were included comprising 273 (51.2%) females. The 4v-MDRD equation without correction for race classified the least number of participants (61.7%) as having an eGFR equivalent to CKD stage G1 compared to 93.6% for CKD-EPI with correction for race. Only age had a statistically significant linear association with eGFR across all equations after performing multiple regression analysis. The multiple correlation coefficients for CKD risk factors were higher for CKD-EPI determined eGFRs.

Conclusions

This study found that eGFR determined using CKD-EPI equations better correlated with a prediction model that included risk factors for CKD and classified fewer asymptomatic black Africans as having a reduced eGFR compared to 4v-MDRD, FAS and CG corrected for body surface area.

Key words

MDRD, CKD-EPI, Cockcroft-Gault, FAS, eGFR

4.2 Background

Routine reporting of estimated glomerular filtration rate (eGFR) by medical laboratories for every serum creatinine request has been encouraged as a way of screening for chronic kidney disease (CKD) and has been shown to increase the absolute number of appropriate referrals to nephrologists (Noble et al., 2008). Patients with early CKD may be asymptomatic and therefore reporting eGFR may help in early detection and appropriate interventions to stop or reverse progression of the disease (Levey, Atkins, et al., 2007).

The use of eGFR equations instead of creatinine clearance (CrCl) is preferred due to the difficulties associated with accurately collecting a 24 hour urine sample. The most popular equations for estimating GFR are Cockcroft-Gault (CG), 4 variable modified diet in renal disease (4v-MDRD) and chronic kidney disease epidemiology collaboration (CKD-EPI). The CG equation was derived from an inpatient population comprising predominantly male patients with CKD (Cockcroft & Gault, 1976). The equation doesn't correct for race and one requires the patient's height and weight to correct for body surface area (BSA) in order to accurately classify CKD. The 4v-MDRD equation was derived from a population with kidney disease comprising a predominantly white population. Despite the low percentage of African-Americans in this study, they determined a correction for race (Levey et al., 1999). It has been assumed, given the ancestral linkage between African Americans and black Africans that this correction should apply to black Africans. However, a study carried out in South Africa that measured GFR in

black South Africans with established CKD or risk factors for developing CKD concluded that eGFR based on the 4v-MDRD equation without correction for race better correlated with measured GFR (mGFR) and had less bias (van Deventer et al., 2008). A similar study carried out in Ghana in a rural African population arrived at a similar conclusion, however, this study compared eGFR to creatinine clearance (Eastwood et al., 2010). Both CG and 4v-MDRD have been shown to underestimate normal and high mGFR values with a greater negative bias seen with CG (Ibrahim et al., 2005; Vervoort, Willems, & Wetzels, 2002). One of the drawbacks of the 4v-MDRD equation is that it doesn't accurately classify patients with early CKD, in particular those with values > 60ml/min/1.73m^2. This is not surprising given that it was derived from patients with CKD who had a mean eGFR of 39.8 ml/min/1.73m^2. The utility of this equation in screening for CKD in an asymptomatic patient with no known risk factors for CKD is questionable given that subjectively healthy individuals generally have an eGFR in the range where the 4v-MDRD equation is inaccurate. The CKD-EPI equation has been found to better correlate with mGFR especially in those with eGFR > 60 ml/min/1.73m^2 (Levey et al., 2009). In a study carried out in Kenya amongst HIV patients with a median mGFR of 115 ml/min/1.73m^2, both CKD-EPI and 4v-MDRD performed better without correction for race with CKD-EPI estimates being more precise (Wyatt et al., 2013). A new equation called the full age spectrum (FAS) equation has been developed by Pottel *et al* which factors in correction for race, age and gender by including population specific mean or median serum creatinine values in the determination of eGFR. This equation has the advantage of being applicable across different age groups from children as young as 2 years to the elderly above 65 years of age. The equation is yet to be validated in a black African population and was derived from population datasets largely comprising Caucasians (Hans Pottel et al., 2016). Most of the studies done in Africa that have concluded that correcting for race is not necessary for a black African population were carried out in populations comprising patients with CKD or at a high risk of developing CKD. The extrapolation of this finding to an asymptomatic population should not be done without evidence that this observation is consistent.

None of the eGFR equations that are routinely used were derived from a black African population and as such their appropriateness in assessing kidney function in this population needs to be evaluated. Since creatinine generation is determined primarily by muscle mass and

dietary intake (Stevens, Coresh, Greene, & Levey, 2006), it is reasonable to assume that the performance of eGFR equations will be influenced by the extent to which the body habitus of an African population differs from the reference populations used when deriving them. Given the paucity of data on the performance of these equations in a subjectively healthy population, we compared eGFR determined using various eGFR equations and assessed which eGFR values best correlated with known risk factors for CKD and the proportion of asymptomatic black Africans with no known risk factors for CKD classified as having a reduced or normal eGFR by each equation.

4.3 Methods

We determined eGFR using single measurements of serum creatinine from Kenyans participating in a cross-sectional multicenter, multinational, global reference interval study which is part of an initiative by the Committee of Reference Intervals and Decision Limits (C-RIDL) under the auspices of the International Federation of Clinical Chemistry (IFCC). The aim of the study is to determine reference intervals for common laboratory tests across different geographical regions and populations, and to explore determinants of variation in reference intervals. Kenya is one of the 3 participating countries in Africa, the other ones being Nigeria and South Africa. For the Kenyan study, we recruited subjectively healthy black African adults who met the inclusion criteria as spelt out in the published protocol (Ozarda et al., 2013). Participants were recruited from Nairobi which is the capital city, Kiambu county which is in central Kenya, Kisii which is in the western part of Kenya, and Nakuru county which is in the Great Rift valley. Recruitment was carried out between January and October 2015. All participants had undergone an overnight fast and written informed consent was sought from each participant after giving a written and verbal explanation of the study. Recruitment was stratified into 4 age groups: 18-29, 30-39, 40-49 and 50-65 years with almost similar numbers of males and females in each age strata. The recruitment procedure, sample handling and analysis has previously been described (Omuse, Maina, Hoffman, et al., 2017). Briefly, all participants had measurements of blood pressure (BP), abdominal circumference and body mass index (BMI) done, samples were collected and centrifuged within 4 hours after collection and stored at -80^{o}c until shipment on dry ice to the Pathcare reference laboratory in South Africa. As part of the

reference interval study, all participating laboratories used a common mini-panel of sera with assigned values to ensure accuracy of reported results and alignment of values if any biases were identified. Urine samples to test for haematuria and proteinuria were not collected. Serum creatinine was determined using a standardized kinetic colour test (Jaffé method) and fasting plasma glucose (FPG) by an enzymatic UV test (hexokinase method) both measured on a Beckman AU5800 (Schizuoka, Japan).

Calculation of body surface area (BSA) was done using the DuBois method (DuBois D, 1916):
BSA (m^2) = [71.84 x weight (kg)$^{0.425}$ x height (cm)$^{0.725}$]/10 000.

Calculation of eGFR (mL/min/1.73 m^2) was done using the following equations:
Re-expressed 4-v MDRD (Levey, Coresh, et al., 2007)
175 x [S-Cr (µmol/L)/88.4]$^{-1.154}$ x age (years)$^{-0.203}$ x (0.742 if female) x (1.212 if African American)

Cockcroft-Gault normalized to 1.73m^2 (Cockcroft & Gault, 1976)
[(140 x age in years) x weight (kg) x (0.85 if female) x 1.73 (m2)]/[S-Cr(µmol/L)x 0.814 x BSA (m^2)]

CKD-EPI for blacks (Levey et al., 2009)
Female with Creatinine ≤ 62 µmol/L; $166 \times (S_{cr}/61.9)^{-0.329} \times (0.993)^{Age}$
Female with Creatinine > 62 µmol/L; $166 \times (S_{cr}/61.9)^{-1.209} \times (0.993)^{Age}$
Male with Creatinine ≤ 80 µmol/L; $163 \times (S_{cr}/79.6)^{-0.411} \times (0.993)^{Age}$
Male with Creatinine > 80 µmol/L; $163 \times (S_{cr}/79.6)^{-1.209} \times (0.993)^{Age}$

CKD-EPI for other races (Levey et al., 2009)
Female with Creatinine ≤ 62 µmol/L; $144 \times (S_{cr}/61.9)^{-0.329} \times (0.993)^{Age}$
Female with Creatinine > 62 µmol/L; $144 \times (S_{cr}/61.9)^{-1.209} \times (0.993)^{Age}$
Male with Creatinine ≤ 80 µmol/L; $141 \times (S_{cr}/79.6)^{-0.411} \times (0.993)^{Age}$
Male with Creatinine > 80 µmol/L; $141 \times (S_{cr}/79.6)^{-1.209} \times (0.993)^{Age}$

FAS equation (Hans Pottel et al., 2016)

2-40 years; 107.3/ (Scr/Q)

> 40 years; $(107.3/ [Scr/Q])0.988^{Age-40}$

Where Scr is serum creatinine, Q is the mean or median population specific serum creatinine, Age is in years

Classification of CKD based on eGFR was done using the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines as follows (KDIGO, 2013):

GFR category	**GFR (ml/min/1.73m^2)**	**Terms**
G1	≥90	Normal or high
G2	60–89	Mildly decreased
G3a	45–59	Mildly to moderately decreased
G3b	30–44	Moderately to severely decreased
G4	15–29	Severely decreased
G5	<15	Kidney failure

A cut off-of 75 ml/min/1.73m^2 was also used to define reduced eGFR for participants less than 40 years of age as proposed by Pottel *et al* (H. Pottel, Hoste, & Delanaye, 2015). Sample size calculation for the primary study was based on recommendations from the Clinical Laboratory Standards Institute (CLSI) which requires a minimum of 120 study participants per stratification in order to get 90% confidence limits around the upper and lower limits of a reference interval (CLSI, 2008). Since we had 4 age stratifications, a minimum sample size of 480 was required to achieve the primary objective for the reference interval study.

Statistical analysis

Descriptive and inferential statistics were performed using SPSS version 20 (IBM corp, Armonk, New York, USA). Medians and interquartile ranges (IQR) were calculated for continuous variables before and after stratification based on gender. Comparison of medians or mean ranks between males and females was done using the Mann-Whitney U test. Classification of eGFR was reported as proportions. Multiple regression analysis (MRA) was also performed to

determine the extent to which the CKD risk factors independently added to the eGFR regression model. Calculation of eGFR, BSA and classification of CKD was done using Microsoft excel 2010 (Redmond, USA). A *p*-value less than 0.05 was considered statistically significant.

4.4 Results

A total of 533 participants were included comprising 260 (48.8%) males and 273 (51.2%) females. The median age, BMI and FPG between males and females wasn't statistically different neither was eGFR except when determined using CG corrected for BSA. Males had higher serum creatinine values. Five participants didn't have FPG assayed due to insufficient samples. A summary of the participants' demographic and clinical descriptive statistics are shown in **Table 4.1**.

Table 4.1: Descriptive characteristics of participants

	Male (n=260)		Female (n=273)		Total (n=533)		Male vs Female
	Median (IQR)	**Min-Max**	**Median (IQR)**	**Min-Max**	**Median (IQR)**	**Min-Max**	***p*-value**
Age (years)	39 (18)	20-65	39 (21)	18-64	39 (20)	18-65	0.971
Height (cms)	172 (8)	156-191	160 (9)	143-191	167 (13)	143-191	0.000
Weight (kg)	74 (19)	46-116	68 (16)	38-109	70 (18)	38-116	0.000
BMI (kg/m^2)	24.9 (5.6)	16.3-34.9	26.1 (6.3)	17.1-38.1	25.5 (5.9)	16.3-38.1	0.051
BSA (m^2)	1.88 (0.24)	1.44-2.36	1.72 (0.20)	1.27-2.24	1.78 (0.22)	1.27-2.36	0.000
Abd. Circ. (cm)	91 (15)	65-124	86 (16)	64-115	89 (17)	64-124	0.005
BP Systolic (mmHg)	127 (18)	84-179	128 (18)	118 (20)	124 (21)	77-194	0.000
BP Diastolic (mmHg)	81 (12)	56-101	79 (14)	57-112	80 (14)	56-112	0.003
*FPG (mmol/L)	4.9 (0.8)	3.0-15.6	4.8 (0.7)	3.3-19.5	4.9 (0.8)	3.0-19.5	0.191
Creatinine (µmol/L)	80 (20)	50-126	61 (15)	30-93	70 (21)	30-126	0.000
4v-MDRD eGFR (mL/min/1.73m^2)	114 (34)	61-184	118 (34)	69-251	115 (33)	61-251	0.126
4v-MDRD^ eGFR (mL/min/1.73m^2)	94 (28)	50-152	97 (28)	57-207	95 (28)	50-207	0.126
CKDEPI eGFR (mL/min/1.73m^2)	118 (25)	60-151	124 (25)	71-168	121 (25)	60-168	0.090
CKDEPI eGFR^ (mL/min/1.73m^2)	102 (21)	52-130	107 (22)	62-146	105 (22)	52-146	0.061
CG eGFR^^ (mL/min/1.73m^2)	103 (26)	55-163	163 (45)	85-345	128 (61)	55-345	0.000
FAS eGFR (mL/min/1.73m^2)	100 (23)	51-163	104 (25)	58-200	102 (25)	51-200	0.309

KEY: IQR-Interquartile range, Abd.Circ.-Abdominal circumference, BMI-Body Mass Index, BP-Blood pressure, FPG-Fasting plasma glucose (*5 males didn't have this test done), ^Not corrected for race, ^^Corrected for body surface area

Overall, women had higher eGFR compared to men. Of the 6 equations, the 4v-MDRD equation without correction for race had the lowest median eGFR and classified the least number of participants (61.7%) as having an eGFR ≥ 90 mL/min/1.73m^2 compared to 93.4% by CKD-EPI with correction for race as shown in table 2. The 4v-MDRD equation without correction for race classified the highest percentage of participants (0.9%) as having an eGFR <60 ml/min/1.73m^2. The number of individuals with an eGFR <60 ml/min/1.73m^2 was less than 1% regardless of the equation used. Using a cut off of 75 ml/min/1.73m^2, the 4v-MDRD equation without correction

for race also classified the highest proportion of participants under the age of 40 years (7.8%) as having reduced eGFR as shown in **Table 4.2**.

After multiple regression analysis, only age had an unstandardized coefficient (B) that was statistically significantly different from zeroacross all the 6 equations. Overall, the correlation between eGFR and risk factors for CKD varied depending on which equation was used. The 6 CKD risk factors evaluated had a stronger association with the CKD-EPI determined eGFRs as evidenced by higher multiple correlation coefficients as shown in **Table 4.2**.

Table 4.2: CKD classification and correlation of eGFR with risk factors

	CG^^	4v-MDRD	4v-MDRD^	CKD-EPI	CKD-EPI^	FAS
CKD classification based on eGFRNo. (%)						
G1	478 (89.7%)	473 (88.7%)	329 (61.7%)	499 (93.6%)	433 (81.2%)	402 (75.4%)
G2	54 (10.1%)	60 (11.3%)	199 (37.3%)	34 (6.4%)	99 (18.6%)	129 (24.2%)
G3a	1 (0.2%)	0 (0.0%)	5 (0.9%)	0 (0.0%)	1 (0.2%)	2 (0.4%)
No. (%) <40 years with eGFR <75 ml/min/1.73m^2(n=270)	0 (0.0%)	0 (0.0%)	21 (7.8%)	0 (0.0%)	1 (0.4%)	5 (1.9%)
eGFR (ml/min/1.73m^2) mean(SD)						
18-29 years (n=135)	149.0 (40.9)	129.5 (21.8)	106.8 (18.0)	135.3 (15.6)	117.2 (13.5)	108.9 (15.5)
30-39 years (n=135)	136.7 (39.2)	112.1 (20.2)	92.6 (16.7)	119.1 (15.8)	103.2 (13.7)	102.9 (15.8)
40-49 years (n=132)	139.8 (43.6)	117.0 (25.4)	96.6 (20.9)	117.6 (14.8)	101.8 (12.8)	106.7 (19.8)
50-65 years (n=131)	120.8 (35.1)	111.9 (25.0)	92.3 (20.6)	107.6 (15.2)	93.2 (13.1)	92.2 (18.4)
Multiple correlation coefficient (R)	0.563	0.305	0.306	0.568	0.568	0.350
Unstandardized (B) coefficients (standard error)						
Age	-0.621*(0.151)	-0.322*(0.102)	-0.267*(0.084)	-0.673*(0.067)	-0.579*(0.058)	-0.403*(0.077)
BMI	7.418*(0.567)	-0.596(0.382)	-0.491(0.315)	-0.188(0.252)	-0.129(0.218)	-0.405(0.289)
Systolic BP	-0.485*(0.140)	-0.121(0.094)	-0.099(0.078)	-0.116(0.062)	-0.102(0.054)	-0.119(0.071)
Diastolic BP	0.237(0.202)	0.139(0.136)	0.115(0.112)	0.142(0.090)	0.124(0.078)	0.117(0.103)
Abd.Circ.	-2.049*(0.231)	-0.178(0.156)	-0.148(0.128)	-0.184(0.103)	-0.172(0.089)	-0.028(0.118)
FPG	0.579(1.170)	1.106(0.788)	0.912(0.650)	0.610(0.519)	0.500(0.450)	0.807(0.596)

KEY: Abd.Circ.-Abdominal circumference, BP-Blood pressure, BMI-Body Mass Index, CKD-Chronic kidney disease, FPG-Fasting plasma glucose, ^Not corrected for race, ^^Corrected for body surface area, * Significant at a *p*-value < 0.05

4.5 Discussion

The prevalence of eGFR < 60 ml/mim/1.73 m^2 was less than 1% according to the 6 equations evaluated in this study which is reflective of the deliberate recruitment of healthy individuals for the reference interval study. In a randomly selected rural and urban adult population in Cameroon, Kaze *et al* found that 10.9% had a CKD-EPI determined eGFR <60 ml/min/1.73m^2 with this being more common in the urban population (Kaze et al., 2015). Both the CKD-EPI and 4v-MDRD equations with correction for race did not classify any participant as having a reduced eGFR in our study largely comprised of an urban population. Assuming that a healthy population should predominantly have an eGFR > 90 ml/min/1.73m^2, the CKD-EPI equation with correction for race classified more participants into stage G1 suggesting that it could be a more ideal equation when estimating eGFR in an asymptomatic population not known to have risk factors for CKD. Unlike the MDRD equation which was derived from individuals with CKD, CKD-EPI was derived from a heterogeneous population that included healthy individuals (Levey et al., 2009). Rule *et al* used different equations to screen for CKD in a general population and found that equations derived from a CKD population gave higher estimates of CKD prevalence compared to equations derived from a population that included healthy individuals (Rule et al., 2006). It is therefore not surprising that the CKD-EPI equation classified less participants as having a reduced eGFR compared to the 4v-MDRD equation.

There has been a debate as to the suitability of the 60 ml/min/1.73m^2 eGFR cut off for defining CKD in young adults and the elderly. Pottel *et al* suggested that a cut off of 75 ml/min/1.73m^2 would be more ideal in young adults under 40 years of age given that one would require a serum creatinine almost 1.8 times above the mean value for this population to achieve an eGFR less than 60 ml/min/1.73m^2 making this cut off quite insensitive to early increments in serum creatinine (H. Pottel et al., 2015). Gharbi *et al* demonstrated that in an Arabic-Berber adult population in Morocco, using a cut off of 60 ml/min/1.73m^2 resulted in under diagnosis of CKD in younger adults and over diagnosis in the elderly population (Benghanem Gharbi et al., 2016). In our study, based on a cut off of 75 ml/min/1.73m^2, no one under the age of 40 years had reduced eGFR when determined using CG corrected for BSA, 4v-MDRD or CKD-EPI equations corrected for race. The FAS equation that can be applied across a wide age spectrum classified 5 (1.9%) individuals less than 40 years of age as having a reduced eGFR compared to 21 (7.8%) for the 4v-MDRD equation without correction for race. In the absence of mGFR, testing for albuminuria or demonstrating persistence of reduced eGFR for at least 3 months, it

is difficult to conclude which individuals truly had CKD and which equation was correctly classifying them as such.

We determined eGFR correlation with known risk factors for CKD such as age, BMI, BP, glycaemia and abdominal circumference. We assumed that in the absence of mGFR, the multiple correlation coefficient would serve as indirect proof of appropriateness for routine reporting of eGFR in an asymptomatic population as it is a measure of linear association between the predicted eGFR after factoring in the CKD risk factors and eGFR determined using the various equations. The CKD-EPI and CG corrected for BSA equations had the highest multiple correlation coefficients suggesting that the the model best fitted these equations. However, given that age is one of the components of the CG equation and both height and weight are used in the calculation of BMI and BSA, there is a possibility that the CG multiple correlation coefficient after correction for BSA is inaccurate due to possible collinearity. Age remained the only risk factor that showed a statistically significant linear association with eGFR across all eGFR equations s. Matsha *et al* demonstrated that age, gender and known history of hypertension were determinants of CKD stage 3-5 in a mixed ancestry population in South Africa. Surprisingly, this was not the case for FPG, BMI, systolic and diastolic BP. However, this study did not correlate eGFR with these potential risk factors but rather used logistic regression to determine the odds of having a reduced eGFR (Tandi E. Matsha et al., 2013). Kaze *et al* concluded that in a rural and urban population in Cameroon, elevated systolic BP, presence of hypertension and diabetes were the main predictors of albuminuria and CKD (Kaze et al., 2015). In the Democratic Republic of Congo, a study by Sumaili *et al* found that age above 65 years and hypertension were independently associated with increased risk of CKD stage 3-5 (Sumaili et al., 2009).

The issue of whether or not one should correct for the black African race when using eGFR equations has not been settled. Deventer *et al* concluded that correction for the African race was not necessary when using the 4v-MDRD equation after they compared eGFR with mGFR in 100 black African patients with CKD and found a greater bias after correcting for race (van Deventer et al., 2008). The mean weight of the participants in this study was 69.5 kg with a BSA of 1.76 m^2 compared to a weight of 79.6 kg and BSA of 1.91 m^2 for the MDRD population which included only 197 (12.1%) African Americans (Levey et al., 1999). The adjustment for the African race is extrapolated from the African-American MDRD study population who had a higher mean weight and BSA compared to the Caucasian population necessitating a correction of the original 4v-MDRD eGFR equation by a factor of

1.18 that was further adjusted to 1.212 after standardization of the creatinine assay. Since serum creatinine levels are affected by muscle mass, it is not surprising that the 4v-MDRD equation without correction for race better approximated mGFR in the South African population given a closer similarity in weight and by extension muscle mass of their study population to the Caucasian population used in deriving the equation. A study carried out in Ghana in a rural population of black Africans also concluded that correction for race was unnecessary for both the 4v-MDRD and CKD-EPI equations. This study compared eGFR to CrCl and the mean weight of the participants was 54.4 kg (Eastwood et al., 2010). Flamant *et al* compared CKD-EPI and 4v-MDRD derived eGFR to mGFR in African Europeans originating from West Africa and concluded that a correction factor of 1.08 was required. However, most of the patients in this study had CKD with a mean mGFR of 57.6 ml/min/1.73m^2 (Flamant et al., 2013). Delanaye *et al* has argued that the correction factor for race when using the 4v-MDRD or CKD-EPI equations should vary depending on whether it is being applied in individuals with CKD and an eGFR <60 ml/min/1.73m^2 or healthy subjects due to the inherent difference in how these 2 populations handle serum creatinine even within the same race (Delanaye, Mariat, Maillard, Krzesinski, & Cavalier, 2011). In our study we didn't compare eGFR to either mGFR or CrCl but rather compared the proportion of healthy black Africans classified as having a reduced or normal eGFR across the different equations and eGFR correlation with a prediction mode comprising risk factors for CKD. Since the correction factor for race for both 4v-MDRD and CKD-EPI is greater than one, it is not surprising that correction for race resulted in higher eGFRs. However, the CKD EPI equation had a higher median eGFR and proportion of individuals with eGFR >90 ml/min/1.73m^2 compared to 4v-MDRD or FAS as well as better correlation with risk factors for CKD. This is not surprising as CKD-EPI is more accurate than the 4v-MDRD equation in individuals with a GFR >60 ml/min/1.73m^2 and would be expected to be more appropriate for the subjectively healthy population recruited in this study (Levey et al., 2009). The presence of a larger proportion of healthy individuals in the CKD-EPI dataset makes it more suitable for reporting eGFR in healthy subjects (Rule et al., 2006). The FAS equation was developed to overcome the potential differences in eGFR associated with adoption of different equations. The equation incorporates population normalized serum creatinine levels and therefore gets rid of the need for further age, gender and race correction. This equation has been shown to be less biased than the CKD-EPI equation when applied on data sets largely comprised of a Caucasian population and therefore requires validation in an ethnic black African population (Hans Pottel et al., 2016). In our study, the FAS determined eGFR had similar correlation coefficients with CKD risk factors as the 4v-MDRD equation and demonstrated a similar pattern in change of mean

eGFR with age where, the mean eGFR in the age group 40-49 years was higher than in the age group 30-39 years. Of the 6 equations, only the CKD-EPI derived mean eGFRs showed a consistent decline with increase in age across the 4 age stratifications in line with the expected reduction of eGFR associated with progressive loss of nephrons above the age of 30 years (Glassock & Rule, 2016).

The median eGFR for female participants calculated using the various equations was consistently higher than male participants in our study though this difference was not statistically significant except for eGFR determined using CG corrected for BSA. This is in contrast to the Ghanaian study by Eastwood *et al* where the female participants had a lower mean eGFR when determined using both the 4v-MDRD and CKD-EPI equations with and without correcting for race but not when CG corrected for BSA was used. Similar to our study, the Ghanaian women had a higher BMI but lower weight and blood pressure compared to the men (Eastwood et al., 2010). No obvious explanation is forthcoming for the relatively better eGFR seen in women compared to men in our study. The Ghanaian rural population had a significantly higher percentage (13.2%) of individuals with reduced eGFR (< 60 ml/min/1.73m^2) compared to < 1% in our study population which can partially be explained by the fact that we recruited a younger population ranging from 18-65 years compared to 40-75 years for the Ghanaian population with 50.8% of our study population being under the age of 40 years. We also had a strict inclusion and exclusion criteria to ensure that as far as possible only healthy individuals were recruited.

A major limitation of our study is that we didn't measure GFR and as such our comparison of various eGFR equations is not validated against a gold standard. In the absence of mGFR, we chose to use correlation with a prediction model of markers known to increase risk of CKD but are not included in the eGFR equations as a basis of comparison. Our assumption was that equations whose eGFR calculations best correlated with these risk factors were potentially better equations to be used when screening for CKD in an asymptomatic black African population. This is not equivalent to mGFR and therefore limits the extent to which conclusions can be made as to which equation is better. Another major limitation is that we didn't carry out any test for albuminuria neither did we repeat serum creatinine measurements after 3 months hence we cannot accurately comment on the prevalence of CKD in this population. Gharbi *et al* found that 32% of the subjects classified as having CKD stage 3a and 7.4% of those classified as 3b had an eGFR >60 ml/min/1.73 m^2 when reinvestigated after 3 months or longer (Benghanem Gharbi et al., 2016). However, the proportion of participants with an

eGFR < 60 ml/min/1.73 m^2 in our study was less than 1% hence the risk of over diagnosing CKD in this healthy population was extremely low. What is more likely is that we may have under diagnosed CKD due to failure to test for albuminuria. We also assumed that diabetes and hypertension are the commonest risk factors for CKD in our population which may not necessarily be the case as demonstrated by Stanifer *et al* in Northern Tanzania where 49.1% of individuals with CKD didn't have hypertension, diabetes or HIV (Stanifer et al., 2015). We also didn't measure cystatin C which has been shown by Meeusen *et al* to significantly improve eGFR estimation across different patient groups including healthy kidney donors when incorporated into the CKD-EPI equation (Meeusen, Rule, Voskoboev, Baumann, & Lieske, 2015).

Conclusion

The CKD-EPI equation with correction for race classified the highest proportion of asymptomatic healthy black Africans as having an eGFR >90 ml/min/1.73m^2 and its eGFR better correlated with risk factors for CKD. For this reason, we recommend the use of the CKD-EPI equation with correction for race for routine reporting of eGFR in an asymptomatic black African population with a low prevalence of risk factors for CKD. Comparing eGFR to mGFR would be the best way to determine the most appropriate equation for CKD screening in an asymptomatic black African population. However, measurement of GFR is a complex and expensive process which serves as a barrier for the validation of eGFR equations especially in sub Saharan Africa. In the absence of mGFR, other ways of evaluating the potential utility and performance of eGFR equations in routine screening for CKD in asymptomatic populations need to be evaluated.

Ethics approval and consent to participate

Ethical approval was obtained from the Aga Khan University, Nairobi Health Research Ethics Committee (2014/REC-46). Written informed consent was obtained from all participants.

Consent for publication

Not applicable

Availability of data and materials

Not shared as it contains confidential participant data.

Competing interests

None

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Author contributions

GO designed the study, collected data, performed statistical analysis and wrote the manuscript. DM designed the study, collected data and critiqued the manuscript, JM and CW designed the study and critiqued the manuscript, AK and EK collected data and critiqued the manuscript, AA, PJ and RE designed the study, planned and supervised its implementation, critiqued the manuscript.

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4.6 References

Benghanem Gharbi, M., Elseviers, M., Zamd, M., Belghiti Alaoui, A., Benahadi, N., Trabelssi, E. H., . . . Hoilund-Carlsen, P. F. (2016). Chronic kidney disease, hypertension, diabetes, and obesity in the adult population of Morocco: how to avoid “over”- and “under”-diagnosis of CKD. *Kidney International*, *89*(6), 1363-1371. doi:10.1016/j.kint.2016.02.019

CLSI. (2008). *Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline* (Third ed.): Wayne PA, Clinical and Laboratory Standards Institute.

Cockcroft, D. W., & Gault, M. H. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron, 16*(1), 31-41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1244564

Delanaye, P., Mariat, C., Maillard, N., Krzesinski, J.-M., & Cavalier, E. (2011). Are the creatinine-based equations accurate to estimate glomerular filtration rate in African American populations? *Clinical Journal of the American Society of Nephrology, 6*(4), 906-912. doi:10.2215/CJN.10931210

DuBois D, D. E. F. (1916). A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med, 17*, 863-871.

Eastwood, J. B., Kerry, S. M., Plange-Rhule, J., Micah, F. B., Antwi, S., Boa, F. G., . . . Cappuccio, F. P. (2010). Assessment of GFR by four methods in adults in Ashanti, Ghana: the need for an eGFR equation for lean African populations. *Nephrology, Dialysis, Transplantation, 25*(7), 2178-2187. doi:10.1093/ndt/gfp765

Flamant, M., Vidal-Petiot, E., Metzger, M., Haymann, J.-P., Letavernier, E., Delatour, V., . . . Haymann, J. P. (2013). Performance of GFR Estimating Equations in African Europeans: Basis for a Lower Race-Ethnicity Factor Than in African Americans. *American Journal of Kidney Diseases, 62*(1), 182-184. doi:10.1053/j.ajkd.2013.03.015

Glassock, R. J., & Rule, A. D. (2016). Aging and the Kidneys: Anatomy, Physiology and Consequences for Defining Chronic Kidney Disease. *Nephron, 134*(1), 25-29. doi:10.1159/000445450

Ibrahim, H., Mondress, M., Tello, A., Fan, Y., Koopmeiners, J., & Thomas, W. (2005). An alternative formula to the Cockcroft-Gault and the modification of diet in renal diseases formulas in predicting GFR in individuals with type 1 diabetes. *Journal of the American Society of Nephrology : JASN, 16*(4), 1051-1060. doi:10.1681/ASN.2004080692

Kaze, F. F., Meto, D. T., Halle, M.-P., Ngogang, J., Kengne, A.-P., Lozano, R., . . . Meena, G. S. (2015). Prevalence and determinants of chronic kidney disease in rural and urban Cameroonians: a cross-sectional study. *BMC Nephrology, 16*(1), 117-117. doi:10.1186/s12882-015-0111-8

KDIGO. (2013). KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney International Supplements, 3*(1), 4-4. doi:10.1038/kisup.2012.76

Levey, A. S., Atkins, R., Coresh, J., Cohen, E. P., Collins, A. J., Eckardt, K. U., . . . Eknoyan, G. (2007). Chronic kidney disease as a global public health problem: approaches and initiatives - a

position statement from Kidney Disease Improving Global Outcomes. *Kidney Int, 72*(3), 247-259. doi:10.1038/sj.ki.5002343

Levey, A. S., Bosch, J. P., Lewis, J. B., Greene, T., Rogers, N., & Roth, D. (1999). A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Annals of Internal Medicine, 130*(6), 461-470.

Levey, A. S., Coresh, J., Greene, T., Marsh, J., Stevens, L. A., Kusek, J. W., & Van Lente, F. (2007). Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clinical Chemistry, 53*(4), 766-772. doi:10.1373/clinchem.2006.077180

Levey, A. S., Stevens, L. A., Schmid, C. H., Zhang, Y. L., Castro, A. F., Feldman, H. I., . . . Coresh, J. (2009). A new equation to estimate glomerular filtration rate. *Annals of Internal Medicine, 150*(9), 604-612. doi:10.7326/0003-4819-150-9-200905050-00006

Matsha, T. E., Yako, Y. Y., Rensburg, M. A., Hassan, M. S., Kengne, A. P., & Erasmus, R. T. (2013). Chronic kidney diseases in mixed ancestry south African populations: prevalence, determinants and concordance between kidney function estimators. *BMC Nephrology, 14*(1), 75-75. doi:10.1186/1471-2369-14-75

Meeusen, J. W., Rule, A. D., Voskoboev, N., Baumann, N. A., & Lieske, J. C. (2015). Performance of cystatin C- and creatinine-based estimated glomerular filtration rate equations depends on patient characteristics. *Clinical Chemistry, 61*(10), 1265-1272. doi:10.1373/clinchem.2015.243030

Noble, E., Johnson, D. W., Gray, N., Hollett, P., Hawley, C. M., Campbell, S. B., . . . Isbel, N. M. (2008). The impact of automated eGFR reporting and education on nephrology service referrals. *Nephrology, Dialysis, Transplantation, 23*(12), 3845-3850. doi:10.1093/ndt/gfn385

Omuse, G., Maina, D., Hoffman, M., Mwangi, J., Wambua, C., Kagotho, E., . . . Erasmus, R. (2017). Metabolic syndrome and its predictors in an urban population in Kenya: A cross sectional study. *BMC Endocrine Disorders, 17*(1). doi:10.1186/s12902-017-0188-0

Ozarda, Y., Ichihara, K., Barth, J. H., & Klee, G. (2013). Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. *Clinical Chemistry and Laboratory Medicine (CCLM), 51*(5), 1027-1040. doi:10.1515/cclm-2013-0249

Pottel, H., Hoste, L., & Delanaye, P. (2015). Abnormal glomerular filtration rate in children, adolescents and young adults starts below 75 mL/min/1.73 m². *Pediatr Nephrol, 30*(5), 821-828. doi:10.1007/s00467-014-3002-5

Pottel, H., Hoste, L., Dubourg, L., Ebert, N., Schaeffner, E., Eriksen, B. O., . . . Delanaye, P. (2016). An estimated glomerular filtration rate equation for the full age spectrum. *Nephrology Dialysis Transplantation, 31*(5), 798-806. doi:10.1093/ndt/gfv454

Rule, A. D., Rodeheffer, R. J., Larson, T. S., Burnett, J. C., Cosio, F. G., Turner, S. T., & Jacobsen, S. J. (2006). Limitations of estimating glomerular filtration rate from serum creatinine in the general population. *Mayo Clinic Proceedings, 81*(11), 1427-1434. doi:10.4065/81.11.1427

Stanifer, J. W., Maro, V., Egger, J., Karia, F., Thielman, N., Turner, E. L., . . . Patel, U. D. (2015). The epidemiology of chronic kidney disease in Northern Tanzania: a population-based survey. *PLOS ONE, 10*(4), e0124506-e0124506. doi:10.1371/journal.pone.0124506

Stevens, L. A., Coresh, J., Greene, T., & Levey, A. S. (2006). Assessing kidney function--measured and estimated glomerular filtration rate. *The New England Journal of Medicine, 354*(23), 2473-2483. doi:10.1056/NEJMra054415

Sumaili, E. K. K., Krzesinski, J. M. M., Zinga, C. V. V., Cohen, E. P. P., Delanaye, P., Munyanga, S. M. M., & Nseka, N. M. M. (2009). Prevalence of chronic kidney disease in Kinshasa: results of a pilot study from the Democratic Republic of Congo. *Nephrol Dial Transplant, 24*(1), 117-122. doi:10.1093/ndt/gfn469

van Deventer, H. E., George, J. A., Paiker, J. E., Becker, P. J., & Katz, I. J. (2008). Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft-Gault equations. *Clinical Chemistry, 54*(7), 1197-1202. doi:10.1373/clinchem.2007.099085

Vervoort, G., Willems, H. L., & Wetzels, J. F. M. (2002). Assessment of glomerular filtration rate in healthy subjects and normoalbuminuric diabetic patients: validity of a new (MDRD) prediction equation. *Nephrology, Dialysis, Transplantation, 17*(11), 1909-1913.

Wyatt, C. M., Schwartz, G. J., Owino Ong'or, W., Abuya, J., Abraham, A. G., Mboku, C., . . . Wools-Kaloustian, K. (2013). Estimating kidney function in HIV-infected adults in Kenya: comparison to a direct measure of glomerular filtration rate by iohexol clearance. *PLOS ONE, 8*(8), e69601-e69601. doi:10.1371/journal.pone.0069601

CHAPTER 5: COMPLETE BLOOD COUNT REFERENCE INTERVALS FROM A HEALTHY ADULT URBAN POPULATION IN KENYA

Omuse, G., Maina, D., Mwangi, J., Wambua, C., Radia, K., Kanyua, A., . . . Erasmus, R. (2018). Complete blood count reference intervals from a healthy adult urban population in Kenya. *PLOS ONE*, 1-19.

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5.1 Abstract

Background

There are racial, ethnic and geographical differences in complete blood count (CBC) reference intervals (RIs) and therefore it is necessary to establish RIs that are population specific. Several studies have been carried out in Africa to derive CBC RIs but many were not conducted with the rigor recommended for RI studies hence limiting the adoption and generalizability of the results.

Method

By use of a Beckman Coulter ACT 5 DIFF CP analyser, we measured CBC parameters in samples collected from 528 healthy black African volunteers in a largely urban population. The latent abnormal values exclusion (LAVE) method was used for secondary exclusion of individuals who may have had sub-clinical diseases. The RIs were derived by both parametric and non-parametric methods with and without LAVE for comparative purposes.

Results

Haemoglobin (Hb) levels were lower while platelet counts were higher in females across the 4 age stratifications. The lower limits for Hb and red blood cell parameters significantly increased after applying the LAVE method which eliminated individuals with latent anemia and inflammation. We adopted RIs by parametric method because 90% confidence intervals of the RI limits were invariably narrower than those by the non-parametric method. The male and female RIs for Hb after applying the LAVE method were 14.5−18.7 g/dL and 12.0−16.5 g/dL respectively while the platelet count RIs were 133−356 and 152−443 x10^3 per µL respectively.

Conclusion

Consistent with other studies from Sub-Saharan Africa, Hb and neutrophil counts were lower than Caucasian values. Our finding of higher Hb and lower eosinophil counts compared to other studies conducted in rural Kenya most likely reflects the strict recruitment criteria and healthier reference population after secondary exclusion of individuals with possible sub-clinical diseases.

5.2 Introduction

Reference intervals (RIs) play an important role in guiding the interpretation of laboratory results. However, several factors influence RIs with the notable sources of variation being age, sex, race, body mass index (BMI) and ethnicity (Ichihara, Ceriotti, Tam, et al., 2013; Ichihara et al., 2008). Use of inappropriate RIs can result in misclassification of patients and subsequent mismanagement (Hershman et al., 2003). For this reason, it is recommended that laboratories determine RIs that are appropriate for the population they serve or at the very least verify any proposed RIs (CLSI, 2008).

Few studies have been carried out in Africa to derive RIs and these have been done mainly while conducting HIV related clinical trials since adopting inappropriate RIs may result in unnecessary exclusion of potential trial volunteers and makes assessment of laboratory adverse events difficult. It is known that there are racial differences in complete blood count (CBC) parameters. Beutler et al. clearly demonstrated that African-Americans had lower haemoglobin (Hb), haematocrit (Hct), white blood cell count (WBC) and absolute granulocyte count compared to European-Americans but higher lymphocyte counts (Beutler & West, 2005). Karita et al. carried out a study to determine RIs for common haematology and chemistry tests in Kenya, Uganda, Zambia and Rwanda. The study found lower Hct, Hb, WBC and neutrophil counts compared to RIs from the United States (US) while eosinophil counts were found to be higher (Karita et al., 2009). A similar study was carried out in Kericho, a rural area situated in the highlands of the Great Rift Valley in Kenya. The derived RIs for males and females were 8.3–11.3g/dL and 5.9–10.0 g/dL respectively. A number of participants were found to have relatively low mean corpuscular volume (MCV) and haemoglobin (MCH), values consistent with iron deficiency especially in women of child bearing age. Lower neutrophil and higher eosinophil counts compared to Caucasians were also found (Kibaya et al., 2008). A genetic deletion of the Duffy antigen receptor for chemokines (DARC-null genotype), a receptor for *Plasmodium vivax,* is thought to contribute to the benign neutropenia seen in Africans and African-Americans (Thobakgale & Ndung'u, 2014). The eosinophilia is thought to be related to increased exposure to environmental allergens and possibly parasite infections but there isn't strong evidence to support this hypothesis.

These studies demonstrate the need to establish population specific RIs. However, establishment of RIs should follow a rigorous process of identifying reference individuals, standardization of sample handling and analysis as well as the use of appropriate statistical methods (Ozarda et al., 2013). Despite

all attempts to ensure that only healthy individuals are included in a RI study, this can never be achieved due to the presence of subclinical disease in subjectively healthy individuals. Reference individuals are also quite heterogeneous in their state of health and there isn't a perfect standard to define health or normality (Petitclerc, 2004). Statistical methods such as latent abnormal values exclusion (LAVE) have been devised in order to secondarily exclude healthy individuals participating in an RI study but who have multiple related laboratory test values outside their RIs suggesting the possibility of sub-clinical diseases (Ichihara, 2014).

A criticism of RI studies especially those done in economically disadvantaged areas is that the recruited population may not necessarily be representative of an ideal reference population. There are different motivators to volunteer in a study and these can result in a selection bias hence compromising the external validity of the study results (McGrath et al., 2001; Tarimo et al., 2011). We set out to determine RIs for complete blood counts (CBCs) in Kenya using data from carefully selected healthy individuals recruited as part of a multicenter, multi-country, global RI study conducted by the Committee of Reference Intervals and Decision Limits (C-RIDL) under the auspices of the International Federation of Clinical Chemistry (IFCC). Further, we compared our RIs with those derived from similar studies in sub-Saharan Africa (SSA).

5.3 Methods

Setting

The study was carried out in Kenyan urban towns located in the counties of Nairobi, Thika, Kiambu, Nakuru and Kisii. Recruitment was largely done from colleges, universities, churches, hospitals, corporations and shopping malls. Awareness of the study was created through use of posters, flyers, emails, church announcements and engaging administrators in the various organizations.

Recruitment

A strict recruitment criteria was used when enrolling individuals for the study as stipulated in the published study protocol and standard operating procedures (Ozarda et al., 2013). Briefly, subjectively healthy black African adults aged 18-65 years who had undergone an overnight fast were recruited between January and October 2015. No financial inducements were given, however, snacks were

provided to those who participated. Recruitment was stratified into 4 age groups: 20-29, 30-39, 40-49 and 50-65 years with a similar distribution of males and females in each age strata. Exclusion criteria included individuals with a body mass index (BMI) greater than 35 kg/m^2, consumption of ethanol greater than or equal to 70 g per day [equivalent to 5 alcoholic drinks], smoking more than 20 tobacco cigarettes per day, taking regular medication for a chronic disease (diabetes mellitus, hypertension, hyperlipidemia, allergic disorders, depression), recent (less than 15 days) recovery from acute illness, injury or surgery requiring hospitalization, known carrier state of Hepatitis B, Hepatitis C or HIV, pregnant or within 1 year after childbirth. Written informed consent was sought from each participant after giving a written and verbal explanation of the study. Individuals with any chronic disease were excluded except for the age group 50-65 years where individuals with well controlled hypertension taking no more than 2 drugs were recruited. All participants had measurements of blood pressure (BP), abdominal circumference and BMI done.

Pre-analytical sample handling

CBC samples were collected by a trained phlebotomist using an evacuated tube system comprising a sterile multi-sample needle, needle holder and plastic evacuated tubes containing ethylene diamine tetra-acetic acid (EDTA) (BD Vacutainer® Blood Collection Tube, US). The samples were transported at refrigerated temperature and analyzed within 12 hours after collection. Serum and plasma samples requiring centrifugation were spun within 4 hours after collection and stored at -80^{o}C until shipment on dry ice to the reference laboratory in South Africa for analysis of tests that were part of the global RI study. All samples arrived in the reference laboratory frozen and were only thawed once prior to sample analysis.

Sample analysis

All the sample analysis for the biochemistries and immunoassays were performed in the PathCare reference laboratory in Cape Town, South Africa, which is an International Organization for Standardization (ISO) 15189 accredited laboratory. All participating countries in Africa had their general biochemistry and immunoassays done in this laboratory so as to standardize analysis, ensure alignment and comparison of results by using a common panel of sera across all participating laboratories globally. The CBC parameters included red blood cell count (RBC), Hb, Hct, MCV, MCH, mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), WBC, platelet (PLT), leukocyte differential counts of neutrophil (Neu), lymphocyte (Lym), monocyte (Mon),

eosinophil (Eos), basophil (Bas). The differential counts were recorded as both % of WBC and absolute count (abs). The CBC was analysed using a Beckman Coulter ACT 5 DIFF CP analyser (Brea, California, US) in the PathCare Kenya laboratory based in Nairobi which is also ISO 15189 accredited by the South African National Accreditation Service (SANAS).

Iron (Fe), transferrin (Tf), albumin (Alb), and ultrasensitive c-reactive protein (hsCRP) were analysed using a Beckman Coulter AU5800 analyser while ferritin levels were determined using a Beckman Coulter DXI analyser. The globulin fraction (Glb) was calculated by subtracting Alb from total protein (TP). These non-CBC parameters were used as part of reference tests in the LAVE procedure for secondary exclusion of individuals with latent anemia or inflammation.

Ethics approval

The study was approved by the Aga Khan University Hospital Nairobi, Health research ethics committee (2014/REC-46) and was carried out in accordance with the Declaration of Helsinki.

Statistical analysis

The sample size from each country participating in the global RI study was set at a minimum of 500 (male and female: 250 × 2), so that country-specific RIs would be obtained in a more reproducible manner. This number was deemed adequate to make between-country comparisons of test results with a power of detecting a difference of two means equivalent to 0.25 times the standard deviation comprising the RI (SD_{RI}), which corresponds to a bias of 0.25 times between-individual variation, allowing errors of α less than 0.05 and β less than 0.2 in the statistical hypothesis testing done separately for each sex. According to the CLSI guideline (CLSI, 2008), at least 120 individuals are required to determine RIs using non-parametric methods for each defined population group and for parameters not influenced by sex (60 females and 60 males). For parameters that are influenced by sex, the minimum recommended number is 240 reference individuals (120 females and 120 males). Therefore, the sample size of 500 well exceeds what is recommended by the CLSI and allows derivation of RIs with higher precision (narrower 90% confidence intervals of the RI limits, or LL and UL). For comparative purposes, RIs were determined using both parametric and non-parametric methods before and after applying the LAVE method as described by Ichihara *et al.* (Ichihara & Boyd, 2010; Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017). Briefly, for the non-parametric method, the reference values coinciding with the 2.5^{th} and 97.5^{th} percentiles after arranging the data in ascending

order were used to identify the RI lower and upper limits (LL and UL). For the parametric method, the data was transformed into a Gaussian form by using a modification of the original Box-Cox power transformation formula as described by Ichihara (Ichihara, 2014), ascertaining the mean and SD, and finally determining the RI as the mean ± 1.96SD, which corresponds to the central 95% limits or LL and UL under transformed scale. Then, the limits are reverse transformed to get the LL and UL in the original scale.

The LAVE method is an iterative optimization procedure which is applied in a situation where mutually related analytes were tested simultaneously. Initially, RIs are determined for each analyte independently using either a parametric (mean ± 1.96 SD after Gaussian transformation) or non-parametric (mid 95% range) method. From the second computation, any individual who has two or more results outside the RIs derived in the previous computation among the reference tests (see below) is excluded. This process was repeated six times at which point the RIs were nearly stable. Of note is that need for exclusion of a reference value is determined using a set of pre-defined reference tests but not the test whose RI is being determined (Ichihara, 2014). In this study we chose the following 11 analytes as the reference tests: Alb, Glb, hsCRP, Fe, Tf, ferritin, Hb, Hct, MCV, WBC, and PLT which can help identify individuals with latent anemia and/or inflammation.

For judging the need to partition reference values by sex and age, we computed the standard deviation ratio (SDR) which represents a ratio of between-subgroup SD (variation of the subgroup means from grand mean) to between-individual SD (approximately 1/4 the width of RI). We performed 2-level nested ANOVA to compute between-sex SD and between-age group SD after partitioning age as 18-29 years, 30-39 years, 40-49 years and 50-65 years. The SDR for between-sex SD (SDRsex) and for between-age SD (SDRage) were computed as a ratio to residual SD (or between-individual SD). Since between-age variation changes by sex, we also computed SDRage for each sex by one way ANOVA. We considered SDR ≥ 0.40 as a guide for judging the need for partitioning reference values by sex or age (Ichihara, 2014). For analysing the relationship between Hb and PLT, we performed least-square linear regression analysis and the degree of the association was expressed by Pearson's correlation coefficient. We quantified the magnitude of change in RI lower limit (LL) and upper limit (UL) before and after applying the LAVE procedure using the formulas SDR-LL = $|LL+ - LL-|/ ((UL+ - LL+)/3.92)$ and SDR-UL = $|UL+ - UL-|/ ((UL+ - LL+))/3.92\}$ where LL+, UL+ are the RI limits with application of LAVE, while LL-, UL- are RI limits without application of LAVE (Borai et al.,

2016). We set 0.3 as a critical value to define a significant change in LL or UL after application of LAVE. Analysis was carried out using a general purpose statistical software, StatFlex version 6.0 (Artech Inc., Osaka, Japan).

5.4 Results

Volunteers recruited

A total of 596 individuals volunteered to participate in the study out of which 63 were excluded for various reasons as shown in **Fig 5.1**. Out of 533 eligible participants, only 528 had CBC results available with 254 (48.1%) being males. Five individuals were excluded because CBCs were performed at a different laboratory using different equipment.

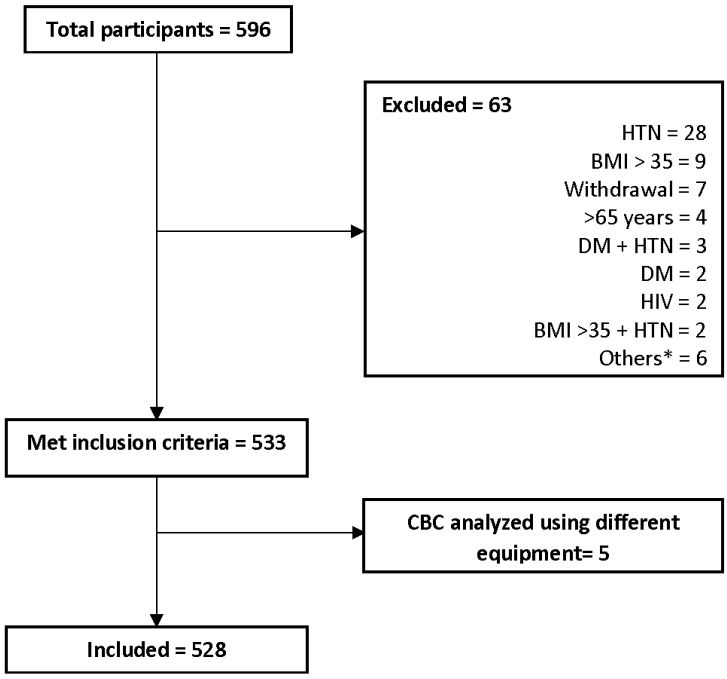


Fig 5.1: Flow diagram for recruitment

Key-BMI: body mass index, CBC: complete blood count, DM: diabetes mellitus, HIV: Human Immunodeficiency Virus, HTN: hypertension
*On antibiotics, blood donation in past 3 months, on treatment for hypothyroidism, > 65 years, > 65 years and hypertensive, rheumatic heart disease, prostate cancer

The median age for the included participants was 39 years with the youngest and oldest being 18 and 65 years respectively as shown in **Table 5.1**. The number of participants in the four age groups were

134 (18-29 years), 136 (30-39 years), 131 (40-49 years) and 127 (50-65 years). The number of smokers was low with only 2 (0.7%) and 13 (5.1%) women and men respectively.

Table 5.1: Descriptive characteristics of participants (Median, 2.5~97.5 percentile)

Parameter	Male (n=255)	Female (n=273)	Total (n=528)
Age (years)	38.0 (21.0~64.0)	39.0 (20.0~61.7)	39.0 (20.0~63.0)
BMI (kg/m^2)	24.9 (18.3~33.6)	26.1 (18.5~34.9)	25.5 (18.3~34.2)
SBP (mmHg)	81.0 (61.0~99.0)	79.0 (61.0~98.7)	80.0 (61.0~99.0)
DBP (mmHg)	127.0 (103.9~151.3)	118.0 (96.3~156.0)	123.5 (98.7~155.0)

Key-BMI: body mass index, DBP: diastolic blood pressure, SBP: systolic blood pressure

Sex and age related changes in reference values

Using a cut-off of 0.4, SDRs revealed that sex was a significant source of variation for RBC count, Hb, Hct, PLT count, absolute monocyte count and percentage while age was a significant source of variation only for absolute monocyte count and percentage in females as shown in **Table 5.2**. For all other CBC parameters, age and sex did not appear to be major sources of variation hence we chose not to partition their RIs.

Table 5.2: Complete blood count standard deviation ratios for age and sex

Item	SDR-sex	SDR-age	SDR-age M	SDR-age F
RBC	**1.02**	0.05	0.08	0.00
Hb	**1.28**	0.10	0.15	0.00
Hct	**1.28**	0.14	0.22	0.00
MCV	0.00	0.00	0.00	0.17
MCH	0.04	0.00	0.00	0.10
MCHC	0.19	0.00	0.00	0.15
RDW	0.13	0.00	0.00	0.00
WBC	0.00	0.13	0.12	0.14
Neu %	0.34	0.16	0.18	0.14
Lym %	0.16	0.08	0.19	0.00
Mon %	**0.46**	0.39	0.33	**0.53**
Eos %	0.13	0.14	0.14	0.13
Bas %	0.27	0.00	0.00	0.05
Neu Abs	0.22	0.00	0.00	0.04
Lym Abs	0.00	0.19	0.21	0.17
Mon Abs	0.33	**0.42**	0.34	**0.53**
Eos Abs	0.12	0.17	0.22	0.07
Bas Abs	0.20	0.08	0.00	0.20
PLT	**0.41**	0.00	0.00	0.00

Key-%: Percentage, RBC: red blood cell count, Hb: haemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red cell distribution width, WBC: white blood cell count, Neu: neutrophil, Lym: lymphocyte, Mon: monocyte, Eos: eosinophil, Bas: basophil, Abs: absolute count, Plt: platelet count, SDR: standard deviation ratio, M: male, F: female. SDRs > 0.4 are in bold

Reference values (RVs) of Hb, Hct and RBC were considerably lower in female participants compared to males while PLT counts were higher in female participants. For the non CBC parameters, RVs of serum iron and ferritin were lower in female participants but those of transferrin were higher as shown in **Fig 5.2**.

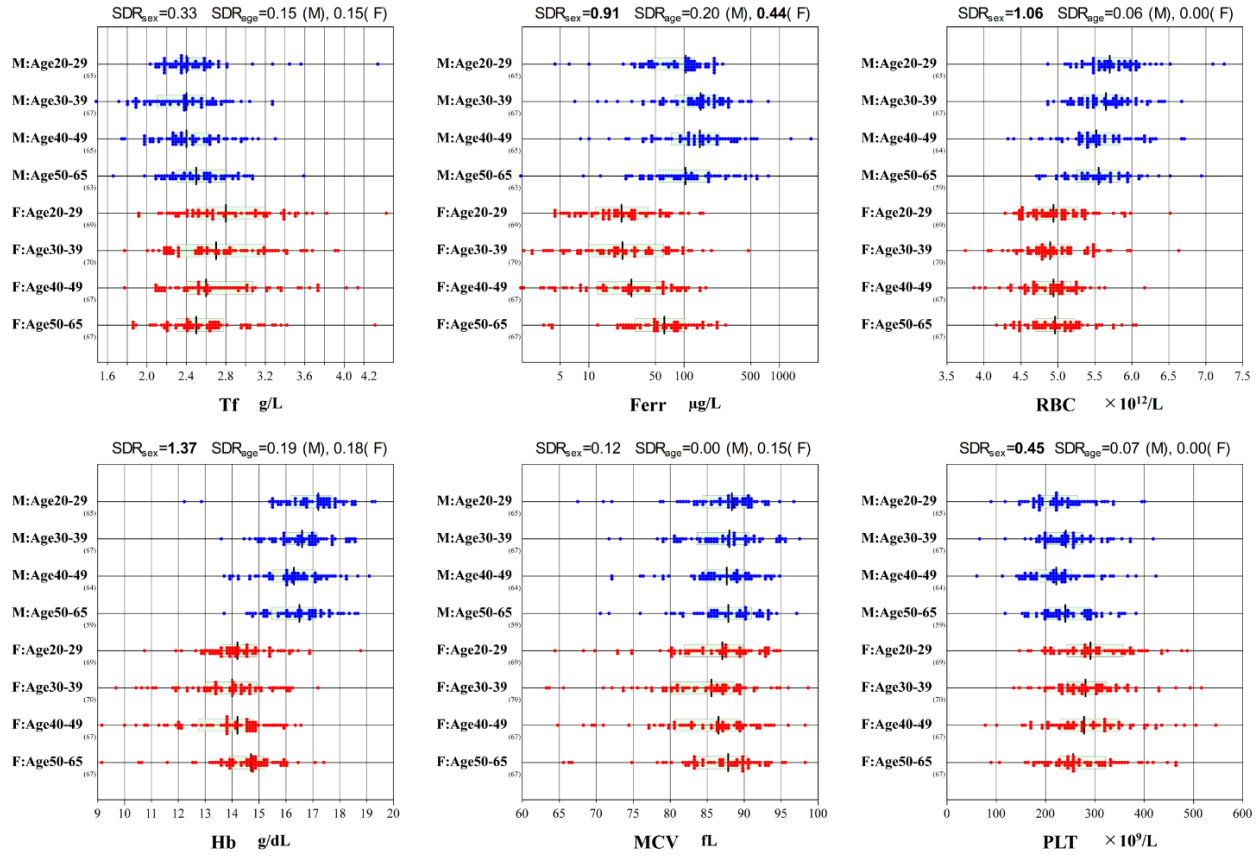


Fig 5.2: Sex and age-related changes for representative parameters

Key-F: female, Ferr: ferritin, Hb: haemoglobin, M: male, MCV: mean corpuscular volume, Plt: platelet count, RBC: red blood cell count, SDR: standard deviation ratio, Tf: transferrin

There was a negative correlation between Hb level and platelet count with the magnitude of the correlation coefficient being greater in females (r=−0.336) than compared to males (r=−0.226) as shown in **Fig 5.3**.

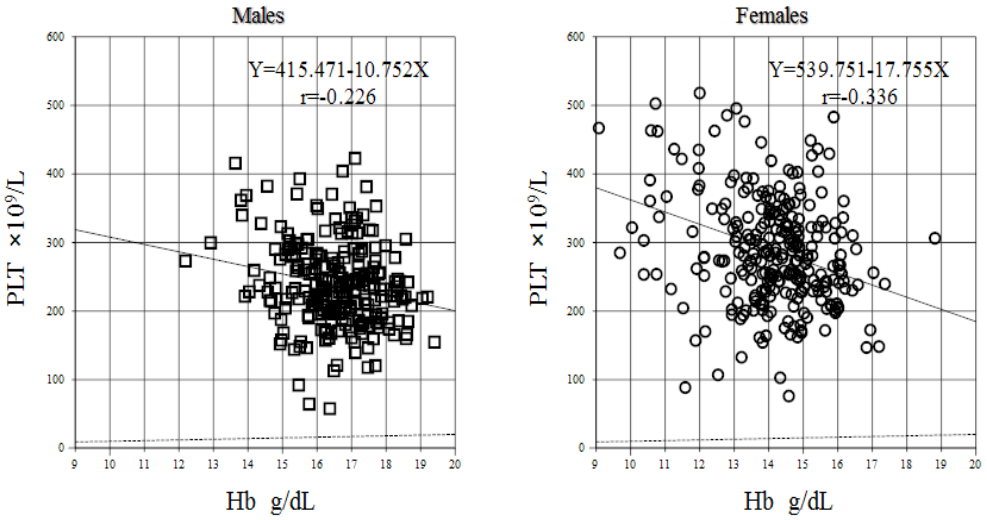


Fig 5.3: Relationship between platelet count and haemoglobin level
Key-Hb: haemoglobin, Plt: platelet

Derivation of reference intervals

Without application of the LAVE method, RIs derived using the non-parametric method generally showed wider ranges and 90% CIs of the RI limits compared to the parametric method. This tendency was more prominently seen in female participants especially for RBC, Hb, Hct, MCV, MCH, RDW and PLT as shown in the graphical representation of RIs in **Fig 5.4** and **Fig S5.1**.

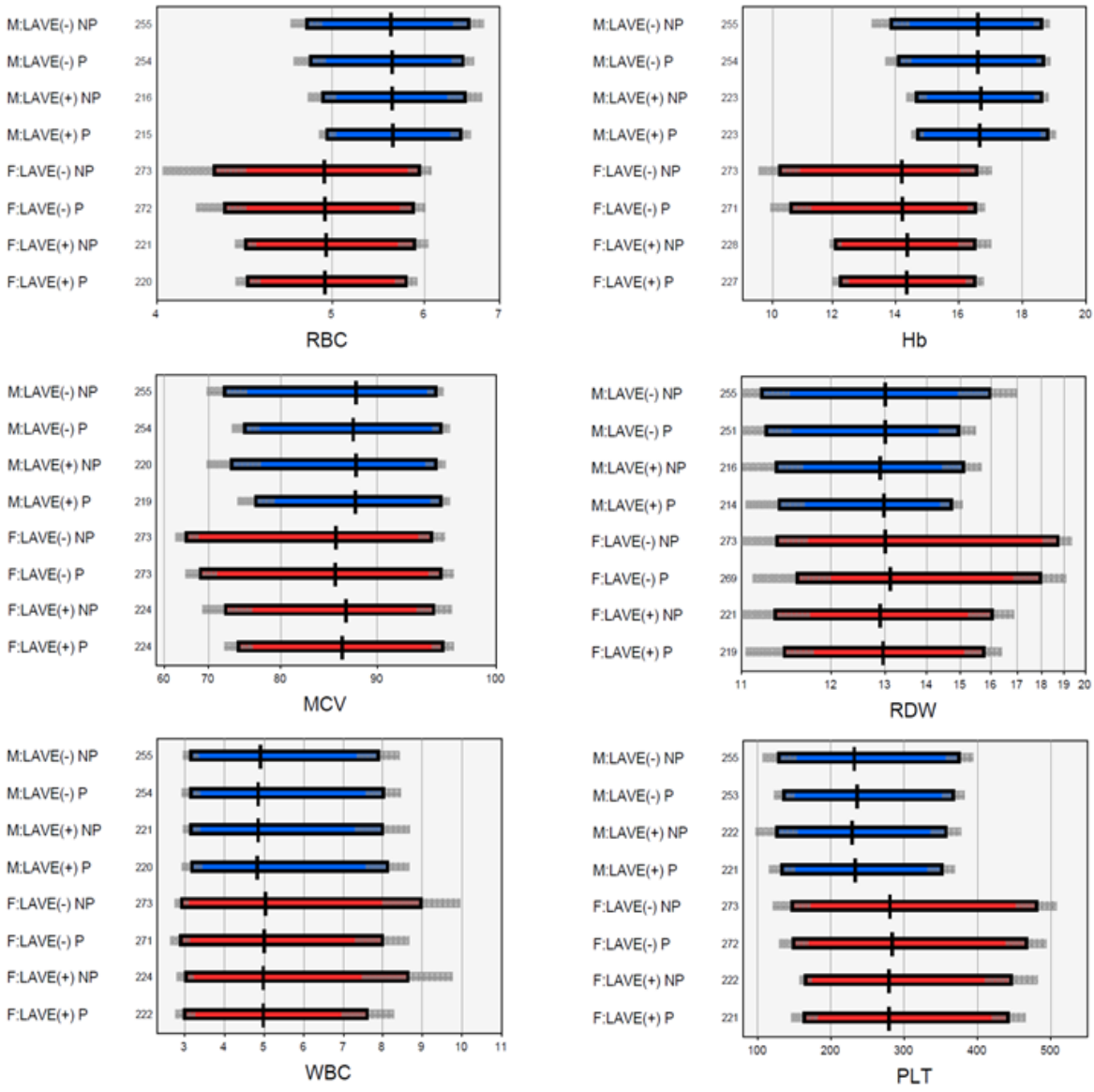


Fig 5.4: Distribution of reference values and reference intervals for selected CBC parameter
Key-F: female, Hb: haemoglobin, LAVE: latent abnormal value exclusion, M: male, MCV: mean corpuscular volume, Plt: platelet count, RBC: red blood cell count, RDW: red cell distribution width, WBC: white blood cells

The LAVE procedure had a greater effect in females where it significantly raised the LLs and narrowed the RIs for Hb, Hct, MCV, MCH and MCHC compared to males where the LAVE resulted in a minor change in the LLs of RBC parameters. **Table S1** shows the SDRs for the change in LLs and ULs after application of the LAVE method. An SDR greater than 0.3 was considered a significant change in LL or UL by use of the LAVE method. On the other hand, the LAVE method didn't significantly alter LLs for WBC parameters but reduced ULs for absolute basophil and neutrophil counts.

The parametrically derived female RI for Hb before applying the LAVE method was 10.6-16.5 g/dL but changed to 12-16.5 g/dL after applying the method. In males, the LAVE method changed the parametrically derived Hb RI from 14.2-18.7 g/dL to 14.5-18.7 g/dL. RIs for RBC related parameters were consistently lower in females as shown in Table 3. RIs for PLT were consistently higher in females with or without the LAVE method as shown in **Table 5.3, Fig S5.2** and **Table S5.2**. The RIs that we chose to adopt based on whether sex was a major source of variation are highlighted in bold in **Table 5.3**.

Table 5.3: Parametric complete blood count reference intervals after latent abnormal value exclusion

	Male + Female		Male		Female	
Item	**N**	**LL- UL**	**N**	**LL-UL**	**N**	**LL-UL**
RBC (x10^12/L)	463	4.41-6.48	226	**4.94-6.52**	229	**4.31-5.76**
Hb (g/dL)	470	12.8-19.0	232	**14.5-18.7**	236	**12.0-16.5**
Hct (L/L)	471	0.38-0.55	232	**0.43-0.55**	237	**0.36-0.49**
MCV (fl)	466	**75.7-95.6**	228	76.5-95.5	232	73.4-95.8
MCH (pg)	462	**24.8-32.8**	227	25.1-32.8	230	24.4-32.7
MCHC (g/dL)	461	**32.2-35.2**	227	32.4-35.4	230	32.0-35.0
RDW (%)	460	**11.3-15.2**	225	11.3-14.7	228	11.4-15.8
WBC (x10^9/L)	464	**3.08-7.83**	229	3.13-8.10	232	2.89-7.72
Neu (%)	463	**28.0-63.3**	227	27.4-60.3	230	29.5-65.4
Lym (%)	463	**27.2-60.0**	227	28.2-60.3	230	25.5-59.3
Mon (%)	461	3.4-13.3	226	**3.5-14.3**	229	**3.2-11.0**
Eos (%)	455	**1.1-11.9**	225	1.2-11.8	227	0.8-9.4
Bas (%)	456	**0.30-1.10**	224	0.40-1.20	228	0.30-1.00
Neu (x10^9/L)	460	**1.05-4.08**	227	1.02-3.92	229	1.07-4.42
Lym (x10^9/L)	461	**1.29-3.40**	226	1.36-3.58	230	1.22-3.24
Mon (x10^9/L)	462	0.14-0.74	227	**0.15-0.76**	229	**0.14-0.68**
Eos (x10^9/L)	460	**0.04-0.59**	226	0.05-0.64	228	0.04-0.49
Bas (x10^9/L)	461	**0.01-0.07**	226	0.01-0.08	224	0.01-0.06
PLT (x10^9/L)	464	144-409	231	**133-356**	232	**152-443**

Key-%: percentage, LL: lower limit, UL: upper limit, RBC: red blood cell count, Hb: haemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red cell distribution width, WBC: white blood cell count, Neu: neutrophil, Lym: lymphocyte, Mon: monocyte, Eos: eosinophil, Bas: basophil, Abs: absolute count, Plt: platelet count. Recommended RIs are shown in bold.

A graphical representation of CBC RIs for selected studies carried out in SSA is given in **Figs 5.5 and 5.6** to enable comparison with our derived RIs. A summary of the same is provided as **Tables S3** and **S4**.

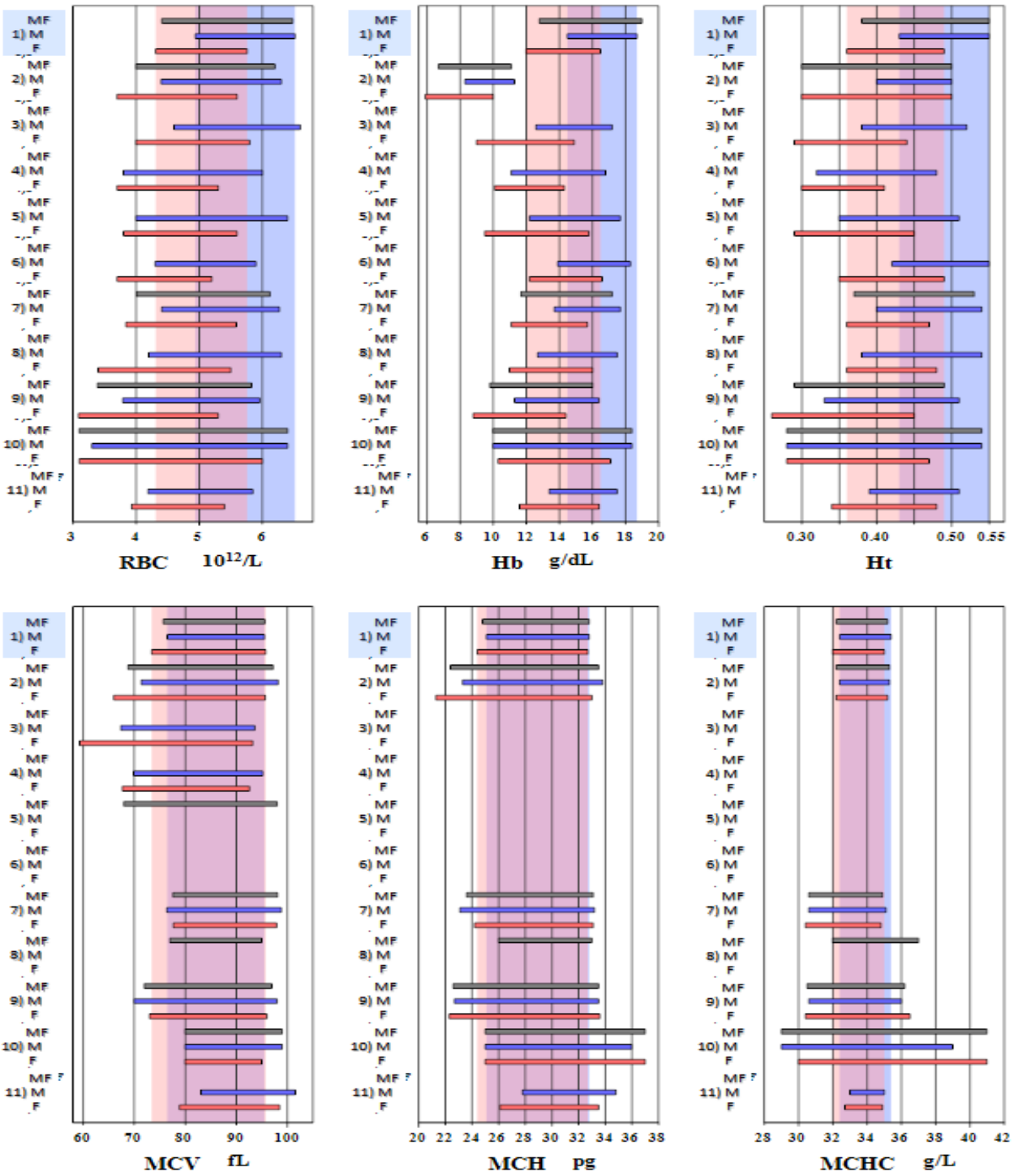


Fig 5.5: Graphical comparison of reference intervals (RIs) for RBC related parameters

Three bars for each study represent RIs for male+female (MF) in grey, male (M) in blue, and female (F) in red. RIs of this study is shown on top of each panel. The background shades in blue and pink represent RIs of this study for males and females.

Key-LL: lower limit, UL: upper limit, RBC: red blood cell count, Hb: haemoglobin, Ht: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration

[1]**Exclusion criteria**: BMI > 35 kg/m^2, consumption of ethanol ≥ 70 g per day, smoking more than 20 tobacco cigarettes per day, chronic illness, recent recovery from acute illness, injury or surgery requiring hospitalization, known carrier state of HBV, HCV or HIV, pregnant or within 1 year after child birth. **CBC analyser**: Beckman Coulter ACT 5 DIFF CP analyser (Brea, California, US).
[2]**Exclusion criteria**: Febrile, pregnant, HIV seropositive, screen positive for syphilis and malaria. **CBC analyser**: ACT 5Diff CP instrument (Beckman Coulter, Fullerton, CA, USA) (Kibaya et al., 2008).
[3]**Exclusion criteri**a: HIV positive, pregnant. **CBC analyser**: Coulter ACT 5Diff CP analyser (Beckman Coulter, France) (Odhiambo et al., 2015).
[4] **Exclusion criteria**: HIV positive, moribund, mentally ill, institutionalized persons, missing personal or laboratory data. **CBC analyser**: Act 5 Diff instrument (Beckman Coulter) (Lugada et al., 2004).
[5] **Exclusion criteria**: Acutely ill, significant findings on physical examination or if laboratory tests revealed that they were pregnant, HIV antibody positive, had evidence of hepatitis B or C infection or suspected syphilis. **CBC analyser**: Beckman Coulter AcT 5 diff CP (Beckman Coulter, USA) (Karita et al., 2009).
[6] **Exclusion criteria**: HIV positive, presence of any illness as defined by the World Health Organization staging systems for HIV infection and disease. **CBC analyser**: Coulter counter T540 (Tsegaye et al., 1999).
[7] **Exclusion criteria**: HIV positive, pregnant or on medication, body temperature ≥37.5^{0}C or if clinical assessment revealed other signs or symptoms of disease that could influence the laboratory parameters of interest. **CBC analyser**: Sysmex KX-21N analyser (Sysmex Corp., Kobe, Japan) (Saathoff et al., 2008).
[8] **Exclusion criteria**: Alcohol abuse, medication, smoking, pregnant, breastfeeding, on oral contraception, on menses. **CBC analyser**: Coulter AcT 5diff and Sysmex KX-21N (Sysmex Corporation, Kobe, Japan) (Gahutu, 2013).
[9] **Exclusion criteria**: Acute or chronic respiratory, cardiovascular, gastrointestinal, hepatic or genitourinary conditions, blood donation or transfusion within the past 3 months, hospitalisation within past 1 month, any findings that would compromise laboratory parameters, pregnant or lactating mothers. **CBC analyser**: Micros 60 analysers (Horiba-ABX, Montpellier, France) (Dosoo et al., 2012).
[10] **Exclusion criteria**: HIV, HBV, and HCV viral infection, malaria, abnormal haemoglobin electrophoresis screening, presence of hypochromia. **CBC analyser**: Sysmex SF-3000 (Sysmex, Kobe, Japan) (Kueviakoe, Segbena, Jouault, Vovor, & Imbert, 2011).
[11] **Exclusion criteria**: HIV, current clinical symptoms, immunosuppressive or corticosteroid medication, chemotherapy, hospitalizations, surgery or blood transfusions in the six months prior to screening, splenomegaly, pregnant, Hb < 12 g/dL. Menstruating women returned in 2 weeks. **CBC analyser**: Beckman Coulter LH 750 (Beckman Coulter, Fullerton, CA, USA) (Lawrie et al., 2009).

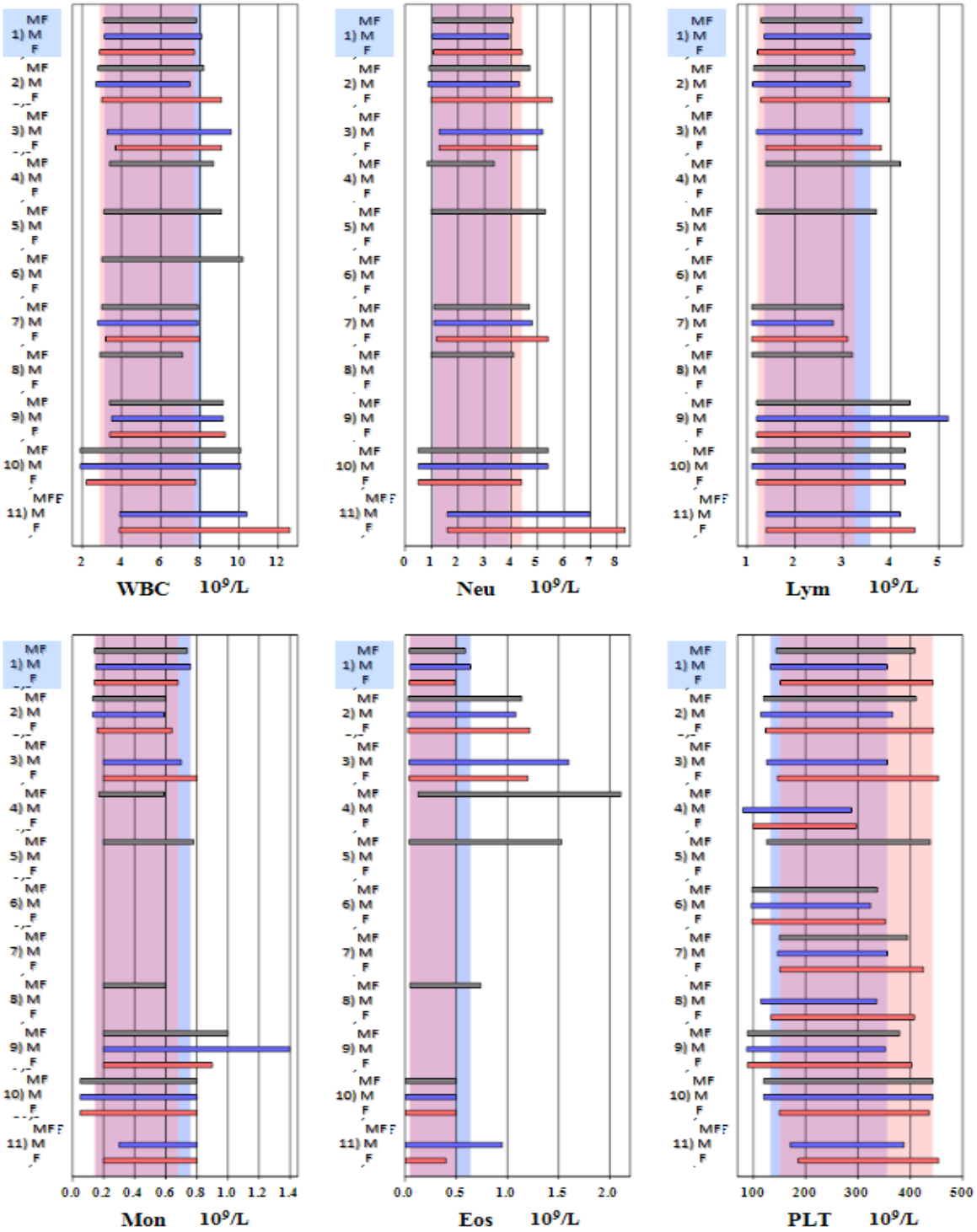


Fig 5.6: Graphical comparison of reference intervals (RIs) for WBC related parameters and platelets

Three bars for each study represent RIs for male+female (MF) in grey, male (M) in blue, and female (F) in red. RIs of this study is shown on top of each panel. The background shades in blue and pink represent RIs of this study for males and females.

Key-WBC: white blood cell count, Neu: Neutrophil, Lym: lymphocyte, Mon: monocyte, Eos: eosinophil, abs-absolute count, Plt-platelet count

[1]**Exclusion criteria**: BMI > 35 kg/m^2, consumption of ethanol ≥ 70 g per day, smoking more than 20 tobacco cigarettes per day, chronic illness, recent recovery from acute illness, injury or surgery requiring hospitalization, known carrier state of HBV, HCV or HIV, pregnant or within 1 year after child birth. **CBC analyser**: Beckman Coulter ACT 5 DIFF CP analyser (Brea, California, US).
[2]**Exclusion criteria**: Febrile, pregnant, HIV seropositive, screen positive for syphilis and malaria. **CBC analyser**: ACT 5Diff CP instrument (Beckman Coulter, Fullerton, CA, USA) (Kibaya et al., 2008).
[3]**Exclusion criteri**a: HIV positive, pregnant. **CBC analyser**: Coulter ACT 5Diff CP analyser (Beckman Coulter, France) (Odhiambo et al., 2015).
[4] **Exclusion criteria**: HIV positive, moribund, mentally ill, institutionalized persons, missing personal or laboratory data. **CBC analyser**: Act 5 Diff instrument (Beckman Coulter) (Lugada et al., 2004).
[5] **Exclusion criteria**: Acutely ill, significant findings on physical examination or if laboratory tests revealed that they were pregnant, HIV antibody positive, had evidence of hepatitis B or C infection or suspected syphilis. **CBC analyser**: Beckman Coulter AcT 5 diff CP (Beckman Coulter, USA) (Karita et al., 2009).
[6] **Exclusion criteria**: HIV positive, presence of any illness as defined by the World Health Organization staging systems for HIV infection and disease. **CBC analyser**: Coulter counter T540 (Tsegaye et al., 1999).
[7] **Exclusion criteria**: HIV positive, pregnant or on medication, body temperature ≥37.5^{0}C or if clinical assessment revealed other signs or symptoms of disease that could influence the laboratory parameters of interest. **CBC analyser**: Sysmex KX-21N analyser (Sysmex Corp., Kobe, Japan) (Saathoff et al., 2008).
[8] **Exclusion criteria**: Alcohol abuse, medication, smoking, pregnant, breastfeeding, on oral contraception, on menses. **CBC analyser**: Coulter AcT 5diff and Sysmex KX-21N (Sysmex Corporation, Kobe, Japan) (Gahutu, 2013).
[9] **Exclusion criteria**: Acute or chronic respiratory, cardiovascular, gastrointestinal, hepatic or genitourinary conditions, blood donation or transfusion within the past 3 months, hospitalisation within past 1 month, any findings that would compromise laboratory parameters, pregnant or lactating mothers. **CBC analyser**: Micros 60 analysers (Horiba-ABX, Montpellier, France) (Dosoo et al., 2012).
[10] **Exclusion criteria**: HIV, HBV, and HCV viral infection, malaria, abnormal haemoglobin electrophoresis screening, presence of hypochromia. **CBC analyser**: Sysmex SF-3000 (Sysmex, Kobe, Japan) (Kueviakoe et al., 2011).
[11] **Exclusion criteria**: HIV, current clinical symptoms, immunosuppressive or corticosteroid medication, chemotherapy, hospitalizations, surgery or blood transfusions in the six months prior to screening, splenomegaly, pregnant, Hb < 12 g/dL. Menstruating women returned in 2 weeks. **CBC analyser**: Beckman Coulter LH 750 (Beckman Coulter, Fullerton, CA, USA) (Lawrie et al., 2009)

5.5 Discussion

Several studies carried out in Africa have reported marked differences in some CBC parameters when compared to Caucasian populations and even between different populations across Africa as shown in S3 and S4 Tables. A fairly consistent finding has been the lower RIs for absolute neutrophil counts in black African populations which is thought to be associated with the DARC-null genotype, an evolutionary adaptation thought to make Africans less susceptible to *Plasmodium vivax* infections (Thobakgale & Ndung'u, 2014). In contrast to RIs from the US where the absolute neutrophil count RI is 1800−7700 cells/µL (Kratz et al., 2004), the parametrically derived neutrophil count RI for males and females in our study was significantly lower at 1050−4080 cells/µL, which is in keeping with what has been found in other African studies (Karita et al., 2009; Kibaya et al., 2008; Saathoff et al., 2008). Neutrophil count RIs with LLs as low as 500 and 840 cells/uL have been reported in Togo and Uganda respectively as shown in S4 Table.

Studies carried out in largely rural populations in SSA have revealed low Hb levels especially in women. A study done in Kericho which is a rural town in Kenya located in The Great Rift Valley at an altitude of over 2000 metres above sea level derived a Hb lower RI limit in women of 5.9 g/dL (Kibaya et al., 2008) while a study by Karita et al. that involved 4 countries in SSA found a LL of 9.1 g/dL (Segolodi et al., 2014). It is inconceivable that a Hb of 5.9 g/dL would be accepted as a LL even if clinically one is not presenting with symptoms of anaemia. In the study by Karita et al., the Hb values from one of the research sites situated in a university teaching hospital in Nairobi, Kenya were excluded as they were significantly higher than the other study sites. Other than the comparatively higher altitude of Nairobi, the authors hypothesized that the Hbs of participants from Nairobi were higher as they comprised mainly medical doctors and students considered to be healthier due to better nutrition and access to healthcare. For this reason, the RVs from Nairobi were subsequently excluded in the derivation of the consensus RI. This secondary exclusion of 'healthier' individuals may partly explain the low RI for Hb they derived (Karita et al., 2009). According to the World Health Organization (WHO), a woman above 15 years of age with a Hb that is less than 12 g/dl is considered anaemic (WHO, 2011a). Therefore, a large proportion of women recruited in a number of studies summarized in Fig 5 were anaemic and given the low RIs for MCV and MCH, the anaemia is in keeping with a microcytic hypochromic picture commonly seen in iron deficiency. In SSA iron deficiency in women is likely due to poor nutrition or blood loss as a result of menses or repeated childbirth.

We carefully recruited an urban population for our study using strict recruitment criteria, and derived a RI for Hb in women of 12.0−16.5 g/dL after application of the LAVE method. This RI for women is higher than that reported in most SSA studies but similar to published RIs from the US of 12.0−16.0 g/dL (Kratz et al., 2004). The UL for the parametrically derived RI for male Hb after LAVE was 18.7 g/dL which is significantly higher than what has been reported from most studies in SSA but similar to the ULs of 18.3 and 18.4 g/dL reported from Ethiopia and Togo respectively (Kueviakoe et al., 2011; Tsegaye et al., 1999). This most likely reflects our careful attempt to recruit well-defined healthy individuals and secondarily exclude individuals with possible sub-clinical diseases by use of the LAVE method. This resulted in an increase in the LLs of RBC parameters and narrowing of RIs especially for female participants. This may explain the higher LLs for these parameters in our study compared to other SSA studies that didn't employ strict recruitment criteria or secondary exclusion. The systematic

removal of individuals with possible sub-clinical disease makes our results more generalizable to an urban healthy black African population in Kenya. The high UL for Hb seen especially in males in this study could be attributed to Nairobi's relatively high altitude of 1700 metres above sea level.

For the comparison of parametric and nonparametric methods, as shown in **Table S5.2**, the nonparametric method generally resulted in a wider RI, and wider 90% CIs of the RI limits. It is attributable to the fact that parametric method has a step of exclusion of values outside mean±3.5SD (probability: 0.00043 or 0.043%) after Gaussian transformation, as shown by a small reduction in data size for the parametric method in most of the CBC parameters. This clearly indicates the presence of extreme values not explained by chance, at the periphery of the distribution. The recommendation by CLSI (CLSI, 2008) that the nonparametric method be used in determining RIs was due to the difficulty of the parametric method to ensure successful Gaussian transformation by use of the Box-Cox power transformation formula. However, Ichihara and Boyd proved that by use of a modified Box-Cox formula with addition of transformation origin in the original formula led to a dramatic change in goodness-of-fit of the transformation (Ichihara & Boyd, 2010). Besides, the optimal value of power for Gaussian transformation predicted by the parametric method is fairly consistent analyte by analyte (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017), a fact supporting the reliability of the parametric method. In fact, for the RVs of all the analytes, the parametric method successfully led to a Gaussian shape judged from values of skewness and kurtosis as well as from the linearity in the probability paper plot (S3 Fig). These are the reasons for our adoption of RIs derived by the parametric method.

A decline in Hb level was associated with an increase in platelet count. Iron deficiency anaemia (IDA) and iron deficient erythropoiesis have been associated with secondary thrombocytosis. Conversely, iron supplementation in iron deficient individuals has been shown to increase Hb levels while reducing platelet count (Kulnigg-Dabsch et al., 2013). This inverse relationship between Hb level and platelet count may explain the higher median platelet count in women in this study as well as some of the other published studies from SSA (Kibaya et al., 2008; Saathoff et al., 2008). Schloesser et al. compared platelet counts in patients with IDA to healthy controls and found an average platelet count of 499,000/µL in IDA patients compared to 242,000/µL in controls (Schloesser et al., 1937). The mechanism behind the relationship between Hb level and platelet count isn't well understood. Various hypothesis have been put forward including homology between erythropoietin and thrombopoietin resulting in proliferation and differentiation of pluripotent erythroid and megakaryocyte precursors,

synergistic action of erythropoietin and thrombopoietin in megakaryocyte maturation and a direct effect of iron on thrombopoiesis (Bilic & Bilic, 2003; Broudy, Lin, & Kaushansky, 1995). Indeed, serum iron and ferritin levels were lower in female participants in our study while transferrin levels were higher, a common finding in iron deficient states. Sex was a significant source of variation in platelet counts (SDRsex=0.45) in this study and therefore we adopted sex specific RIs for PLT. As shown in **Fig 5.6**, women generally have higher platelet counts than men.

The UL for the parametrically derived absolute eosinophil count in our study was 590 cells/ µL which is much lower than that reported by Karita et al. (1530 cells/µL) and Kibaya et al. (1140 cells/ µL) both of whom did studies in Kenya but recruited largely a rural population (Karita et al., 2009; Kibaya et al., 2008). It is possible that the higher eosinophil counts in the rural population is due to a bigger burden of parasite infection or greater exposure to environmental allergens. Karita et al. mentions that in a sub-study done in Lusaka-Zambia, Entebbe-Uganda and Kigali-Rwanda, up to one third of stool samples had ova or parasites possibly explaining the higher eosinophil counts (Karita et al., 2009). Given that our study population was largely comprised of an urban working population, the prevalence of stool parasites would be expected to be low. Measurement of serum IgE levels and evaluation of stool samples would have provided objective evidence to explore this hypothesis.

We used SDRs to determine whether sex or age were significant sources of variation necessitating partitioning of RIs. This is a method widely adopted in the studies by Ichihara *et al.* and is useful when evaluating more than one source of variation, especially when any source of variation consists of more than 2 categories (Ichihara, 2014; Ichihara et al., 2008). An unexpected finding was the decline in monocyte levels with increase in age for female participants (**Fig S5.1**). With lack of reports on similar observation, no obvious explanation is forthcoming, hence further studies are required to investigate the consistency of this finding and its significance.

RIs are used in developing guidelines for defining laboratory adverse events to be used when enrolling and following up patients in a clinical trial. In many HIV clinical trials, the Division of AIDS (DAIDS), National Institute of Allergy and Infectious Disease/National Institutes of Health adverse event tables are used to evaluate toxicities (NIH, 2014). As highlighted by Karita et al., adopting the 2004 DAIDS grading criteria would inadvertently misclassify many healthy study volunteers as having adverse events at the time of clinical trial recruitment. In the study, 319 (15.2%) individuals would have been

misclassified as having a haematology laboratory adverse event which would have resulted in their unnecessary exclusion from the study (Karita et al., 2009). Given the marked variability in certain CBC parameters across different populations, population specific RIs should be used when defining laboratory based adverse events for clinical trials. Failure to do so will not only result in inappropriate exclusion or inclusion of study participants but misreporting on the incidence of toxicities.

The major strength of this study is the careful recruitment of healthy individuals with an even distribution of age and sex. Unlike most of the other RI studies conducted in SSA, we had strict exclusion criteria and further carried out secondary exclusion which significantly reduced the influence of individuals with latent anaemia or inflammation. This increases the external validity of the results in so far as extrapolating to an adult urban black African population in Kenya is concerned.

One of the limitations of this study was that sample analysis was done only on a Beckman Coulter analyzer hence the transferability of the RIs is limited if between-analyzer bias exists in test results. Secondly, our recruitment comprised largely an urban population, the vast majority from the capital city Nairobi and surrounding counties whose socio-economic status and lifestyles are different from a rural population. Some of the RIs may therefore not be directly applicable to a rural community. Another limitation is that we relied on self-reporting of chronic illnesses including those of an infectious aetiology. It is possible that some participants may not have provided accurate information and as such may have ended up being included inadvertently. We however tried to minimize the effect of such inclusion by secondarily excluding individuals whose test results suggested the possibility of sub-clinical disease.

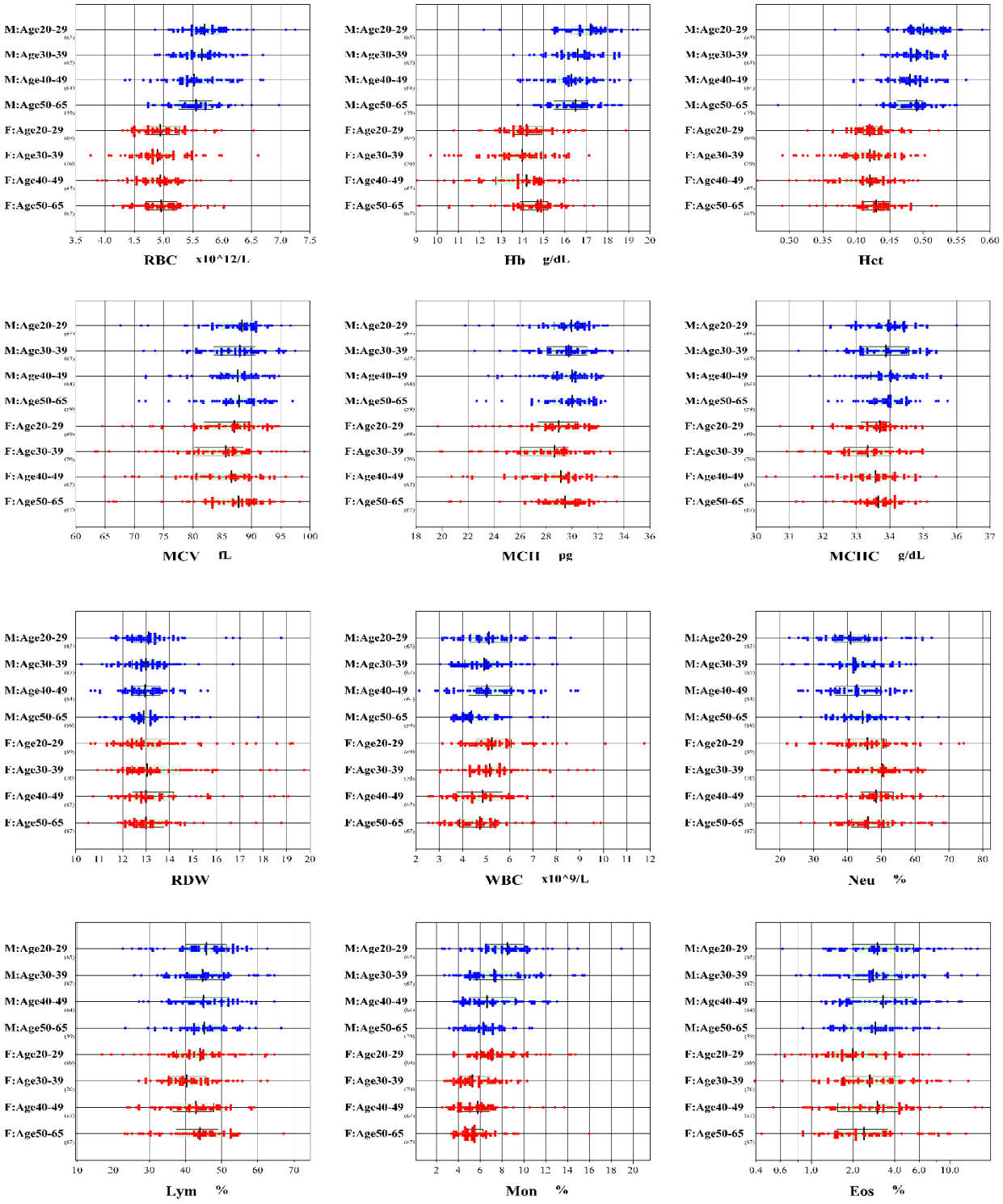
Conclusion

The present study highlights marked differences in certain CBC parameters such as Hb, eosinophil and platelet counts compared to other SSA countries and lower neutrophil counts compared to the US. To the best of our knowledge this is the first study from Africa that has used the LAVE procedure to determine RIs for CBC and we do believe that the secondary exclusion of individuals with possible sub-clinical disease makes our RIs more representative of a healthy urban Kenyan population. Our study results can serve as a reference for laboratories in SSA especially in situations where conducting a formal RI study may not be feasible.

Acknowledgments

Jared Oseko and Patricia Ingato of Aga Khan University Hospital Nairobi assisted in sample collection, bar coding and processing. Benjamin Matheka, marketing manager PathCare Kenya Limited, assisted in participant recruitment and sample collection. Arno Theron carried out sample analysis, quality assurance and data handling at the PathCare reference laboratory in Cape Town, South Africa.

5.6 Supporting information



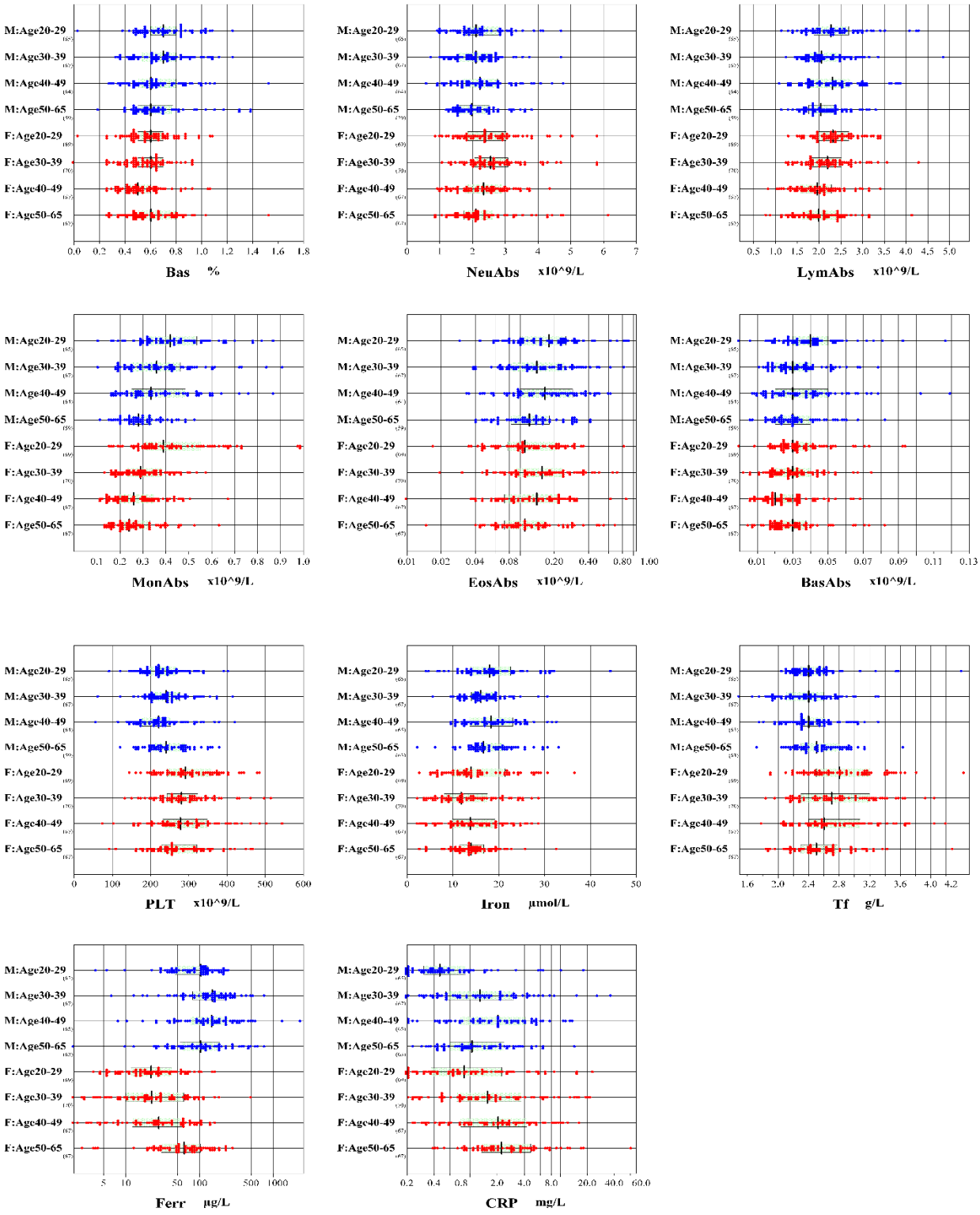
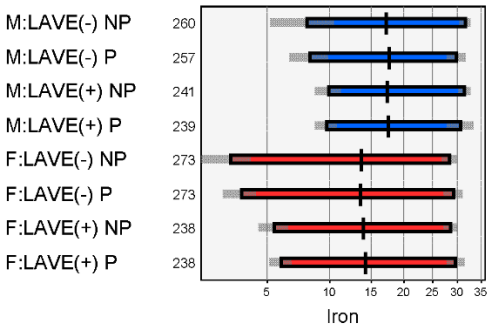
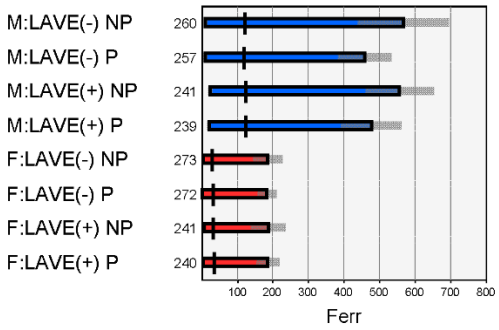
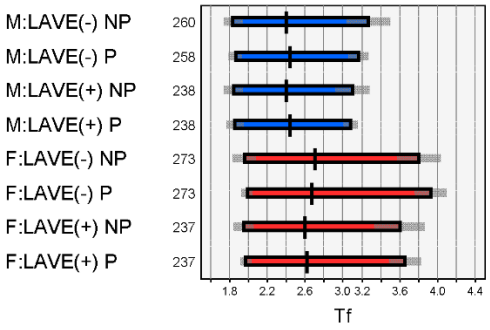
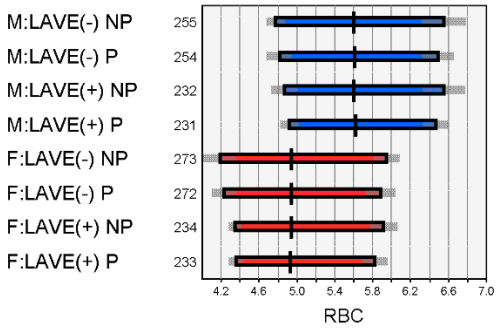


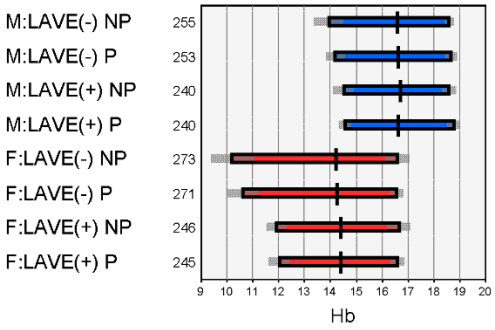
Fig S5.1: Age and sex distribution of anaytes

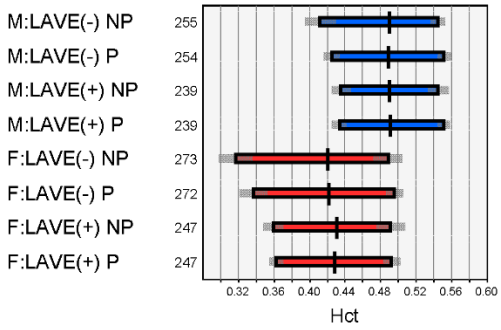


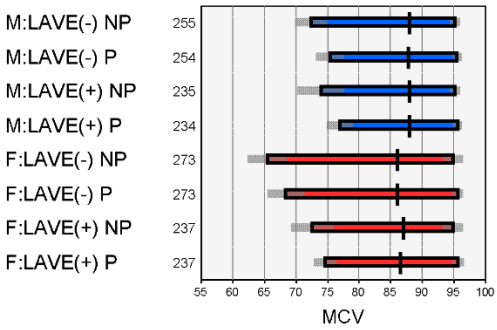


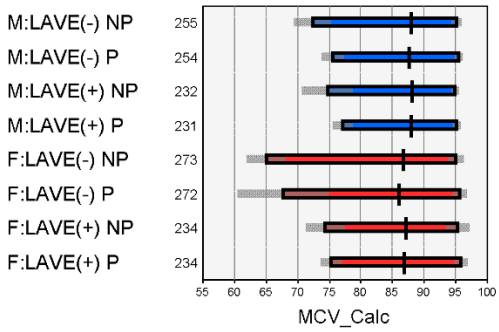












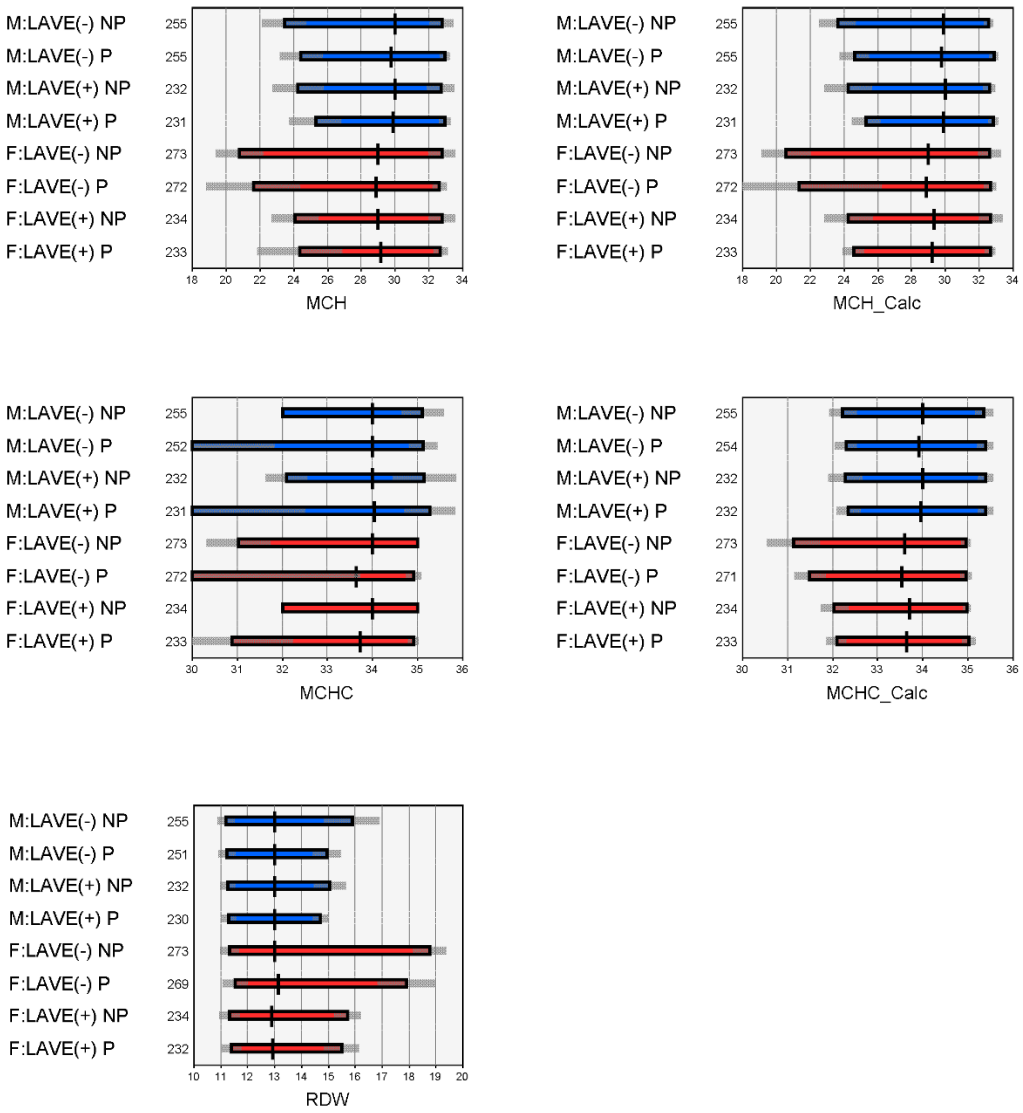
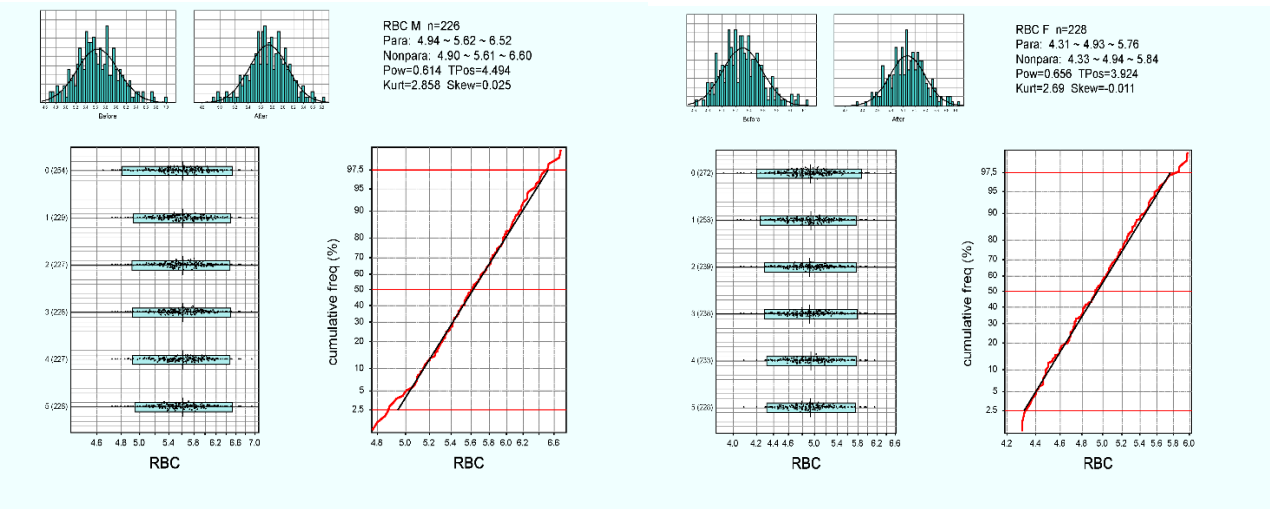
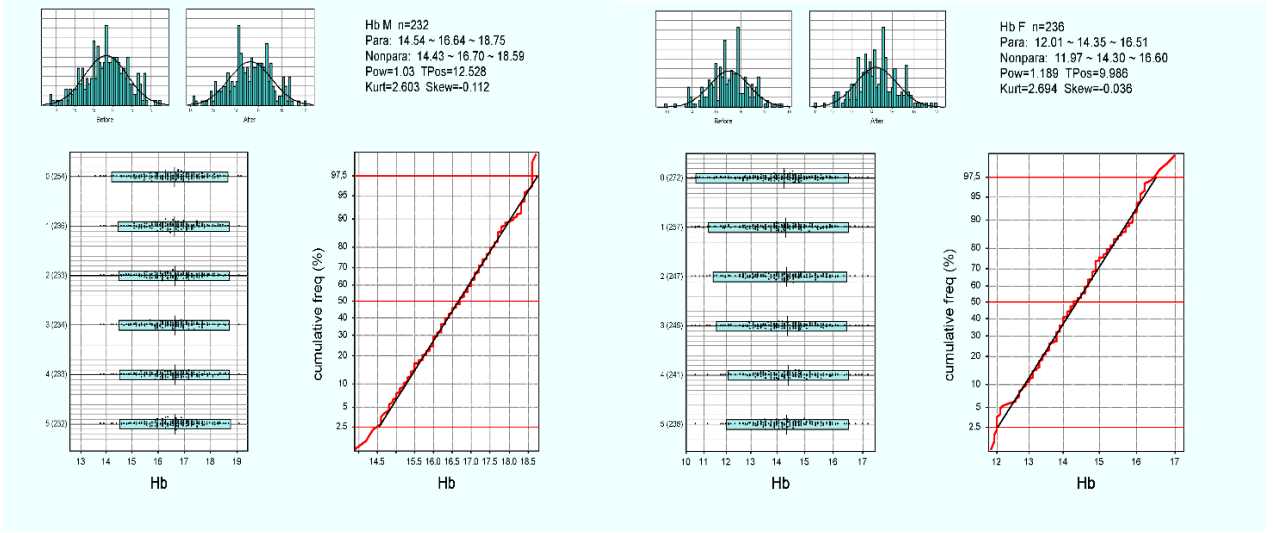
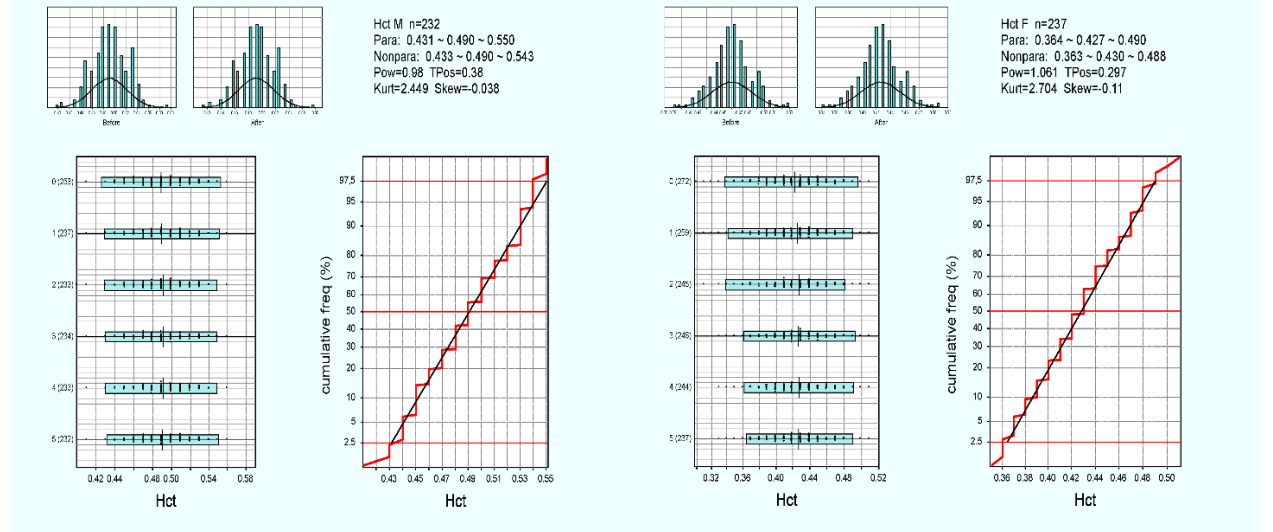
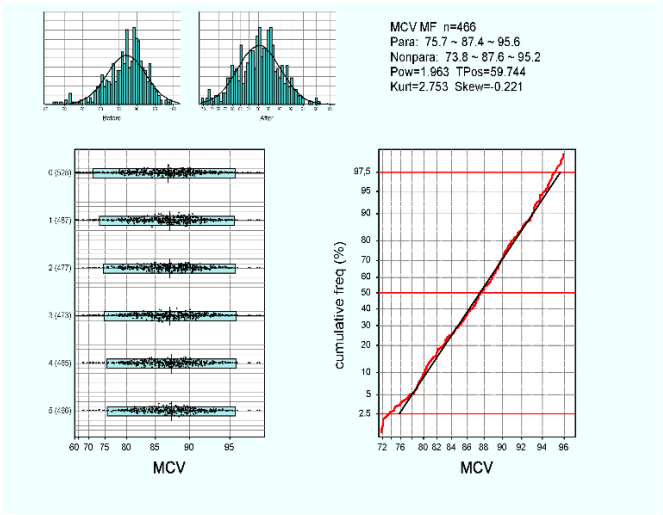


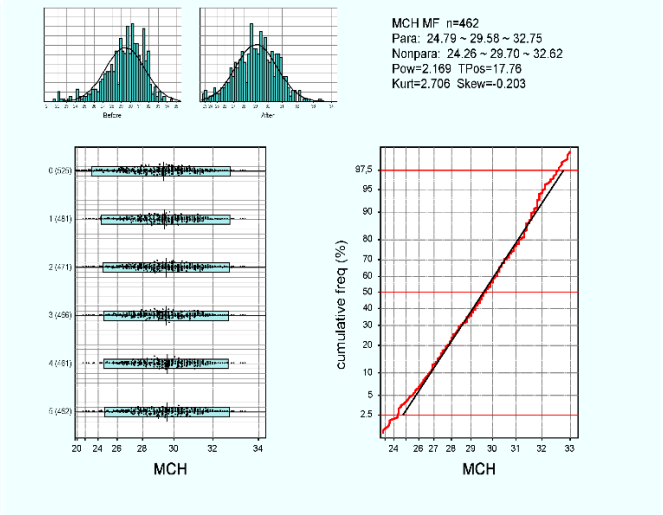
Fig S5.2: Reference intervals before and after latent abnormal value exclusion

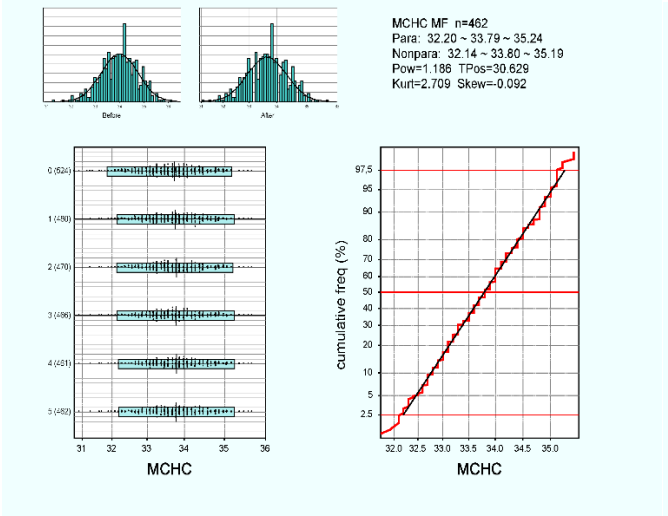


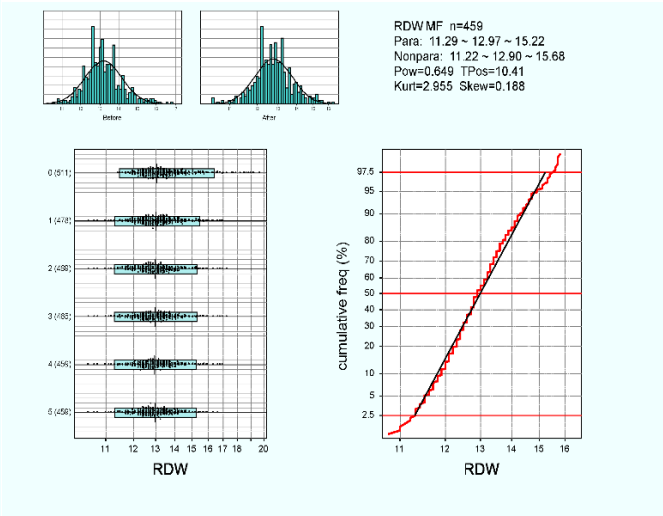


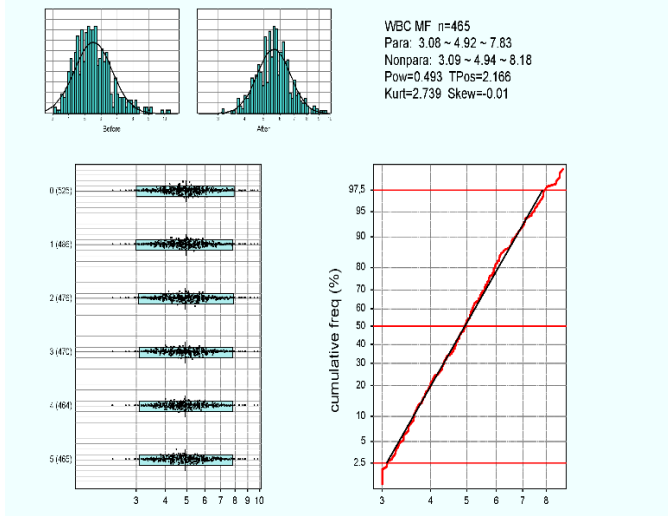


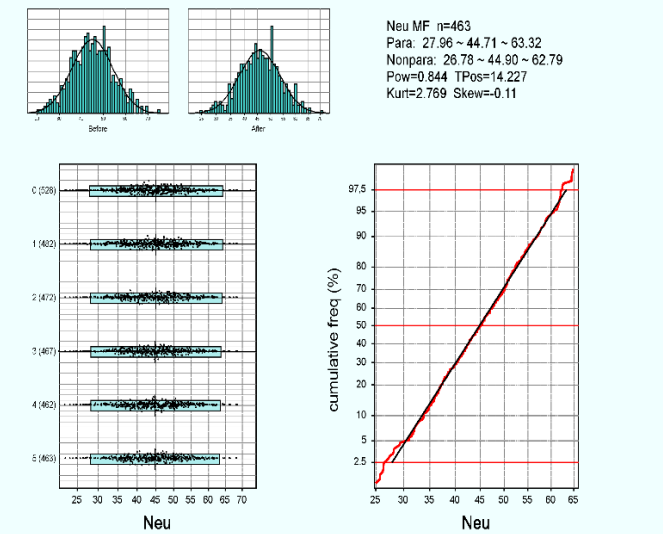


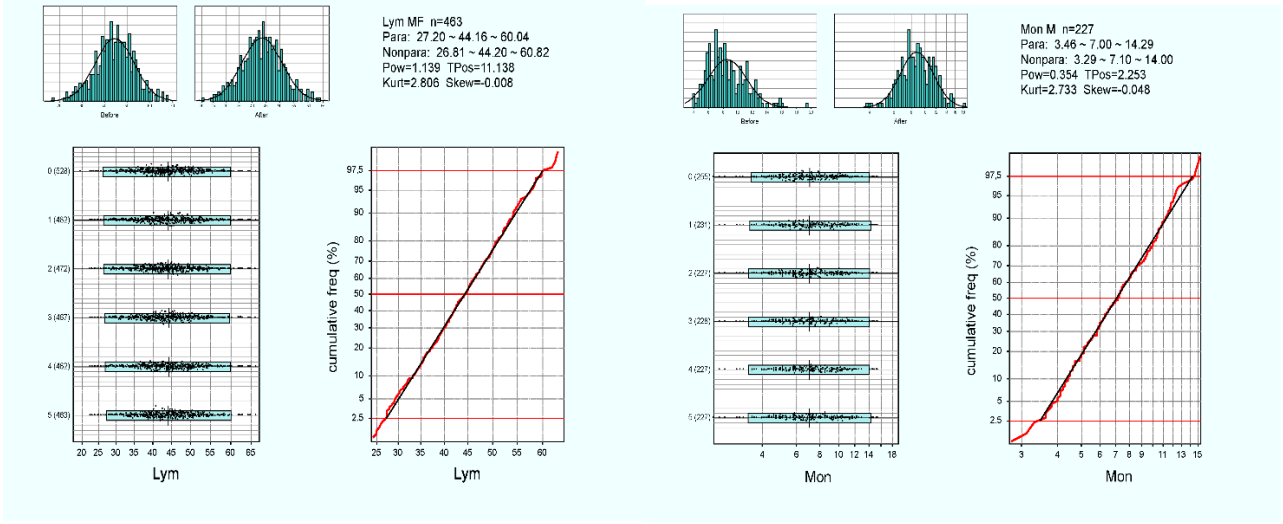


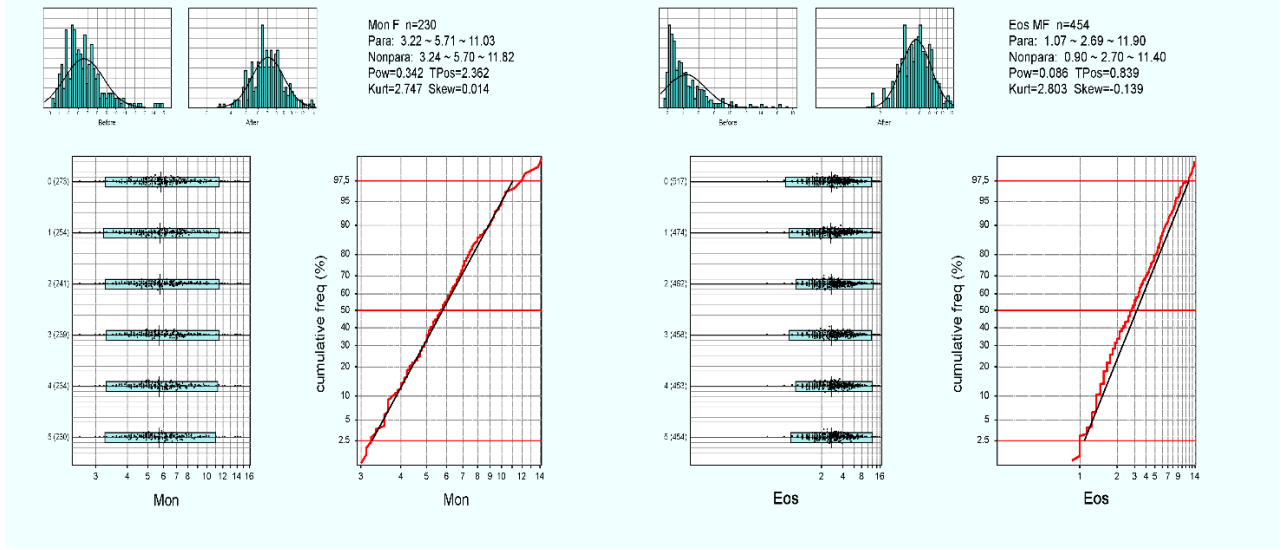


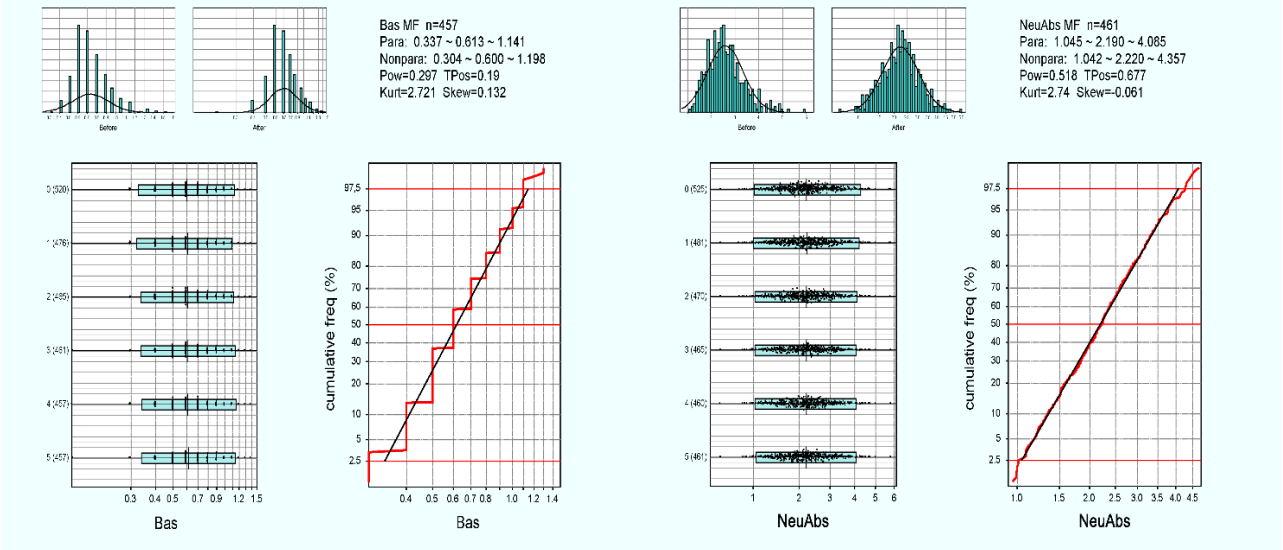


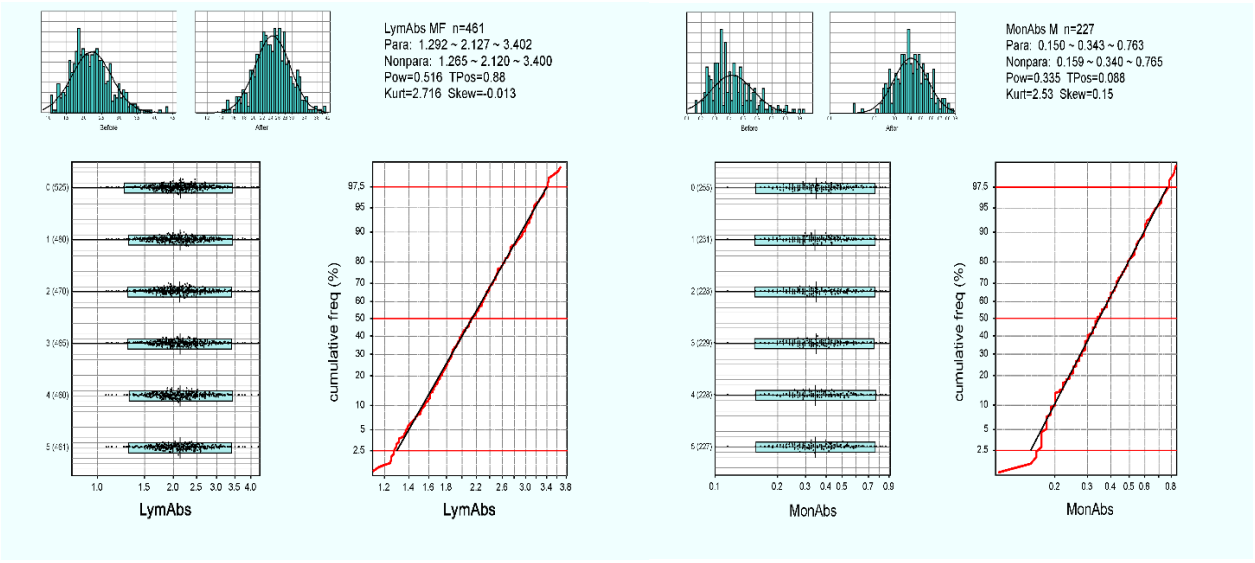


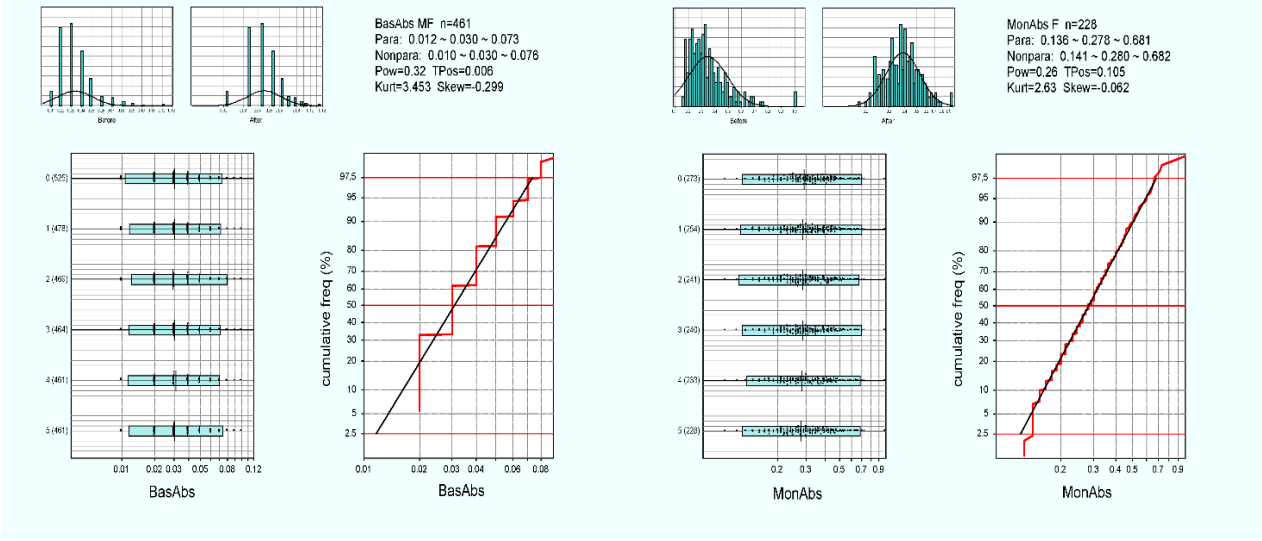


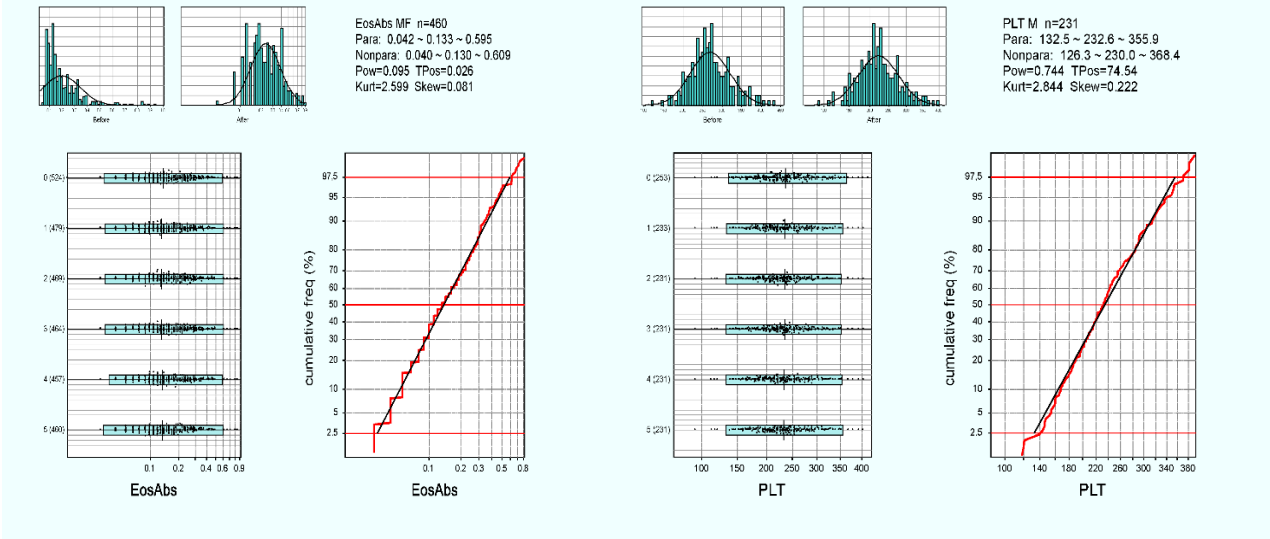












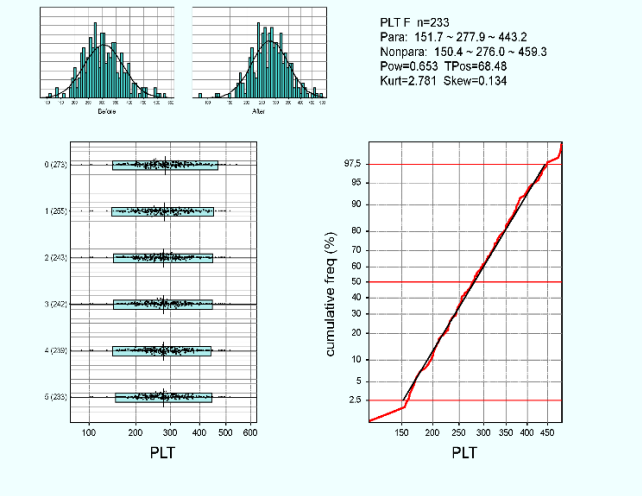


Fig S5.3: Normalization of analyte data

Table S5.1: Standard deviation ratios for change in upper and lower limits of reference intervals by use of the LAVE method

	Parametric						Non-parametric					
	Male + Female		Male		Female		Male + Female		Male		Female	
Item	**LL**	**UL**	**LL**	**UL**	**LL**	**UL**	**LL**	**UL**	**LL**	**UL**	**LL**	**UL**
RBC	0.25	0.09	**0.32**	0.00	**0.30**	**0.30**	0.22	0.06	0.28	0.02	**0.47**	0.23
Hb	**1.01**	0.32	0.28	0.00	**1.22**	0.00	**1.28**	0.00	0.37	0.00	**1.53**	0.09
Hct	**0.69**	0.00	0.00	0.00	**0.60**	**0.30**	**0.98**	0.00	**0.71**	0.00	**1.21**	0.00
MCV	**0.85**	0.00	0.19	0.02	**1.00**	0.04	**1.04**	0.00	0.18	0.04	**1.10**	0.03
MCH	**0.78**	0.00	**0.31**	0.05	**1.13**	0.00	**1.09**	0.00	0.28	0.00	**1.11**	0.04
MCHC	**0.52**	0.00	0.13	0.00	**0.65**	0.00	**0.63**	0.00	0.00	0.13	**0.91**	0.13
RDW	0.20	**1.21**	0.12	0.23	0.09	**1.87**	0.00	**1.92**	0.10	**0.83**	0.00	**2.25**
WBC	0.06	0.09	0.02	0.09	0.00	0.22	0.06	0.18	0.02	0.07	0.02	0.25
Neu %	0.02	0.09	0.05	0.01	0.02	0.05	0.01	0.03	0.02	0.01	0.10	0.03
Lym %	0.12	0.01	0.05	0.09	0.06	0.07	0.07	0.02	0.08	0.07	0.02	0.08
Mon %	0.04	0.08	0.04	0.07	0.00	0.25	0.04	0.04	0.07	0.11	0.05	0.27
Eos %	0.04	0.15	0.00	0.04	0.00	0.23	0.00	0.19	0.00	0.20	0.00	0.00
Bas %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Neu Abs	0.04	0.27	0.05	0.05	0.01	**0.33**	0.05	0.31	0.04	0.18	0.01	**0.47**
Lym Abs	0.09	0.07	0.04	0.12	0.14	0.10	0.07	0.13	0.02	0.29	0.07	0.04
Mon Abs	0.00	0.07	0.00	0.00	0.00	0.07	0.00	0.07	0.00	0.06	0.00	0.07
Eos Abs	0.00	0.07	0.07	0.13	0.00	0.09	0.00	0.00	0.00	0.21	0.00	0.16
Bas Abs	0.00	0.00	0.00	**0.56**	0.00	**0.78**	0.00	**0.56**	0.00	0.00	0.00	**0.78**
PLT	0.05	**0.33**	0.07	0.17	0.08	**0.33**	0.01	**0.51**	0.06	0.08	0.11	0.26

Key-%: percentage, LL: lower limit, Me: median, UL: upper limit, RBC: red blood cell count, Hb: haemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red cell distribution width, WBC: white blood cell count, Neu: neutrophil, Lym: lymphocyte, Mon: monocyte, Eos: eosinophil, Bas: basophil, Abs: absolute count, Plt: platelet count, SDRs > 0.3 are in bold

Table S5.2: Complete blood count reference intervals before and after latent abnormal value exclusion

		Parametric								
		M+F			M			F		
Item	LAVE	n	LL	UL	n	LL	UL	n	LL	UL
RBC	LAVE(-)	528	4.3	6.4	254	4.8	6.5	272	4.2	5.9
	LAVE(+)	463	4.4	6.5	226	4.9	6.5	229	4.3	5.8
Hb	LAVE(-)	527	11.2	18.5	254	14.2	18.7	272	10.6	16.5
	LAVE(+)	470	12.8	19.0	232	14.5	18.7	236	12.0	16.5
Hct	LAVE(-)	528	0.35	0.55	253	0.43	0.55	272	0.34	0.50
	LAVE(+)	471	0.38	0.55	232	0.43	0.55	237	0.36	0.49
MCV	LAVE(-)	527	71.4	95.6	254	75.6	95.4	273	67.7	95.6
	LAVE(+)	466	75.7	95.6	228	76.5	95.5	232	73.4	95.8
MCH	LAVE(-)	526	23.2	32.8	255	24.5	32.9	272	22.0	32.7
	LAVE(+)	462	24.8	32.8	227	25.1	32.8	230	24.4	32.7
MCHC	LAVE(-)	524	31.8	35.2	254	32.3	35.4	271	31.5	35.0
	LAVE(+)	461	32.2	35.2	227	32.4	35.4	230	32.0	35.0
RDW	LAVE(-)	517	11.5	16.4	251	11.2	14.9	270	11.5	17.9
	LAVE(+)	460	11.3	15.2	225	11.3	14.7	228	11.4	15.8
WBC	LAVE(-)	525	3.01	7.94	255	3.15	7.99	270	2.89	7.99
	LAVE(+)	464	3.08	7.83	229	3.13	8.1	232	2.89	7.72
Neu	LAVE(-)	528	27.8	64.1	255	27.0	60.4	273	29.3	65.9
	LAVE(+)	463	28.0	63.3	227	27.4	60.3	230	29.5	65.4
Lym	LAVE(-)	528	26.2	59.9	255	28.6	61.0	273	25.0	58.7
	LAVE(+)	463	27.2	60.0	227	28.2	60.3	230	25.5	59.3
Mon	LAVE(-)	527	3.3	13.1	254	3.6	14.1	272	3.2	11.5
	LAVE(+)	461	3.4	13.3	226	3.5	14.3	229	3.2	11.0
Eos	LAVE(-)	520	1.0	11.5	253	1.2	11.9	270	0.8	9.9
	LAVE(+)	455	1.1	11.9	225	1.2	11.8	227	0.8	9.4
Bas	LAVE(-)	520	0.3	1.1	251	0.4	1.2	270	0.3	1.0
	LAVE(+)	456	0.3	1.1	224	0.4	1.2	228	0.3	1.0
NeuAbs	LAVE(-)	524	1.02	4.29	255	0.98	3.96	271	1.08	4.70
	LAVE(+)	460	1.05	4.08	227	1.02	3.92	229	1.07	4.42
LymAbs	LAVE(-)	526	1.24	3.44	254	1.34	3.65	272	1.15	3.29
	LAVE(+)	461	1.29	3.40	226	1.36	3.58	230	1.22	3.24
MonAbs	LAVE(-)	527	0.14	0.73	255	0.15	0.76	272	0.14	0.69
	LAVE(+)	462	0.14	0.74	227	0.15	0.76	229	0.14	0.68
EosAbs	LAVE(-)	523	0.04	0.58	254	0.04	0.62	270	0.04	0.48
	LAVE(+)	460	0.04	0.59	226	0.05	0.64	228	0.04	0.49
BasAbs	LAVE(-)	525	0.01	0.07	253	0.01	0.07	272	0.01	0.07
	LAVE(+)	461	0.01	0.07	226	0.01	0.08	224	0.01	0.06
PLT	LAVE(-)	525	141	431	254	137	365	272	146	468
	LAVE(+)	464	144	409	231	133	356	232	152	443

Key-%: percentage, LL: lower limit, Me: Median, UL: upper limit, RBC: Red blood cell count, Hb: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RDW: Red cell distribution width, WBC: White blood cell count, Neu: Neutrophil, Lym: Lymphocyte, Mon: Monocyte, Eos: Eosinophil, Bas: Basophil, Abs: absolute count, Plt: platelet count, LAVE: Latent abnormal values exclusion. (-): without LAVE, (+): with LAVE. Reference tests used in applying the LAVE method were Iron, Ferritin, Transferrin, Hb, Hct, MCV, Albumin, Globulins, CRP, WBC and PLT

		Nonparametric								
		M+F			M			F		
Item	LAVE	n	LL	UL	n	LL	UL	n	LL	UL
RBC	LAVE(-)	528	4.3	6.4	255	4.8	6.6	273	4.2	5.9
	LAVE(+)	463	4.4	6.4	227	4.9	6.6	230	4.3	5.8
Hb	LAVE(-)	528	10.8	18.5	255	14.0	18.6	273	10.2	16.5
	LAVE(+)	470	12.7	18.5	232	14.4	18.6	237	12.0	16.6
Hct	LAVE(-)	528	0.34	0.54	255	0.41	0.54	273	0.32	0.49
	LAVE(+)	471	0.38	0.54	232	0.43	0.54	237	0.36	0.49
MCV	LAVE(-)	528	68.1	95.2	255	72.7	95.0	273	64.9	95.2
	LAVE(+)	466	73.8	95.2	228	73.7	95.2	232	71.6	95.4
MCH	LAVE(-)	528	22.0	32.6	255	23.5	32.6	273	20.7	32.6
	LAVE(+)	463	24.3	32.6	227	24.1	32.6	230	23.3	32.5
MCHC	LAVE(-)	528	31.6	35.2	255	32.3	35.3	273	31.2	35.0
	LAVE(+)	463	32.1	35.2	227	32.3	35.4	230	31.9	34.9
RDW	LAVE(-)	528	11.2	17.9	255	11.2	15.9	273	11.3	18.7
	LAVE(+)	463	11.2	15.7	227	11.3	15.1	230	11.3	16.0
WBC	LAVE(-)	528	3.01	8.42	255	3.18	7.87	273	2.92	9.1
	LAVE(+)	467	3.09	8.18	230	3.15	7.96	234	2.95	8.73
Neu	LAVE(-)	528	26.7	63.1	255	26.2	59.9	273	28.0	66.0
	LAVE(+)	463	26.8	62.8	227	26.4	60.0	230	28.9	65.7
Lym	LAVE(-)	528	26.2	60.6	255	27.7	61.2	273	25.0	59.7
	LAVE(+)	463	26.8	60.8	227	27.0	60.6	230	25.2	60.4
Mon	LAVE(-)	528	3.3	13.3	255	3.5	13.7	273	3.3	12.4
	LAVE(+)	463	3.4	13.2	227	3.3	14.0	230	3.2	11.8
Eos	LAVE(-)	528	0.9	10.9	255	1.1	11.2	273	0.7	9.5
	LAVE(+)	463	0.9	11.4	227	1.1	10.7	230	0.7	9.5
Bas	LAVE(-)	528	0.3	1.2	255	0.3	1.3	273	0.3	1.0
	LAVE(+)	463	0.3	1.2	227	0.3	1.3	230	0.3	1.0
NeuAbs	LAVE(-)	528	1.00	4.62	255	0.99	4.05	273	1.03	5.18
	LAVE(+)	463	1.04	4.36	227	1.02	3.92	230	1.04	4.74
LymAbs	LAVE(-)	528	1.23	3.47	255	1.29	3.73	273	1.18	3.34
	LAVE(+)	463	1.27	3.40	227	1.28	3.56	230	1.22	3.32
MonAbs	LAVE(-)	528	0.15	0.73	255	0.16	0.78	273	0.14	0.69
	LAVE(+)	463	0.15	0.74	227	0.16	0.77	230	0.14	0.68
EosAbs	LAVE(-)	528	0.04	0.61	255	0.04	0.64	273	0.04	0.52
	LAVE(+)	463	0.04	0.61	227	0.04	0.61	230	0.04	0.54
BasAbs	LAVE(-)	528	0.01	0.07	255	0.01	0.08	273	0.01	0.07
	LAVE(+)	463	0.01	0.08	227	0.01	0.08	230	0.01	0.06
PLT	LAVE(-)	528	141	450	255	130	373	273	142	480
	LAVE(+)	467	142	414	232	126	368	233	150	459

Key-%: percentage, LL: lower limit, Me: Median, UL: upper limit, RBC: Red blood cell count, Hb: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RDW: Red cell distribution width, WBC: White blood cell count, Neu: Neutrophil, Lym: Lymphocyte, Mon: Monocyte, Eos: Eosinophil, Bas: Basophil, Abs: absolute count, Plt: platelet count, LAVE: Latent abnormal values exclusion. (-): without LAVE, (+): with LAVE. Reference tests used in applying the LAVE method were Iron, Ferritin, Transferrin, Hb, Hct, MCV, Albumin, Globulins, CRP, WBC and PLT

Table S5.3: Summary of selected reference interval studies for erythrocyte related parameters in sub-Saharan Africa

Study	Inclusion criteria	Statistical method	Sex	RBC (x 10^12/L)			Hb (g/dL)			Hct (L/L)			MCV (fl)			MCH (pg)			MCHC (g/dL)			RDW (%)		
				n	LL	UL	n	LL	UL	n	LL	UL	n	LL	UL	n	LL	UL	n	LL	UL	n	LL	UL
[1]Kenya (Nairobi, Thika, Kiambu, Nakuru, Kisii), Jan-Oct 2015, (This study)	Healthy volunteers, 18-65 years, urban towns in Kenya	Parametric + LAVE method	M+F	463	4.41	6.48	470	12.8	19.0	471	0.38	0.55	466	75.7	95.6	462	24.8	32.8	461	32.2	35.2	460	11.3	15.2
			M	226	4.94	6.52	232	14.5	18.7	232	0.43	0.55	228	76.5	95.5	227	25.1	32.8	227	32.4	35.4	225	11.3	14.7
			F	229	4.31	5.76	236	12.0	16.5	237	0.36	0.49	232	73.4	95.8	230	24.4	32.7	230	32.0	35.0	228	11.4	15.8
[2]Kenya (Kericho), Jul-Dec 2004, Kibaya *et al*	Healthy participants of a natural history cohort evaluating HIV-1 infection, 18-55 years	Parametric or non-parametric (mid 95%) depending on distribution of data	M+F	1541	4.00	6.20	1541	6.7	11.1	1541	0.30	0.50	1541	68.8	97.2	1541	22.4	33.5	1541	32.2	35.3			
			M	1020	4.40	6.30	1020	8.3	11.3	1020	0.40	0.50	1020	71.4	98.2	1020	23.3	33.8	1020	32.4	35.3			
			F	521	3.70	5.60	521	5.9	10.0	521	0.30	0.50	521	66.0	95.7	521	21.3	33.0	521	32.2	35.2			
[3]Kenya (Kisumu), Jan 2007-Jun 2010 (Results for 18-34 years), Odhiambo *et al*	Healthy sexually active individuals participating in a prospective study estimating the incidence of HIV seroconversion, 16-34 years	Non-parametric (mid 95%)	M+F																					
			M	389	4.60	6.60	389	12.6	17.2	389	0.38	0.52	389	67.4	93.6									
			F	322	4.00	5.80	322	9.0	14.9	322	0.29	0.44	322	59.3	93.2									
[4]Uganda (Eastern Uganda), Jan-Sep 2002 (Results for > 24 years), Lugada *et al*	Healthy individuals participating in a study to investigate risk factors for human herpes virus 8 infection, 1 week-92 years	Non-parametric (mid 90%)	M+F																					
			M	410	3.80	6.00	410	11.1	16.8	410	0.32	0.48	410	69.9	95.2									
			F	435	3.70	5.30	435	10.1	14.3	435	0.30	0.41	435	67.7	92.6									
[5]Kenya (Nairobi, Kilifi), Uganda(Entebe, Masaka) Zambia (Lusaka), Rwanda (Kigali), Dec 2004-Oct 2006, Karita *et al*	Healthy volunteers, participating in studies prior to HIV vaccine safety and efficacy trials, 18–60 years, HIV-negative test	Non-parametric (mid 95%) , for some parameters certain study sites were excluded as outliers	M+F										2105	68.0	98.0									
			M	1083	4.00	6.40	1083	12.2	17.7	799	0.35	0.51												
			F	846	3.80	5.60	1022	9.5	15.8	846	0.29	0.45												
[6]Ethiopia (Akaki) , Published in 1998, Tsegaye *et al*	Healthy factory workers, 15-45 years	Non-parametric (mid 95%)	M+F																					
			M	280	4.30	5.90	280	13.9	18.3	280	0.42	0.55												
			F	205	3.70	5.20	205	12.2	16.6	205	0.35	0.49												
[7]Tanzania (Mbeya), Dec 2004-Jul 2005 Saathof *et al*	Healthy volunteers, 19-48 years, participating in a larger study called COPE that employed population based sampling	Non-parametric (mid 95%) after removal of outliers	M+F	272	4.01	6.12	276	11.7	17.2	276	0.37	0.53	274	77.6	98.1	272	23.6	33.1	275	30.6	34.9			
			M	145	4.41	6.27	148	13.7	17.7	149	0.40	0.54	149	76.4	98.8	147	23.1	33.2	148	30.6	35.1			
			F	127	3.84	5.59	128	11.1	15.7	127	0.36	0.47	125	77.7	97.9	125	24.2	33.1	127	30.4	34.8			
[8]Rwanda (Buhanda,Ruhango, Nyaruteja, Butare), Aug-Dec 2011, Gahutu *et al*	Blood donors, 18-40 years	Non-parametric (mid 95%) after removal of outliers	M+F										296	77.0	95.0	296	26.0	33.0	296	32.0	37.0			
			M	206	4.20	6.30	206	12.7	17.5	206	0.38	0.54												
			F	90	3.40	5.50	90	11.0	16.0	90	0.36	0.48												
[9]Ghana (Kintampo), published in 2012, Dosoo *et al*	Healthy individuals randomly selected from a Health and Demographic Surveillance System (HDSS)	Non-parametric (mid 95%) after removal of outliers	M+F	623	3.39	5.83	624	9.8	16.0	625	0.29	0.49	625	72.0	97.0	623	22.6	33.5	620	30.5	36.2	625	11.5	16.7
			M	316	3.79	5.96	316	11.3	16.4	316	0.33	0.51	316	70.0	98.0	316	22.7	33.5	315	30.6	36.0	316	11.5	16.7
			F	307	3.09	5.30	308	8.8	14.4	309	0.26	0.45	309	73.0	96.0	307	22.3	33.6	305	30.4	36.5	309	11.4	16.8
[10]Togo (Lome), Apr-Sep 2008, Kueviakoe *et al*	Blood donors, 17-58 years	Non-parametric (mid 95%) after removal of outliers	M+F	1349	3.10	6.40	1349	10.0	18.4	1349	0.28	0.54	1349	80.0	99.0	1349	25.0	37.0	1349	29.0	41.0			
			M	1047	3.30	6.40	1047	10.0	18.4	1047	0.28	0.54	1047	80.0	99.0	1047	25.0	36.0	1047	29.0	39.0			
			F	302	3.10	6.00	302	10.3	17.1	302	0.28	0.47	302	80.0	95.0	302	25.0	37.0	302	30.0	41.0			
[11]South Africa (Gauteng), Published in 2009, Lawrie *et al*	Healthy volunteers involved in a study assessing morbidity prior to HIV vaccine trials, 18-60 years	Non-parametric (mid 95%)	M+F																					
			M	88	4.19	5.85	88	13.4	17.5	88	0.39	0.51	88	83.1	101.6	88	27.8	34.8	88	33.0	35.0	88	12.1	16.3
			F	631	3.93	5.40	631	11.6	16.4	631	0.34	0.48	631	78.9	98.5	631	26.1	33.5	631	32.7	34.9	631	12.4	17.3

Key-LL: lower limit, UL: upper limit, RBC: red blood cell count, Hb: haemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red cell distribution width, LAVE: latent abnormal values exclusion

[1]**Exclusion criteria**: BMI > 35 kg/m^2, consumption of ethanol ≥ 70 g per day, smoking more than 20 tobacco cigarettes per day, chronic illness, recent recovery from acute illness, injury or surgery requiring hospitalization, known carrier state of HBV, HCV or HIV, pregnant or within 1 year after child birth. **CBC analyser**: Beckman Coulter ACT 5 DIFF CP analyser (Brea, California, US).
[2]**Exclusion criteria**: Febrile, pregnant, HIV seropositive, screen positive for syphilis and malaria. **CBC analyser**: ACT 5Diff CP instrument (Beckman Coulter, Fullerton, CA, USA) (Kibaya et al., 2008).
[3]**Exclusion criteri**a: HIV positive, pregnant. **CBC analyser**: Coulter ACT 5Diff CP analyser (Beckman Coulter, France) (Odhiambo et al., 2015).
[4] **Exclusion criteria**: HIV positive, moribund, mentally ill, institutionalized persons, missing personal or laboratory data. **CBC analyser**: Act 5 Diff instrument (Beckman Coulter) (Lugada et al., 2004).
[5] **Exclusion criteria**: Acutely ill, significant findings on physical examination or if laboratory tests revealed that they were pregnant, HIV antibody positive, had evidence of hepatitis B or C infection or suspected syphilis. **CBC analyser**: Beckman Coulter AcT 5 diff CP (Beckman Coulter, USA) (Karita et al., 2009).
[6] **Exclusion criteria**: HIV positive, presence of any illness as defined by the World Health Organization staging systems for HIV infection and disease. **CBC analyser**: Coulter counter T540 (Tsegaye et al., 1999).
[7] **Exclusion criteria**: HIV positive, pregnant or on medication, body temperature ≥37.5^{0}C or if clinical assessment revealed other signs or symptoms of disease that could influence the laboratory parameters of interest. **CBC analyser**: Sysmex KX-21N analyser (Sysmex Corp., Kobe, Japan) (Saathoff et al., 2008).
[8] **Exclusion criteria**: Alcohol abuse, medication, smoking, pregnant, breastfeeding, on oral contraception, on menses. **CBC analyser**: Coulter AcT 5diff and Sysmex KX-21N (Sysmex Corporation, Kobe, Japan) (Gahutu, 2013).
[9] **Exclusion criteria**: Acute or chronic respiratory, cardiovascular, gastrointestinal, hepatic or genitourinary conditions, blood donation or transfusion within the past 3 months, hospitalisation within past 1 month, any findings that would compromise laboratory parameters, pregnant or lactating mothers. **CBC analyser**: Micros 60 analysers (Horiba-ABX, Montpellier, France) (Dosoo et al., 2012).
[10] **Exclusion criteria**: HIV, HBV, and HCV viral infection, malaria, abnormal haemoglobin electrophoresis screening, presence of hypochromia. **CBC analyser**: Sysmex SF-3000 (Sysmex, Kobe, Japan) (Kueviakoe et al., 2011).
[11] **Exclusion criteria**: HIV, current clinical symptoms, immunosuppressive or corticosteroid medication, chemotherapy, hospitalizations, surgery or blood transfusions in the six months prior to screening, splenomegaly, pregnant, Hb < 12 g/dL. Menstruating women returned in 2 weeks. **CBC analyser**: Beckman Coulter LH 750 (Beckman Coulter, Fullerton, CA, USA) (Lawrie et al., 2009).

Table S5.4: Summary of selected reference interval studies for platelets and white blood cell related parameters in sub-Saharan Africa

Study	Inclusion criteria	Statistical method	Sex	WBC (x 10^9/L)			Neu Abs (x 10^9/L)			Lym Abs (x 10^9/L)			Mon Abs (x 10^9/L)			Eos Abs (x 10^9/L)			Bas Abs (x 10^9/L)			Plt (x 10^9/L)		
				n	LL	UL	n	LL	UL	n	LL	UL	n	LL	UL	n	LL	UL	n	LL	UL	n	LL	UL
[1]Kenya (Nairobi, Thika, Kiambu, Nakuru, Kisii), Jan-Oct 2015, (This study)	Healthy volunteers, 18-65 years, urban towns in Kenya	Parametric + LAVE method	M+F	464	3.08	7.83	460	1.05	4.08	461	1.29	3.40	462	0.14	0.74	460	0.04	0.59	461	0.01	0.07	464	144	409
			M	229	3.13	8.10	227	1.02	3.92	226	1.36	3.58	227	0.15	0.76	226	0.05	0.64	226	0.01	0.08	231	133	356
			F	232	2.89	7.72	229	1.07	4.42	230	1.22	3.24	229	0.14	0.68	228	0.04	0.49	224	0.01	0.06	232	152	443
[2]Kenya (Kericho), Jul-Dec 2004, Kibaya *et al*	Healthy participants of a natural history cohort evaluating HIV-1 infection, 18-55 years	Parametric or non-parametric (mid 95%) depending on distribution of data	M+F	1541	2.80	8.20	1541	0.91	4.72	1541	1.14	3.45	1541	0.13	0.60	1541	0.03	1.14	1541	0.01	0.08	1541	120	411
			M	1020	2.70	7.50	1020	0.87	4.32	1020	1.12	3.16	1020	0.13	0.59	1020	0.03	1.08	1020	0.01	0.09	1020	115	366
			F	521	3.00	9.10	521	0.99	5.56	521	1.29	3.96	521	0.16	0.64	521	0.03	1.22	521	0.01	0.07	521	124	444
[3]Kenya (Kisumu), Jan 2007-Jun 2010 (Results for 18-34 years), Odhiambo *et al*	Healthy sexually active individuals participating in a prospective study estimating the incidence of HIV seroconversion, 16-34 years	Non-parametric (mid 95%)	M+F																				
			M	389	3.30	9.60	389	1.30	5.20	389	1.20	3.40	389	0.20	0.70	389	0.04	1.60	389	0.01	0.14	389	126	356
			F	322	3.70	9.10	322	1.30	5.00	322	1.40	3.80	322	0.20	0.80	322	0.04	1.20	322	0.02	0.09	322	147	454
[4]Uganda (Eastern Uganda), Jan-Sep 2002 (Results for > 24 years), Lugada *et al*	Healthy individuals participating in a study to investigate risk factors for human herpes virus 8 infection, 1 week-92 years	Non-parametric (mid 90%)	M+F	845	3.40	8.70	845	0.84	3.37	845	1.40	4.20	845	0.17	0.59	845	0.13	2.11	845	0.01	0.07			
			M																		410	80	288
			F																		435	100	297
[5]Kenya (Nairobi, Kilifi), Uganda(Entebe, Masaka) Zambia (Lusaka), Rwanda (Kigali), Dec 2004-Oct 2006, Karita *et al*	Healthy volunteers, participating in studies prior to HIV vaccine safety and efficacy trials, 18–60 years, HIV-negative test	Non-parametric (mid 95%) , for some parameters certain study sites were excluded as outliers	M+F	2105	3.10	9.10	2103	1.00	5.30	2105	1.20	3.70	2103	0.20	0.78	2104	0.04	1.53	1750	0.01	0.15	2105	126	438
			M																				
			F																				
[6]Ethiopia (Akaki) , Published in 1998, Tsegaye *et al*	Healthy factory workers, 15-45 years	Non-parametric (mid 95%)	M+F	485	3.00	10.20															485	98	337
			M																		280	97	324
			F																		205	98	352
[7]Tanzania (Mbeya), Dec 2004-Jul 2005 Saathof *et al*	Healthy volunteers, 19-48 years, participating in a larger study called COPE that employed population based sampling	Non-parametric (mid 95%) after removal of outliers	M+F	279	3.00	7.90	276	1.10	4.70	278	1.10	3.00										274	150	395
			M	150	2.80	7.90	150	1.10	4.80	149	1.10	2.80										147	147	356
			F	129	3.20	8.00	126	1.20	5.40	129	1.10	3.10										127	151	425
[8]Rwanda (Buhanda,Ruhango, Nyaruteja, Butare), Aug-Dec 2011, Gahutu *et al*	Blood donors, 18-40 years	Non-parametric (mid 95%) after removal of outliers	M+F	296	2.90	7.11	296	1.00	4.10	296	1.10	3.20	296	0.20	0.60	296	0.05	0.74	296	0.01	0.08			
			M																		206	115	336
			F																		90	134	408
[9]Ghana (Kintampo), published in 2012, Dosoo *et al*	Healthy individuals randomly selected from a Health and Demographic Surveillance System (HDSS)	Non-parametric (mid 95%) after removal of outliers	M+F	620	3.40	9.20				624	1.20	4.40	624	0.20	1.00							625	89	380
			M	311	3.50	9.20				316	1.20	5.20	316	0.20	1.40							316	88	352
			F	309	3.40	9.30				308	1.20	4.40	308	0.20	0.90							309	89	403
[10]Togo (Lome), Apr-Sep 2008, Kueviakoe *et al*	Blood donors, 17-58 years	Non-parametric (mid 95%) after removal of outliers	M+F	1349	1.90	10.10	1349	0.50	5.40	1349	1.10	4.30	1349	0.05	0.80	1349	0.00	0.50	1349			1349	120	443
			M	1047	1.90	10.10	1047	0.50	5.40	1047	1.10	4.30	1047	0.05	0.80	1047	0.00	0.50				1047	120	443
			F	302	2.20	7.80	302	0.50	4.40	302	1.20	4.30	302	0.05	0.80	302	0.00	0.50				302	150	436
[11]South Africa (Gauteng), Published in 2009, Lawrie *et al*	Healthy volunteers involved in a study assessing morbidity prior to HIV vaccine trials, 18-60 years	Non-parametric (mid 95%)	M+F																				
			M	88	3.92	10.40	88	1.60	6.98	88	1.40	4.20	88	0.30	0.80	88	0.00	0.95	88	0.00	0.10	88	171	388
			F	631	3.90	12.60	631	1.60	8.30	631	1.40	4.50	631	0.20	0.80	631	0.00	0.40	631	0.00	0.10	631	186	454

Key-LL: lower limit, UL: upper limit, RBC: red blood cell count, Hb: haemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red cell distribution width, LAVE: latent abnormal values exclusion

[1]**Exclusion criteria**: BMI > 35 kg/m^2, consumption of ethanol ≥ 70 g per day, smoking more than 20 tobacco cigarettes per day, chronic illness, recent recovery from acute illness, injury or surgery requiring hospitalization, known carrier state of HBV, HCV or HIV, pregnant or within 1 year after child birth. **CBC analyser**: Beckman Coulter ACT 5 DIFF CP analyser (Brea, California, US).
[2]**Exclusion criteria**: Febrile, pregnant, HIV seropositive, screen positive for syphilis and malaria. **CBC analyser**: ACT 5Diff CP instrument (Beckman Coulter, Fullerton, CA, USA) (Kibaya et al., 2008).
[3]**Exclusion criteri**a: HIV positive, pregnant. **CBC analyser**: Coulter ACT 5Diff CP analyser (Beckman Coulter, France) (Odhiambo et al., 2015).
[4] **Exclusion criteria**: HIV positive, moribund, mentally ill, institutionalized persons, missing personal or laboratory data. **CBC analyser**: Act 5 Diff instrument (Beckman Coulter) (Lugada et al., 2004).
[5] **Exclusion criteria**: Acutely ill, significant findings on physical examination or if laboratory tests revealed that they were pregnant, HIV antibody positive, had evidence of hepatitis B or C infection or suspected syphilis. **CBC analyser**: Beckman Coulter AcT 5 diff CP (Beckman Coulter, USA) (Karita et al., 2009).
[6] **Exclusion criteria**: HIV positive, presence of any illness as defined by the World Health Organization staging systems for HIV infection and disease. **CBC analyser**: Coulter counter T540 (Tsegaye et al., 1999).
[7] **Exclusion criteria**: HIV positive, pregnant or on medication, body temperature ≥37.5^{0}C or if clinical assessment revealed other signs or symptoms of disease that could influence the laboratory parameters of interest. **CBC analyser**: Sysmex KX-21N analyser (Sysmex Corp., Kobe, Japan) (Saathoff et al., 2008).
[8] **Exclusion criteria**: Alcohol abuse, medication, smoking, pregnant, breastfeeding, on oral contraception, on menses. **CBC analyser**: Coulter AcT 5diff and Sysmex KX-21N (Sysmex Corporation, Kobe, Japan) (Gahutu, 2013).
[9] **Exclusion criteria**: Acute or chronic respiratory, cardiovascular, gastrointestinal, hepatic or genitourinary conditions, blood donation or transfusion within the past 3 months, hospitalisation within past 1 month, any findings that would compromise laboratory parameters, pregnant or lactating mothers. **CBC analyser**: Micros 60 analysers (Horiba-ABX, Montpellier, France) (Dosoo et al., 2012).
[10] **Exclusion criteria**: HIV, HBV, and HCV viral infection, malaria, abnormal haemoglobin electrophoresis screening, presence of hypochromia. **CBC analyser**: Sysmex SF-3000 (Sysmex, Kobe, Japan) (Kueviakoe et al., 2011).
[11] **Exclusion criteria**: HIV, current clinical symptoms, immunosuppressive or corticosteroid medication, chemotherapy, hospitalizations, surgery or blood transfusions in the six months prior to screening, splenomegaly, pregnant, Hb < 12 g/dL. Menstruating women returned in 2 weeks. **CBC analyser**: Beckman Coulter LH 750 (Beckman Coulter, Fullerton, CA, USA) (Lawrie et al., 2009).

5.7 References

Beutler, E., & West, C. (2005). Hematologic differences between African-Americans and whites: The roles of iron deficiency and alpha-thalassemia on hemoglobin levels and mean corpuscular volume. *Blood, 106*(2), 740-745. doi:10.1182/blood-2005-02-0713

Bilic, E., & Bilic, E. (2003). Amino acid sequence homology of thrombopoietin and erythropoietin may explain thrombocytosis in children with iron deficiency anemia. *Journal of Pediatric Hematology/Oncology, 25*(8), 675-676.

Borai, A., Ichihara, K., Al Masaud, A., Tamimi, W., Bahijri, S., Armbuster, D., . . . Abdelaal, M. (2016). Establishment of reference intervals of clinical chemistry analytes for the adult population in Saudi Arabia: A study conducted as a part of the IFCC global study on reference values. *Clinical Chemistry and Laboratory Medicine, 54*(5), 843-855. doi:10.1515/cclm-2015-0490

Broudy, V. C., Lin, N. L., & Kaushansky, K. (1995). Thrombopoietin (c-mpl ligand) acts synergistically with erythropoietin, stem cell factor, and interleukin-11 to enhance murine megakaryocyte colony growth and increases megakaryocyte ploidy in vitro. *Blood, 85*(7), 1719-1726.

CLSI. (2008). *Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline* (Third ed.): Wayne PA, Clinical and Laboratory Standards Institute.

Dosoo, D. K., Kayan, K., Adu-gyasi, D., Kwara, E., Ocran, J., Osei, K., . . . Owusu-agyei, S. (2012). Haematological and Biochemical Reference Values for Healthy Adults in the Middle Belt of Ghana. *PLOS ONE, 7*(4), 1-9. doi:10.1371/journal.pone.0036308

Gahutu, J. B. (2013). Hematological Values at Moderate Altitude in a Low-Income Population. *International Blood Research & Reviews, 1*(27 juni 2013), 22-28.

Hershman, D., Weinberg, M., Rosner, Z., Alexis, K., Tiersten, A., Grann, V. R., . . . Neugut, A. I. (2003). Ethnic neutropenia and treatment delay in African American women undergoing chemotherapy for early-stage breast cancer. *Journal of the National Cancer Institute, 95*(20), 1545-1548. doi:10.1093/JNCI/DJG073

Ichihara, K. (2014). Statistical considerations for harmonization of the global multicenter study on reference values. *Clinica Chimica Acta, 432*, 108-118. doi:10.1016/j.cca.2014.01.025

Ichihara, K., & Boyd, J. C. (2010). An appraisal of statistical procedures used in derivation of reference intervals. *Clinical Chemistry and Laboratory Medicine, 48*(11), 1537-1551. doi:10.1515/CCLM.2010.319

Ichihara, K., Ceriotti, F., Tam, T. H., Sueyoshi, S., Poon, P. M. K., Thong, M. L., . . . Panteghini, M. (2013). The Asian project for collaborative derivation of reference intervals: (1) strategy and major results of standardized analytes. *Clinical Chemistry and Laboratory Medicine, 51*(7), 1429-1442. doi:10.1515/cclm-2012-0421

Ichihara, K., Itoh, Y., Lam, C. W. K., Poon, P. M. K., Kim, J.-H., Kyono, H., . . . Muliaty, D. (2008). Sources of variation of commonly measured serum analytes in 6 Asian cities and consideration of common reference intervals. *Clinical Chemistry, 54*(2), 356-365. doi:10.1373/clinchem.2007.091843

Ichihara, K., Ozarda, Y., Barth, J. H., Klee, G., Qiu, L., Erasmus, R., . . . Yadav, B. (2017). A global multicenter study on reference values: 1. Assessment of methods for derivation and comparison of reference intervals. *Clinica Chimica Acta, 467*, 70-82. doi:10.1016/J.CCA.2016.09.016

Karita, E., Ketter, N., Price, M. A., Kayitenkore, K., Kaleebu, P., Nanvubya, A., . . . Kamali, A. (2009). CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. *PLOS ONE, 4*(2), e4401. doi:10.1371/journal.pone.0004401

Kibaya, R. S., Bautista, C. T., Sawe, F. K., Shaffer, D. N., Sateren, W. B., Scott, P. T., . . . de Souza, M. S. (2008). Reference ranges for the clinical laboratory derived from a rural population in Kericho, Kenya. *PLOS ONE, 3*(10), e3327-e3327. doi:10.1371/journal.pone.0003327

Kratz, A., Ferraro, M., Sluss, P. M., & Lewandrowski, K. B. (2004). Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Laboratory reference values. *The New England Journal of Medicine, 351*(15), 1548-1563. doi:10.1056/NEJMcpc049016

Kueviakoe, I. M., Segbena, A. Y., Jouault, H., Vovor, A., & Imbert, M. (2011). Hematological reference values for healthy adults in togo. *ISRN Hematology, 2011*(February 2016), 736062-736062. doi:10.5402/2011/736062

Kulnigg-Dabsch, S., Schmid, W., Howaldt, S., Stein, J., Mickisch, O., Waldhör, T., . . . Gasche, C. (2013). Iron Deficiency Generates Secondary Thrombocytosis and Platelet Activation in IBD. *Inflammatory Bowel Diseases, 19*(8), 1-1. doi:10.1097/MIB.0b013e318281f4db

Lawrie, D., Coetzee, L. M., Becker, P., Mahlangu, J., Stevens, W., & Glencross, D. K. (2009). Local reference ranges for full blood count and CD4 lymphocyte count testing. *S Afr Med J., 99*(4), 243-248.

Lugada, E. S., Mermin, J., Kaharuza, F., Ulvestad, E., Were, W., Langeland, N., . . . Downing, R. (2004). Population-based hematologic and immunologic reference values for a healthy Ugandan population. *Clinical and Diagnostic Laboratory Immunology, 11*(1), 29-34.

McGrath, J. W., George, K., Svilar, G., Ihler, E., Mafigiri, D., Kabugo, M., & Mugisha, E. (2001). Knowledge about vaccine trials and willingness to participate in an HIV/AIDS vaccine study in the Ugandan military. *Journal of Acquired Immune Deficiency Syndromes (1999), 27*(4), 381-388.

NIH. (2014). U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS (DAIDS). Table for Grading the Severity of Adult and Pediatric Adverse Events. *Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0.* Retrieved from http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf

Odhiambo, C., Oyaro, B., Odipo, R., Otieno, F., Alemnji, G., Williamson, J., & Zeh, C. (2015). Evaluation of locally established reference intervals for hematology and biochemistry parameters in Western Kenya. *PLOS ONE, 10*(4), e0123140-e0123140. doi:10.1371/journal.pone.0123140

Ozarda, Y., Ichihara, K., Barth, J. H., & Klee, G. (2013). Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. *Clinical Chemistry and Laboratory Medicine (CCLM), 51*(5), 1027-1040. doi:10.1515/cclm-2013-0249

Petitclerc, C. (2004). Normality: the unreachable star? *Clinical Chemistry and Laboratory Medicine, 42*(7), 698-701. doi:10.1515/CCLM.2004.119

Saathoff, E., Schneider, P., Kleinfeldt, V., Geis, S., Haule, D., Maboko, L., . . . Hoelscher, M. (2008). Laboratory reference values for healthy adults from southern Tanzania. *Tropical Medicine & International Health, 13*(5), 612-625. doi:10.1111/j.1365-3156.2008.02047.x

Schloesser, L. L., Kipp, M. A., Wenzel, F. J., Olef, I., Wintrobe, M. M., Desforges, J. F., . . . Harris, J. W. (1937). Thrombocytosis in iron-deficiency anemia. *The Journal of Laboratory and Clinical Medicine, 66*(1), 107-114. doi:10.5555/URI:PII:0022214365900831

Segolodi, T. M., Henderson, F. L., Rose, C. E., Turner, K. T., Zeh, C., Fonjungo, P. N., . . . Paxton, L. A. (2014). Normal laboratory reference intervals among healthy adults screened for a HIV pre-exposure prophylaxis clinical trial in Botswana. *PLOS ONE, 9*(4), e93034-e93034. doi:10.1371/journal.pone.0093034

Tarimo, E. A. M., Thorson, A., Kohi, T. W., Bakari, M., Mhalu, F., & Kulane, A. (2011). Reasons for declining to enroll in a phase I and II HIV vaccine trial after randomization among eligible volunteers in Dar es Salaam, Tanzania. *PLOS ONE, 6*(2), e14619-e14619. doi:10.1371/journal.pone.0014619

Thobakgale, C. F., & Ndung'u, T. (2014). Neutrophil counts in persons of African origin. *Current Opinion in Hematology, 21*(1), 50-57. doi:10.1097/MOH.0000000000000007

Tsegaye, A., Messele, T., Tilahun, T., Hailu, E., Sahlu, T., Doorly, R., . . . Rinke de Wit, T. F. (1999). Immunohematological reference ranges for adult Ethiopians. *Clinical and Diagnostic Laboratory Immunology, 6*(3), 410-414.

WHO. (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. *Vitamin and Mineral Nutrition Information System, WHO/NMH/NH*. Retrieved from http://www.who.int/vmnis/indicators/haemoglobin.pdf?ua=1

CHAPTER 6: DETERMINATION OF REFERENCE INTERVALS FOR COMMON CHEMISTRY AND IMMUNOASSAY TESTS FOR KENYAN ADULTS BASED ON AN INTERNATIONALLY HARMONIZED PROTOCOL AND UP-TO-DATE STATISTICAL METHODS

Omuse, G., Ichihara, K., Maina, D., Hoffman, M., Kagotho, E., Kanyua, A., . . . Erasmus, R. (2020). Determination of reference intervals for common chemistry and immunoassay tests for Kenyan adults based on an internationally harmonized protocol and up-to-date statistical methods. PLOS ONE, 15(7), e0235234.

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6.1 Abstract

Background: Due to a lack of reliable reference intervals (RIs) for Kenya, we set out to determine RIs for 40 common chemistry and immunoassay tests as part of the IFCC global RI project.

Methods: Apparently healthy adults aged 18-65 years were recruited according to a harmonized protocol and samples analyzed using Beckman-Coulter analyzers. Value assigned serum panels were measured to standardize chemistry results. The need for partitioning reference values by sex and age was based on between-subgroup differences expressed as standard deviation ratio (SDR) or bias in lower or upper limits (LLs and ULs) of the RI. RIs were derived using a parametric method with/without latent abnormal value exclusion (LAVE).

Results: Sex-specific RIs were required for uric acid, creatinine, total bilirubin (TBil), total cholesterol (TC), ALT, AST, CK, GGT, transferrin, transferrin saturation (TfSat) and immunoglobulin-M. Age-specific RIs were required for glucose and triglyceride for both sexes, and for urea, magnesium, TC, HDL-cholesterol ratio, ALP, and ferritin for females. LAVE was effective in optimizing RIs for AST, ALT, GGT iron-markers and CRP by reducing influence of latent anemia and metabolic diseases. Thyroid profile RIs were derived after excluding volunteers with anti-thyroid antibodies. Kenyan RIs were comparable to those of other countries participating in the global study with a few exceptions such as higher ULs for TBil and CRP.

Conclusions: Kenyan RIs for major analytes were established using harmonized protocol from well-defined reference individuals. Standardized RIs for chemistry analytes can be shared across sub-Saharan African laboratories with similar ethnic and life-style profile.

6.2 Introduction

Reference intervals (RIs) are an integral part of laboratory reports as they assist clinicians in interpretation of results. RIs should be population specific to ensure appropriate interpretation. Unfortunately, many clinical laboratories in sub-Saharan Africa (SSA) adopt RIs provided by manufacturers of laboratory reagents/equipment without verifying them as recommended by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2008). This could result in inaccurate interpretation of quantitative laboratory results leading to medical errors. Saathoff *et al* carried out a study in the Mbeya region, south-western Tanzania and found marked differences in RIs from the United States (US), Tanzania and other SSA countries. Overall, only 80.9% of reference values (RVs) for clinical chemistry tests from healthy individuals in Tanzania would have been classified as normal as per the US RIs published by Kratz *et al* (Kratz et al., 2004).

The International Federation of Clinical Chemistry (IFCC) under its Committee on Reference Intervals and Decision Limits (C-RIDL) has been carrying out a global RI study using a protocol that harmonizes the pre-analytical, analytical and post-analytical study processes to ensure ease of comparison of derived RIs across different regions, countries and ethnicities (Ozarda et al., 2013).

An interim report of the global RI study comprising data from 12 countries identified between ethnic group differences in both males and females for serum total protein (TP), albumin (Alb), total bilirubin (TBil), high density lipoprotein cholesterol (HDL-C), magnesium (Mg), C-reactive protein (CRP), IgG, complement 3 (C3), vitamin B12, and folate. Females were found to generally have more pronounced age-related changes in RVs. Ethnic differences in BMI-related changes was also demonstrated. The only African country whose data were included in the interim report was South Africa where comparisons between black South Africans and Caucasian / mixed race showed much higher levels of CRP in the black South Africans (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017).

The Kenyan study was undertaken to explore sources of variation of RVs, to derive country specific RIs and to standardize the RIs by use of a value-assigned panel of sera (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017) intended for nationwide use and international comparison.

6.3 Materials and methods

The methodology used in recruiting participants for the study, sample collection, handling and analysis has previously been published (Omuse et al., 2018). The study was approved by the Aga Khan University Hospital Nairobi (2014/REC-46) and Stellenbosch University (S16/10/219) Health Research Ethics Committees. The study was conducted in conformity with the Declaration of Helsinki.

Study population

Recruitment of study participants in Kenya was carried out between January and October 2015 in several counties. Majority were urban dwellers from the capital city Nairobi, Kiambu county in central Kenya, Kisii County in western Kenya, and Nakuru County based in the Great Rift Valley.

Inclusion and exclusion criteria

Inclusion of participants was limited to healthy adults aged 18-65 years stratified into 4 age groups: 18-29, 30-39, 40-49 and 50-65 years. Exclusion criteria included individuals with a body mass index (BMI) >35 kg/m^2, consumption of ethanol ≥70 g per day, smoking >20 cigarettes per day, taking regular medication for a chronic disease (diabetes mellitus, hypertension, hyperlipidemia, allergic disorders, depression), recent (< 15 days) recovery from acute illness, injury or surgery requiring hospitalization, carrier of HBV, HCV or HIV, pregnant or within 1 year after delivery. Written informed consent was obtained after written/verbal explanation of the study. Those with any chronic disease were excluded except for individuals aged 50-65 years who had well controlled hypertension taking up to 2 drugs. A single measurement of blood pressure, abdominal circumference and BMI was done after filling the study questionnaire.

Blood collection and handling

Blood samples were collected by trained phlebotomists into a serum separator tube for all analytes tested in serum, lithium heparin tube for troponin I, sodium fluoride tube for plasma glucose. Serum and plasma samples requiring centrifugation were spun 2-4 hours after collection and stored at −80ºC at the Aga Khan University Hospital, Nairobi (AKUHN). Centrifugation was done at 2000g, for 10 mins in a non-refrigerated centrifuge (Beckman coulter, Allegra X-30, Brea, California, US) These were subsequently shipped frozen to the PathCare reference laboratory in Cape Town, South Africa for analysis. We also drew 2mL of whole blood for testing hematology parameters using Beckman-Coulter ACT5-DIFF-CP analyser (Brea, California, US), tested in PathCare Nairobi. The test results were

primarily used for establishing RIs for hematology parameters (Omuse et al., 2018), but they were referred to in this study for secondary exclusion of individuals with latent anemia or inflammation.

Measurements

The analysis of all serum specimens was performed in batches on the Beckman Coulter AU 5800 (Brea, California, US) for chemistry assays and DXI (Brea, California, US) for immunoassays as summarized in **Table 6.1**.

Table 6.1: Summary of tests, equipment, assay methods and analytical performance

Analyte	Abbreviation	Method	Units	Between Run CV
Sodium	Na	Ion selective electrode / diluted (indirect)	mmol/L	1.3
Potassium	K	Ion selective electrode / diluted (indirect)	mmol/L	3.8
Chloride	Cl	Ion selective electrode / diluted (indirect)	mmol/L	1.4
Urea	Urea	Urease	mmol/L	2.4
Creatinine	Cre	Modified kinetic Jaffè	µmol/L	1.6
Total Protein	TP	Biuret	g/L	4.6
Albumin	Alb	Bromocresol Green dye binding	g/L	2.8
Total Bilirubin	TBil	Diazonium salt	µmol/L	7.7
Gamma-glutamyl transferase	GGT	Gamma-glutamyl-3-carboxy-4-nitroanilide	IU/L	3.3
Alkaline phosphatase	ALP	P-nitro-phenylphosphate hydrolysis	IU/L	4.0
Lactate dehydrogenase	LDH	Lactate to Pyruvate	IU/L	3.2
Calcium	Ca	Arsenazo III dye	mmol/L	1.4
Magnesium	Mg	Xylidyl blue	mmol/L	1.9
Phosphate	IP	Molybdate hydolysis	mmol/L	1.8
Lipase	Lip	1, 2-Diglyceride hydrolysis	U/L	8.0
Total cholesterol	TC	Cholesterol oxidase	mmol/L	1.2
Triglycerides	TG	Glycerol phosphate oxidase	mmol/L	4.1
High density lipoprotein cholesterol	HDL-C	Two phase selective accelerator detergent	mmol/L	2.0
Low density lipoprotein cholesterol	LDL-C	Two phase selective accelerator detergent	mmol/L	1.6
Uric acid	UA	Modified Trinder reaction with Uricase	mmol/L	1.2
High sensitive c reactive protein	CRP	Turbidimetry	mg/L	1.8
Amylase	AMY	2-chloro-4-nitrophenyl-α-D-maltotrioside	U/L	8.1
Immunoglobulin A	IgA	Turbidimetry	g/L	1.3
Immunoglobulin G	IgG	Turbidimetry	g/L	1.1
Immunoglobulin M	IgM	Turbidimetry	g/L	7.0
Alanine aminotransferase	ALT	NADH (without P-5'-P)	U/L	28.1
Aspartate aminotransferase	AST	NADH (without P-5'-P)	U/L	5.6
Creatinine kinase	CK	Creatine phosphate dephosphorilysation	U/L	6.5
Iron	Fe	2, 4, 6-Tri-(2-pyridyl)-5-triazine chromogen	µmol/L	4.2
Transferrin	Tf	Turbidimetry	g/L	2.6
Anti-thyroglobulin	TgAb	Two-site immune – enzymatic immunoassay	IU/ml	4.8
Anti-thyroid peroxidase	TPOAb	Two-site immune – enzymatic immunoassay	IU/ml	28.8
Thyroid stimulating hormone	TSH	Two-site immune – enzymatic immunoassay	mIU/L	13.0
Free thyroxine	FT4	Two-site immune – enzymatic immunoassay	pmol/L	8.1
Free tri-iodothyronine	FT3	Two-site immune – enzymatic immunoassay	pmol/L	4.8
Ferritin	Fer	Turbidimetry	µg/L	8.2
Prostate specific antigen	PSA	Two-site immune – enzymatic immunoassay	µg/L	2.3

As calculated parameters, globulin (Glb) was computed as TP – Alb; non-HDL-C as TC – HDL-C; HDL-C ratio (HDLrat) as TC/HDL-C.

Quality control

The PathCare reference laboratory is accredited by the South African National Accreditation System. For purposes of the global RI study, all participating laboratories received a panel of sera produced by the C-RIDL in 2014 that had assigned values (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017). This panel was measured by participating laboratories to enable recalibration of RVs using linear regression analysis. It also allowed for alignment of RVs across different countries by all-pairwise comparison of test results.

Statistical analysis

In order to assess sex, age and BMI as sources of variation, we adopted the standard deviation ratio (SDR), which represents a ratio of between-subgroup SD (variation of the subgroup means from grand mean) to between-individual SD (approximately 0.25 the width of RI). For calculating SDRs, we first performed 2-level nested ANOVA to compute between-sex SD and between-age SD after partitioning age at 30, 40, and 50 years. With the results, the SDR for between-sex SD (SDRsex) and between-age SD (SDRage) were calculated as a ratio to the residual SD that corresponds to roughly between-individual SD or SD comprizing RI (SD_{RI}. Since between-age variation changes by sex, we also computed SDRage for each sex by one way ANOVA. We considered SDR≥0.40 as a primary criterion for judging the need for partitioning RVs by sex and/or age (Omuse et al., 2018).

However, SDR represents between-subgroup difference at the center of the RV distribution, which may not reflect the difference (bias) at LL or UL (ΔLL or ΔUL). Therefore, we also evaluated ΔLL or ΔUL as its ratio to SD comprising RI (SD_{RI}) [= |UL−LL|/3.92] and expressed it as bias ratio (BR) at LL or UL (BR_{LL} or BR_{UL}). For example, the formula for determining BR for sex was:

$$BR_{LL} = \frac{|LL_M - LL_F|}{(UL_{MF} - LL_{MF})/3.92} \quad , \quad BR_{UL} = \frac{|UL_M - UL_F|}{(UL_{MF} - LL_{MF})/3.92}$$

where subscripts of MF, M, and F attached to LL or UL indicate the RI without partition by sex (for male+female) and the RIs after partition by male and female, respectively (Ozarda et al., 2017). The same calculation was done for judging the need for age-specific RIs by setting LL, UL with/without partitioning by age. The distinction of the concepts between SDR and BR is illustrated in **Fig 6.1**.

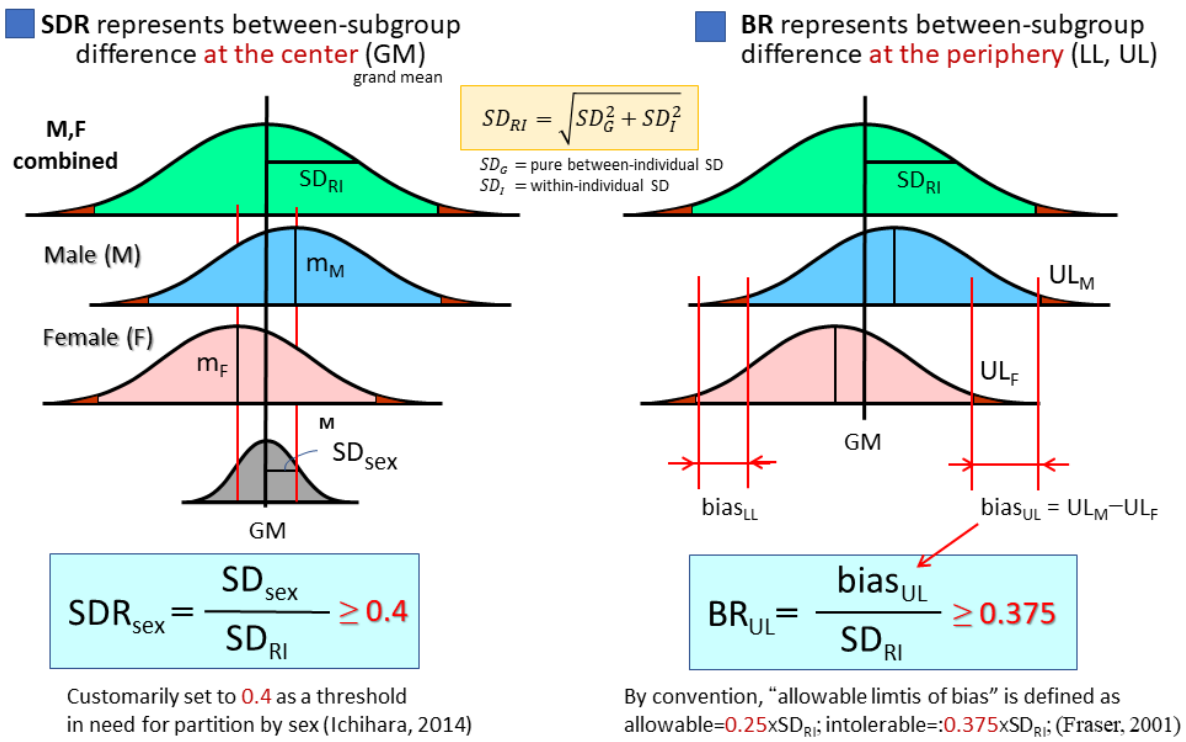
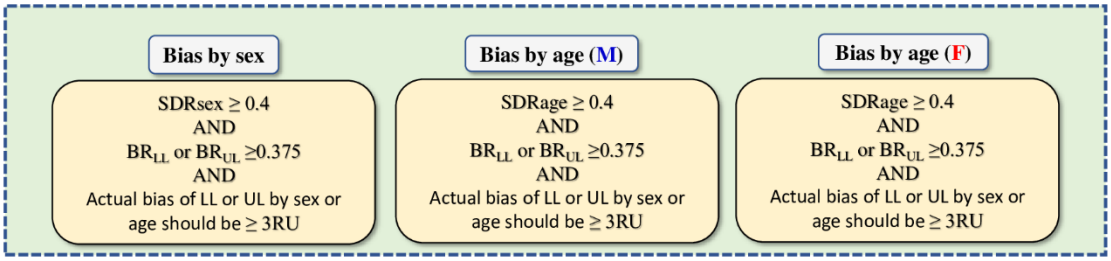


Fig 6.1: SD ratio (SDR) vs. bias ratio (BR) as a measure of between-subgroup differences

SDR represents between-subgroup differences at the center of distributions, while **BR** represents between-subgroup differences at the periphery (LL or UL) of the distributions. The numerator of SDR is between-subgroup SD (or SDsex, if sub-grouped by sex), while that of BR is a difference of LLs or ULs.

In setting the threshold for the bias ratios, we followed the convention of allowable limits of bias in measurements: $\sqrt{SD_I^2 + SD_G^2} \leq 0.375$, where SD_I and SD_G represent within-individual and between-individual SD (7). Since SD_{RI} or the denominator of BR is composed of both SD_I and SD_G and equal to $\sqrt{SD_I^2 + SD_G^2}$, we set 0.375 as a threshold for BR. This scheme was adopted in recent papers (Borai et al., 2020; Evgina et al., 2020). On the other hand, both SDR and BR depend on their common denominator, SD_{RI}. For example, when the RI is narrow, both ratios can be inflated. Conversely, when the RI is wide, both ratios are suppressed. In order to cope with such situations, we set a pragmatic third criterion that between-subgroup bias at LL or UL (ΔLL or ΔUL) should be equal to or more than 3 times the "reporting unit (RU)" to allow partitioning of RVs. RU represents a unit of value for reporting test results. If the number of digits below the decimal point in reporting test results is 2, 1, or

0, RU is 0.01, 0.1, or 1, respectively. The flow of logic in deciding the need for partitioning by sex or age is shown in **Fig 6.2**.



Eight categories of RIs partitioned by sex and age

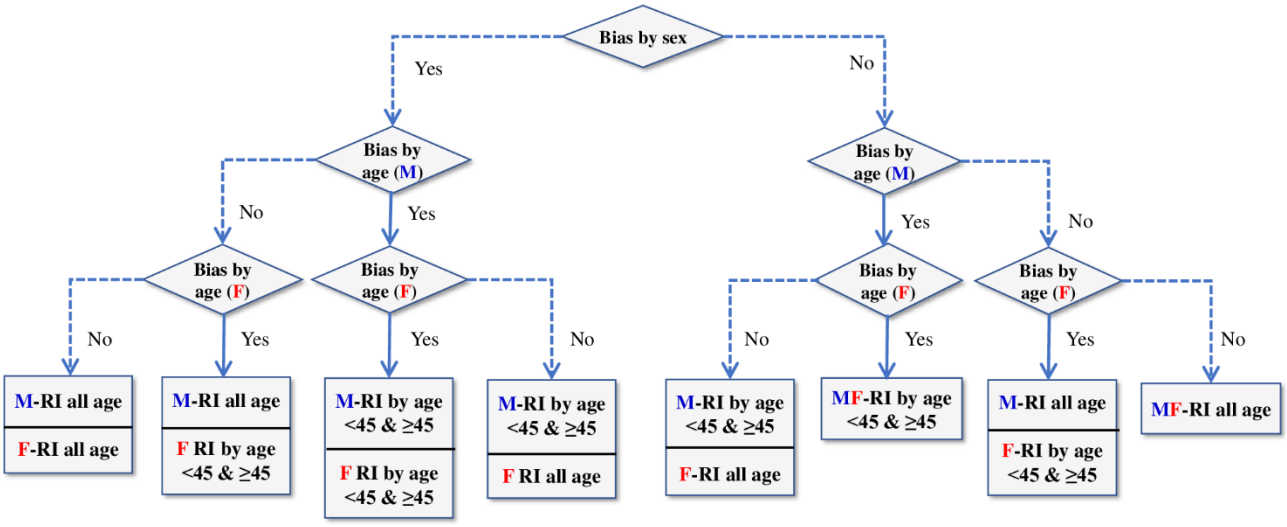


Fig 6.2: Scheme for partitioning RVs by sex and age

We adopted this flow-chart in judging the needs for partitioning RVs by sex and age. We defined between-sex (or -age) subgroup bias in reference to the three points: 1) SDR>0.4 that represents the between-subgroup bias at the center of RV distribution, 2) BR>0.375 that represents the between-subgroup bias at the limits (LL or UL) of RV distribution, and 3) the actual difference (bias) ≤ three reporting unit (RP). There were eight possible choices for the partitioning.

RIs were determined using both parametric and non-parametric methods before and after applying the latent abnormal values exclusion (LAVE) method (Ichihara, 2014; Ichihara & Boyd, 2010). For the non-parametric method, the RVs coinciding with the 2.5^{th} and 97.5^{th} percentiles after sorting the data in ascending order were used as the lower and upper limits (LL, UL) of the RI. For the parametric method, the RVs were transformed into a Gaussian form by the Box-Cox power transformation formula, and then mean±1.96 SD was computed as the central 95% limits (LL−UL) under the transformed scale, which were then reverse transformed to get the LL and UL in the original scale (Ichihara, 2014).

As a measure for secondary exclusion of abnormal values, we tried to apply LAVE in deriving the RI both by parametric and nonparametric methods (Ichihara, 2014). For LAVE, we primarily used the following set of reference tests: Alb, Glb, UA, Glu, non-HDL, TG, ALT, AST, LDH, CK, GGT, and CRP, which were meant for excluding individuals with inappropriate values in the nutritional, inflammatory, or muscular damage markers. As an exception, for iron related analytes (Fe, Ferr, Tf, and TfSat), we set hemoglobin (Hb), hematocrit (Hct), and mid-corpuscular volume (MCV) in addition to the four iron markers as the reference tests. For proteins (TP, Alb, Glb, IgG/A/M, CRP), we set all the seven tests plus white blood cell and platelet counts as the reference tests.

On the other hand, for determination of RIs for the thyroid panel, the LAVE procedure was not applied because reference tests associated with the thyroid panel were not available. Rather, we first estimated the cutoff values for anti-thyroglobulin antibody (TgAb), and anti-thyroid peroxidase antibody (TPOAb) from the probability paper plot drawn with x-axis in logarithmic scale, as an intersection of the central linear part with the horizontal line at 97.5 percentile as shown in **Fig 6.3**.

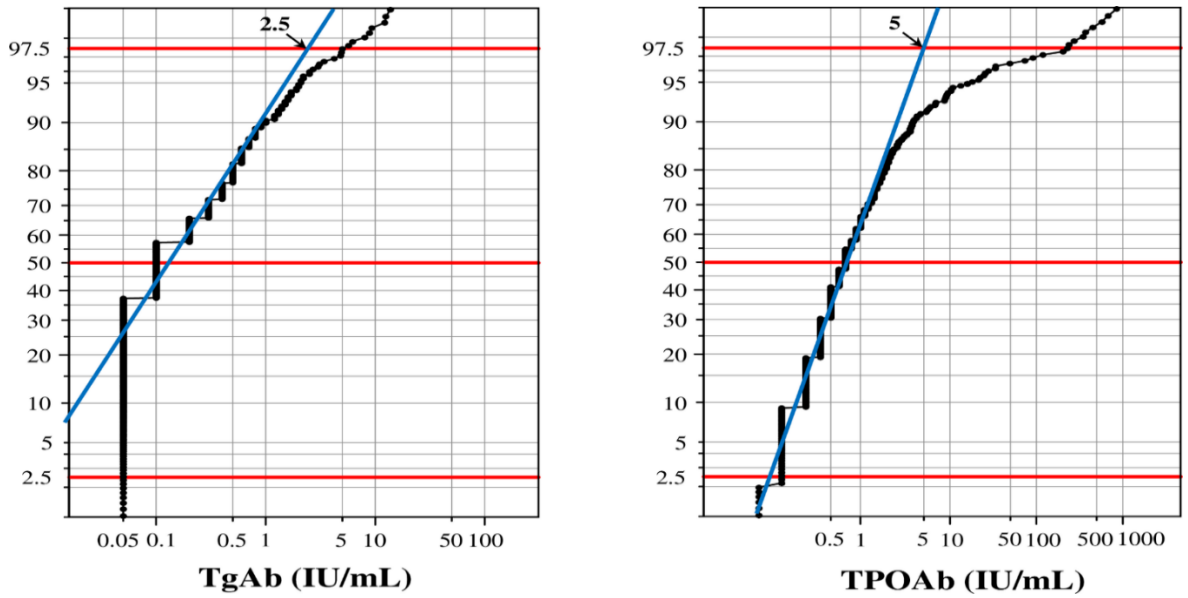


Fig 6.3: Determination of cutoff values for TgAb and TPOAb based on the probability paper plot

Cutoff values for thyroglobulin antibody (TgAb), and thyroid peroxidase antibody (TPOAb) were empirically determined by use of the probability paper plot. Y-axis represents cumulative frequencies of RV distribution, while x-axis was drawn in logarithmic scale. We assumed that both antibodies are specific to autoimmune thyroiditis (AIT), and thus extreme values in the tail constitutes a group of values from AIT cases. Therefore, we determined the cutoff value as an intersection of central linear part with the horizontal line at 97.5%.

For judging the need for adopting LAVE, we computed BR_{LL} and BR_{UL} by setting LLs and ULs of RIs with/without LAVE: i.e., SD_{RI} in the denominator was set to the RI by the LAVE procedure. The 90%

confidence intervals (CIs) of LL and UL were derived by the bootstrap method with resampling of the final set of RVs and repeated computations of LL and UL for 50 iterations. Accordingly, final RIs were determined as the averages of LL and UL thus derived.

6.4 Results

Profile of the subjects

Out of 596 volunteers, 533 met the inclusion criteria: 260 (48.8%) males and 273 (51.2%) females. The main reason for exclusion was hypertension as shown in **Fig 6.4**.

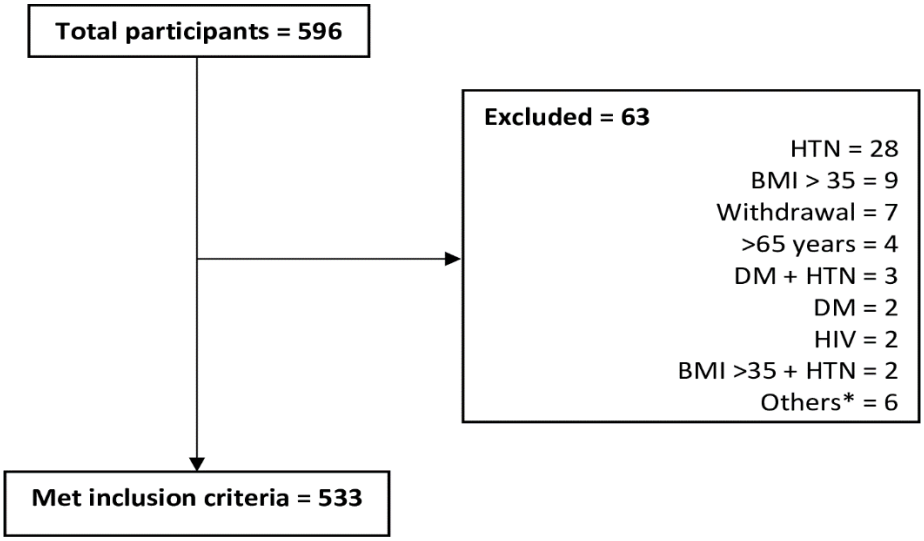


Fig 6.4: Study flowchart

BMI: body mass index, CBC: complete blood count, DM: diabetes mellitus, HIV: Human Immunodeficiency Virus, HTN: hypertension. *On antibiotics, blood donation in past 3 months, on treatment for hypothyroidism, > 65 years, > 65 years and hypertensive, rheumatic heart disease, prostate cancer

The median age was 39 years with a range of 18-65 years. The participant characteristics are summarized in **Table 6.2**.

Table 6.2: Descriptive characteristics of participants

	Male (n=260)		Female (n=273)		Total (n=533)	
	Median (IQR)	Min-Max	Median (IQR)	Min-Max	Median (IQR)	Min-Max
Age (years)	39 (18)	20-65	39 (21)	18-64	39 (20)	18-65
Height (cms)	172 (8)	156-191	160 (9)	143-191	167 (13)	143-191
Weight (kg)	74 (19)	46-116	68 (16)	38-109	70 (18)	38-116
BMI (kg/m^2)	24.9 (5.6)	16.3-34.9	26.1 (6.3)	17.1-38.1	25.5 (5.9)	16.3-38.1
BSA (m^2)	1.88 (0.24)	1.44-2.36	1.72 (0.20)	1.27-2.24	1.78 (0.22)	1.27-2.36
AC (cm)	91 (15)	65-124	86 (16)	64-115	89 (17)	64-124
BP Systolic (mmHg)	127 (18)	84-179	128 (18)	118 (20)	124 (21)	77-194
BP Diastolic (mmHg)	81 (12)	56-101	79 (14)	57-112	80 (14)	56-112

AC: abdominal circumference; BMI: body mass index; BSA: body surface area; IQR: interquartile range

Sources of variation

Sex, age and BMI as sources of variation were explored with SDR≥0.4 regarded as being significant as shown in **Table 6.3**.

Table 6.3: Standard deviation ratios for between sex and age

	SDRsex	SDRage		
	M+F	M+F	M	F
TP	0.00	0.36	0.38	0.35
Alb	**0.50**	**0.54**	**0.56**	**0.53**
Glb	0.32	0.00	0.00	0.00
Urea	0.30	0.32	0.21	**0.42**
eGFR	0.00	0.32	0.34	0.29
UA	**0.89**	0.38	**0.41**	0.33
Cre	**1.19**	0.24	0.30	0.14
TBil	**0.48**	0.27	0.21	0.32
Na	0.00	0.38	0.14	**0.47**
K	0.00	0.23	0.22	0.25
Cl	**0.44**	0.30	0.38	0.18
Ca	0.16	0.29	0.31	0.26
IP	0.21	0.20	0.22	0.17
Mg	0.00	**0.46**	0.38	**0.52**
Glu	0.00	**0.41**	**0.44**	0.39
TC	0.00	**0.41**	**0.41**	**0.42**
TG	0.22	**0.58**	**0.56**	**0.60**
HDL-C	0.33	0.00	0.00	0.00
HDLrat	0.33	0.38	0.39	0.37
nonHDL	0.00	**0.45**	**0.44**	**0.46**
LDL-C	0.00	**0.42**	0.40	**0.43**
Lip	0.07	0.16	0.17	0.15

	SDRsex	SDRage		
	M+F	M+F	M	F
ALT	**0.56**	**0.42**	0.31	**0.52**
AST	**0.48**	0.26	0.09	0.34
ALP	0.00	**0.47**	0.16	**0.66**
AMY	0.05	0.00	0.00	0.00
LDH	0.00	0.22	0.00	0.31
CK	**0.50**	0.08	0.02	0.12
GGT	**0.51**	0.28	0.35	0.14
Iron	0.39	0.09	0.10	0.08
Ferr	**0.78**	0.26	0.22	**0.45**
TF	**0.43**	0.18	0.18	0.18
TFSat	**0.50**	0.00	0.00	0.00
CRP	0.10	0.31	0.37	0.25
IgA	0.00	0.24	0.15	0.31
IgG	0.10	0.12	0.17	0.07
IgM	**0.48**	0.12	0.15	0.08
FT3	0.12	0.04	0.00	0.09
FT4	0.00	0.36	0.35	0.37
TSH	0.00	0.00	0.00	0.03
TgAb	0.00	0.28	0.27	0.28
TPOAb	0.09	0.04	0.06	0.03
Trop I	0.00	0.08	0.00	0.09
PSA			0.39	

SDR≧0.4 shown in bold; SDR≧0.5 oange background

Between-sex differences exceeded that level in Alb, UA, Cre, TBil, Cl, ALT, AST, CK, GGT, Ferr, Tf, TfSat, and IgM. Similarly, between-age subgroup differences were significant for Alb, Cl, Glu, and TG in males, and for Alb, urea, Na, Mg, Glu, TC, TG, HDLrat, LDL-C, ALT, ALP, and Ferr in females. BMI was an independent source of variation for UA, Glu, TC, HDLrat, nonHDL-C, LDL-C, ALT, AST, LDH, and GGT in males, and for CRP only in females. Graphical representations of reference value distribution sub-grouped by sex and age are shown for 12 representative analytes in **Fig 6.5** and for all analytes in **Fig S6.1.**

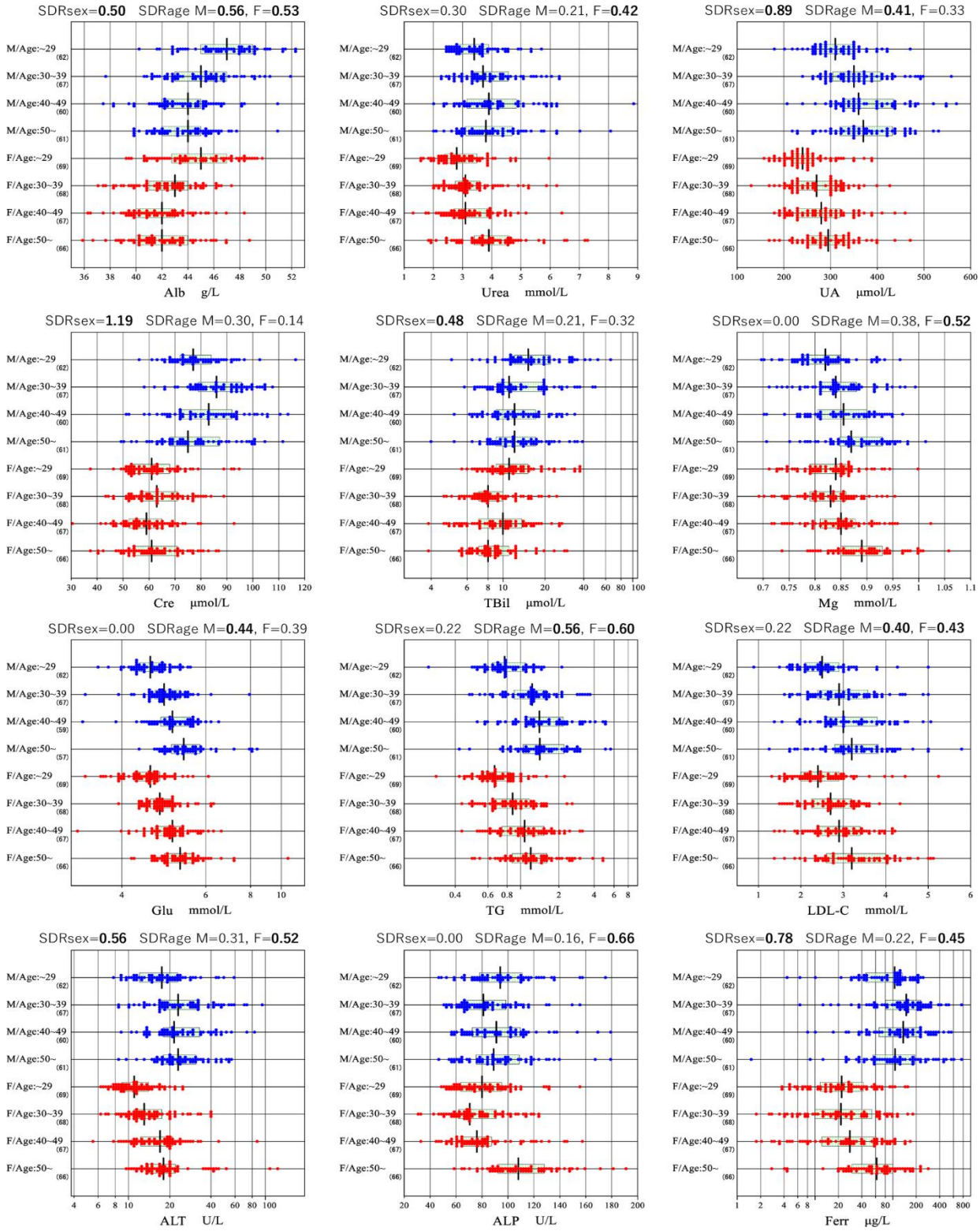


Fig 6.5: Sex and age-related changes for 12 analytes

The distributions of reference values are shown based on age and sex stratification for 12 analytes. The SDRsex and SDRage for each sex are shown at the top of each analyte chart. No secondary exclusion was performed in plotting the data. The box in each scattergram represents central 50% range and the vertical bar in the middle represents a median point.

Reference intervals

Generally, the parametric method resulted in similar or lower RI ULs, and narrower 90% CIs for the LL and UL of the RIs compared to the non-parametric method as shown in **Fig S6.2.** Besides, the accuracy of Gaussian transformation by the parametric method was confirmed as shown in **Fig S6.3.** Therefore, we adopted the RIs derived by the parametric method exclusively. In addition, we could not calculate a RI for Trop I because 94.7% of RVs were below detection limit. For TgAb and TPOAb, we determined cutoff values by use of the probability paper plot as 2.5 IU/ml and 5.0 IU/mL respectively as shown in **Fig 6.3**. We regarded individuals who had antibody values exceeding either of the cutoffs (7.7% of males and 13.6% of females) as possible autoimmune thyroiditis (AIT), and excluded them when calculating RIs for thyroid function tests.

The LAVE method resulted in significant differences in LL or UL (BR_{UL} or $BR_{LL} > 0.375$) for some analytes as shown in **Table S6.1**. For example, UL lowered for AST, ALT, CRP; LL raised for Fe.

Based on the decision flow chart shown in **Fig 6.2**, RIs for TP, Alb, Glb, Na, K, Cl, Ca, IP, Glu, TG, HDL-C, nonHDL, Lip, AMY, LDH, CRP, Fe, IgG and IgA were not partitioned by sex as shown in **Table 6.4**. For age partitions, although the mean age of menopause for females is about 50 years, we chose 45 years as the borderline because there were limited number of subjects above the age of 50.

Table 6.4: Proposed reference intervals

			M + F			M			F		
Item	Units	Age	n	LL	UL	n	LL	UL	n	LL	UL
TP	g/L	All	526	67	83						
Alb	g/L	~45	349	39	50						
		45~	150	39	47						
Glb	g/L	All	524	24	38						
Urea	mmol/L	All				258	2.4	6.6			
		~45							145	1.8	5.2
		45~							83	1.8	6.4
UA	mmol/L	All				259	238	532	272	178	417
Cre	µmol/L	All				260	58	109	271	45	86
TBil	µmol/L	All				257	6	43	271	5	27
Na	mmol/L	All	531	134	142						
K	mmol/L	All	531	3.4	4.8						
Cl	mmol/L	All	501	100	110						
Ca	mmol/L	All	531	2.19	2.57						
IP	mmol/L	All	533	0.78	1.42						
Mg	mmol/L	All				260	0.73	0.98			
		~45							174	0.71	0.93
		45~							99	0.76	1.01
Glu	mmol/L	~45	345	3.9	5.8						
		45~	172	4.4	7.3						
TC	mmol/L	All				260	3.1	6.9			
		~45							174	3.1	6.2
		45~							99	3.2	7.1
TG	mmol/L	~45	289	0.47	2.60						
		45~	154	0.55	3.70						
HDL-C	mmol/L	All	530	0.7	1.7						
HDLrat		All				259	2.6	7.6			
		~45							174	2.4	5.9
		45~							98	2.7	6.7
nonHDL	mmol/L	All	532	2.0	5.6						
LDL-C	mmol/L	All				260	1.6	4.8			
		~45							174	1.5	4.2
		45~							99	1.7	4.9
Lip	U/L	All	527	9	68						
ALT	IU/L	All				202	10	55	229	7	30
AST	IU/L	All				206	17	40	227	15	29
ALP	IU/L	All				260	53	153			
		~45							172	47	130
		45~							99	55	174
AMY	IU/L	All	530	47	164						
LDH	IU/L	All	530	138	257						
CK	IU/L	All				259	72	460	268	53	260
GGT	IU/L	All				199	13	90	269	10	50
CRP	mg/L	All	425	0.21	14.67						
Fe	µmol/L	All	412	8.8	28.9						
Ferr	µg/L	All				253	10	475			
		~45							173	2	150
		45~							99	0	222
Tf	g/L	All				254	1.9	3.2	206	2.0	3.5
TfSat	%	All				208	15	49	211	10	44
IgG	g/L	All	525	11.1	20.0						
IgA	g/L	All	526	1.05	4.63						
IgM	g/L	All				254	0.38	2.29	272	0.51	2.77
FT3	pmol/L	All	467	3.9	6.3						
FT4	pmol/L	All	468	7.8	14.1						
TSH	mIU/L	All	461	0.61	4.86						
PSA	µg/L	All				243	0.29	2.92			

A comparison of our RIs with those recommended by Beckman coulter and those derived from IFCC studies conducted in India, Saudi Arabia and Turkey found much higher RIs for TBil as shown in **Table 6.5**. A similar comparison that also includes studies carried out in other African countries is shown in **Table S6.2.**

Table 6.5: Comparison of reference intervals

		PRESENT STUDY KENYA							BECKMAN AU[1]							SAUDI ARABIA[2]							TURKEY[3]							INDIA[4]						
			M+F		M		F			M+F		M		F			M+F		M		F			M+F		M		F			M+F		M		F	
Test	Units	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL
TP	g/L	18-65	67	83					NA	66	83					18-65	66	83					20-79	66	82					18-65	68	86				
Alb	g/L	18-65							NA	35	52					18-65	39	50					20-79	41	49					18-65					36	47
		18-44			40	51	38	49																												
		45-65			40	47	38	46																												
Urea	mmol/L	18-65			2.4	6.6			NA	2.8	7.2					18-65			2.8	7.3	2.1	6.4								18-65			2.2	6.0		
		18-44					1.8	5.3															20-49			2.95	7.20	2.21	6.12	18-45					1.9	5.1
		45-65					1.9	6.2															50-79					2.85	7.96	46-65					2.4	6.7
UA	µmol/L	18-65			243	507	178	417	NA			208.3	428.4	154.7	357	18-65			223	444	148	321	20-79			226	458	166	345	18-65			248	509	159	404
Cre	µmol/L	18-65			58	109	45	86	NA			59	104	45	84	18-65			66	111	50	74	20-79			59	92	50	71	18-65			58	95	35	74
TBil	µmol/L	18-65			6	43	5	27	NA	5	21					18-65			3.6	22.4	2.2	15.5	20-79			3.8	24.1	2.7	15.9	18-65			6.2	23.7	4	17.3
Na	mmol/L	18-65	134	142					NA	136	146					18-65	135	144					20-79	137	144					18-65	135	146				
K	mmol/L	18-65	3.4	4.8					NA	3.5	5.1					18-65	3.7	4.9					20-79	3.7	4.9					18-65	3.8	5				
Cl	mmol/L	18-65	100	110					NA	101	109					18-65	101	111					20-79	99	107					18-65	102	113				
Ca	mmol/L	18-65	2.19	2.57					NA	2.2	2.65					18-65	2.11	2.56					20-79	2.15	2.47					18-65	2.1	2.44				
IP	mmol/L	18-65	0.78	1.42					NA	0.81	1.45					18-65	0.81	1.44					20-79	0.8	1.4					18-65	0.8	1.43				
Mg	mmol/L	18-65			0.73	0.98			NA			0.73	1.06	0.77	1.03	18-65	0.71	0.96					20-79	0.77	1.06					18-65	0.77	1.07				
		18-44					0.71	0.93																												
		45-65					0.76	1.01																												
Glu	mmol/L	18-65							NA	4.1	5.9					18-65	4	5.9					20-79	3.96	5.88					18-65						
		18-44	3.9	5.8																										18-45	4.1	5.5				
		45-65	4.4	7.3																										46-65	4.3	6.0				
TC	mmol/L	18-65							NA		5.2					18-65	3.5	6.36												18-65					2.9	6.6
		18-45	3.10	6.10																			20-49	3.22	6.45	3.20	6.42	3.20	6.38	18-45			3.1	6.2		
		46-65	3.20	7.20																			50-79					3.93	7.92	46-65			2.5	6.7		
TG	mmol/L	18-65							NA		1.7					18-65			0.5	3.58	0.39	1.6								18-65			0.6	2.7	0.5	2.1
		18-45			0.50	2.88	0.45	2.03															20-49			0.53	3.39	0.46	2.52	18-45						
		46-65			0.53	3.87	0.55	3.24															50-79					0.64	3.55	46-65						
HDL-C	mmol/L	18-65	0.70	1.70					NA	1.03	1.55					18-65			0.74	1.76	0.98	2.19				0.85	1.52	0.95	1.56	18-65			0.7	1.5	0.8	1.8
LDL-C	mmol/L	18-65			1.60	4.80			NA		2.60					18-65	1.8	4.34												18-65	1.7	4.4				
		18-44					1.50	4.20															20-49	1.47	3.92	1.60	4.01	1.32	3.92	18-45						
		45-65					1.70	4.90															50-79					1.78	4.91	46-65						
Lip	U/L	18-65			8	75	10	63	NA		67																			18-65						
ALT	IU/L	18-65			10	55	7	30	NA				50		35	18-65			7	39	5	18				9	57	7	28	18-65			15	74	10	37
AST	IU/L	18-65			17	40	15	29	NA				50		35	18-65			11	28	10	24				13	30	11	25	18-65			20	53	17	39
ALP	IU/L	18-65			53	153			NA	30	120						39	114												18-65			41	111		
		18-44					47	130															20-49	38	112	43	116	34	97	18-45					35	100
		45-65					55	174															50-79					47	133	46-65					43	117
AMY	IU/L	18-65	47	164					NA	22	80					18-65	31	117						34	119					18-65	36	135				
LDH	IU/L	18-65	138	257					NA				248		247	18-65	10	238						126	220					18-65	104	206				
CK	IU/L	18-65			72	460	53	260	NA				171		145	18-65			54	266	27	138				48	227	34	131	18-65			48	304	36	184
GGT	IU/L	18-65			13	90	10	47	NA				55		38	18-65			11	65	7	21				11	69	7	33	18-65			14	62	11	40
Fe	µmol/L	18-65	8.8	28.9					NA			12.5	32.2	10.7	32.2	18-65			7.9	29.6	3.7	26				5.9	31.6	3.5	27.8	18-65			7	33	4	26
Ferr	µg/L	18-65			20	457			NA			20	250	10	120								18-79					4.7	136	18-65						
		18-44					5	150															18-44			13	276	4.3	91							
		45-65					12	232															45-79					5.9	175							
Tf	g/L	18-65			1.9	3.2	2.0	3.5	NA	2	3.6					18-65			2	3.2	2.1	3.9	18-79	1.8	3.3	1.8	3.3	1.9	3.5	18-65	2.2	4				
TfSat	%	18-65	13	47																										18-65						
CRP	mg/L	18-65	0.21	14.7					NA		1					18-65	0.2	11.8												18-65			0.33	7.34	0.35	11.9
IgA	g/L	18-65	1.05	4.63					NA	0.7	4																			18-65	0.94	4.35				
IgG	g/L	18-65	11.1	20.0					NA	7	16																			18-65	9.1	20.4				
IgM	g/L	18-65			0.38	2.29	0.51	2.77	NA	0.4	2.3																			18-65			0.4	2.54	0.51	3.1
FT3	pmol/L	18-65	3.9	6.3					NA	3.8	6																			18-65						
FT4	pmol/L	18-65	7.8	14.1					NA	7.86	14.41																			18-65						
TSH	mIU/L	18-65	0.61	4.86					NA	0.38	5.33																			18-65						
PSA	µg/L	18-65			0.25	2.95																								18-65						

Key: M+F-Male & Female; M-Male; F-Female; [1](*AU Reagent Quick Reference Guide*, 2018), [2](Borai et al., 2016), [3](Ozarda et al., 2014), [4](S. Shah, Ichihara, Dherai, & Ashavaid, 2018)

Standardization of the RIs

Since this study utilized a serum panel provided by C-RIDL which comprised 50 samples with values assigned to 25 chemistry analytes, we confirmed the standardized status of the assays as shown in **S6.4 Fig**. For the method comparison, major-axis regression was used to express the structural relationship between our test results and the panel assigned values by calculating BR_{LL} or BR_{UL} as a difference of LL or UL before and after the recalibration using the regression coefficients. As a result, we found it necessary to recalibrate our RIs for HDL-C and LDH. For Na, we could not get a good linear relationship because of poor precision of the assay with the narrow reference interval.

6.5 Discussion

There have been controversies over the statistical methods used in deriving RIs. They include: selection between parametric and nonparametric methods, how and when to exclude RVs secondarily, and when to partition RVs into subgroups by sex and age. In this study we sought optimal options for each. We found the nonparametric method of limited use with its wider 90% CI for RIs and frequent bias in UL (**Fig S6.1**), while the parametric method was found to be more reliable after successful Gaussian transformation (**Fig S6.3)** For secondary exclusion, we found LAVE procedure effective for reducing the influence of over nutrition for those analytes with high association with BMI such as TG, ALT, AST, and CRP. It was also effective in reducing the influence of latent inflammation and anemia on Fe, Tf and TfSat.

In order to decide the need for partitioning RVs into subgroups by sex or age, we primarily used SDR, but it tended to provide an inflated value when the width of the RI was narrow. Another problem of SDR is that it reflects between-subgroup difference at the central part which may not reflect between-subgroup bias at the LL or UL, hence the use of BR_{LL} or BR_{UL}. Furthermore, we found it necessary to confirm the appropriateness of BR_{LL} or BR_{UL} by quantitating the actual difference at LL or UL by use of the reporting unit (RU). We chose to adopt partitioned RIs only when the difference at LL or UL was ≥3RU. We believe this three-way consideration ensured optimal judgement in partitioning RIs by sex and age as shown in **Table S6.1**.

RIs can vary appreciably across different populations as demonstrated in the interim report of the global study analyzing RVs of 12 countries (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017). They identified ethnic differences in many analytes such as Alb, urea, TBil, HDL-C, CRP, IgG, C3, and PTH. In reference to the RV profiles, we noted certain unique features of Kenyan RIs. For instance, our urea RIs are lower than those from Turkey and Saudi Arabia. Although most of our RIs for liver function tests were similar to published reports from Saudi Arabia and Turkey (Borai et al., 2016; Ozarda et al., 2014), our TBil RI of 6-43 µmol/L for males and 5-27 µmol/L for females is almost double what has been reported in published studies from outside the African continent as shown in **Table S6.2** (Borai et al., 2016; Ichihara, Ceriotti, Kazuo, et al., 2013; Kratz et al., 2004; Ozarda et al., 2014; S. Shah et al., 2018). We hypothesize that Gilbert's syndrome, the commonest genetic cause of asymptomatic unconjugated hyperbilirubinaemia, could be quite prevalent in our population. Genetic studies would thus be useful in elucidating the cause of hyperbilirubinaemia observed in our study.

Our electrolytes didn't differ much from other published IFCC studies except that Mg levels increased with age in females. It has been documented that reduced levels of oestrogen are associated with increased Mg levels in post-menopausal women as well as in the follicular phase of the menstrual cycle in women of reproductive age (Dullo & Vedi, 2008; Lindsay, Hart, & Forrest, 1980).

Our TC and TG RIs were higher than those reported by Kibaya *et al* who carried out a similar study in a rural Kenyan population (Kibaya et al., 2008). Our study population was primarily composed of urban dwellers of whom 25.6% had metabolic syndrome (Omuse, Maina, Hoffman, et al., 2017). According to the third report of the National Cholesterol Education Program (NCEP), the desirable LDL-C level for low risk adults is <4.1 mmol/L (NCEP, 2001). Our UL for LDL-C in males (4.8 mmol/L) and in females (4.2 and 4.9 mmol/L) are higher than the NCEP targets. However, the NCEP clinical decision limits (CDLs) are used for diagnosis of hyperlipidaemia and serve as treatment targets for reducing cardiovascular risk. Aside from the risk of over-nutrition, the RI ULs for TC and LDL-C are important in diagnosing cholestatic conditions and hypothyroidism. These are relatively short-term conditions unrelated to occurrence of atherosclerosis hence the RIs are more relevant than CDLs in their diagnosis. On the other hand, determination of LLs for TC or LDL-C RIs are essential for diagnosing malnutrition or thyrotoxicosis. Therefore, we are not replacing the derived RIs with those CDLs, rather providing CDLs in the footnote of test result report sheet.

We obtained fasting plasma glucose (FPG) RIs of 3.9−5.8 and 4.4−7.3 mmol/L for those aged <45 and ≥45 years respectively. The former RI is comparable to what was obtained in Turkey (3.96−5.88 mmol/L) and Saudi Arabia (4.0−5.9 mmol/L) (Borai et al., 2016; Ozarda et al., 2014). The American Diabetes Association (ADA) uses FPG ≥ 5.6 and 7.0 mmol/L to define pre-diabetes and diabetes respectively. Based on the ADA criteria, a total of 63 out of 528 participants would have been classified as having elevated values compared to 20 if the ULs of the derived RIs were used.

Our RIs for immunoglobulins are higher than those derived in the US but very similar to those derived in India (Kratz et al., 2004; S. Shah et al., 2018). Karita *et al* also found high levels of IgG in several SSA countries (Karita et al., 2009). We hypothesize that this may be due to inflammation caused by increased exposure to either infectious disease agents or environmental allergens. The increased inflammation is further evidenced by the very high RI UL for CRP of 14.7 mg/L compared to 1 mg/L recommended by Beckman Coulter (*AU Reagent Quick Reference Guide*, 2018). Ichihara *et al* in a similar study carried out in Asia found that the closer the country or region was to the equator, the higher the serum concentrations of positive inflammatory markers (IgG, C3, CRP) a phenomenon they ascribed to increased exposure to infectious agents (Ichihara, Ceriotti, Tam, et al., 2013). Similar to the IFCC study in India, IgM was significantly higher in female participants requiring the determination of sex specific RIs (S. Shah et al., 2018). We hypothesize that this could be linked to the role that estrogen plays in enhancing humoral immunity (Taneja, 2018).

Ichihara *et al* has demonstrated that BMI is a major source of variation in RVs for many analytes and that the magnitude of this association varies across different populations (Ichihara, Ozarda, Barth, Klee, Shimizu, et al., 2017). For example, a similar change in BMI resulted in a greater decline in HDL cholesterol amongst the Japanese (r = −0.39) compared to people from Pakistan (r = −0.05). On the other hand, an increase in BMI was associated with a greater increase in ALT values (r=0.48) amongst non-black South Africans compared to black South Africans (r=0.02) (Ichihara, Ozarda, Barth, Klee, Shimizu, et al., 2017). BMI was a source of variation for several analytes in males especially those known to be associated with the presence of metabolic syndrome such as Glu, LDL-C, and ALT. We did not partition our RIs by BMI because the influence of BMI was suppressed by use of the LAVE method.

For iron markers, we also applied LAVE to reduce the influence of latent anemia. Although it was very effective in raising their LLs, the female LL for ferritin was still lower than the WHO cutoff value of <15 µg/L for iron deficiency (WHO, 2011b).

The strengths of our study include the deliberate recruitment of healthy individuals using a harmonized protocol to ensure pre-analytical confounders were minimized, centralized analysis of samples in an accredited laboratory with excellent internal quality control, standardization of the RIs by use of a value-assigned panel of sera and use of the LAVE procedure to reduce the influence of sub-clinical disease on the derived RIs.

The weaknesses include failure to perform infectious serology to rule out chronic infections such as HIV, HBV or HCV, and over representation of an urban population hence limiting the generalizability of our findings to a rural population.

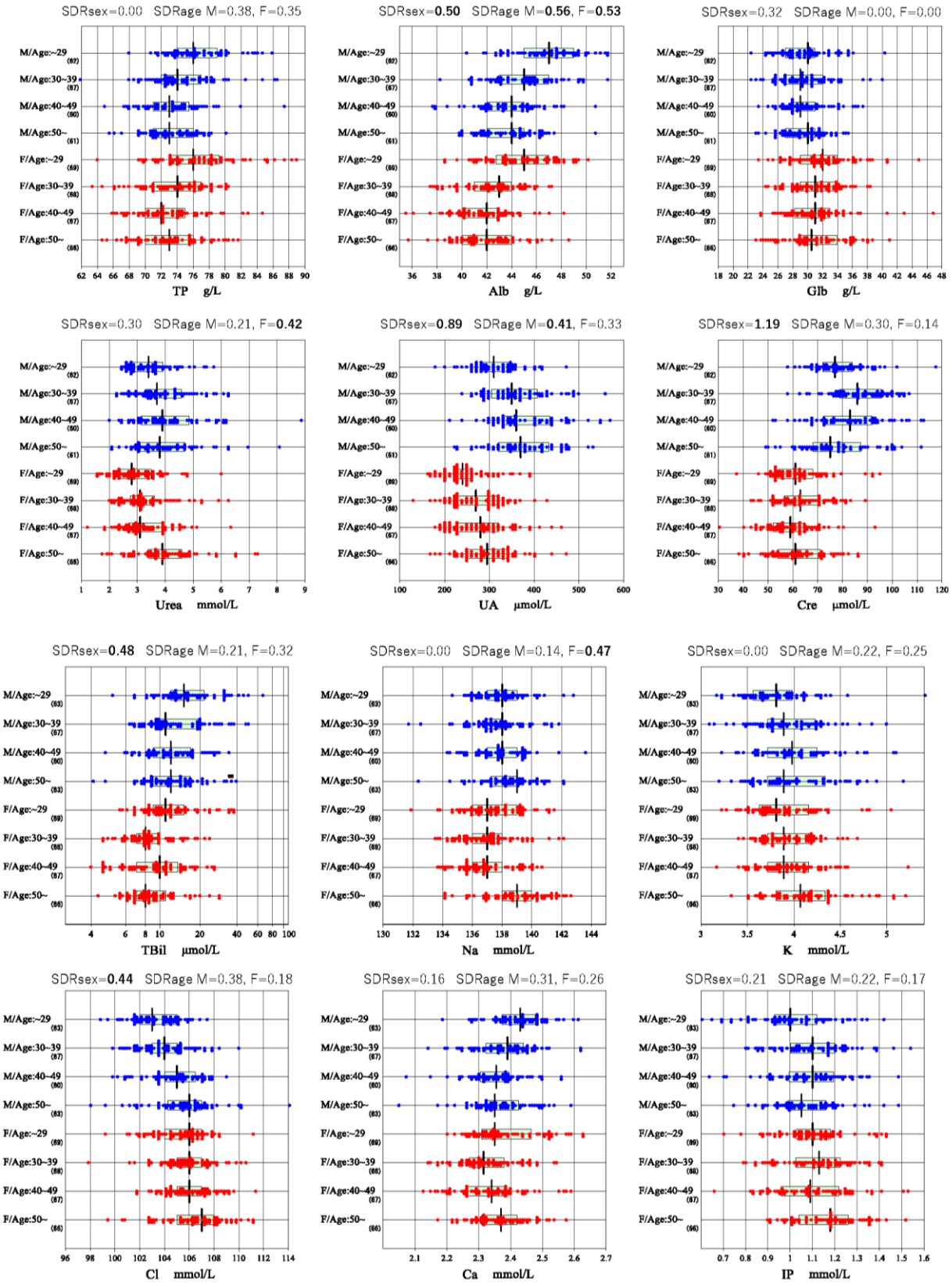
Conclusion

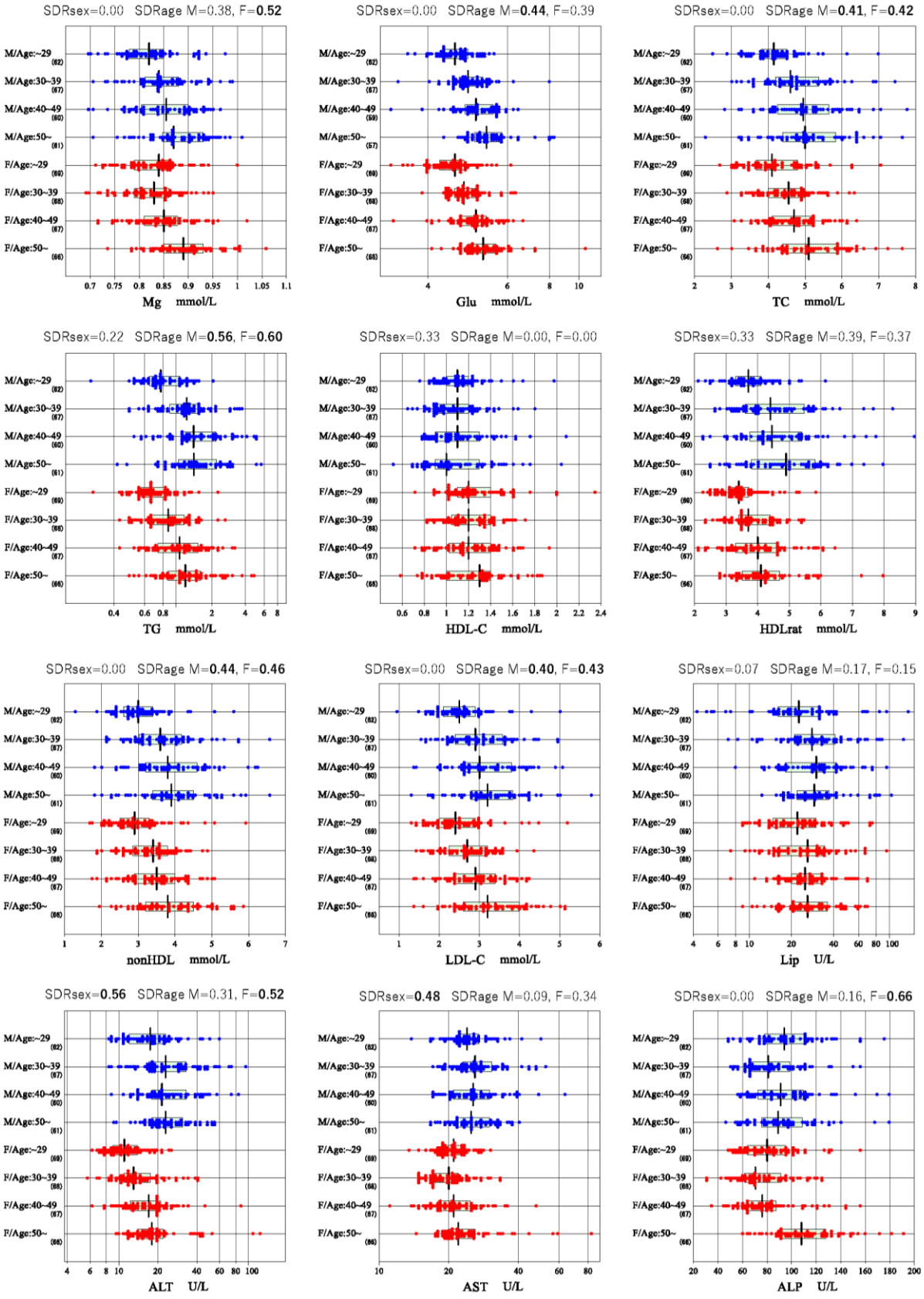
According to the harmonized IFCC-C-RIDL protocol, we established RIs for 40 major chemistry and immunoassay tests from well-defined healthy Kenyan volunteers by use of the up-to-date statistical methods for the first time in Africa. The LAVE method was effective in reducing the influence on RIs of latent anemia and metabolic disorders. Based on SD ratio and bias ratios, we developed a flow chart to judge the need for partitioning RVs by sex and age subgroups, which we believe is helpful for the future RI study. RIs for chemistry analytes were standardized by use of a value assigned serum panel, and thus could be shared across sub-Saharan African laboratories with similar ethnic and life-style profile. As a whole, Kenyan RIs were comparable to those of other countries participating in the global study with a few exceptions such as higher ULs for TBil and CRP.

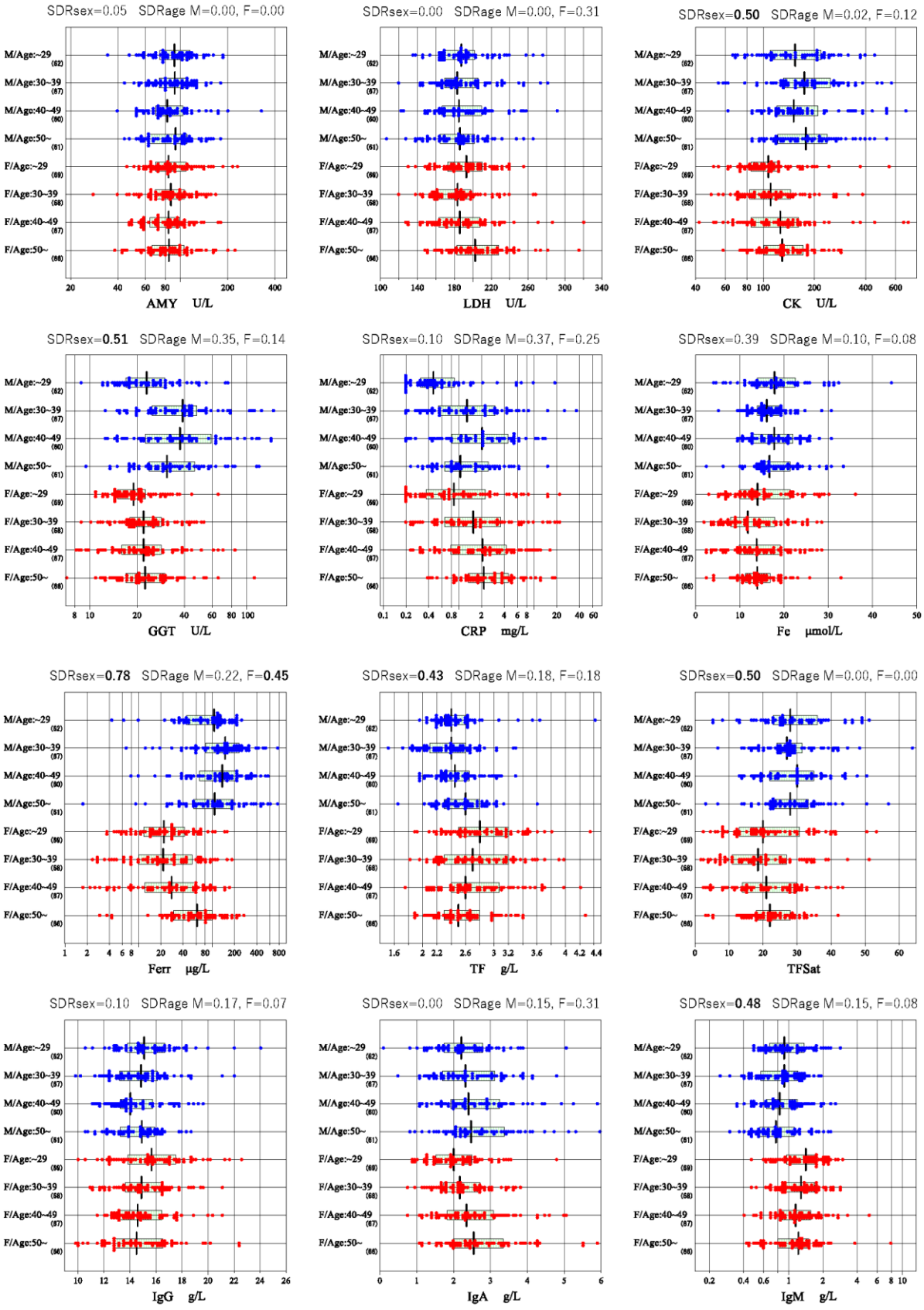
Acknowledgments

Jared Oseko and Patricia Ingato of Aga Khan University Hospital Nairobi assisted in sample collection, bar coding and processing. Benjamin Matheka, marketing manager PathCare Kenya Limited, assisted in participant recruitment and sample collection. Arno Theron carried out sample analysis, quality assurance and data handling at the PathCare reference laboratory in Cape Town, South Africa.

6.6 Supporting information



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M/Age:30~39 (67)
M/Age:40~49 (60)
M/Age:50~ (61)
F/Age:~29 (69)
F/Age:30~39 (68)
F/Age:40~49 (67)
F/Age:50~ (66)
20 40 60 80 200 400
AMY U/L
SDRsex=0.00 SDRage M=0.00, F=0.31
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LDH U/L
SDRsex=0.50 SDRage M=0.02, F=0.12
40 60 80 100 200 400 600
CK U/L
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8 10 20 40 60 80 100
GGT U/L
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CRP mg/L
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1 2 4 6 8 20 40 80 200 400 800
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IgM g/L

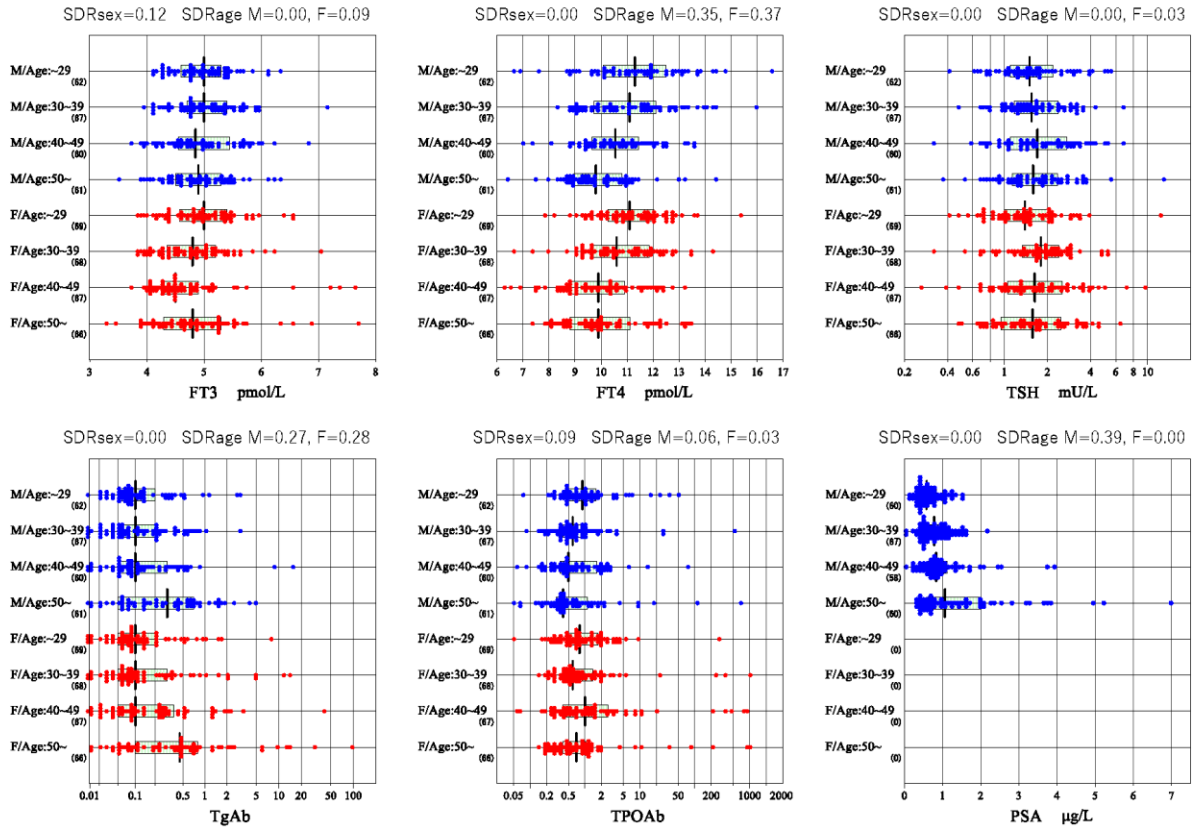
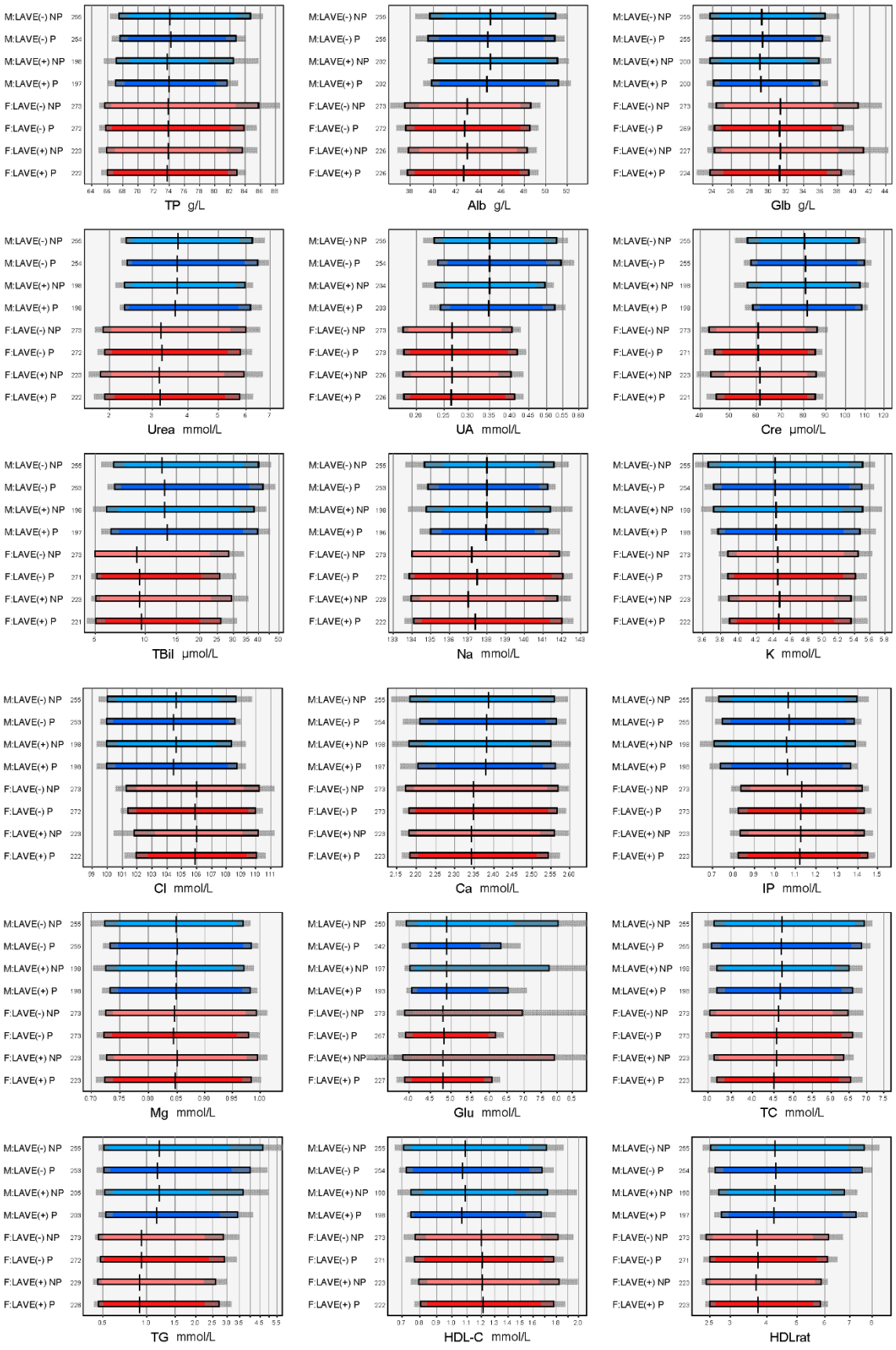


Fig S6.1: Sex and age-related changes

The distributions of reference values are shown based on age and sex stratification for all analytes. The SDRsex and SDRage for each sex are shown at the top of each analyte chart. No secondary exclusion was performed in plotting the data. The box in each scattergram represents central 50% range and the vertical bar in the middle represents a median point.



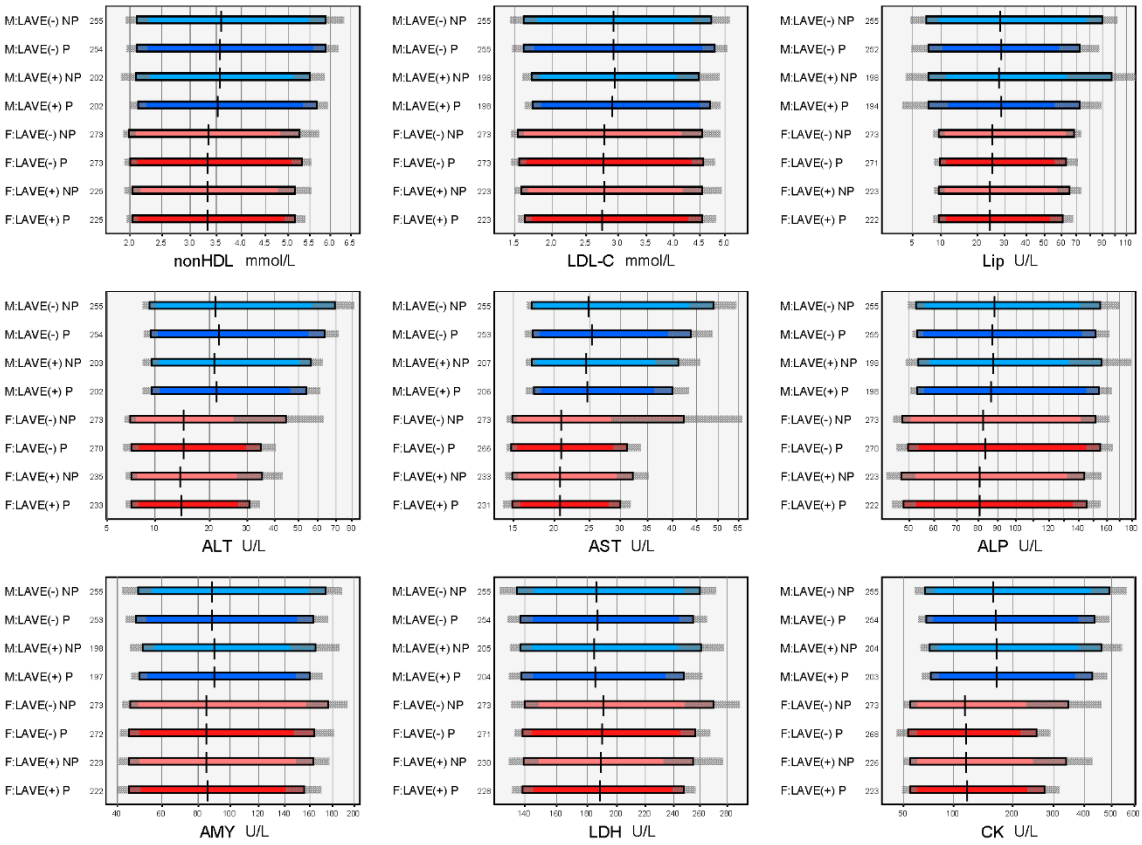
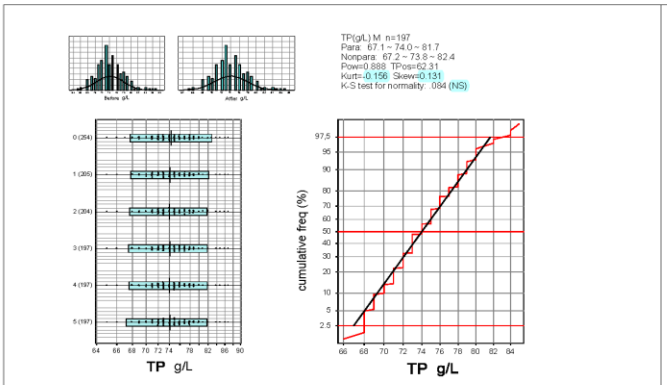
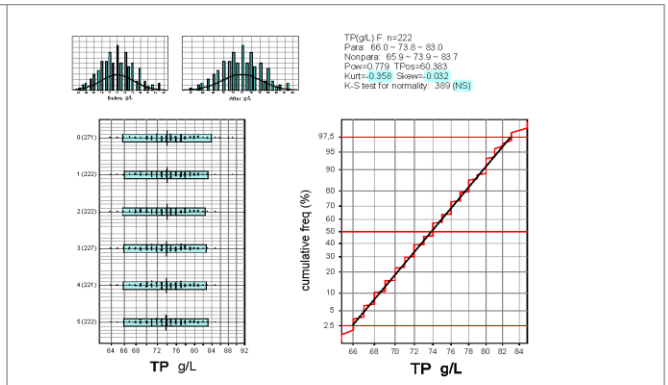
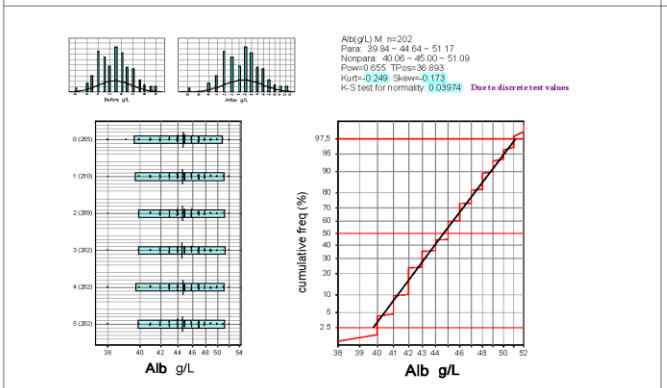


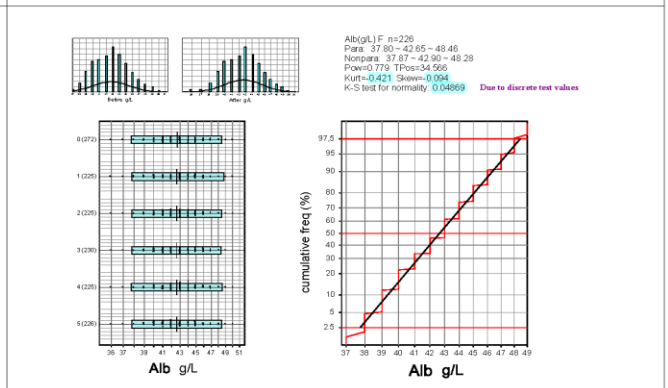
Fig S6.2: Bar-chart comparison of reference interval calculation methods

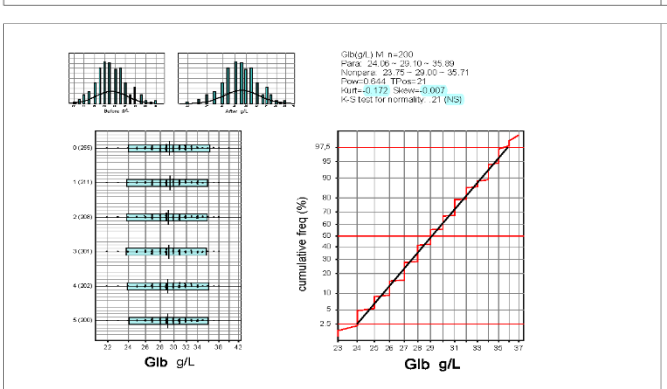
The RIs of all analytes were derived in four ways by parametric (P) or nonparametric (NP) method with/without the LAVE procedure, separately in males+Females (MF), males, and females. Each horizontal bar represents the range of the RI, and the vertical line in the center corresponds to the mid-point. The shades on both ends of the bar represent 90%CI for the limits of the RI predicted by the bootstrap method.

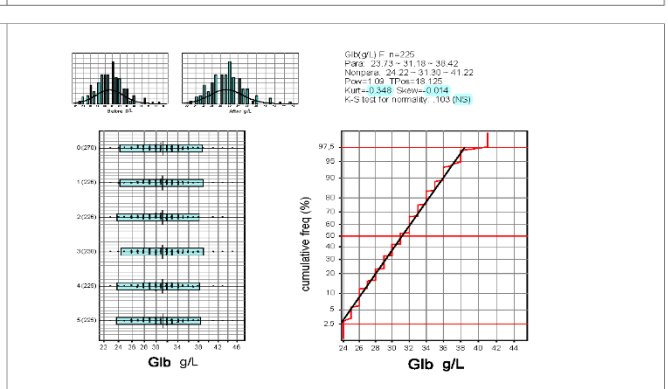


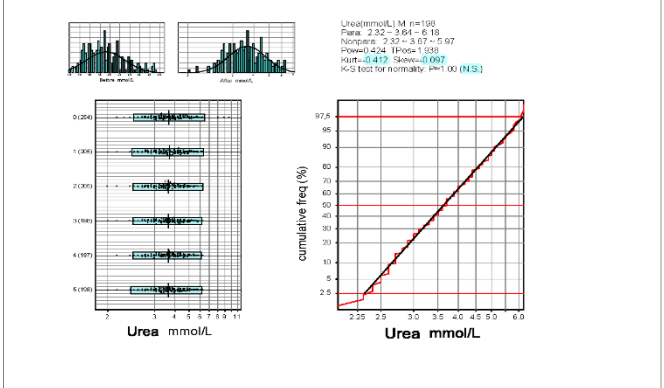


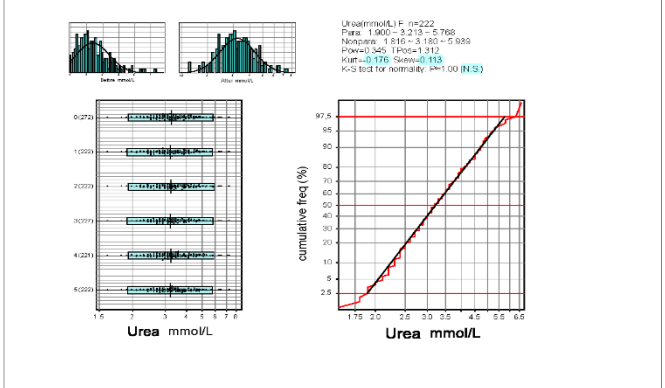


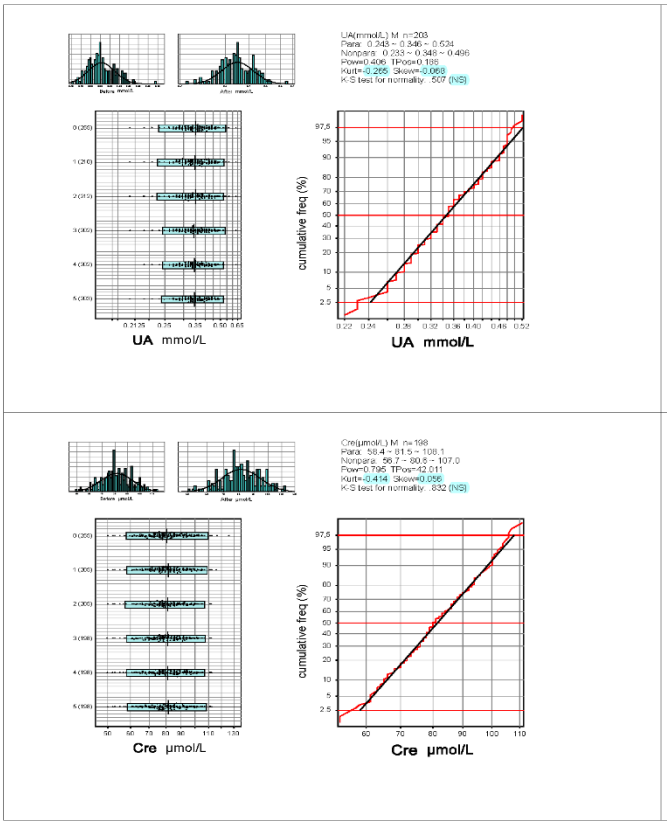


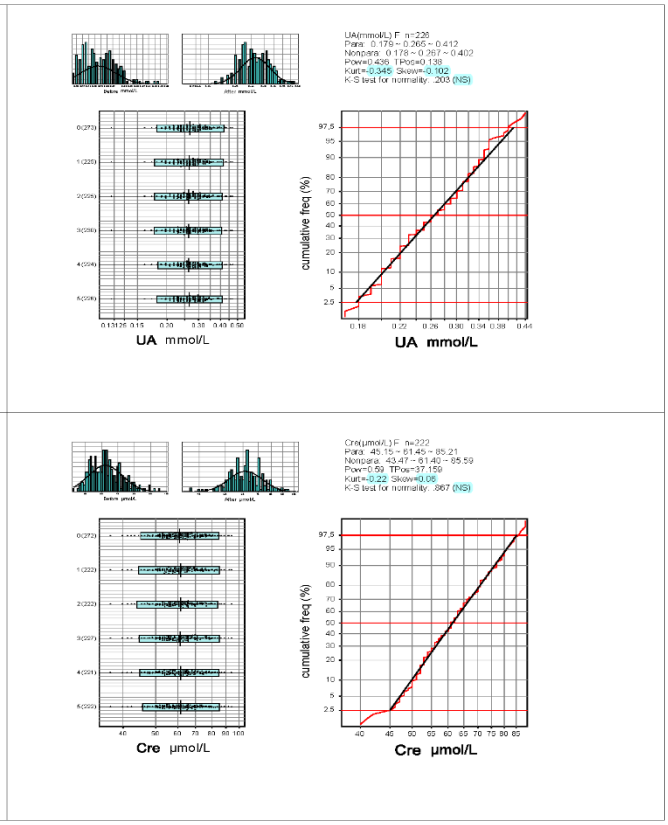


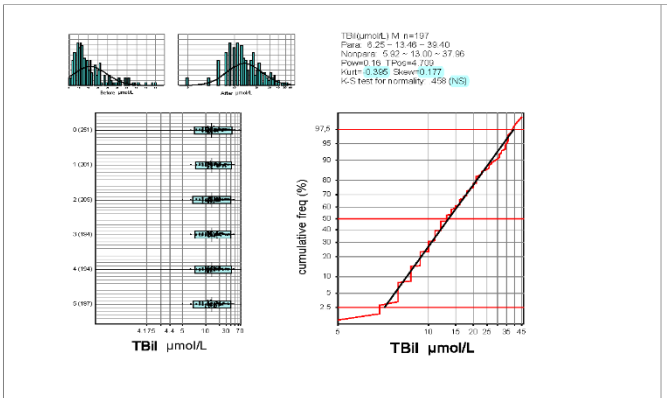


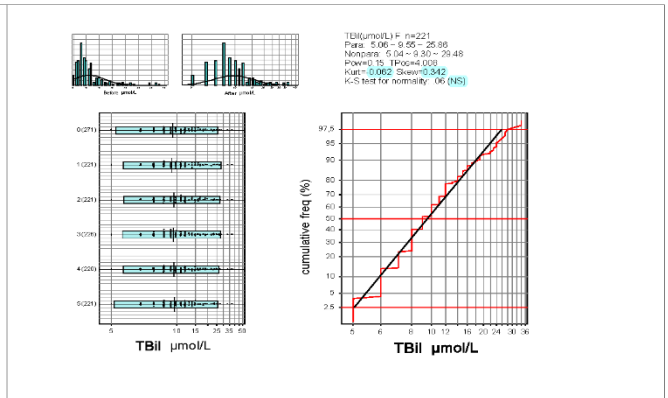


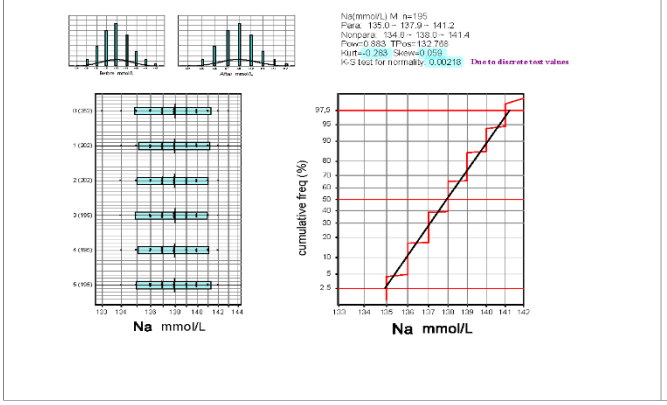


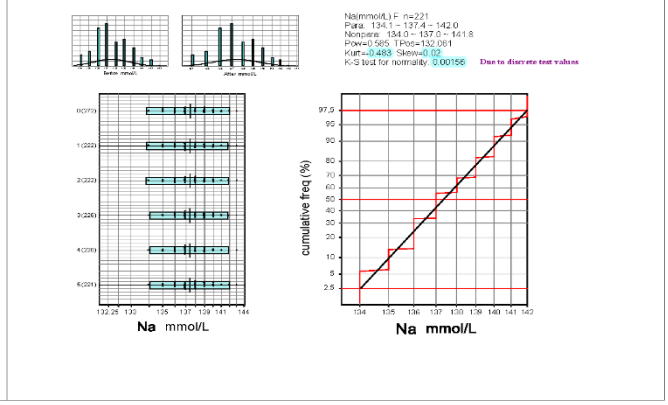


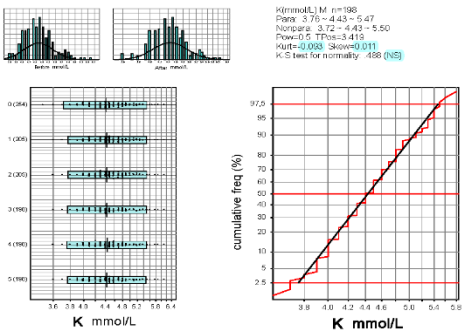


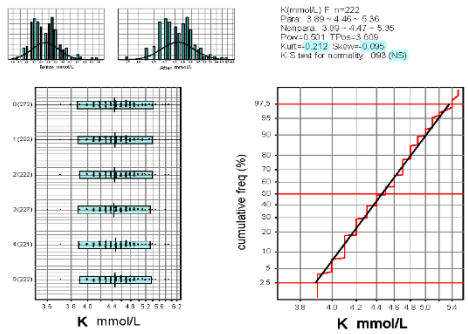


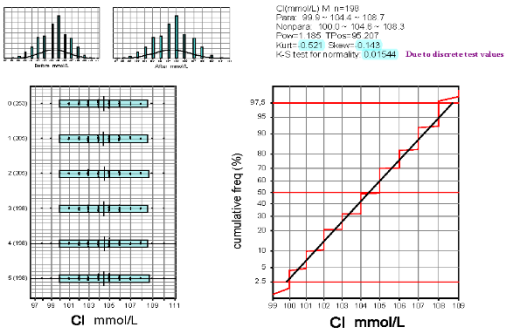


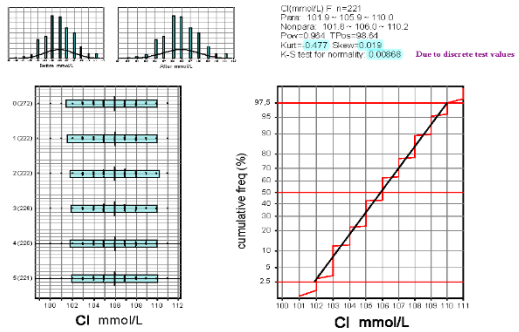


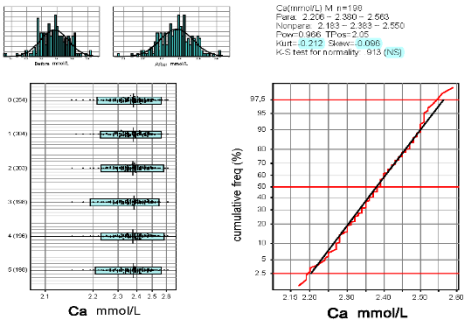


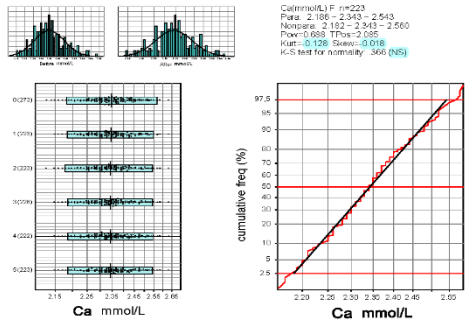


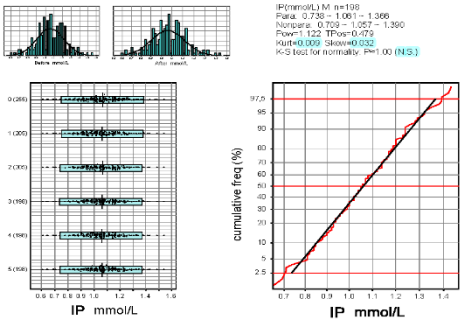


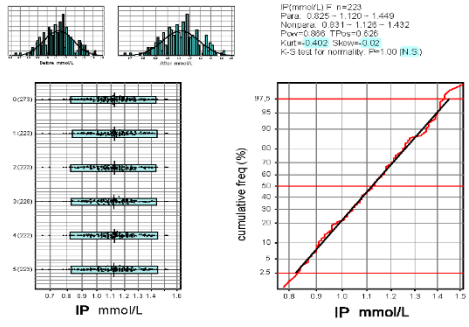


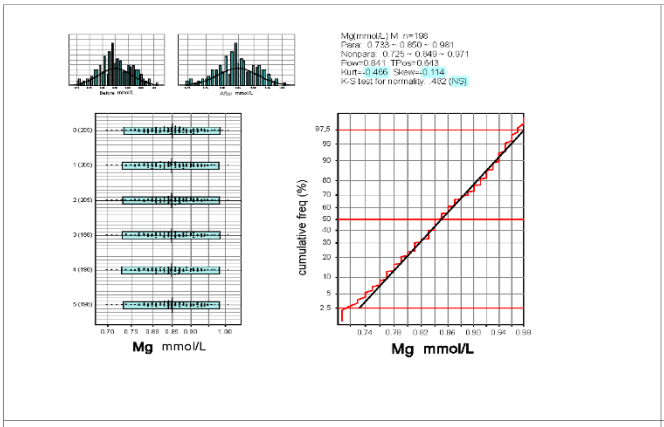


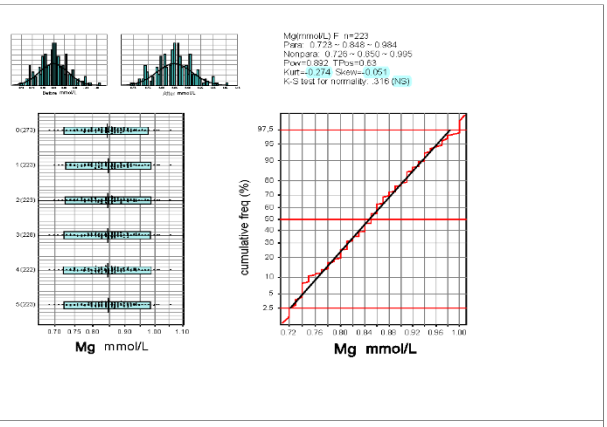


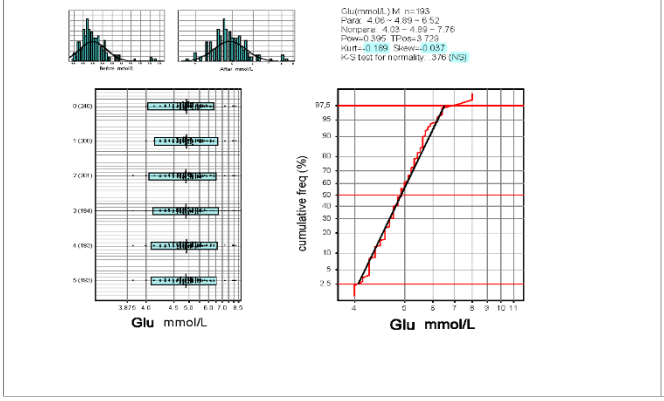


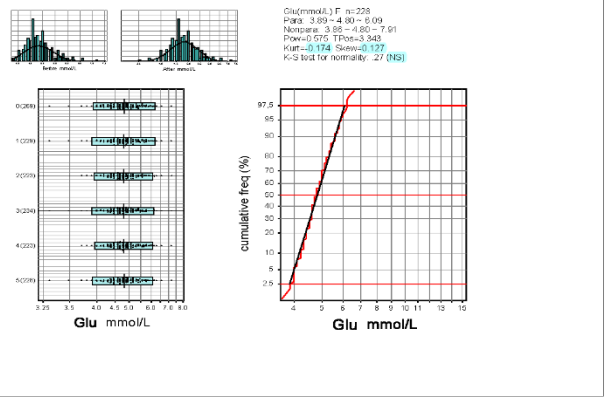


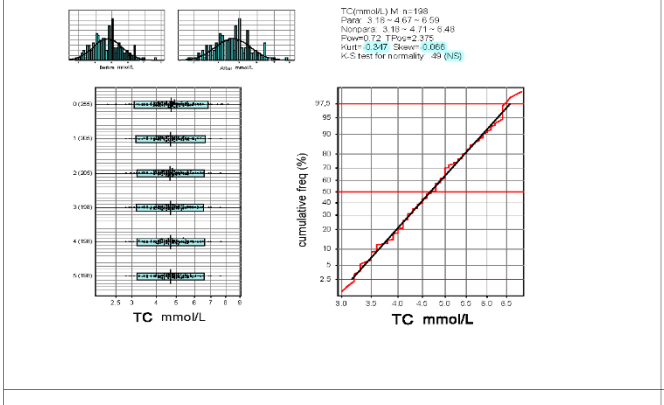


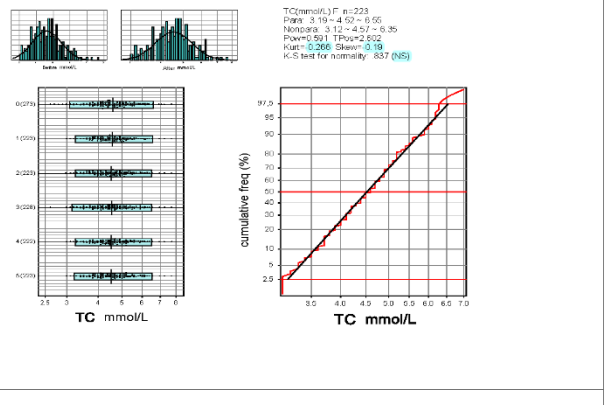


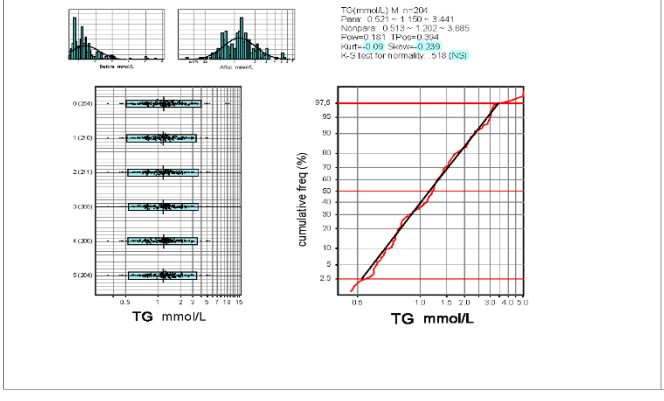


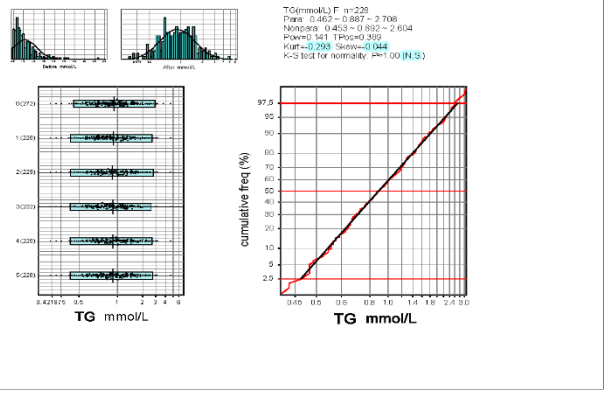


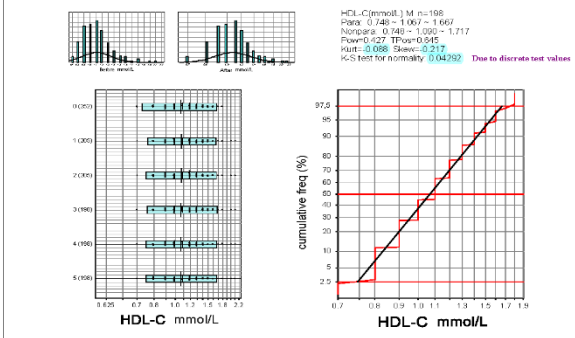


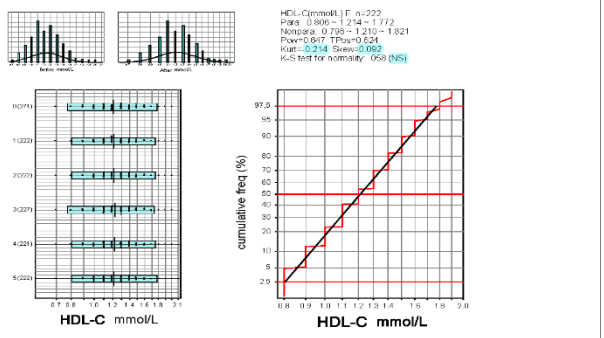


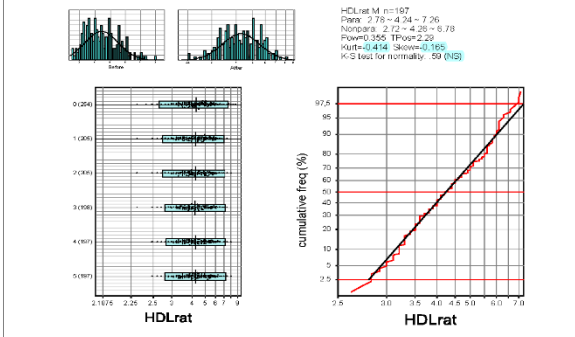


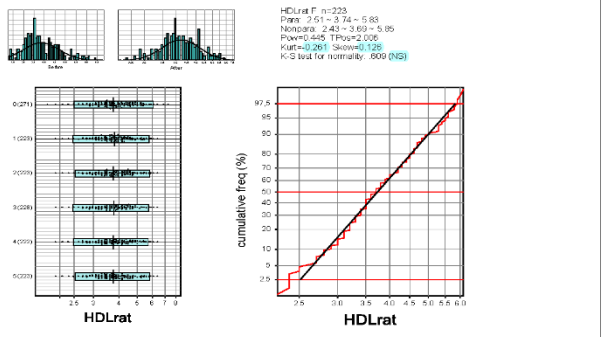


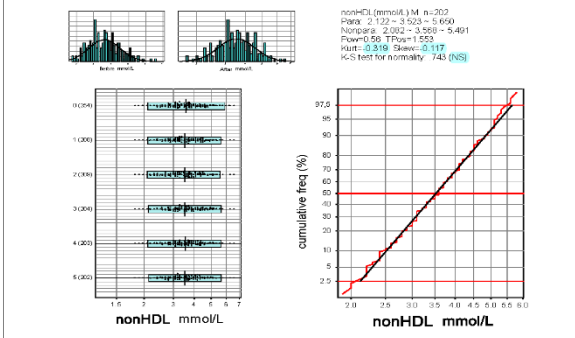


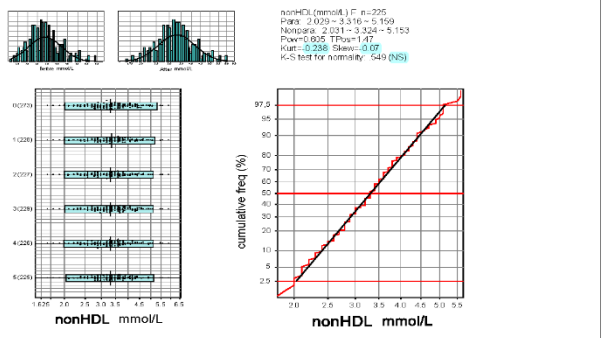

HDL-C(mmol/L) M n=198
Para: 0.748 ~ 1.067 ~ 1.667
Nonpara: 0.748 ~ 1.090 ~ 1.717
Pow=0.627 TPos=0.945
Kurt=-0.088 Skew=-0.217
K-S test for normality: 0.04292 Due to discrete test values
HDL-C mmol/L
cumulative freq (%)
HDL-C mmol/L

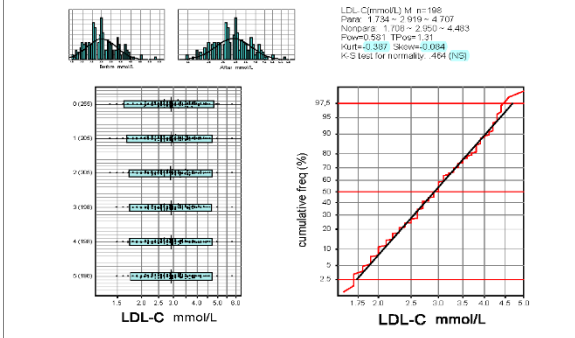

HDL-C(mmol/L) F n=222
Para: 0.806 ~ 1.214 ~ 1.772
Nonpara: 0.798 ~ 1.210 ~ 1.821
Pow=0.647 TPos=0.624
Kurt=-0.214 Skew=0.092
K-S test for normality: .058 (NS)
HDL-C mmol/L
cumulative freq (%)
HDL-C mmol/L

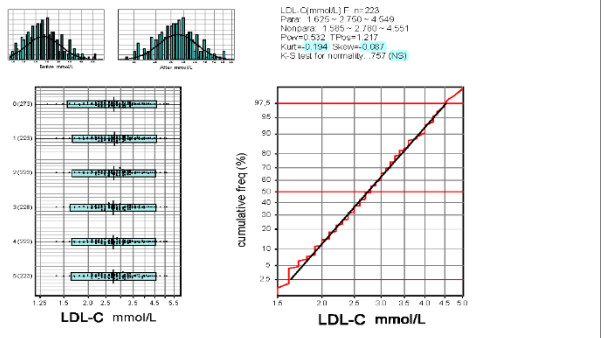

HDLrat M n=197
Para: 2.78 ~ 4.24 ~ 7.26
Nonpara: 2.72 ~ 4.28 ~ 6.78
Pow=0.355 TPos=2.29
Kurt=0.414 Skew=0.165
K-S test for normality: .59 (NS)
HDLrat
cumulative freq (%)
HDLrat

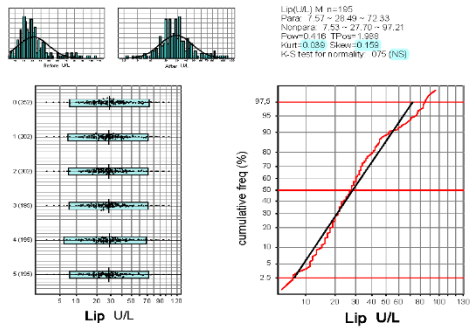

HDLrat F n=223
Para: 2.51 ~ 3.74 ~ 5.83
Nonpara: 2.43 ~ 3.69 ~ 5.85
Pow=0.445 TPos=2.006
Kurt=-0.261 Skew=0.126
K-S test for normality: .609 (NS)
HDLrat
cumulative freq (%)
HDLrat

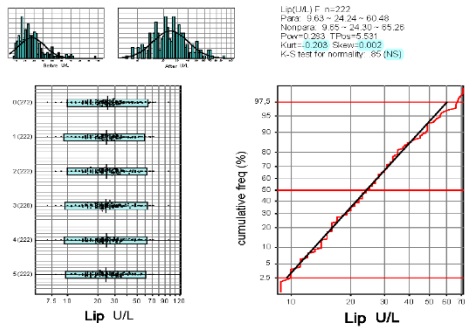

nonHDL(mmol/L) M n=202
Para: 2.122 ~ 3.523 ~ 5.650
Nonpara: 2.092 ~ 3.568 ~ 5.491
Pow=0.56 TPos=1.553
Kurt=-0.319 Skew=-0.117
K-S test for normality: .743 (NS)
nonHDL mmol/L
cumulative freq (%)
nonHDL mmol/L

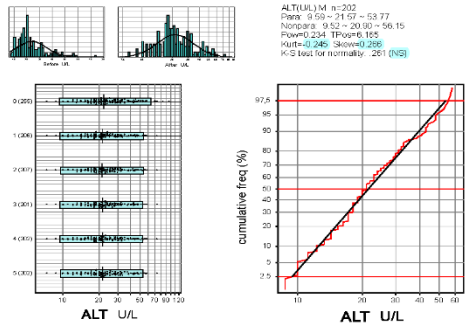

nonHDL(mmol/L) F n=225
Para: 2.029 ~ 3.316 ~ 5.159
Nonpara: 2.031 ~ 3.324 ~ 5.153
Pow=0.605 TPos=1.47
Kurt=-0.238 Skew=-0.07
K-S test for normality: .549 (NS)
nonHDL mmol/L
cumulative freq (%)
nonHDL mmol/L

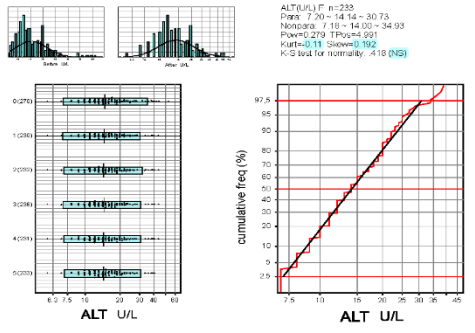

LDL-C(mmol/L) M n=198
Para: 1.734 ~ 2.919 ~ 4.707
Nonpara: 1.708 ~ 2.950 ~ 4.483
Pow=0.581 TPos=1.31
Kurt=-0.387 Skew=0.084
K-S test for normality: .464 (NS)
LDL-C mmol/L
cumulative freq (%)
LDL-C mmol/L

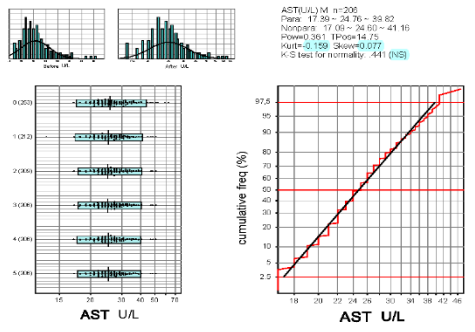

LDL-C(mmol/L) F n=223
Para: 1.625 ~ 2.760 ~ 4.549
Nonpara: 1.585 ~ 2.780 ~ 4.551
Pow=0.532 TPos=1.217
Kurt=-0.194 Skew=-0.087
K-S test for normality: .757 (NS)
LDL-C mmol/L
cumulative freq (%)
LDL-C mmol/L

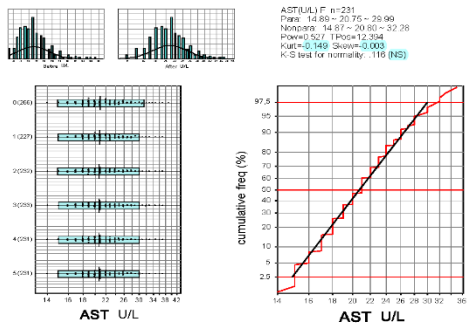


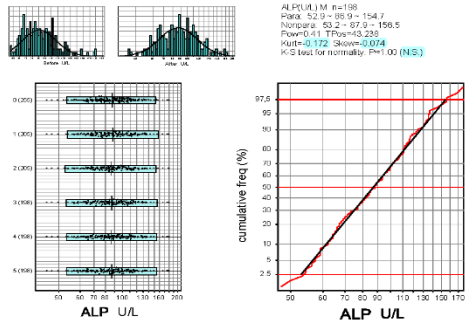


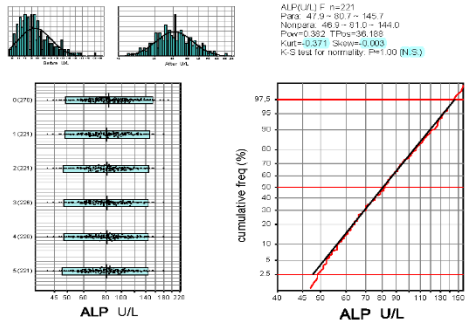


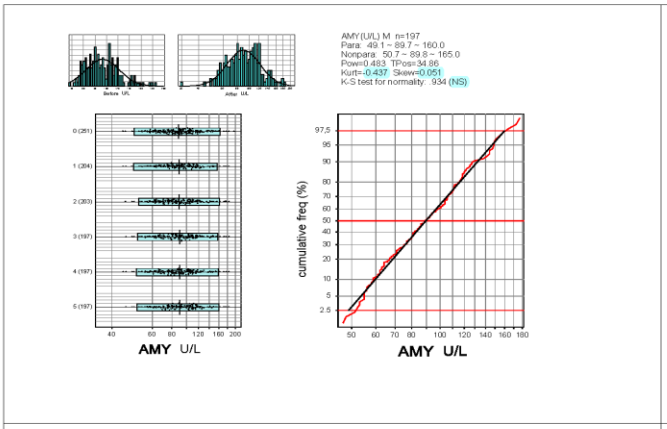


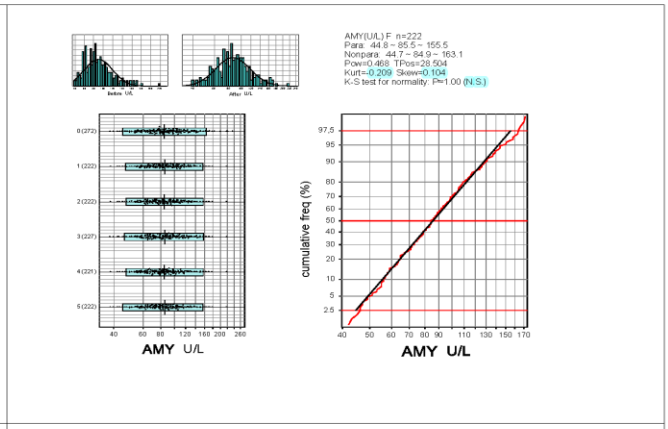


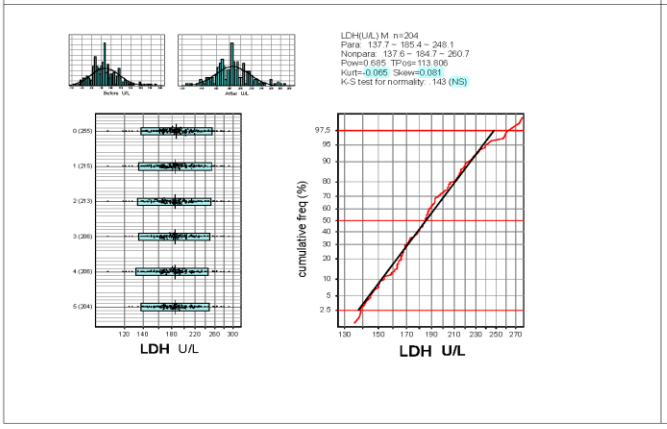


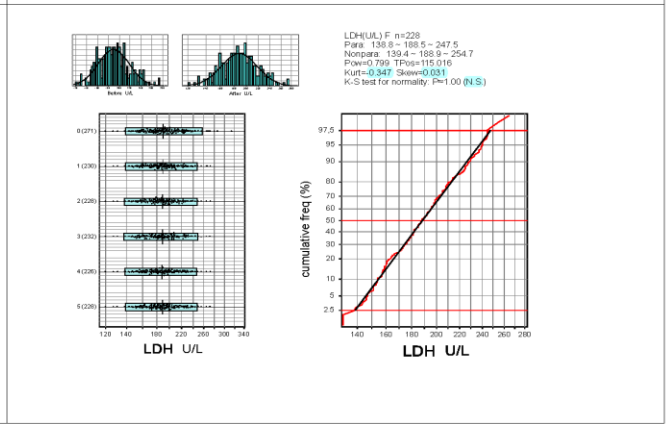


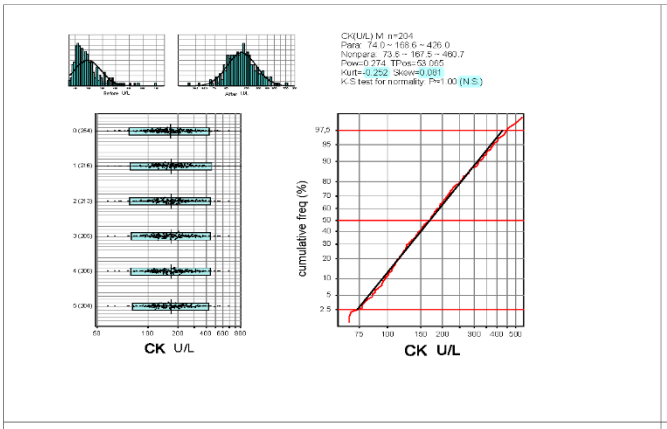


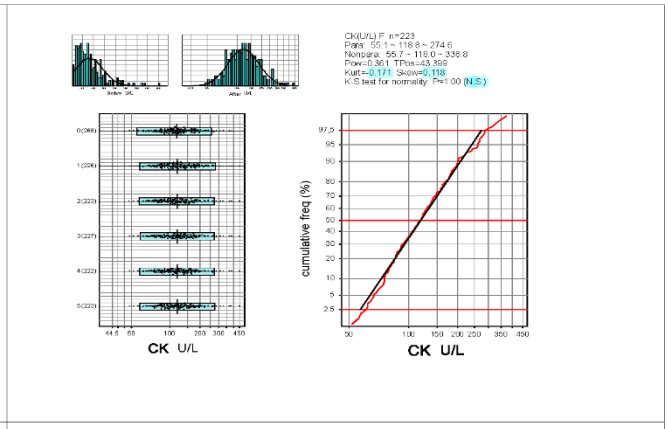


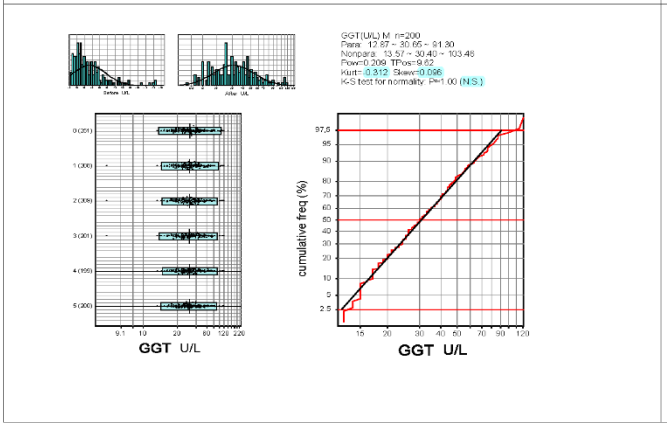


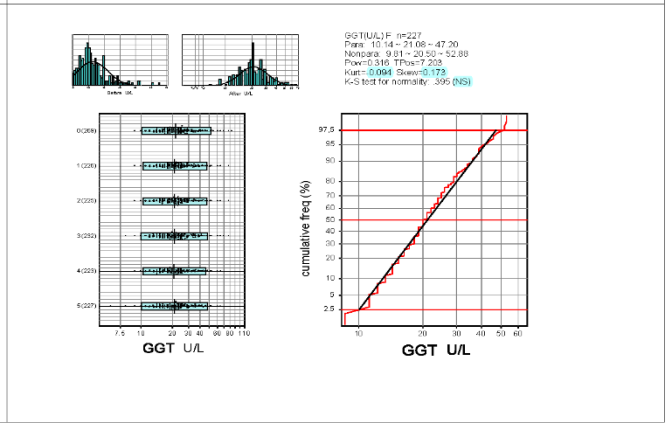












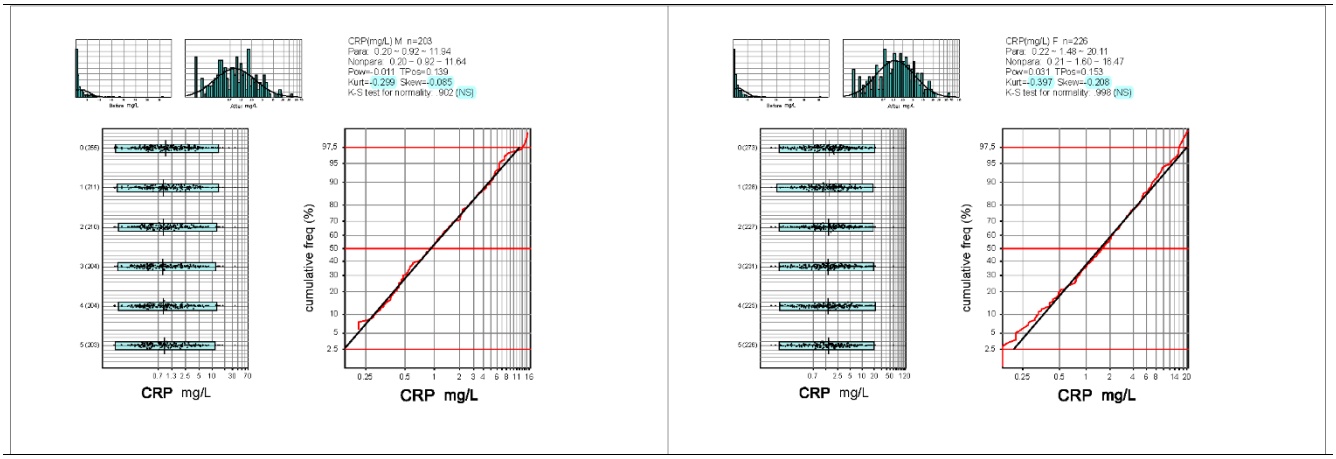
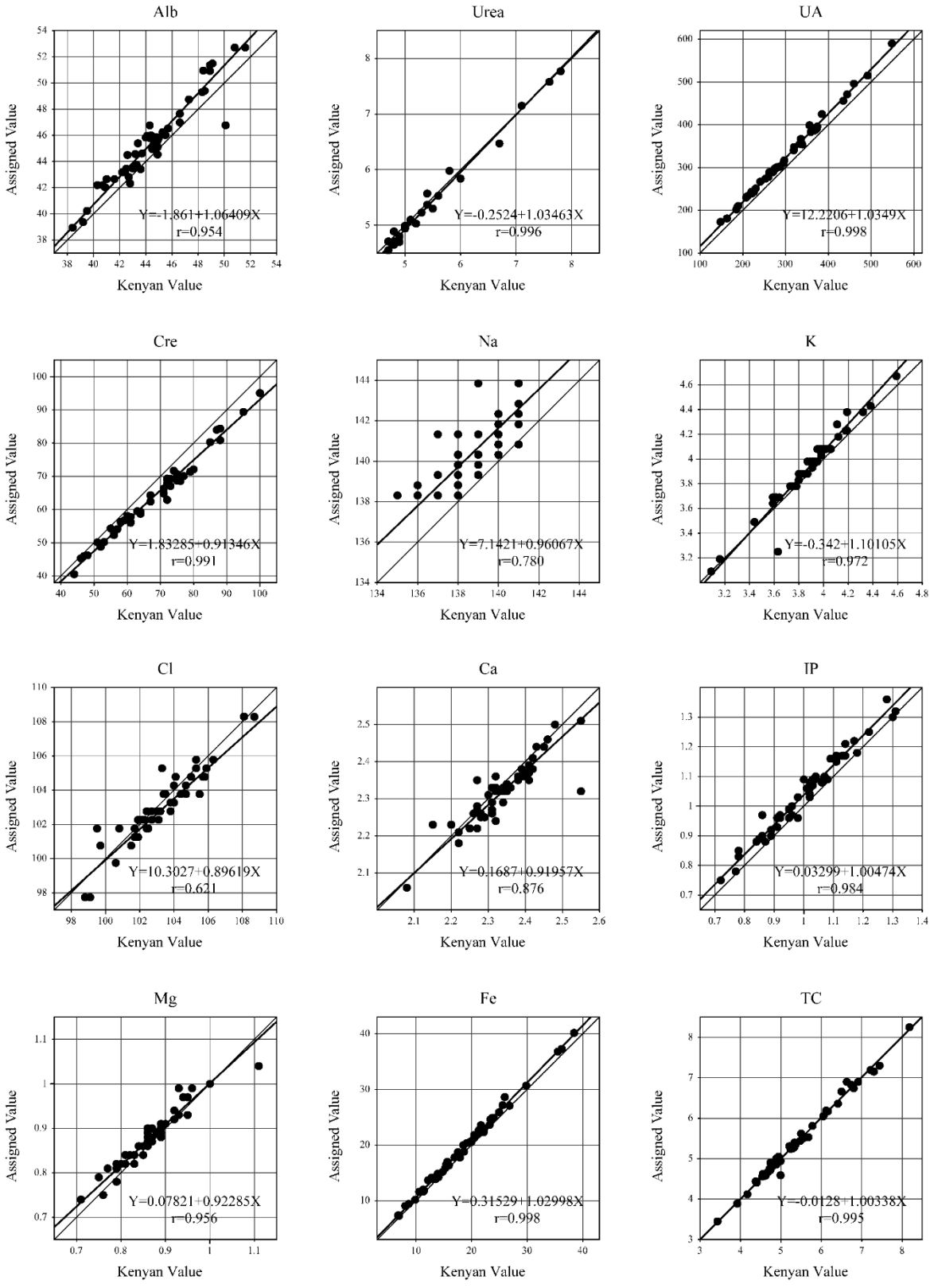


Fig S6.3: Results of Gaussian transformation by the parametric method

The accuracy of Gaussian transformation by Box-Cox formula can be assessed from theoretical Gaussian curves in two histograms shown on left top (before and after the transformation) of each panel. Accuracy can be also seen from the linearity in probability paper plot on the right. The limits of the RI by nonparametric method corresponds to the points where red zigzag line intersect with horizontal 2.5 and 97.5 % red lines of cumulative frequencies.



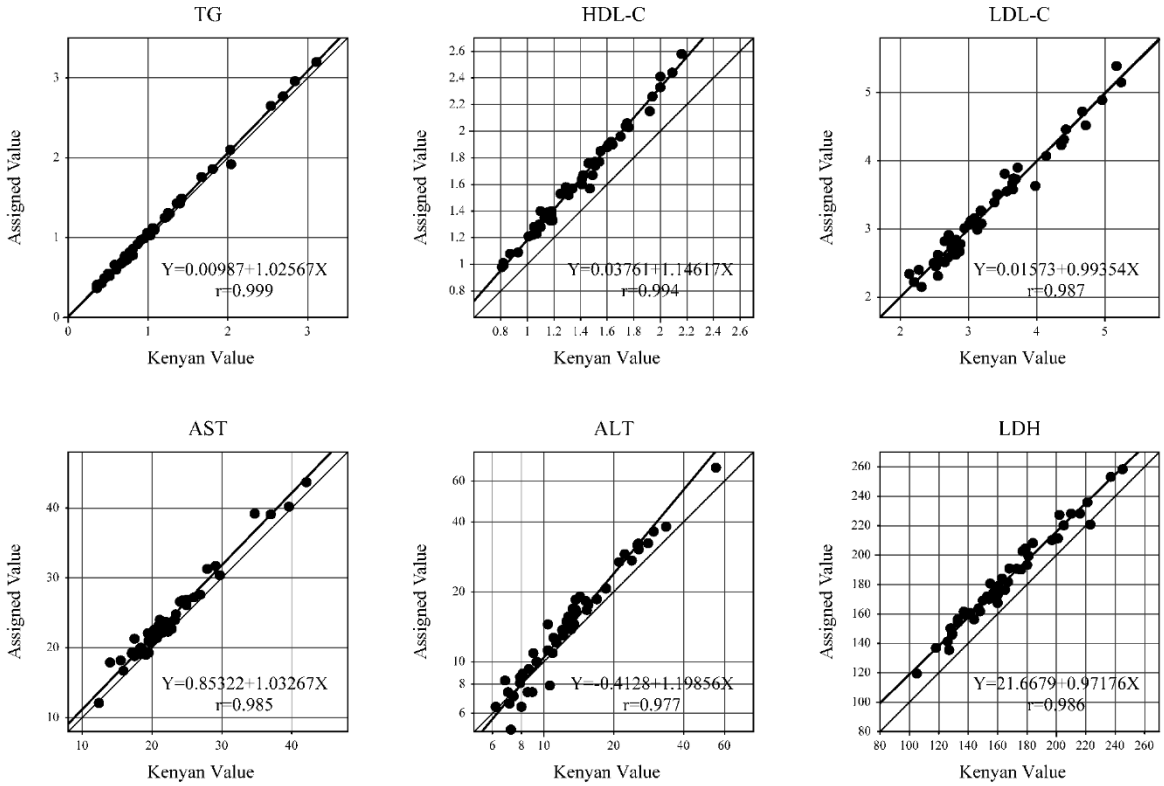


Fig S6.4: Panel test results for assessment of standardized status of assays

The panel of sera from 50 healthy volunteers, each of which were value assigned for 40 chemistry analytes were measured. Our measured values were plotted on Y-axis and assigned values on X-axis. Major axis linear regression was used as a structural relationship for the method comparison. The Y=X line is shown as a diagonal broken line.

Table S6.1: Reference intervals and determination of method, sex and age bias

									RIs by parametric method													LAVE effect on LL			LAVE effect on UL			btw-sex diff		btw-age diff		btw-age diff		Decision
				M	F				M + F				M				F																	
Item	Units	RU	SDR sex	SDR age		LAVE criteria	LAVE	Age	n	LL	Me	UL	n	LL	Me	UL	n	LL	Me	UL		M+F	M	F	M+F	M	F						
																						BR_{LL}			BR_{UL}			BR_{LL}	BR_{UL}	BR_{LL}	BR_{UL}	BR_{LL}	BR_{UL}	
TP	g/L	1	0.00	0.38	0.35	2	(-)	All	526	67	74	83	254	68	74	83	271	66	74	84								**0.49**	-0.25					No partition by sex/age, LAVE-
						2	(+)		414	67	74	82	195	68	74	82	215	66	74	82	0.00	0.00	0.00	0.26	0.28	**0.49**	**0.52**	0.00					
Alb	g/L	1	**0.52**	**0.55**	**0.52**	2	(-)	All	527	38	44	50	255	40	45	51	272	38	43	48								**0.65**	**0.98**					Partition by age, LAVE- for < 45 and LAVE + > 45
						2	(+)		423	39	44	50	197	41	45	51	216	38	43	49	-0.36	**-0.39**	0.00	0.00	0.00	-0.36	**1.07**	**0.71**					
						2	(-)	~45	349	39	44	50	176	40	45	51	173	38	43	49								**0.71**	**0.71**	0.00	**0.71**	0.00	**0.78**	
						2	(+)		292	39	44	50	148	40	45	51	145	38	43	49	0.00	0.00	0.00	0.00	0.00	0.00	**0.71**	**0.71**	0.00	**1.57**	0.00	**1.07**	
						2	(-)	45~	183	38	43	48	84	40	44	49	99	38	42	47								**0.78**	**0.78**					
						2	(+)		150	39	43	47	66	40	43	47	83	38	42	46	**-0.49**	0.00	0.00	**0.49**	**1.12**	**0.49**	**0.98**	**0.49**					
Glb	g/L	1	0.34	0.00	0.00	2	(-)	All	524	24	30	38	255	24	29	36	270	24	31	39								0.00	**-0.84**					No partition by sex/age. LAVE-
						2	(+)		435	24	30	37	204	24	29	36	221	24	31	37	0.00	0.00	0.00	0.30	0.00	**0.60**	0.00	-0.30					
Urea	mmol/L	0.1	0.30	0.22	**0.41**	1	(-)	All	530	2.0	3.5	6.1	258	**2.4**	3.7	**6.6**	272	1.9	3.2	5.8								**0.48**	**0.76**					Partition by age for female. LAVE-
						1	(+)		395	2.0	3.4	6.0	198	2.3	3.6	6.3	218	1.9	3.2	5.9	0.00	0.10	0.00	0.10	0.29	-0.10	**0.39**	**0.39**					
						1	(-)	~45	349	2.0	3.3	5.8	175	2.4	3.6	6.5	174	1.8	3.1	5.3								**0.62**	**1.24**	0.00	**-0.47**	-0.10	**-0.90**	
						1	(+)		284	2.0	3.3	5.6	145	2.4	3.5	6.3	145	**1.8**	3.0	**5.2**	0.00	0.00	0.00	0.22	0.20	0.12	**0.65**	**1.20**	0.10	0.00	0.00	**-1.18**	
						1	(-)	45~	182	2.1	3.7	6.6	84	2.4	3.9	7.0	99	1.9	3.6	6.2								**0.44**	**0.70**					
						1	(+)		149	2.1	3.7	6.4	66	2.3	3.8	6.3	83	**1.8**	3.6	**6.4**	0.00	0.10	0.09	0.18	**0.69**	-0.17	**0.46**	-0.09					
UA	mmol/L	1	**0.94**	0.39	0.31	1	(-)	All	532	183	308	489	259	**238**	350	**532**	272	**178**	268	**417**								**0.77**	**1.47**					Partition by sex. LAVE-
						1	(+)		407	184	297	459	204	243	349	507	221	178	264	403	-0.01	-0.07	0.00	**0.43**	0.37	0.24	**0.93**	**1.48**					
Cre	µmol/L	1	**1.21**	0.27	0.14	1	(-)	All	531	47	70	108	260	**58**	80	**109**	271	**45**	61	**86**								**0.84**	**1.48**					Partition by sex. Use LAVE-
						1	(+)		394	46	69	105	198	58	81	108	218	44	61	84	0.07	0.00	0.10	0.20	0.08	0.20	**0.93**	**1.59**					
TBil	µmol/L	1	**0.48**	0.21	0.30	1	(-)	All	530	5	11	35	257	**6**	13	**43**	271	**5**	9	**27**								0.13	**2.09**					Partition by sex. Use LAVE-
						1	(+)		394	5	11	35	197	6	13	40	217	5	10	26	0.00	0.00	0.00	0.00	0.35	0.19	0.13	**1.83**					
Na	mmol/L	1	0.00	0.09	**0.49**	1	(-)	All	531	**134**	138	**142**	258	135	138	141	272	134	137	142								**0.49**	**-0.49**					No partiion by sex/age. with actual bias by age in females ≤2RU. LAVE -
						1	(+)		394	134	138	142	197	135	138	141	218	134	137	142	0.00	0.00	0.00	0.00	0.00	0.00	**0.49**	**-0.49**					
						1	(-)	~45	348	134	137	141	175	135	138	141	173	134	137	141								**0.52**	-0.03	**0.54**	-0.29	-0.05	**-0.65**	
						1	(+)		283	134	138	141	144	135	138	141	144	134	137	141	-0.15	-0.06	-0.12	-0.06	0.06	0.03	**0.46**	-0.05	0.33	-0.11	0.05	**-0.65**	
						1	(-)	45~	183	134	138	142	84	134	138	142	99	134	138	143								0.00	**-0.46**					
						1	(+)		149	134	138	142	66	134	138	141	83	134	138	143	0.05	-0.24	0.00	0.08	0.21	0.02	0.20	**-0.61**					
K	mmol/L	0.1	0.00	0.20	0.22	1	(-)	All	531	**3.4**	3.9	**4.8**	259	3.3	3.9	4.9	273	3.5	4.0	4.8								**-0.49**	0.24					No partiion by sex/age. LAVE-
						1	(+)		394	3.4	3.9	4.8	198	3.4	3.9	4.9	218	3.5	4.0	4.8	0.00	-0.23	0.00	0.00	0.00	0.00	-0.24	0.24					
Cl	mmol/L	1	**0.45**	**0.41**	0.21	1	(-)	All	531	**100**	105	**110**	258	100	104	109	272	101	106	110								**-0.39**	**-0.39**					No partiion by sex/age. LAVE- with actual bias by sex, age ≤2RU
						1	(+)		395	101	105	110	198	100	104	109	218	102	106	110	**-0.44**	0.00	**-0.49**	0.00	0.00	0.00	**-0.87**	**-0.44**					
						1	(-)	~45	349	100	105	109	173	101	105.7	110	134	100	104	108								0.10	**0.61**	**-0.62**	-0.34	**-0.71**	**-1.22**	
						1	(+)		183	101	106	110	99	102	106.2	111	65	101	106	109	-0.25	-0.13	-0.18	**-0.54**	**-0.52**	**-0.42**	0.22	0.37	-0.35	**-0.50**	**-0.60**	**-1.19**	
						1	(-)	45~	175	100	104	108	266	101	104.9	109	134	102	106	110								-0.03	**-0.38**					
						1	(+)		83	100	105	110	137	101	106	110	79	102	106	111	-0.13	-0.35	0.17	**-0.67**	**-0.52**	**-0.51**	-0.07	**-0.40**					
Ca	mmol/L	0.01	0.15	0.31	0.26	1	(-)	All	531	**2.19**	2.36	**2.57**	259	2.21	2.38	2.56	273	2.18	2.35	2.57								0.31	-0.10					No partiion by sex/age. LAVE-
						1	(+)		395	2.20	2.36	2.55	197	2.21	2.38	2.57	219	2.19	2.34	2.54	-0.11	0.00	-0.11	0.22	-0.11	0.34	0.22	0.34					
IP	mmol/L	0.01	0.21	0.22	0.16	1	(-)	All	533	**0.78**	1.10	**1.42**	260	0.75	1.07	1.38	273	0.82	1.12	1.44								**-0.43**	-0.37					No partiion by sex/age. LAVE-
						1	(+)		396	0.77	1.09	1.43	198	0.74	1.06	1.36	219	0.82	1.12	1.44	0.06	0.06	0.00	-0.06	0.13	0.00	**-0.48**	**-0.48**					
Mg	mmol/L	0.01	0.00	0.37	**0.54**	1	(-)	All	533	0.73	0.85	0.98	260	**0.73**	0.85	**0.98**	273	0.72	0.85	0.98								0.16	0.00					Partition by age for female. LAVE-
						1	(+)		396	0.72	0.85	0.98	198	0.73	0.85	0.98	219	0.72	0.85	0.98	0.15	0.00	0.00	0.00	0.00	0.00	0.15	0.00					
						1	(-)	~45	350	0.72	0.83	0.95	176	0.72	0.83	0.97	174	**0.71**	0.83	**0.93**								0.10	**0.61**	**-0.62**	-0.34	**-0.71**	**-1.22**	
						1	(+)		285	0.72	0.83	0.95	145	0.73	0.83	0.96	145	0.72	0.83	0.94	0.00	-0.17	-0.05	0.00	0.17	-0.08	0.22	0.37	-0.35	**-0.50**	**-0.60**	**-1.19**	
						1	(-)	45~	183	0.76	0.88	1.00	84	0.76	0.88	0.99	99	**0.76**	0.88	**1.01**								-0.03	**-0.38**					
						1	(+)		149	0.76	0.88	1.01	66	0.75	0.88	0.99	83	0.76	0.88	1.02	0.00	0.11	0.06	-0.16	0.00	-0.04	-0.07	**-0.40**					
Glu	mmol/L	0.1	0.00	**0.62**	**0.59**	1	(-)	All	512	3.9	4.9	6.2	247	4.0	4.9	6.3	268	3.9	4.8	6.1								0.17	0.34					Partition by age, LAVE-
						1	(+)		395	3.9	4.8	6.1	195	4.1	4.9	6.4	224	3.9	4.8	6.1	0.00	-0.17	0.00	0.18	-0.17	0.00	0.36	**0.53**					
						1	(-)	~45	345	**3.9**	4.7	**5.8**	173	3.9	4.8	5.8	172	3.8	4.6	5.7								0.21	0.21	**-1.02**	**-3.75**	**-0.89**	**-1.78**	
						1	(+)		287	3.9	4.7	5.7	146	3.9	4.7	5.8	151	3.8	4.6	5.8	0.00	0.00	0.00	0.22	0.00	-0.20	0.22	0.00	**-1.02**	**-4.94**	**-0.89**	**-1.43**	
						1	(-)	45~	172	**4.4**	5.2	**7.3**	77	4.5	5.3	8.0	97	4.3	5.2	6.7								0.27	**1.76**					
						1	(+)		144	4.4	5.2	7.1	62	4.5	5.2	8.7	83	4.3	5.2	6.6	0.00	0.00	0.00	0.29	**-0.65**	0.17	0.29	**3.05**					
TC	mmol/L	0.1	0.00	0.37	**0.41**	1	(-)	All	532	3.1	4.6	6.7	260	**3.1**	4.7	**6.9**	273	3.1	4.6	6.6								0.00	0.33					Partition by age for female. LAVE -
						1	(+)		396	3.2	4.5	6.6	198	3.2	4.7	6.7	219	3.3	4.6	6.6	-0.12	-0.11	-0.24	0.12	0.22	0.00	-0.12	0.12					
						1	(-)	~45	350	3.1	4.5	6.4	176	3.1	4.5	6.6	174	**3.1**	4.4	**6.2**								0.00	**0.48**	0.00	**-0.83**	-0.11	**-1.01**	
						1	(+)		285	3.1	4.4	6.1	145	3.2	4.5	6.3	145	3.1	4.3	6.1	0.00	-0.13	0.00	**0.39**	**0.38**	0.13	0.13	0.26	-0.34	**-1.01**	**-0.48**	**-1.07**	
						1	(-)	45~	183	3.2	5.0	7.2	84	3.1	5.1	7.4	99	**3.2**	4.9	**7.1**								-0.10	0.29					
						1	(+)		149	3.5	4.9	6.9	66	3.5	5.0	7.2	83	3.5	4.8	7.0	-0.35	**-0.42**	-0.34	0.35	0.21	0.11	0.00	0.23					
TG	mmol/L	0.01	0.23	**0.55**	**0.59**	1	(-)	All	528	0.49	1.03	3.49	258	0.50	1.17	4.14	272	0.47	0.91	2.85								0.04	**1.69**					Partition by age, LAVE+
						1	(+)		403	0.49	0.96	2.95	204	0.52	1.19	3.51	225	0.47	0.89	2.70	0.00	-0.03	0.00	**0.86**	**0.83**	0.26	0.08	**1.29**					
						1	(-)	~45	346	0.49	0.90	2.99	175	0.51	1.07	3.29	173	0.46	0.78	2.29								0.08	**1.57**	-0.05	**-2.53**	-0.15	**-1.94**	
						1	(+)		289	**0.47**	0.86	**2.60**	149	0.50	1.02	2.88	146	0.45	0.75	2.03	0.04	0.02	0.02	**0.72**	**0.68**	**0.65**	0.09	**1.56**	-0.04	**-1.30**	-0.18	**-2.13**	
						1	(-)	45~	181	0.55	1.32	4.26	83	0.56	1.49	5.64	99	0.55	1.18	3.47								0.01	**2.29**					
						1	(+)		154	**0.55**	1.25	**3.70**	71	0.53	1.41	3.87	84	0.55	1.17	3.24	0.00	0.04	0.00	**0.70**	**2.08**	0.34	-0.02	**0.78**					

				M	F				RIs by parametric method												LAVE effect on LL			LAVE effect on UL			btw-sex diff		btw-age diff M		btw-age diff F		Decision
									M + F				M				F				M+F	M	F	M+F	M	F							
Item	Units	RU	SDR sex	SDR age		LAVE criteria	LAVE	Age	n	LL	Me	UL	n	LL	Me	UL	n	LL	Me	UL	BR_{LL}			BR_{UL}			BR_{LL}	BR_{UL}	BR_{LL}	BR_{UL}	BR_{LL}	BR_{UL}	
HDL-C	mmol/L	0.1	0.31	0.00	0.00	1	(-)	All	530	**0.7**	1.1	**1.7**	258	0.7	1.1	1.7	271	0.8	1.2	1.8							**-0.39**	**-0.39**					No partition by age or sex. LAVE- with its effect of ≤2RU
						1	(+)		395	0.8	1.2	1.8	198	0.7	1.1	1.7	218	0.8	1.2	1.8	**-0.39**	0.00	0.00	**-0.39**	0.00	0.00	**-0.39**	**-0.39**					
HDLrat		0.1	0.34	0.38	**0.45**	1	(-)	All	530	2.5	4.0	7.0	259	**2.6**	4.3	**7.6**	271	2.5	3.8	6.1							0.09	**1.31**					Partition by age (female). LAVE-
						1	(+)		396	2.6	3.9	6.3	198	2.8	4.3	7.2	219	2.5	3.8	5.8	-0.11	-0.18	0.00	**0.74**	0.36	0.36	0.32	**1.48**					
						1	(-)	~45	348	2.5	3.9	6.6	175	2.7	4.2	7.2	174	**2.4**	3.6	**5.9**							0.29	**1.24**	0.16	**-0.47**	-0.33	**-0.87**	
						1	(+)		285	2.6	3.8	6.1	144	2.8	4.1	6.7	145	2.4	3.6	5.7	-0.11	-0.10	0.00	**0.56**	**0.50**	0.24	**0.45**	**1.12**	0.27	**-0.53**	-0.36	**-0.83**	
						1	(-)	45~	182	2.7	4.3	7.5	83	2.5	4.7	7.8	98	**2.7**	4.0	**6.7**							-0.16	**0.90**					
						1	(+)		149	2.7	4.2	7.4	66	2.5	4.6	7.3	82	2.7	4.0	6.4	0.00	0.00	0.00	0.08	**0.41**	0.32	-0.17	**0.75**					
nonHDL	mmol/L	0.1	0.00	0.32	0.35	1	(-)	All	532	**2.0**	3.4	**5.6**	260	2.1	3.5	5.9	273	2.0	3.3	5.3							0.11	**0.65**					No partition by sex or age. LAVE-
						1	(+)		409	2.0	3.4	5.3	203	2.1	3.5	5.6	223	2.0	3.3	5.2	0.00	0.00	0.00	0.36	0.34	0.12	0.12	**0.48**					
LDL-C	mmol/L	0.1	0.00	0.35	**0.43**	1	(-)	All	533	1.5	2.8	4.7	260	**1.6**	2.9	**4.8**	273	1.6	2.8	4.6							0.00	0.25					Partition by age for female. LAVE-.
						1	(+)		396	1.7	2.8	4.5	198	1.7	2.9	4.8	219	1.6	2.8	4.5	-0.28	-0.13	0.00	0.28	0.00	0.14	0.14	**0.42**					
						1	(-)	~45	350	1.5	2.7	4.5	176	1.6	2.8	4.7	174	**1.5**	2.6	**4.2**							0.13	**0.65**	-0.12	-0.37	-0.26	**-0.91**	
						1	(+)		285	1.6	2.7	4.3	145	1.7	2.8	4.6	145	1.5	2.6	4.1	-0.15	-0.14	0.00	0.29	0.14	0.15	0.29	**0.73**	-0.25	**-0.76**	**-0.54**	**-1.08**	
						1	(-)	45~	183	1.7	3.1	5.0	84	1.7	3.2	5.0	99	**1.7**	3.0	**4.9**							0.00	0.12					
						1	(+)		149	1.9	3.1	5.0	66	1.9	3.2	5.2	83	1.9	3.0	4.9	-0.25	-0.24	-0.26	0.00	-0.24	0.00	0.00	**0.38**					
Lip	U/L	1	0.06	0.16	0.14	1	(-)	All	527	**9**	27	**68**	257	8	29	75	272	10	25	63							-0.13	**0.80**					No partition by sex or age. LAVE-
						1	(+)		392	9	26	64	194	8	29	71	218	9	24	60	0.00	0.00	0.08	0.29	0.25	0.23	-0.07	**0.78**					
ALT	IU/L	1	**0.59**	0.20	**0.51**	1	(-)	All	528	8	18	55	259	10	22	64	269	7	15	35							0.25	**2.42**					Partition by sex, LAVE+. No partiition by age
						1	(+)		412	8	16	39	202	**10**	22	**55**	229	**7**	14	**30**	0.00	0.00	0.00	**2.02**	**0.78**	**0.85**	**0.38**	**3.16**					
						1	(-)	~45	347	7	16	51	176	9	21	61	173	7	13	33							0.18	**2.49**	-0.22	**-0.65**	0.00	**-0.42**	
						1	(+)		283	8	15	38	144	9	20	47	144	7	13	29	-0.13	0.00	0.00	**1.70**	**1.44**	**0.71**	0.26	**2.35**	-0.35	**-1.31**	-0.17	0.00	
						1	(-)	45~	181	10	20	60	84	12	25	70	96	7	18	36							**0.39**	**2.67**					
						1	(+)		148	10	19	48	66	13	25	62	81	8	17	29	0.00	-0.08	-0.19	**1.24**	**0.64**	**1.31**	**0.52**	**3.40**					
AST	IU/L	1	**0.49**	0.00	0.35	1	(-)	All	525	16	23	39	258	17	25	45	266	15	21	32							0.34	**2.22**					Partition by sex. LAVE+
						1	(+)		409	16	22	33	206	**17**	25	**40**	227	**15**	21	**29**	0.00	0.00	0.00	**1.38**	**0.85**	**0.84**	**0.46**	**2.54**					
ALP	IU/L	1	0.00	0.17	**0.69**	1	(-)	All	531	50	85	153	260	**53**	88	**153**	270	49	83	156							0.15	-0.11					Partition by age for females. LAVE-.
						1	(+)		394	50	84	151	198	54	87	153	218	48	81	148	0.00	-0.04	0.04	0.08	0.00	0.31	0.23	0.19					
						1	(-)	~45	347	50	81	142	176	53	86	153	172	**47**	77	**130**							0.26	**0.98**	0.04	0.08	-0.29	**-1.61**	
						1	(+)		282	50	80	142	145	54	87	156	144	47	75	129	0.00	-0.04	0.00	0.00	-0.12	0.05	0.30	**1.15**	0.16	0.00	-0.24	**-1.41**	
						1	(-)	45~	183	54	95	163	84	52	92	151	99	**55**	100	**174**							-0.11	**-0.83**					
						1	(+)		149	53	94	160	66	50	90	156	83	53	96	165	0.04	0.07	0.07	0.11	-0.18	0.32	-0.11	-0.33					
AMY	IU/L	1	0.02	0.00	0.00	1	(-)	All	530	**47**	87	**164**	258	48	87	163	272	45	85	160							0.10	0.10					No partiion by sex or age. LAVE-
						1	(+)		394	47	87	154	197	49	90	161	218	44	85	154	0.00	-0.04	0.04	0.37	0.07	0.21	0.18	0.26					
LDH	IU/L	1	0.00	0.00	0.28	1	(-)	All	530	**138**	189	**257**	259	136	187	255	271	139	190	259							-0.10	-0.13					No partiion by sex or age. LAVE-
						1	(+)		409	136	187	249	207	139	186	250	225	138	189	250	0.07	-0.11	0.04	0.28	0.18	0.32	0.03	0.00					
CK	IU/L	1	**0.52**	0.00	0.08	1	(-)	All	529	61	139	380	259	**72**	167	**460**	268	**53**	119	**260**							0.23	**2.46**					Partition by sex. LAVE-
						1	(+)		406	58	135	332	203	76	172	440	219	54	119	277	0.04	-0.04	-0.02	**0.69**	0.22	-0.30	0.31	**2.33**					
GGT	IU/L	1	**0.54**	0.29	0.13	1	(-)	All	524	12	25	88	257	12	31	105	269	**10**	21	**50**							0.10	**2.84**					Partition by sex. LAVE+ for males. LAVE- females
						1	(+)		405	11	24	65	199	**13**	31	**90**	224	10	21	47	0.07	-0.05	0.00	**1.67**	**0.76**	0.32	0.22	**3.12**					
CRP	mg/L	0.01	0.11	0.35	0.17	1	(-)	All	528	0.22	1.22	18.86	255	0.20	1.01	14.35	273	0.23	1.54	20.2							-0.01	**-1.23**					No partiion by sex or age. LAVE+
						1	(+)		425	**0.21**	1.10	**14.67**	200	0.20	0.97	12.97	216	0.23	1.41	13.9	0.00	0.00	0.00	**1.14**	**0.42**	**1.82**	-0.01	-0.24					
Fe	µmol/L	0.1	0.39	0.10	0.04	3	(-)	All	526	4.3	15.8	29.0	253	7.7	17.5	29.9	273	3.9	13.7	29.3							**0.60**	0.10					No partiion by sex or age. LAVE+
						3	(+)		412	**8.8**	16.1	**28.9**	207	9.8	17.4	28.7	207	7.5	14.6	29.3	**-0.88**	**-0.44**	**-0.65**	0.02	0.25	0.00	**0.45**	-0.12					
Ferr	µg/L	**1**	**0.81**	0.20	**0.44**	3	(-)	All	526	1	63	404	253	**10**	120	**475**	272	2	31	184							0.08	**2.83**					Partition by sex and age for female. LAVE-
						3	(+)		404	9	74	413	202	20	124	457	206	7	38	193	-0.08	-0.09	-0.11	-0.09	0.16	-0.19							
						3	(-)	~45	348	1	58	358	174	9	121	366	173	**2**	24	**150**							0.08	**2.37**	0.03	**-2.54**	0.04	**-1.55**	
						3	(+)		281	6	71	366	147	18	123	371	141	5	28	150	-0.05	-0.10	-0.08	-0.09	-0.06	0.00			0.02	**-2.50**	-0.15	**-1.73**	
						3	(-)	45~	177	1	71	450	78	6	122	667	99	**0**	48	**222**							0.05	**3.89**					
						3	(+)		134	13	81	452	65	16	131	650	72	12	62	232	-0.11	-0.06	-0.21	-0.02	0.11	-0.18							
Tf	g/L	0.1	**0.44**	0.15	0.18	3	(-)	All	525	1.9	2.5	3.7	254	**1.9**	2.4	**3.2**	273	2.0	2.7	3.9							-0.22	**-1.52**					Partition by sex. LAVE+ for female
						3	(+)		408	1.9	2.5	3.3	203	1.8	2.4	3.1	206	**2.0**	2.6	**3.5**	0.00	0.30	0.00	**1.12**	0.30	**1.05**							
TfSat	%	1	**0.50**	0.00	0.00	3	(-)	All	524	5	25	46	252	11	29	48	273	4	20	44							**0.67**	**0.38**					Partition by sex. LAVE+
						3	(+)		416	13	26	47	208	**15**	28	**49**	211	**10**	22	**44**	**-0.92**	**-0.46**	**-0.69**	-0.12	-0.12	0.00							
IgG	g/L	0.1	0.11	0.18	0.00	2	(-)	All	525	**11.1**	14.7	**20.0**	254	11.1	14.6	19.4	270	10.9	15.0	20.5							0.07	**-0.52**					No partiion by sex or age. LAVE-
						2	(+)		428	11.1	14.7	19.4	201	11.5	14.7	19.3	218	11.2	14.8	19.5	-0.04	-0.21	-0.10	0.29	0.05	**0.51**							
IgA	g/L	0.01	0.00	0.15	0.30	2	(-)	All	526	**1.05**	2.32	**4.63**	254	1.02	2.37	4.72	273	1.03	2.28	4.54							-0.01	0.20					No partiion by sex or age. LAVE-
						2	(+)		423	1.02	2.35	4.42	198	0.99	2.40	4.44	218	1.02	2.23	4.35	0.03	0.03	0.01	0.24	0.32	0.22							
IgM	g/L	0.01	**0.50**	0.16	0.07	2	(-)	All	526	0.41	1.02	2.58	254	**0.38**	0.85	**2.29**	272	**0.51**	1.21	**2.77**							-0.23	**-0.87**					Partition by sex. LAVE-
						2	(+)		417	0.41	1.02	2.60	197	0.40	0.85	2.37	217	0.51	1.20	2.68	0.00	-0.04	0.00	-0.04	-0.16	0.16							
FT3	pmol/L		0.12	0.00	0.09	4	(-)	All	467	**3.9**	4.8	**6.3**	235	4.0	4.9	6.2	233	3.8	4.8	6.4							0.33	-0.33					No partiion by sex or age
FT4	pmol/L		0.00	0.34	0.38	4	(-)	All	468	**7.8**	10.6	**14.1**	235	7.7	10.7	14.3	234	7.8	10.5	13.8							-0.06	0.31					No partiion by sex or age
TSH	mIU/L		0.00	0.00	0.00	4	(-)	All	461	**0.61**	1.60	**4.86**	231	0.63	1.63	5.10	232	0.51	1.59	4.53							0.11	**0.53**					No partiion by sex or age
PSA	µg/L			0.39			(-)	All					243	**0.29**	0.77	**2.92**																	No partiion by age

Table S6.2: Comparison of reference intervals

		PRESENT STUDY KENYA							BECKMAN AU[1]							SAUDI ARABIA[2]							TURKEY[3]						
			M+F		M		F			M+F		M		F				M+F		M		F			M+F		M		F	
Test	Units	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL
TP	g/L	18-65	67	83					NA	66	83					18-65	66	83					20-79	66	82				
Alb	g/L	18-65							NA	35	52					18-65	39	50					20-79	41	49				
		18-44			40	51	38	49																					
		45-65			40	47	38	46																					
Urea	mmol/L	18-65			2.4	6.6			NA	2.8	7.2					18-65			2.8	7.3	2.1	6.4							
		18-44					1.8	5.3																20-49			2.95	7.20	2.21	6.12
		45-65					1.9	6.2																50-79					2.85	7.96
UA	µmol/L	18-65			243	507	178	417	NA			208.3	428.4	154.7	357	18-65			223	444	148	321	20-79			226	458	166	345
Cre	µmol/L	18-65			58	109	45	86	NA			59	104	45	84	18-65			66	111	50	74	20-79			59	92	50	71
TBil	µmol/L	18-65			6	43	5	27	NA	5	21					18-65			3.6	22.4	2.2	15.5	20-79			3.8	24.1	2.7	15.9
Na	mmol/L	18-65	134	142					NA	136	146					18-65	135	144					20-79	137	144				
K	mmol/L	18-65	3.4	4.8					NA	3.5	5.1					18-65	3.7	4.9					20-79	3.7	4.9				
Cl	mmol/L	18-65	100	110					NA	101	109					18-65	101	111					20-79	99	107				
Ca	mmol/L	18-65	2.19	2.57					NA	2.2	2.65					18-65	2.11	2.56					20-79	2.15	2.47				
IP	mmol/L	18-65	0.78	1.42					NA	0.81	1.45					18-65	0.81	1.44					20-79	0.8	1.4				
Mg	mmol/L	18-65			0.73	0.98			NA			0.73	1.06	0.77	1.03	18-65	0.71	0.96					20-79	0.77	1.06				
		18-44					0.71	0.93																					
		45-65					0.76	1.01																					
Glu	mmol/L	18-65							NA	4.1	5.9					18-65	4	5.9					20-79	3.96	5.88				
		18-44	3.9	5.8																									
		45-65	4.4	7.3																									
TC	mmol/L	18-65							NA		5.2					18-65	3.5	6.36											
		18-45	3.10	6.10																			20-49	3.22	6.45	3.20	6.42	3.20	6.38
		46-65	3.20	7.20																			50-79					3.93	7.92
TG	mmol/L	18-65							NA		1.7					18-65			0.5	3.58	0.39	1.6							
		18-45			0.50	2.88	0.45	2.03															20-49			0.53	3.39	0.46	2.52
		46-65			0.53	3.87	0.55	3.24															50-79					0.64	3.55
HDL-C	mmol/L	18-65	0.70	1.70					NA	1.03	1.55					18-65			0.74	1.76	0.98	2.19				0.85	1.52	0.95	1.56
LDL-C	mmol/L	18-65			1.60	4.80			NA		2.60					18-65	1.8	4.34											
		18-44					1.50	4.20															20-49	1.47	3.92	1.60	4.01	1.32	3.92
		45-65					1.70	4.90															50-79					1.78	4.91
Lip	U/L	18-65			8	75	10	63	NA		67																		
ALT	IU/L	18-65			10	55	7	30	NA				50		35	18-65			7	39	5	18				9	57	7	28
AST	IU/L	18-65			17	40	15	29	NA				50		35	18-65			11	28	10	24				13	30	11	25
ALP	IU/L	18-65			53	153			NA	30	120						39	114											
		18-44					47	130															20-49	38	112	43	116	34	97
		45-65					55	174															50-79					47	133
AMY	IU/L	18-65	47	164					NA	22	80					18-65	31	117						34	119				
LDH	IU/L	18-65	138	257					NA				248		247	18-65	10	238						126	220				
CK	IU/L	18-65			72	460	53	260	NA				171		145	18-65			54	266	27	138				48	227	34	131
GGT	IU/L	18-65			13	90	10	47	NA				55		38	18-65			11	65	7	21				11	69	7	33
Fe	µmol/L	18-65	8.8	28.9					NA			12.5	32.2	10.7	32.2	18-65			7.9	29.6	3.7	26				5.9	31.6	3.5	27.8
Ferr	µg/L	18-65			20	457			NA			20	250	10	120								18-79					4.7	136
		18-44					5	150															18-44			13	276	4.3	91
		45-65					12	232															45-79					5.9	175
Tf	g/L	18-65			1.9	3.2	2.0	3.5	NA	2	3.6					18-65			2	3.2	2.1	3.9	18-79	1.8	3.3	1.8	3.3	1.9	3.5
TfSat	%	18-65	13	47																									
CRP	mg/L	18-65	0.21	14.7					NA		1					18-65	0.2	11.8											
IgA	g/L	18-65	1.05	4.63					NA	0.7	4																		
IgG	g/L	18-65	11.1	20.0					NA	7	16																		
IgM	g/L	18-65			0.38	2.29	0.51	2.77	NA	0.4	2.3																		
FT3	pmol/L	18-65	3.9	6.3					NA	3.8	6																		
FT4	pmol/L	18-65	7.8	14.1					NA	7.86	14.41																		
TSH	mIU/L	18-65	0.61	4.86					NA	0.38	5.33																		
PSA	µg/L	18-65			0.25	2.95																							

		INDIA[4]							US-MGH[5]							KENYA-KERICHO[6]							KENYA-NAIROBI, KILIFI[7]						
			M+F		M		F			M+F		M		F			M+F		M		F			M+F		M		F	
Test	Units	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL	Age	LL	UL	LL	UL	LL	UL
TP	g/L	18-65	68	86					NA	55	80												18-60	58	88	59	88	58	87
Alb	g/L	18-65					36	47	NA	35	55					18-55	35.8	48.1	36.9	48.5	34.4	47.5	18-60	35	52	36	52	35	51
Urea	mmol/L	18-65			2.2	6.0			NA	3.6	7.1					18-55	1.4	4.6	1.5	4.6	1.4	4.6							
		18-45					1.9	5.1	NA																				
		46-65					2.4	6.7	NA																				
UA	µmol/L	18-65			248	509	159	404	NA			150	480	90	360														
Cre	µmol/L	18-65			58	95	35	74	NA		133					18-55	55	102	62	106	51	91	18-60	47	109	52	114	43	99
TBil	µmol/L	18-65			6.2	23.7	4	17.3	NA	5.1	17					18-55	4.9	39.9	5.6	41.9	4.4	26.8	18-60	2.9	37	3.6	41.9	2.7	31.5
Na	mmol/L	18-65	135	146					NA	136	145					18-55	141	153	142	152	140	155							
K	mmol/L	18-65	3.8	5					NA	3.5	5.0					18-55	3.9	5.8	3.9	5.8	3.8	5.8							
Cl	mmol/L	18-65	102	113					NA	98	106					18-55	100.5	112	100	111	101	113							
Ca	mmol/L	18-65	2.1	2.44					NA	2.2	2.6																		
IP	mmol/L	18-65	0.8	1.43					NA	1.0	1.4																		
Mg	mmol/L	18-65	0.77	1.07					NA	0.8	1.2																		
Glu	mmol/L	18-65							NA	4.2	6.4																		
		18-45	4.1	5.5					NA																				
		46-65	4.3	6.0					NA																				
TC	mmol/L	18-65					2.9	6.6	NA							18-55	2.6	5.7	2.5	5.5	2.6	5.9							
		18-45			3.1	6.2			NA																				
		46-65			2.5	6.7			NA																				
TG	mmol/L	18-65			0.6	2.7	0.5	2.1	NA		1.8					18-55	0.4	2.6	0.4	2.7	0.4	2.5							
		18-45							NA																				
		46-65							NA																				
HDL-C	mmol/L	18-65			0.7	1.5	0.8	1.8	NA	1.03																			
LDL-C	mmol/L	18-65	1.7	4.4					NA																				
		18-45							NA																				
		46-65							NA																				
Lip	U/L	18-65							NA	0	160																		
ALT	IU/L	18-65			15	74	10	37	NA	0	35					18-55	9.6	52	10.8	53.9	8.6	47	18-60	8	61	9	67	8	48
AST	IU/L	18-65			20	53	17	39	NA	0	35					18-55	13.8	42.3	14.9	45.3	13.1	38.1	18-60	14	60	15	71	13	43
ALP	IU/L	18-65			41	111			NA	30	120												18-60	48	164	48	138	46	179
		18-45					35	100	NA																				
		46-65					43	117	NA																				
AMY	IU/L	18-65	36	135					NA	60	180					18-55	38.3	163.0	40.0	171.3	36.0	147.8	18-60	35	159	37	167	33	140
LDH	IU/L	18-65	104	206					NA	100	190					18-55	126.0	263.9	124.0	259.0	131.5	295.4	18-60	213	678	209	691	217	652
CK	IU/L	18-65			48	304	36	184	NA			60	400	40	150								18-60	53	552	60	709	49	354
GGT	IU/L	18-65			14	62	11	40	NA	1	94																		
Fe	µmol/L	18-65			7	33	4	26	NA	9	27																		
Ferr	µg/L	18-65							NA			30	300	10	200														
Tf	g/L	18-65	2.2	4					NA	2.3	3.9																		
TfSat	%	18-65							NA	20	45																		
CRP	mg/L	18-65			0.33	7.34	0.35	11.9	NA	0.02	8																		
IgA	g/L	18-65	0.94	4.35					NA	0.6	3.09																		
IgG	g/L	18-65	9.1	20.4					NA	6.14	12.95												18-60	7.74	28.33	7.56	28.04	7.92	28.95
IgM	g/L	18-65			0.4	2.54	0.51	3.1	NA	0.53	3.34																		
FT3	pmol/L	18-65							NA	0.22	6.78																		
FT4	pmol/L	18-65							NA	10.3	35																		
TSH	mIU/L	18-65							NA	0.5	4.7																		
PSA	µg/L	18-65							NA	0	4																		

Key:[1](*AU Reagent Quick Reference Guide*, 2018), [2](Borai et al., 2016), [3](Ozarda et al., 2014), [4](S. Shah et al., 2018), [5](Kratz et al., 2004), [6](Kibaya et al., 2008), [7](Karita et al., 2009)

6.7 References

AU Reagent Quick Reference Guide. (2018). Beckman Coulter.

Borai, A., Ichihara, K., Al Masaud, A., Tamimi, W., Bahijri, S., Armbuster, D., . . . Abdelaal, M. (2016). Establishment of reference intervals of clinical chemistry analytes for the adult population in Saudi Arabia: A study conducted as a part of the IFCC global study on reference values. *Clinical Chemistry and Laboratory Medicine, 54*(5), 843-855. doi:10.1515/cclm-2015-0490

Borai, A., Ichihara, K., Masaud, A., Tamimi, W., Bahijri, S., Armbuster, D., . . . Almohammadi, M. (2020). Establishment of reference intervals for immunoassay analytes of adult population in Saudi Arabia. *Clinical Chemistry and Laboratory Medicine*, Forthcoming-Forthcoming. doi:10.1515/cclm-2019-1049

CLSI. (2008). *Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline* (Third ed.): Wayne PA, Clinical and Laboratory Standards Institute.

Dullo, P., & Vedi, N. (2008). Changes in serum calcium, magnesium and inorganic phosphorus levels during different phases of the menstrual cycle. *Journal of Human Reproductive Sciences, 1*(2), 77-77.

Evgina, S., Ichihara, K., Ruzhanskaya, A., Skibo, I., Vybornova, N., Vasiliev, A., . . . Emanuel, V. (2020). Establishing reference intervals for major biochemical analytes for the Russian population: a research conducted as a part of the IFCC global study on reference values. *Clin Biochem, 81*, 47-58. doi:10.1016/j.clinbiochem.2020.04.001

Ichihara, K. (2014). Statistical considerations for harmonization of the global multicenter study on reference values. *Clinica Chimica Acta, 432*, 108-118. doi:10.1016/j.cca.2014.01.025

Ichihara, K., & Boyd, J. C. (2010). An appraisal of statistical procedures used in derivation of reference intervals. *Clinical Chemistry and Laboratory Medicine, 48*(11), 1537-1551. doi:10.1515/CCLM.2010.319

Ichihara, K., Ceriotti, F., Kazuo, M., Huang, Y.-Y., Shimizu, Y., Suzuki, H., . . . Okubo, Y. (2013). The Asian project for collaborative derivation of reference intervals: (2) results of non-standardized analytes and transference of reference intervals to the participating laboratories on the basis of cross-comparison of test results. *Clinical Chemistry and Laboratory Medicine, 51*(7), 1443-1457. doi:10.1515/cclm-2012-0422

Ichihara, K., Ceriotti, F., Tam, T. H., Sueyoshi, S., Poon, P. M. K., Thong, M. L., . . . Panteghini, M. (2013). The Asian project for collaborative derivation of reference intervals: (1) strategy and major results of standardized analytes. *Clinical Chemistry and Laboratory Medicine, 51*(7), 1429-1442. doi:10.1515/cclm-2012-0421

Ichihara, K., Ozarda, Y., Barth, J. H., Klee, G., Qiu, L., Erasmus, R., . . . Yadav, B. (2017). A global multicenter study on reference values: 1. Assessment of methods for derivation and comparison of reference intervals. *Clinica Chimica Acta, 467*, 70-82. doi:10.1016/J.CCA.2016.09.016

Ichihara, K., Ozarda, Y., Barth, J. H., Klee, G., Shimizu, Y., Xia, L., . . . Takahashi, A. (2017). A global multicenter study on reference values: 2. Exploration of sources of variation across the countries. *Clinica Chimica Acta, 467*, 83-97. doi:10.1016/j.cca.2016.09.015

Karita, E., Ketter, N., Price, M. A., Kayitenkore, K., Kaleebu, P., Nanvubya, A., . . . Kamali, A. (2009). CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. *PLOS ONE, 4*(2), e4401. doi:10.1371/journal.pone.0004401

Kibaya, R. S., Bautista, C. T., Sawe, F. K., Shaffer, D. N., Sateren, W. B., Scott, P. T., . . . de Souza, M. S. (2008). Reference ranges for the clinical laboratory derived from a rural population in Kericho, Kenya. *PLOS ONE, 3*(10), e3327-e3327. doi:10.1371/journal.pone.0003327

Kratz, A., Ferraro, M., Sluss, P. M., & Lewandrowski, K. B. (2004). Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Laboratory reference values. *The New England journal of medicine, 351*(15), 1548-1563. doi:10.1056/NEJMcpc049016

Lindsay, R., Hart, D. M., & Forrest, C. (1980). Effect of a natural and artificial menopause on serum, urinary and erythrocyte magnesium. *Clinical science (London, England : 1979), 58*(3), 255-257. doi:10.1042/CS0580255

NCEP. (2001). Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA, 285*(19), 2486-2497. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11368702

Omuse, G., Maina, D., Hoffman, M., Mwangi, J., Wambua, C., Kagotho, E., . . . Erasmus, R. (2017). Metabolic syndrome and its predictors in an urban population in Kenya: A cross sectional study. *BMC Endocrine Disorders, 17*(1). doi:10.1186/s12902-017-0188-0

Omuse, G., Maina, D., Mwangi, J., Wambua, C., Radia, K., Kanyua, A., . . . Erasmus, R. (2018). Complete blood count reference intervals from a healthy adult urban population in Kenya. *PLOS ONE*, 1-19.

Ozarda, Y., Ichihara, K., Aslan, D., Aybek, H., Ari, Z., Taneli, F., . . . Degirmen, E. (2014). A multicenter nationwide reference intervals study for common biochemical analytes in Turkey using Abbott analyzers. *Clinical Chemistry and Laboratory Medicine (CCLM), 52*(12). doi:10.1515/cclm-2014-0228

Ozarda, Y., Ichihara, K., Bakan, E., Polat, H., Ozturk, N., Baygutalp, N. K., . . . Eker, P. (2017). A nationwide multicentre study in Turkey for establishing reference intervals of haematological parameters with novel use of a panel of whole blood. *Biochemia Medica, 27*(2), 350-377. doi:10.11613/BM.2017.038

Ozarda, Y., Ichihara, K., Barth, J. H., & Klee, G. (2013). Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. *Clinical Chemistry and Laboratory Medicine (CCLM), 51*(5), 1027-1040. doi:10.1515/cclm-2013-0249

Shah, S., Ichihara, K., Dherai, A. J., & Ashavaid, T. F. (2018). Reference intervals for 33 biochemical analytes in healthy Indian population: C-RIDL IFCC initiative. *Clinical Chemistry and Laboratory Medicine, 56*(12), 2093-2103. doi:10.1515/cclm-2018-0152

Taneja, V. (2018). Sex Hormones Determine Immune Response. *Frontiers in Immunology, 9*, 1931-1931. doi:10.3389/fimmu.2018.01931

WHO. (2011). Serum ferritin concentrations for assessment of iron status and iron deficiency in populations. Retrieved from https://www.who.int/vmnis/indicators/serum_ferritin.pdf

CHAPTER 7: COMPARISON OF REFERENCE INTERVALS AND SOURCES OF VARIATION BETWEEN KENYA AND SOUTH AFRICA

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7.1 Abstract

Introduction: Variation of reference intervals (RIs) across different populations has been well documented. Several reasons have been postulated to contribute to these variations including differences in population structure, race, environment, diet, sample handling, analytical methods and statistical analysis approaches. RI studies published from Africa have highlighted several differences across countries even in populations thought to be homogeneous. We set out to compare reference values of 31 analytes and their sources of variation between black Africans in Kenya and South Africa using data collected as part of the IFCC global study on RIs.

Methodology: Participants in Kenya and South Africa were enrolled using a harmonized protocol. Recruitment was targeted at enrolling adults aged 18-65 years with similar numbers of males and females to enable determination of RIs using a parametric approach with sex and age stratification where appropriate. Reference values of the two populations were standardized before merging based on the test results of the value-assigned serum panel measured in common. Comparison of RIs was done by determining bias ratios for RI lower and upper limits.

Results: A total of 1130 participants had sufficient data for inclusion and further analysis. This included 597 and 533 participants from South Africa and Kenya respectively. South African RIs for UA, TC, LDL-C, ALT, LDH, ALP, ALB, Mg, TSH and PSA were lower than the Kenyan RIs. Sex was an important source of variation for UA, Cre, AST, CK and Tf. Age was a significant source for variation for UA, Glu and TG. BMI had a significant positive correlation with UA, TG, ALT and CRP for both males and females.

Conclusion: In conclusion, the findings of this study highlight the difficulties in harmonizing RIs even for populations thought to be similar despite standardizing study processes to enable direct comparison of RIs. For most analytes, harmonization of RIs between Kenyans and South Africans of African ancestry will not be ideal as they could result in misclassification of individuals as either diseased or healthy.

7.2 Introduction

Variation of reference intervals (RIs) across different populations has been well documented. Several reasons have been postulated to contribute to these variations including differences in population genetic structure, race and ethnicity, environment, diet, sample handling, analytical methods and statistical analysis approaches. In order to explore the possibility of harmonizing RIs globally, the International Federation of Clinical Chemistry (IFCC) through its Committee of Reference Intervals and Decision Limits (C-RIDL) planned a study to determine RIs using a harmonized protocol (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017; Ichihara, Ozarda, Barth, Klee, Shimizu, et al., 2017; Ozarda et al., 2013). In Africa, the first countries to join the study were South Africa, Nigeria and Kenya representing South, West and East Africa respectively.

RI studies published from Africa have highlighted several differences across countries even in populations thought to be homogeneous. Karita et al determined RIs from Kenya, Uganda, Rwanda and Zambia and noted significant differences compared to RIs from the United States of America (USA). Differences were also seen across the participating countries such as low haemoglobin levels in women from Kilifi in Kenya, increased neutrophil counts in men and women from Nairobi and Kilifi in Kenya, increased eosinophil counts and immunoglobulin G (IgG) levels in men and women from Masaka in Uganda as well as increased total bilirubin in men and women from Masaka and Entebbe in Uganda (Karita et al., 2009). The authors hypothesized that some of the causes for the variations in RIs seen include poor nutrition, high prevalence of parasitic infections, differences in altitude and the possibility of inherited diseases such as Gilbert’s syndrome.

In South Africa, differences in RIs across races have been described. Francois et al highlighted differences in neutrophil counts among people of African, Caucasian and mixed ancestry with Africans having significantly lower total white blood cell and neutrophil counts while those of mixed ancestry were generally found to have similar blood counts to participants of Caucasian ancestry (Smit et al., 2019). Ichihara et al compared preliminary data on biochemistry reference values between black and non-black South Africans and found that black South Africans had lower median reference values (RVs) for creatinine, high density lipoprotein cholesterol (HDL-C) and uric acid (UA) (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017).

The main factors considered when partitioning RIs are age and sex which are generally considered important sources of variation in reference values. Ichihara et al has shown that RVs vary across different countries and that other sources of variation need to be considered given that variations are seen even in people of the same race but residing in different countries (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017). Body-mass index (BMI) has emerged as an important source of variation especially for tests such as liver enzymes, glucose, markers of inflammation and lipids which are associated with metabolic syndrome. Furthermore, it has been demonstrated that this BMI effect varies across races and ethnicities. For instance, in South Africa, an increase in BMI was associated with minimal increase in alanine transaminase (ALT) (r=0.02) in blacks compared to non-blacks (r=0.48) (Ichihara, Ozarda, Barth, Klee, Shimizu, et al., 2017).

Reference intervals can be derived using either parametric or non-parametric methods. The non-parametric method is recommended by the Clinical Laboratory Standards Institutes (CLSI) as it is simple, doesn't require transformation of data and often times outlier data is treated as a variation of normal as opposed to being abnormal (CLSI, 2010). Parametric method has been used as well but it only applies to data whose distribution is Gaussian. This often requires transformation of non-Gaussian data which is not always successful. However, Ichihara has demonstrated that any dataset can be transformed into a Gaussian distribution provided one defines the origin of the transformation relative to the data distribution and not from zero as has been the case for the original Box-Cox transformation (Box & Cox, 1964; Ichihara, 2014).

We set out to compare reference values and sources of variation between black Africans in Kenya and South Africa using data collected as part of the global study. South Africa is a multi-racial society hence the comparison was limited to black South Africans in order to control for race which is a known source of variation in RVs.

7.3 Methodology

Participants in Kenya and South Africa were enrolled using a harmonized protocol (Ozarda et al., 2013). The specifics of the recruitment process in Kenya and South Africa have previously been

published (Omuse, Maina, Hoffman, et al., 2017; Omuse et al., 2018; Smit et al., 2019). In Kenya, healthy adults aged 18-65 years from selected counties (Nairobi, Kiambu, Nakuru and Kisii) were enrolled. This was done between January and October 2015. For South Africa, the study was conducted between October 2012 and February 2015 as a multicenter- study involving three core institutions located in three different provinces in South Africa: PathCare Laboratories in Cape Town, Western Cape Province, which acted as the central laboratory, Water Sisulu University in Mthatha, Eastern Cape Province, and Wits University in Johannesburg, Gauteng Province. Recruitment was stratified into 4 age groups: 18-29, 30-39, 40-49 and 50-65 years with a similar distribution of males and females in each age strata. In South Africa, a few participants above the age of 65 years were also recruited but were excluded for this comparison. Informed consent was obtained from all participants after confirming that they had fasted overnight. Measurement of blood pressure (BP), WC and body mass index (BMI) was carried out after filling in a questionnaire which took 15-20 minutes. All blood samples were collected and centrifuged within 4 hours and stored at -80^{o}c until shipment on dry ice to the Pathcare reference laboratory in Cape town, South Africa.

Ethical approval was obtained from Aga Khan University-Nairobi Health Research Ethics Committee (2014/REC-46), Wellness Sciences' Research and Ethical Committee of Cape Peninsula University of Technology (CPUT/NHREC: REC-230408-014) as well as the University of Stellenbosch's Health Research Ethics Committee (S12/05/147).

The chemistry tests were analyzed using an automated Beckman Coulter DXC 700 analyser (Brea, CA USA) for specimens obtained between 2012 and 2013 and AU 600 analyser (Beckman Coulter, Tokyo, Japan) for the specimens obtained from 2014. Immunoassays were measured using a Beckman Coulter DxI 800 analyser (Brea, CA USA). The test abbreviations, methodologies and imprecision are shown in **Table 7.1**.

Table 7.1: Test abbreviations, methodologies and imprecision

Analyte	Abbreviation	Method	Units	Between Run CV
Sodium	Na	Ion selective electrode / diluted (indirect)	mmol/L	1.3
Potassium	K	Ion selective electrode / diluted (indirect)	mmol/L	3.8
Chloride	Cl	Ion selective electrode / diluted (indirect)	mmol/L	1.4
Urea	Urea	Urease	mmol/L	2.4
Creatinine	Cre	Modified kinetic Jaffè	µmol/L	1.6
Total Protein	TP	Biuret	g/L	4.6
Albumin	Alb	Bromocresol Green dye binding	g/L	2.8
Gamma-glutamyl transferase	GGT	Gamma-glutamyl-3-carboxy-4-nitroanilide	IU/L	3.3
Alkaline phosphatase	ALP	P-nitro-phenylphosphate hydrolysis	IU/L	4.0
Lactate dehydrogenase	LDH	Lactate to Pyruvate	IU/L	3.2
Calcium	Ca	Arsenazo III dye	mmol/L	1.4
Magnesium	Mg	Xylidyl blue	mmol/L	1.9
Phosphate	IP	Molybdate hydolysis	mmol/L	1.8
Lipase	Lip	1, 2-Diglyceride hydrolysis	U/L	8.0
Total cholesterol	TC	Cholesterol oxidase	mmol/L	1.2
Triglycerides	TG	Glycerol phosphate oxidase	mmol/L	4.1
High density lipoprotein cholesterol	HDL-C	Two phase selective accelerator detergent	mmol/L	2.0
Low density lipoprotein cholesterol	LDL-C	Two phase selective accelerator detergent	mmol/L	1.6
Uric acid	UA	Modified Trinder reaction with Uricase	mmol/L	1.2
High sensitive c reactive protein	CRP	Turbidimetry	mg/L	1.8
Amylase	AMY	2-chloro-4-nitrophenyl-α-D-maltotrioside	U/L	8.1
Immunoglobulin A	IgA	Turbidimetry	g/L	1.3
Immunoglobulin G	IgG	Turbidimetry	g/L	1.1
Immunoglobulin M	IgM	Turbidimetry	g/L	7.0
Alanine aminotransferase	ALT	NADH (without P-5′-P)	U/L	28.1
Aspartate aminotransferase	AST	NADH (without P-5′-P)	U/L	5.6
Creatinine kinase	CK	Creatine phosphate dephosphorilysation	U/L	6.5
Iron	Fe	2, 4, 6-Tri-(2-pyridyl)-5-triazine chromogen	µmol/L	4.2
Transferrin	Tf	Turbidimetry	g/L	2.6
Anti-thyroglobulin	TgAb	Two-site immune – enzymatic immunoassay	IU/ml	4.8
Anti-thyroid peroxidase	TPOAb	Two-site immune – enzymatic immunoassay	IU/ml	28.8
Thyroid stimulating hormone	TSH	Two-site immune – enzymatic immunoassay	mIU/L	13.0
Prostate specific antigen	PSA	Two-site immune – enzymatic immunoassay	µg/L	2.3

In order to allow for direct comparison of reference values, all participating laboratories received a panel of sera produced by the C-RIDL in 2011 and in 2014 that had values assigned. This panel was measured by participating laboratories to aid with recalibration of reference values using linear regression analysis. Further, a mini panel of sera derived from 5 healthy individuals analyzed alongside the study samples in order to assess for between-day variations and recalibrate the results if the variation exceeded acceptable limits (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017).

Statistical analysis

Continuous variables were presented as medians with their corresponding interquartile ranges. In order to compare RIs between South Africa and Kenya, we determined RIs using a parametric approach after

latent abnormal value exclusion (LAVE) as described by Ichihara et al (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017). LAVE was based on the following reference tests: Alb, UA, Glu, TG, HDL-C, LDL-C, AST, ALT, LDH, GGT, CK and CRP, which were used to exclude individuals with outlier values tough to be related to nutrition status, inflammation, or muscular damage. For proteins we set TP, Alb, Urea, Cre, Na, Ca, Fe, CRP, IgG, IgA, and Tf as the reference tests when applying LAVE. For comparison of RIs, we excluded all individuals with a BMI > 35 kg/m^2, missing BMI data and age > 65 years.

Sources of variation were assessed using standard deviation ratios (SDRs) calculated as between country (SDRcountry), between sex (SDRsex) and between age (SDRage). These were computed as a ratio to the residual SD (or between-individual SD). Since between-age and between-country variation changes by sex, we also computed SDRage and SDRcountry for males, females and both males and females by one way ANOVA. We considered SDR ≥ 0.40 as a guide for judging the important sources of variation for the different analytes. For SDRage, age was stratified as 18-29, 30-39, 40-49 and 50-65 years. This analysis was carried out using a general purpose statistical software, StatFlex version 6.0 (Artech Inc., Osaka, Japan). We also evaluated the RI lower limit (LL) and upper limit (UL) biases between Kenya and South African RIs expressed as bias ratio (BR) at LL or UL (BR_{LL} or BR_{UL}) as shown below:

$$BR_{LL} = \frac{|LL_{ZA} - LL_{KE}|}{(UL_{KE/ZA} - LL_{KE/ZA})/3.92} \quad , \quad BR_{UL} = \frac{|UL_{ZA} - UL_{KE}|}{(UL_{KE/ZA} - UL_{KE/ZA})/3.92}$$

The subscripts of KE and ZA refer to LL or UL of Kenyan and South African RIs respectively and KE/ZA refers to combined RIs for Kenya and South Africa. Multiple regression analysis (MRA) was performed to identify factors possibly influencing the test results, including country, age, BMI, smoking, exercise and alcohol intake. The level of cigarette smoking was categorized as none, ≤ 20, and > 20 cigarettes/day; exercise was categorized as none, 1–7 days/week; alcohol intake was categorized as none, <12.5, 12.5−25, 25−50, >50 g ethanol/day. A given explanatory variable was considered to be of practical importance when its standardized partial regression coefficient (r_p) was ≥ 0.20 in its absolute value. Linear regression was used to demonstrate the association between BMI and selected analytes. This analysis was carried out using a general purpose statistical software SPSS® version 23 (IBM, USA).

7.4 Results

A total of 1130 participants had sufficient data for inclusion and further analysis. This included 597 and 533 participants from South Africa and Kenya respectively. Participant characteristics were similar except for alcohol intake in where the Kenyan participants had a much higher percentage as shown in **Table 7.2**.

Table 7.2: Participant characteristics

Country		South Africa			Kenya			All		
Sex		Male	Female	All	Male	Female	All	Male	Female	All
Age	n	312	285	597	259	274	533	571	559	1130
(Years)	Median (IQR)	36 (21)	34 (22)	35 (21)	39 (19)	39 (20)	39 (20)	38 (21)	37 (21)	37 (20)
BMI	n	306	273	579	259	274	533	565	547	1112
(Kg/m^2)	Median (IQR)	25.2 (7.3)	26.9 (6.8)	26.3 (7.0)	24.9 (5.6)	26.1 (6.2)	25.5 (5.9)	25.1 (6.3)	26.4 (6.6)	25.8 (6.6)
SBP	n	165	232	389	259	274	530	424	506	930
(mmHg)	Median (IQR)	127 (21)	123 (24)	125	128 (18)	118 (20)	124	127 (18)	120 (22)	124 (21)
DBP	n	165	229	394	259	274	532	424	506	930
(mmHg)	Median (IQR)	82 (16)	82 (15)	82	81 (12)	79 (14)	80	81 (13)	80 (15)	80 (14)
Alcohol intake	n	86	97	183	259	274	533	345	371	716
	No. (%)	19 (22%)	11 (11%)	30 (16%)	101 (39%)	73 (27%)	174 (33%)	120 (35%)	84 (23%)	204 (28%)
Smoking	n	93	117	210	259	274	533	352	391	743
	No. (%)	6 (6%)	1 (1%)	7 (3%)	12 (5%)	2 (1%)	14 (3%)	18 (6%)	3 (1%)	21 (3%)

Using an SDR cut-off of 0.4 to evaluate sources of variation, sex was an important source of variation for UA, Cre, AST, CK and Tf without considering between country variation. Between country variation was significant for Alb, Mg, Glu, LDL-C, ALT, GGT, CK and IgM while age was a significant source of variation for UA, Glu and TG without partitioning by sex as shown in **Table 7.3**.

Table 7.3: Standard deviation ratios for between sex, country and age

Analytes	SDRsex	SDRcountry			SDRage		
		MF	M	F	MF	M	F
TP	0.000	0.000	0.392	0.000	0.220	0.197	0.237
Alb	0.000	**0.800**	**0.472**	**0.769**	0.374	**0.405**	0.339
Urea	0.187	0.306	0.000	0.341	0.306	0.232	0.362
UA	**0.771**	0.000	**0.829**	0.000	**0.406**	0.360	**0.456**
Cre	**1.050**	0.025	**0.534**	0.000	0.158	0.188	0.111
Na	0.000	0.000	0.261	0.000	0.299	0.328	0.273
K	0.000	0.211	0.190	0.283	0.154	0.155	0.153
Cl	0.137	0.000	0.076	0.000	0.242	0.316	0.131
Ca	0.189	0.141	0.110	0.200	0.221	0.224	0.218
IP	0.173	0.157	0.000	0.268	0.262	0.324	0.175
Mg	0.000	**0.466**	**0.427**	**0.442**	0.277	0.277	0.278
Fe	0.357	0.198	0.000	0.104	0.051	0.114	0.000
Glu	0.000	**0.481**	0.000	**0.488**	**0.659**	**0.655**	**0.662**
TC	0.000	0.198	**0.490**	0.000	0.394	0.372	**0.418**
TG	0.219	0.000	0.276	0.000	**0.551**	**0.488**	**0.625**
HDL-C	0.263	0.105	**0.453**	0.159	0.000	0.057	0.000
LDL-C	0.000	**0.538**	0.290	**0.435**	0.339	0.301	0.375
AST	**0.455**	0.000	0.000	0.000	0.254	0.251	0.258
ALT	0.000	**1.154**	**0.619**	**1.107**	0.269	0.204	0.339
LDH	0.000	**0.636**	0.299	**0.597**	0.126	0.074	0.165
ALP	0.000	0.394	0.000	**0.406**	0.376	0.202	**0.488**
GGT	0.379	0.272	**1.191**	0.369	0.314	0.326	0.301
CK	**0.412**	0.057	**0.670**	0.000	0.088	0.114	0.042
AMY	0.076	0.000	0.379	0.053	0.044	0.051	0.034
CRP	0.000	0.329	0.134	0.238	0.307	0.292	0.322
IgG	0.025	0.033	0.085	0.000	0.082	0.043	0.107
IgA	0.000	0.103	0.000	0.097	0.316	0.234	0.390
IgM	0.347	0.219	**0.406**	0.035	0.170	0.088	0.225
Tf	**0.405**	0.075	0.060	0.112	0.206	0.168	0.227
TSH	0.000	0.190	0.107	0.193	0.063	0.057	0.069
PSA			0.308			0.338	

Key: F-Female, M-Male, MF-Male and Female

MRA revealed that drinking, exercise and smoking had minimal influence on reference values for all the analytes. Compared to Kenyan data, reference values for Alb, Na, Cl, Mg, TC, LDL-C, ALT and LDH were much lower for the South Africans denoted by r_p values less than -0.2. BMI had a significant positive correlation (r_p>0.2) for UA, TG, ALT and CRP while age had a positive correlation with Glu and TG for both males and females. Age also had a positive correlation with PSA as shown in **Table 7.4**.

Table 7.4: Multiple regression analysis and partial correlation coefficients for all analytes

Male								Female							
Analyte	**R**	**ZA**	**Age**	**BMI**	**DrkLvl**	**SmkLvl**	**ExerLvl**	**Analyte**	**R**	**ZA**	**Age**	**BMI**	**DrkLvl**	**SmkLvl**	**ExerLvl**
TP	0.294	-0.216	-0.220	0.019	-0.019	-0.035	-0.023	TP	0.280	-0.153	-0.169	-0.044	-0.068	-0.025	0.092
Alb	0.513	-0.436	-0.377	0.013	-0.076	-0.077	0.014	Alb	0.558	-0.453	-0.211	-0.229	-0.082	0.039	0.021
Urea	0.320	0.152	0.156	0.150	0.125	0.054	-0.055	Urea	0.357	0.216	0.290	0.015	0.081	0.035	0.035
UA	0.515	-0.086	0.175	0.393	0.113	0.018	-0.021	UA	0.462	-0.161	0.180	0.295	0.071	0.000	-0.139
Cre	0.298	-0.099	-0.121	0.181	0.162	0.029	0.006	Cre	0.171	-0.044	-0.053	0.112	0.085	-0.045	0.037
Na	0.470	-0.415	0.068	-0.096	0.139	-0.048	-0.027	Na	0.444	-0.353	0.189	-0.110	0.041	0.029	0.007
K	0.311	0.278	0.135	0.016	0.087	0.095	0.024	K	0.338	0.262	0.095	0.132	-0.044	0.028	0.015
Cl	0.510	-0.420	0.228	-0.070	0.070	0.009	-0.092	Cl	0.453	-0.392	0.080	-0.044	0.080	0.030	0.012
Ca	0.283	-0.193	-0.227	0.032	0.003	0.044	0.017	Ca	0.375	-0.308	0.000	-0.100	0.029	0.072	0.044
IP	0.269	-0.165	0.015	0.095	0.137	0.025	0.020	IP	0.307	-0.221	0.099	-0.115	0.103	0.011	0.001
Mg	0.522	-0.376	0.306	-0.017	0.059	0.003	0.015	Mg	0.477	-0.348	0.242	-0.001	-0.104	0.024	0.015
Fe	0.234	-0.202	-0.036	-0.074	-0.061	0.041	-0.084	Fe	0.181	-0.167	-0.025	0.041	0.034	0.009	-0.060
Glu	0.595	-0.114	0.340	0.357	-0.132	-0.044	-0.068	Glu	0.548	-0.114	0.423	0.087	-0.019	-0.028	-0.080
TC	0.520	-0.267	0.223	0.284	0.125	-0.050	-0.002	TC	0.398	0.123	0.308	0.036	0.069	0.043	0.053
TG*	0.560	-0.070	0.315	0.375	0.038	0.051	0.003	TG*	0.519	-0.035	0.342	0.214	-0.105	-0.042	-0.010
HDL-C	0.328	-0.102	0.069	-0.291	0.087	-0.023	0.047	HDL-C	0.298	-0.090	0.083	-0.161	0.020	0.082	0.170
LDL-C	0.551	-0.339	0.202	0.304	0.108	-0.067	-0.010	LDL-C	0.451	-0.229	0.304	0.062	0.089	0.043	0.015
AST*	0.240	-0.061	-0.072	0.224	-0.079	-0.049	-0.012	AST*	0.278	0.043	0.122	0.125	-0.107	-0.042	0.037
ALT*	0.633	-0.546	-0.009	0.383	0.027	0.003	-0.047	ALT*	0.736	-0.654	0.259	0.203	0.056	0.046	0.009
LDH	0.390	-0.355	-0.095	0.165	0.039	0.005	-0.028	LDH	0.401	-0.305	0.080	0.150	0.038	-0.021	0.030
ALP	0.233	-0.225	-0.029	0.021	-0.074	0.006	-0.061	ALP	0.426	-0.196	0.269	0.081	-0.069	-0.035	0.014
GGT*	0.457	0.138	0.061	0.366	0.126	0.044	-0.075	GGT*	0.283	0.215	0.133	0.091	0.058	0.002	0.065
CK*	0.206	0.039	-0.071	0.193	0.002	-0.010	0.046	CK*	0.229	-0.039	0.048	0.155	-0.068	0.029	0.077
AMY	0.218	0.009	-0.014	-0.125	-0.107	-0.089	0.042	AMY	0.183	-0.058	0.014	-0.122	-0.062	0.083	0.037
CRP*	0.473	0.192	0.147	0.330	0.157	0.054	0.006	CRP*	0.529	0.257	0.156	0.394	0.055	0.063	0.159
IgG	0.181	-0.099	-0.056	-0.035	-0.084	-0.096	0.023	IgG	0.137	0.041	-0.092	0.051	-0.080	-0.040	0.036
IgA	0.231	0.046	0.187	0.038	0.081	0.029	-0.026	IgA	0.322	0.044	0.278	0.006	-0.022	0.025	-0.003
IgM	0.222	0.117	-0.076	-0.116	0.042	-0.033	0.027	IgM	0.151	0.081	-0.039	-0.066	-0.062	-0.017	0.062
Tf	0.194	-0.139	0.074	0.035	-0.052	0.018	0.013	Tf	0.260	-0.121	-0.130	-0.060	0.029	-0.015	0.090
TSH	0.210	-0.172	0.027	0.076	-0.004	0.030	0.000	TSH	0.148	-0.098	-0.049	0.079	-0.049	-0.032	0.039
PSA	0.294	-0.039	0.257	-0.067	-0.043	-0.008	-0.076								

Key: *used log transformed data; shaded red background rP < -0.2; shaded green background rP > 0.2; DrkLvl-Drink level; SmkLvl-Smoking level; ZA-South Africa

A comparison of linear regression between Kenya and South African data for selected analytes with BMI is shown in **Fig 7.1**. Both between country and sex differences are seen in the effect of BMI on the selected analytes as evidenced by the differences in the slope of the regression lines.

Not all participants had test results for all the analytes hence RIs were determined using available data as shown in **Table 7.5**. South African RIs for UA, TC, LDL-C, ALT, LDH, ALP, ALB, Mg, TSH and PSA were lower than the Kenyan RIs as seen by the negative biases for both LLs and ULs. For instance, the RIs for Na in Kenyan males and females were 135-141 mmol/L and 134-142 mmol/L respectively, while that for South African males and females were 129-144 mmol/L and 129-147 mmol/L respectively. Glucose was excluded from the RI comparison given the very few reference values available from South Africa compared to Kenya (66 vs 522).

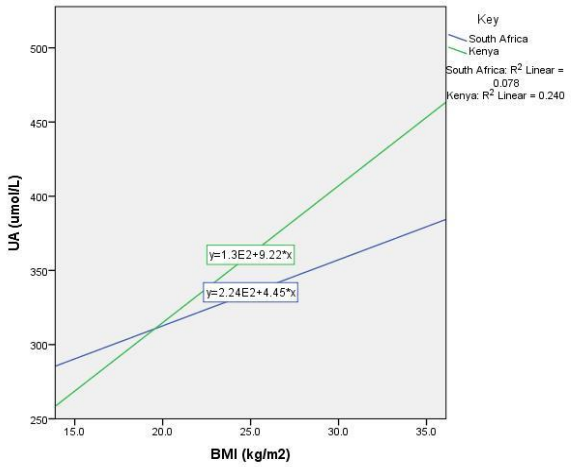

Figure 7.1a: Linear regression of UA and BMI in males

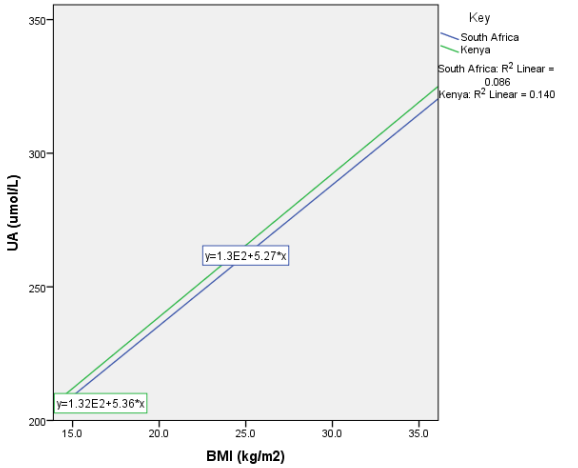

Figure 7.1b: Linear regression of UA and BMI in females

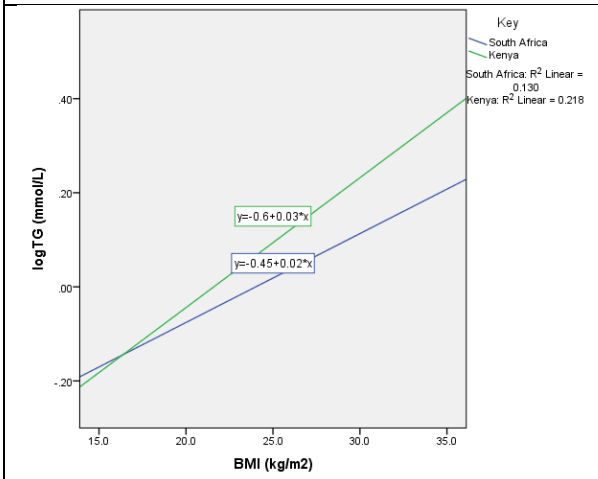

Figure 7.1c: Linear regression of logTG and BMI in males

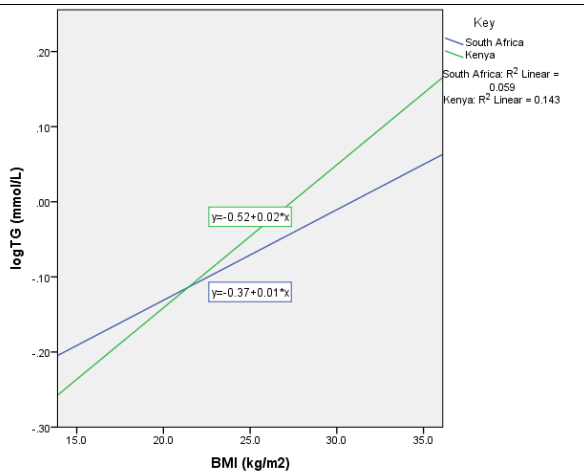

Figure 7.1d: Linear regression of logTG and BMI in females

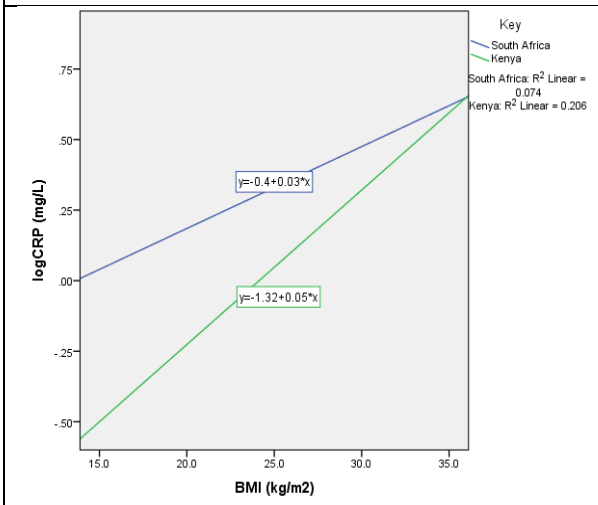

Figure 7.1e: Linear regression of CRP and BMI in males

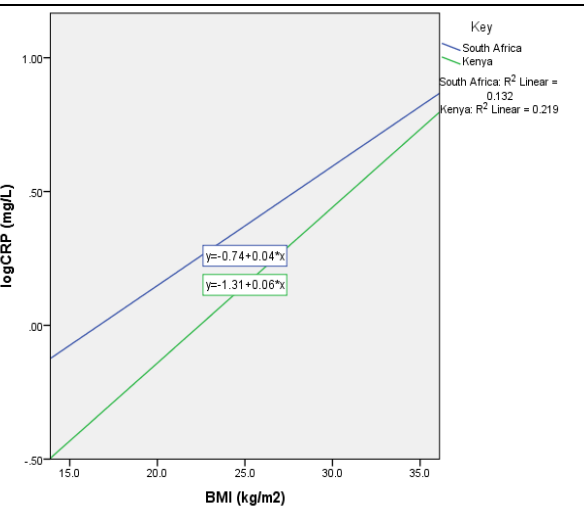

Figure 7.1f: Linear regression of CRP and BMI females

Fig 7.1: Linear regression of BMI and selected analytes

Table 7.5: Comparison of reference intervals between Kenya and South Africa

	Male						Female						Male + Female					
	KE		ZA		ZA vs KE		KE		ZA		ZA vs KE		KE		ZA		ZA vs KE	
Item	**LL**	**UL**	**LL**	**UL**	**BR_{LL}**	**BR_{UL}**	**LL**	**UL**	**LL**	**UL**	**BR_{LL}**	**BR_{UL}**	**LL**	**UL**	**LL**	**UL**	**BR_{LL}**	**BR_{UL}**
UA	248	506	198	468	**-0.73**	**-0.57**	175	392	154	406	-0.35	0.24	187	459	160	463	-0.37	0.06
TC	3.19	6.67	2.78	6.35	**-0.44**	-0.34	3.21	6.50	2.60	6.36	**-0.68**	-0.15	3.14	6.45	2.66	6.33	**-0.55**	-0.14
TG	0.54	3.32	0.49	3.42	-0.07	0.14	0.46	2.40	0.44	2.44	-0.04	0.07	0.49	2.82	0.46	3.11	-0.05	**0.46**
HDL-C	0.76	1.63	0.69	1.62	-0.27	-0.03	0.82	1.78	0.74	1.82	-0.29	0.14	0.78	1.75	0.72	1.75	-0.20	0.02
LDL-C	1.72	4.82	0.92	3.80	**-0.99**	**-1.25**	1.57	4.59	0.94	3.96	**-0.80**	**-0.80**	1.57	4.57	0.95	3.86	**-0.77**	**-0.88**
AST	17	39	15	42	-0.30	**0.43**	15	29	14	33	-0.19	**0.84**	15	33	14	40	-0.18	**1.24**
ALT	10	51	4	25	**-0.52**	**-2.30**	7	28	2	17	**-0.83**	**-1.79**	8	40	3	21	**-0.58**	**-2.13**
LDH	137	248	93	218	**-1.33**	**-0.89**	139	252	112	230	**-0.84**	**-0.66**	136	247	97	224	**-1.20**	**-0.68**
ALP	52	150	43	125	-0.37	**-0.99**	48	146	39	124	-0.36	**-0.90**	50	147	39	125	**-0.44**	**-0.92**
GGT	14	85	14	92	0.04	0.30	10	45	12	84	0.18	**3.09**	12	66	13	96	0.09	**1.68**
CK	75	427	57	505	-0.18	**0.76**	52	271	46	266	-0.11	-0.08	58	350	50	419	-0.10	**0.84**
AMY	50	162	48	166	-0.09	0.14	46	162	43	159	-0.10	-0.12	48	161	43	162	-0.15	0.04
TP	67.9	80.7	66.2	82.7	**-0.46**	**0.55**	66.2	82.5	66.2	88.4	-0.01	**1.28**	67.2	81.8	65.7	85.5	-0.35	**0.88**
Alb	40.1	50.2	37.3	47.7	**-0.98**	**-0.89**	37.8	47.8	34.0	47.2	**-1.19**	-0.18	39.1	49.6	34.9	47.8	**-1.29**	**-0.55**
Urea	2.36	6.47	2.66	6.93	0.26	**0.41**	1.89	5.85	1.99	6.69	0.09	**0.75**	2.08	6.03	2.26	6.91	0.16	**0.78**
Cre	57	109	55	108	-0.17	-0.13	44	86	44	86	0.03	0.03	48	107	46	104	-0.11	-0.22
Na	135	141	129	144	**-2.53**	**0.93**	134	142	129	147	**-1.67**	**1.67**	134	142	128	145	**-2.16**	**1.12**
K	3.3	4.8	3.6	5.3	**0.66**	**1.16**	3.4	4.8	3.6	5.4	**0.43**	**1.41**	3.4	4.8	3.6	5.3	**0.52**	**1.21**
Cl	100	109	95	112	**-1.82**	**1.18**	102	110	98	115	**-1.12**	**1.79**	101	109	96	113	**-1.51**	**1.19**
Ca	2.22	2.55	2.17	2.58	**-0.56**	**0.38**	2.18	2.54	2.07	2.64	**-1.01**	**0.83**	2.22	2.55	2.10	2.60	**-1.13**	**0.51**
IP	0.76	1.40	0.76	1.36	-0.01	-0.21	0.83	1.42	0.76	1.38	**-0.41**	-0.24	0.78	1.42	0.75	1.36	-0.20	-0.33
Mg	0.73	0.99	0.66	0.96	**-1.01**	-0.37	0.71	0.98	0.47	0.93	**-3.26**	**-0.63**	0.73	0.98	0.58	0.95	**-2.04**	**-0.45**
Fe	8.9	29.7	7.2	28.8	-0.30	-0.17	3.6	29.0	3.7	26.7	0.03	-0.37	5.6	28.6	4.6	28.3	-0.18	-0.05
CRP	0.19	11.80	0.36	16.68	0.04	**1.28**	0.22	19.68	0.32	28.70	0.02	**1.65**	0.22	16.11	0.35	25.83	0.03	**1.97**
IgG	11.40	18.89	9.96	21.52	**-0.61**	**1.13**	11.21	20.53	10.52	26.53	-0.25	**2.22**	11.22	19.55	10.15	23.25	**-0.42**	**1.44**
IgA	0.92	4.74	1.14	5.00	0.22	0.26	1.00	4.35	1.13	5.26	0.14	**0.99**	0.98	4.54	1.10	5.13	0.13	**0.62**
IgM	0.37	2.23	0.42	2.57	0.10	**0.67**	0.50	2.87	0.55	2.92	0.09	0.08	0.40	2.50	0.45	2.78	0.10	**0.51**
Tf	1.88	3.08	1.83	3.11	-0.17	0.09	2.01	3.90	1.91	3.76	-0.20	-0.30	1.93	3.48	1.85	3.48	-0.20	-0.01
TSH	0.58	4.81	0.41	3.79	-0.16	**-0.98**	0.51	4.99	0.47	3.01	-0.04	**-2.07**	0.57	5.02	0.46	3.62	-0.11	**-1.38**
PSA	0.27	2.87	0.22	2.27	-0.08	**-0.91**												

Key: KE-Kenya; ZA-South Africa; Red shade-negative bias; Green shade-positive bias

7.5 Discussion

We compared RIs between Kenya and South Africa and assessed the extent to which potential sources of RI variation impacted reference values. This was an attempt at determining the possibility of harmonizing RIs as the 2 countries used a harmonized study protocol including analysis of samples in a central laboratory using appropriate quality control procedures to ensure comparability of results (Ozarda et al., 2013). A study by Karita et al demonstrated significant inter-country variation in RIs for some analytes among East African countries (Karita et al., 2009). However, there was no harmonization of pre-analytical sample handling and sample analysis was not centralized which could have contributed to some of the differences seen. Generally, there is scanty published data on chemistry and immunoassay RIs from sub-Saharan Africa. Some of the published data was derived from participants screened as part of HIV clinical trials and often times the requisite rigor expected of a RI study wasn't met (Segolodi et al., 2014). There are concerns that individuals volunteering to participate in HIV related clinical trials may not be representative of a healthy reference population, hence compromising their external validity. The IFCC has a fairly strict criteria that should be followed when identifying reference individuals for a RI study (PetitClerc & Solberg, 1987).

In our study, we found several differences in RIs between Kenya and South Africa that we didn't expect. For example, the LL for Na in the South African population was 128 mmol/L for combined males and females compared to 134 mmol/L in the Kenyan participants. The LL for Cl for both males and females in South Africans was similarly very low at 95 and 98 mmol/L respectively compared to 100 and 102 mmol/L for Kenya. The RIs we obtained for electrolytes in Kenya are in keeping with what has previously been published (Kibaya et al., 2008). A search for published literature on RIs for electrolytes from South Africa didn't yield citable references hence we are unable to find an explanation for the unexpected low Na and Cl. However, a study from Botswana which borders South Africa reported RIs for Na (135-143 mmol/L) and Cl (98-107 mmol/L) that are similar to what has been found in other countries in Africa (Segolodi et al., 2014). Given that the Na values obtained from South Africa are low enough to be clinically relevant and that they are at variance with what has been widely published (Burtis et al., 2005), verification of the same is required. Given the association between hyponatremia and

tuberculosis, it would be desirable to find out whether some of the participants could have had undiagnosed tuberculosis (Jafari et al., 2012).

Lipid levels were consistently lower in the black South Africans compared to Kenyans. Various factors can contribute to variations in lipid levels across populations including differences in environment, physiology and genetics (Bentley & Rotimi, 2012). In our study, no significant differences were seen in age and BMI between participants from the 2 countries. The impact of exercise on reference values for all the tests was also minimal. Therefore, it is unlikely that the differences seen in lipid levels can be attributed to differences in age, BMI or exercise. CRP and immunoglobulin levels were higher in the South African participants which may partially explain the lower cholesterol levels seen given that inflammation may cause a dyslipidemia characterized by low HDL-C levels (Esteve, Ricart, & Fernandez-Real, 2005). We have previously published analysis of the same Kenyan data set that revealed a prevalence of metabolic syndrome of 25.6% (Omuse, Maina, Hoffman, et al., 2017). Metabolic syndrome is associated with increased visceral adipose tissue and a dyslipidemia characterized by increased LDL-C, TGs and reduced HDL-C. We hypothesize that a high prevalence of metabolic syndrome in the Kenyan participants may contribute to the differences seen in lipid levels. Differences in diet have been shown to contribute to variation in lipid levels. Kakarmath et al demonstrated that a combination of using palm oil for cooking, eating more than one serving of meat per day and fewer than five servings of fruits and vegetables per day, was associated with higher serum total cholesterol in Dar es Salaam, Tanzania (Kakarmath et al., 2017). We did collect data on food intake however, we did not use a standardized and validated questionnaire to objectively determine its association with lipid levels. There are also known inter-ethnic variations in genes associated with lipids such as APOL1 whose prevalence varies across sub-Saharan Africa and is thought to influence the distribution of HDL particles (Freedman et al., 2011). Genetic testing to determine the prevalence of the APOL1 gene would aid in further elucidating reasons for the variation in lipid levels. Kesswell et al examined differences in body fat distribution between premenopausal black and white South African women and found that black women had less central and more peripheral (lower-body) but had lower serum lipid concentrations. Central and peripheral fat deposition was shown to be independently associated with the presence of insulin resistance in both the black and white women, and with TG concentrations in the black women (Keswell,

Tootla, & Goedecke, 2016). We did not collect data on body composition hence do not know whether this could have contributed to some of the differences seen in lipid levels between Kenya and South African blacks.

Differences in liver function tests were found with South Africans having much lower albumin, ALT, LDH and ALP levels in both males and females but higher GGT. We didn't have data on total and direct bilirubin from South Africa to be able to evaluate whether the increased GGT could be associated with cholestasis or hepatobiliary disease though the low ALP levels make this unlikely. Alcohol consumption was lower in South Africans hence would not explain the increased GGT. Given that CRP and IgG levels in South Africans were much higher compared to Kenyans, the low albumin may be due to inflammation given that it is a negative acute phase reactant (Gulhar, Ashraf, & Jialal, 2020). We didn't test for the presence of viral hepatitis hence cannot speculate on whether this may have contributed to the differences in liver enzymes. Further studies are recommended to investigate the reasons for the differences in liver function tests seen.

Several factors are thought to contribute to intra-ethnic differences in inflammatory markers. We found higher levels of CRP and immunoglobulins in the South African participants compared to their Kenyan counterparts. It is often assumed that infections contribute to the increased inflammation seen in Africans but non-communicable diseases also contribute to chronic inflammation especially with urbanization taking root in the continent (Bickler et al., 2018). South Africa is a more urbanized country compared to Kenya and differences in diet, lifestyle and environment could contribute to the increased inflammation seen. Urbanization is thought to influence gene expression through unknown mechanisms. For example, in Morocco, a comparison of rural and urban populations demonstrated differences in gene expression of G protein-coupled receptors (Bickler, Lizardo, Cauvi, & De Maio, 2016). These receptors impact on almost every aspect of biology in humans hence differential expression could possibly translate into physiological differences including inflammatory responses. Chronic inflammation is a known cardiovascular disease risk factor hence identifying the reason for the increased inflammatory markers may provide an opportunity to mitigate this risk.

Little is known about sources of RI variation in sub-Saharan Africa and whether BMI impacts on reference values especially those associated with metabolic syndrome. Not surprisingly, an increase in BMI was associated with an increase in TC, TG, LDL-C, UA, CRP, GGT and a decrease in HDL-C as seen by absolute r_p values > 0.2. Of note was that the slope of the regression lines for UA, TG and CRP seen in **Fig 7.1** was different between Kenya and South Africa indicating that factors beyond ethnicity influence this relationship, further adding to the complexity in determining harmonized RIs. Ichihara et al has also demonstrated similar variations across several countries with change in BMI having the greatest impact in Asian countries compared to other races and ethnicities (Ichihara, 2014). This suggests that BMI should be an important consideration when determining RIs, however, there is need for robust evidence to guide what BMI cut off should be used as an inclusion or exclusion criteria.

Our study had various limitations. Despite all attempts at standardizing pre-analytical, analytical and post-analytical processes, discrepancies were found in the actual implementation of the recruitment criteria with individuals with BMIs greater than 35 kg/m^2 being recruited so long as they were subjectively healthy. However, for purposes of comparing Kenya and South Africa data, everyone with a BMI above 35 kg/m^2 was secondarily excluded. Given the challenge of coordinating sample collection from various sites, it is possible that sample handling may have differed especially with regards to time to centrifugation, aliquoting and storage. Analytes such as potassium and glucose which are sensitive to sample handling might have been affected for some samples. In order to minimize the impact of any delay in centrifuging on glucose, samples for glucose analysis were collected in fluoride tubes. We relied on participants to truthfully report on their health status when filling in the questionnaire including whether they had any infection such as HIV, HBV and HCV. It is possible that a few of our participants may have been recruited as healthy individuals despite this not being true thereby impacting on derived RIs. However, we used LAVE to secondarily exclude individuals with outlier values based on a combination of analytes used in evaluating nutritional and inflammatory status.

In conclusion, the findings of this study highlight the difficulties in harmonizing RIs even for populations thought to be similar despite standardizing study processes to enable direct comparison of RIs. For most analytes, harmonization of RIs between Kenyans and South Africans of African ancestry will not be ideal as they could result in misclassification of individuals as either diseased or healthy. We therefore recommend separate RIs for the respective reference populations. Furthermore, BMI was shown to be a major source of variation and future studies should be sufficiently powered to factor this as an independent variable that influences RIs and also determine the ideal BMI to use as an inclusion or exclusion criteria given the subjectivity of defining health.

Author contributions

GO designed the study, collected data, performed statistical analysis and wrote the manuscript. FS designed the study, collected data and critiqued the study. KI designed the study, planned and supervised its implementation, assisted with statistical analysis and critiqued the manuscript. DM designed the study, collected data and critiqued the manuscript. ZP critiqued the manuscript. RE designed the study, planned and supervised its implementation, critiqued the manuscript.

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Jared Oseko and Patricia Ingato of Aga Khan University Hospital Nairobi assisted in sample collection, bar coding and processing. Benjamin Matheka, marketing manager PathCare Kenya Limited, assisted in participant recruitment and sample collection. Arno Theron carried out sample analysis, quality assurance and data handling at the PathCare reference laboratory in Cape Town, South Africa.

7.6 References

Bentley, A. R., & Rotimi, C. N. (2012). Interethnic Variation in Lipid Profiles: Implications for Underidentification of African-Americans at risk for Metabolic Disorders. *Expert Rev Endocrinol Metab, 7*(6), 659-667. doi:10.1586/eem.12.55

Bickler, S. W., Lizardo, E., Cauvi, D. M., & De Maio, A. (2016). The transition from a rural to an urban environment in Africa alters G protein-coupled receptor signaling. *Med Hypotheses, 95*, 49-53. doi:10.1016/j.mehy.2016.08.004

Bickler, S. W., Wang, A., Amin, S., Halbach, J., Lizardo, R., Cauvi, D. M., & De Maio, A. (2018). Urbanization in Sub-Saharan Africa: Declining Rates of Chronic and Recurrent Infection and Their Possible Role in the Origins of Non-communicable Diseases. *World J Surg, 42*(6), 1617-1628. doi:10.1007/s00268-017-4389-5

Burtis, C., Ashwood, E., & Bruns, D. (2005). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*.

Esteve, E., Ricart, W., & Fernandez-Real, J. M. (2005). Dyslipidemia and inflammation: an evolutionary conserved mechanism. *Clin Nutr, 24*(1), 16-31. doi:10.1016/j.clnu.2004.08.004

Freedman, B. I., Langefeld, C. D., Murea, M., Ma, L., Otvos, J. D., Turner, J., . . . Parks, J. S. (2011). Apolipoprotein L1 nephropathy risk variants associate with HDL subfraction concentration in African Americans. *Nephrol Dial Transplant, 26*(11), 3805-3810. doi:10.1093/ndt/gfr542

Gulhar, R., Ashraf, M. A., & Jialal, I. (2020). Physiology, Acute Phase Reactants. In *StatPearls*. Treasure Island (FL).

Ichihara, K. (2014). Statistical considerations for harmonization of the global multicenter study on reference values. *Clinica Chimica Acta, 432*, 108-118. doi:10.1016/j.cca.2014.01.025

Ichihara, K., Ozarda, Y., Barth, J. H., Klee, G., Qiu, L., Erasmus, R., . . . Yadav, B. (2017). A global multicenter study on reference values: 1. Assessment of methods for derivation and comparison of reference intervals. *Clinica Chimica Acta, 467*, 70-82. doi:10.1016/J.CCA.2016.09.016

Ichihara, K., Ozarda, Y., Barth, J. H., Klee, G., Shimizu, Y., Xia, L., . . . Takahashi, A. (2017). A global multicenter study on reference values: 2. Exploration of sources of variation across the countries. *Clinica Chimica Acta, 467*, 83-97. doi:10.1016/j.cca.2016.09.015

Jafari, N. J., Izadi, M., Sarrafzadeh, F., Heidari, A., Ranjbar, R., & Saburi, A. (2012). Hyponatremia Due to Pulmonary Tuberculosis: Review of 200 Cases. *Nephrourol Mon., 5*(1), 687–691. doi:10.5812/numonthly.7091

Kakarmath, S. S., Zack, R. M., Leyna, G. H., Fahimi, S., Liu, E., Fawzi, W. W., . . . Danaei, G. (2017). Dietary determinants of serum total cholesterol among middle-aged and older adults: a population-based cross-sectional study in Dar es Salaam, Tanzania. *BMJ open, 7*(5), e015028. doi:10.1136/bmjopen-2016-015028

Karita, E., Ketter, N., Price, M. A., Kayitenkore, K., Kaleebu, P., Nanvubya, A., . . . Kamali, A. (2009). CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. *PLOS ONE, 4*(2), e4401. doi:10.1371/journal.pone.0004401

Keswell, D., Tootla, M., & Goedecke, J. H. (2016). Associations between body fat distribution, insulin resistance and dyslipidaemia in black and white South African women. *Cardiovasc J Afr, 27*(3), 177-183. doi:10.5830/CVJA-2015-088

Kibaya, R. S., Bautista, C. T., Sawe, F. K., Shaffer, D. N., Sateren, W. B., Scott, P. T., . . . de Souza, M. S. (2008). Reference ranges for the clinical laboratory derived from a rural population in Kericho, Kenya. *PLOS ONE, 3*(10), e3327-e3327. doi:10.1371/journal.pone.0003327

Omuse, G., Maina, D., Hoffman, M., Mwangi, J., Wambua, C., Kagotho, E., . . . Erasmus, R. (2017). Metabolic syndrome and its predictors in an urban population in Kenya: A cross sectional study. *BMC Endocrine Disorders, 17*(1). doi:10.1186/s12902-017-0188-0

Omuse, G., Maina, D., Mwangi, J., Wambua, C., Radia, K., Kanyua, A., . . . Erasmus, R. (2018). Complete blood count reference intervals from a healthy adult urban population in Kenya. *PLOS ONE*, 1-19.

Ozarda, Y., Ichihara, K., Barth, J. H., & Klee, G. (2013). Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. *Clinical Chemistry and Laboratory Medicine (CCLM), 51*(5), 1027-1040. doi:10.1515/cclm-2013-0249

PetitClerc, C., & Solberg, H. E. (1987). Approved recommendation (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values. *Clinica Chimica Acta, 170*(2-3), S1-S11. doi:10.1016/0009-8981(87)90150-1

Segolodi, T. M., Henderson, F. L., Rose, C. E., Turner, K. T., Zeh, C., Fonjungo, P. N., . . . Paxton, L. A. (2014). Normal laboratory reference intervals among healthy adults

screened for a HIV pre-exposure prophylaxis clinical trial in Botswana. *PLOS ONE, 9*(4), e93034-e93034. doi:10.1371/journal.pone.0093034

Smit, F. C., Davison, G. M., Hoffmann, M., Erasmus, R. T., Davids, S., & Matsha, T. E. (2019). Full blood count and white cell differential count reference ranges obtained from a healthy urban South African population residing in the Western Cape of South Africa. *Int J Lab Hematol, 41*(5), 635-641. doi:10.1111/ijlh.13076

CHAPTER 8: DISCUSSION

8.1 Integrated summary

Establishing RIs for any population is a herculean task that requires attention to detail at every step of the testing cycle. The global study on reference intervals (RIs) adopted a harmonized protocol to enable comparison of results across participating countries by using a panel of sera to recalibrate and align reference values (RVs) (Ichihara, Ozarda, Barth, Klee, Qiu, et al., 2017; Ozarda et al., 2013). Despite all attempts at ensuring that we recruited healthy individuals, it is clear from the prevalence of metabolic syndrome (MetS) of 25.6% that a sizeable proportion had increased waist circumference, hypertension, dysglycaemia or dyslipidemia (Omuse, Maina, Hoffman, et al., 2017). People with MetS are at an increased risk for cardiovascular disease or type 2 diabetes and one would argue that they should have been secondarily excluded when determining our RIs. We reduced the influence of MetS and increased body mass index (BMI) on derived RIs by use of the latent abnormal value exclusion (LAVE) method as well as restricting recruitment to individuals with a BMI $\leq$ 35 kg/m^2. LAVE was effective in optimizing RIs for AST, ALT, GGT iron-markers and CRP by reducing the influence of latent anemia and metabolic diseases (Omuse et al., 2020). LAVE was also useful in reducing the influence of anaemia and inflammation when deriving RIs for complete blood count parameters and this was seen by the higher lower limits (LLs) and narrowing of the RIs for red blood cell (RBC) related parameters especially for females (Omuse et al., 2018).

Less than 1% of participants recruited in our study had reduced eGFR, a testimony to the relatively good health of the recruited individuals and successful effort in recruiting a sample representative of an ideal reference population. We found that eGFR determined using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (with or without correcting for race) better correlated with a prediction model that included risk factors for chronic kidney disease (CKD) and classified fewer asymptomatic healthy Kenyan adults as having a reduced eGFR (Omuse, Maina, Mwangi, et al., 2017). Correction for race further improved the correlation hence we recommend the use of the CKD-EPI equation in screening for CKD in asymptomatic adults in Kenya.

Our derived RIs for CBCs provide a suitable reference for the East African population that can be used to guide interpretation of respective laboratory tests given the paucity of well conducted RIs studies in the East African region. We have provided a comprehensive comparison with similar studies in Africa clearly highlighting the differences and possible explanations for the same. Our RIs for haematological parameters such as haemoglobin and haematocrit were higher than what has previously been reported from Kenya (Karita et al., 2009). We are cognizant that these differences could reflect recruitment of a much healthier population but at the same time could be partially explained by the difference in altitude between Nairobi and some of the other study sites. Ozarda et al describes regional variations in haematological parameters in Turkey attributable to altitude differences despite aefforts at standardizing all aspects of the testing cycle (Ozarda et al., 2017). This observation highlights the influence of the environment on RIs and may explain some of the geographical differences seen even in populations thought to be fairly homogeneous. Similar to other studies, we have demonstrated the need to adjust our RIs for neutrophil counts to reflect the inherent low counts in the African population (Omuse et al., 2018). The use of manufacturer provided RIs or those found in text books which are largely derived from Caucasian populations could result in sub-optimal patient management. Laboratories should at the very least verify these RIs as recommended by the Clinical Laboratory Standards Institute (CLSI, 2010).

Our derived RIs for some of the chemistry and immunoassay tests highlight the need for sex specific RIs for analytes which traditionally are reported without sex stratification. These include uric acid (UA), creatinine, total bilirubin (TBil), total cholesterol, alanine transaminase (ALT), aspartate transaminase (AST), creatinine kinase (CK), gamma-glutamyl transferase (GGT), transferrin (Tf), transferrin saturation (TfSat) and immunoglobulin-M (Omuse et al., 2020). Whether this proposed change in what has previously been accepted as a norm will be adopted by laboratories in Kenya and any other users of our RIs is a question that can only be answered over time. Indeed, a lot of effort will be required to explain and convince users on the need for sex stratification. Our TBil RI of 6-43 µmol/L for males and 5-27 µmol/L for females is almost double what has been reported in published studies from Asia and North America. This finding of increased TBil with normal transaminases has been described in other studies carried out in East Africa (Kibaya et al., 2008; Saathoff et al., 2008). This needs to be investigated further in

order to advise doctors and patients what this finding actually means and to what extent an asymptomatic adult with elevated TBil but normal transaminases needs to be investigated.

The differences seen between our RIs between and those from other countries suggests that similar differences may be observed with CDLs. Unfortunately, most countries adopting CDLs for diagnosis of pre-diabetes and diabetes do so without verifying their appropriateness for their respective populations. It is possible that this results in misclassification of individuals as diseased or not diseased as shown by Zemlin et al who evaluated the utility of the recommended HbA1c cut-off for diagnosing diabetes of 6.5% and found that 6.1% was the most appropriate cut-off in mixed ancestry South Africans (Zemlin, Matsha, Hassan, & Erasmus, 2011). Such differences emphasize the need for population specific CDLs or at the very least verification of what is found in guidelines before adoption. Zemlin et al carried out a study to derive and validate a HbA1c cut-off for diagnosing pre-diabetes (Zemlin, Matsha, Kengne, & Erasmus, 2015). A cut-off of 5.75% was determined to be most optimal which is very similar to the American Diabetes Association recommended cut-off of 5.7%. Similarly, the use of CDLs that are not population specific for lipid lowering targets may result in treatment dosages that are not optimal or inaccurate evaluation of cardiovascular risk when risk scores that incorporate lipid values are used. To determine our own CDLs for lipids, longitudinal studies are required where various cut-offs and their ability to predict cardiovascular risk are evaluated.

The use of various statistical approaches such as standard deviation ratios (SDRs) and bias ratios (BRs) presented challenges when deciding when to partition an RI. Each statistical method has its limitations and sometimes may create a dilemma when they give conflicting findings (Ichihara, 2014; Ichihara & Boyd, 2010). We used an SDR $\geq$0.4 to determine the need for partitioning of RIs by sex or age. SDR gives an indication of the extent to which a source of variation such as sex contributes to the overall (between-individual) imprecision seen in a data set but doesn't provide much information about data at the extremes which is crucial when determining lower and upper limits (ULs) of an RI. For this reason, we also adopted lower and upper limit bias ratios (BR_{LL} and BR_{UL}) with an absolute cut-off of 0.375 to further guide us on the need for partitioning data. BR represents between subgroup differences at the periphery (LL or UL) of the distributions. In order to avoid over partitioning, we further added a third criteria

based on the absolute bias in relation to the reporting units and published the first paper that proposes the use of a partitioning flowchart combining the three criteria (Omuse et al., 2020). We do propose the adoption of this scheme when evaluating the need for partitioning data based on sources of variation as it takes into consideration variation at the centre and periphery of a data set when comparing groups.

The extent to which BMI influences RIs and whether BMI specific RIs should be adopted is an ongoing debate. There is no doubt that there is an association between BMI and analytes related to MetS such as glucose, lipids, UA and transaminases based on an absolute partial correlation co-efficient (r_p)> 0.2 as shown in our multiple regression analysis. We did restrict recruitment to individuals with a BMI≤35 kg/m^2. However, other similar studies used lower BMI cut-offs such 32 kg/m^2 in Saudi Arabia (Borai et al., 2016) and 28 kg/m^2 in Turkey (Ozarda et al., 2014). The complexity that arises with incorporating BMI as a source of variation is that its effect on analytes varies across populations as demonstrated by Ichihara et al (Ichihara, Ozarda, Barth, Klee, Shimizu, et al., 2017). Furthermore, BMI can be influenced by so many variables including food intake and activity hence can change quite rapidly over time. It is therefore not practical to derive BMI specific RIs as this could further complicate the utility of RIs. Our study was also not powered to sufficiently to allow stratification by BMI.

Comparison of our data with data from black South Africans collected using the same study protocol including sample analysis done in the same laboratory revealed interesting findings some of which require further studies to determine their veracity. For instance, the hyponatremia seen in some of the South Africans is inexplicable and requires further verification. What is clear from the comparison is that having harmonized RIs will not be possible as the data reveals significant sex and age differences. We therefore recommend country specific RIs for Kenya and black South Africans. South Africa being a multi-racial country, there is need to analyze the effect of race and ethnicity on the derived RIs.

We used both parametric and non-parametric methods to derive the Kenyan RIs but adopted the parametric method which resulted in narrower 90% confidence intervals around the LL and UL as well as lower ULs for chemistry tests which can be attributed to removal of outliers (Omuse et

al., 2020). For CBC parameters, LAVE resulted in an increase in the LLs and narrowing of RIs (Omuse et al., 2018). However, the non-parametric method is simpler to use as it simply involves listing reference values from the lowest to the highest value and identifying the LL and UL as the values corresponding to the 2.5^{th} and 97.5^{th} percentiles. The use of LAVE significantly impacted on RIs for analytes associated with the presence of MetS, anaemia or inflammation with ULs for analytes such as ALT, AST, Tf, c-reactive protein and triglycerides reducing while LLs for iron and TfSat increasing (Omuse et al., 2020). The use of statistical approaches like LAVE to secondarily exclude reference individual's data based on out of range values for related analytes further helps in ensuring that RIs are reflective of a healthy population by reducing the likelihood of including individuals with sub-clinical or overt disease in a reference data set. We therefore advocate its use in derivation of RIs.

The totality of the presented work supports the need for population specific RIs. However, beyond sex and age, it is clear that other sources of variation such as BMI and possibly altitude need to be considered for specific analytes. The difficulty in identifying an ideal reference population is also emphasized given the prevalence of sub-clinical conditions that confer increased risk for disease. It is also impossible to rule out all possible disease conditions that may impact on an analyte. No inclusion or exclusion criteria can be perfect hence any RIs can only be extrapolated to populations that to a large extent are similar to the reference population from whom the RIs were derived.

8.2 Future directions

Our RIs need to be verified and their appropriateness for different populations across Kenya and the East African region needs to be determined. Nevertheless, given the rigour with which the study was conducted, the published studies will provide a very useful reference for laboratories in SSA that are looking for RIs for common haematology, chemistry and immunoassay tests. We will carry out transference studies to determine the transferability of our RIs to analyzers from other manufacturers given that Beckman analyzers for chemistry and immunoassay aren't very common in Kenya.

There is a need to verify widely adopted CDLs and determine how they relate to population specific RIs to find out whether population specific RIs for tests like fasting plasma glucose, uric acid and lipids may be used to derive population specific CDLs without necessarily carrying out a longitudinal study which would are expensive and require long term follow-up to collect outcome data.

We did not explore the impact of genetic diversity on RIs given the costs associated with carrying out genetic studies. Genetic variation might have contributed to some of the differences seen in RIs between Kenya and South Africa given that genetic diversity is thought to be more prevalent in the African continent compared to any other continent. Future studies should therefore explore the association between genetic diversity at an individual or population level and RIs as this would be more scientifically sound as opposed to comparisons based on race which is a poor surrogate for genetic diversity.

The utility of our flowchart in determining whether to partition RIs by sex and/or age needs to be verified using other datasets. We intend to do so when analyzing data from other countries that have participated in the global RI study and evaluate to what extent it impacts on previously derived RIs. The impact of adopting our RIs on reporting out of reportable range values in comparison to manufacturer recommended RIs needs to be evaluated. This can be assessed by monitoring the flagging rate of 'abnormal' values using either archived laboratory data or data collected prospectively.

Given the rigour, technical expertise, cost and time required to carry out a reference interval study, alternative ways of deriving RIs such as use of indirect methods need to be evaluated. Indirect methods rely on mining routine laboratory data that is readily available and this can be an alternative especially where time and financial resources are constrained as well as for deriving RIs for rare sample types like CSF and for populations such as children, expectant women and the elderly.

We do intend to engage the Ministry of Health in Kenya and present our RIs for adoption as a key reference for both public and private laboratories in Kenya and the East African region.

8.3 References

Borai, A., Ichihara, K., Al Masaud, A., Tamimi, W., Bahijri, S., Armbuster, D., . . . Abdelaal, M. (2016). Establishment of reference intervals of clinical chemistry analytes for the adult population in Saudi Arabia: A study conducted as a part of the IFCC global study on reference values. *Clinical Chemistry and Laboratory Medicine, 54*(5), 843-855. doi:10.1515/cclm-2015-0490

CLSI. (2010). *EP28-A3C: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline* (Third ed.): Clinical Laboratory Standards Institute.

Ichihara, K. (2014). Statistical considerations for harmonization of the global multicenter study on reference values. *Clinica Chimica Acta, 432*, 108-118. doi:10.1016/j.cca.2014.01.025

Ichihara, K., & Boyd, J. C. (2010). An appraisal of statistical procedures used in derivation of reference intervals. *Clinical Chemistry and Laboratory Medicine, 48*(11), 1537-1551. doi:10.1515/CCLM.2010.319

Ichihara, K., Ozarda, Y., Barth, J. H., Klee, G., Qiu, L., Erasmus, R., . . . Yadav, B. (2017). A global multicenter study on reference values: 1. Assessment of methods for derivation and comparison of reference intervals. *Clinica Chimica Acta, 467*, 70-82. doi:10.1016/J.CCA.2016.09.016

Ichihara, K., Ozarda, Y., Barth, J. H., Klee, G., Shimizu, Y., Xia, L., . . . Takahashi, A. (2017). A global multicenter study on reference values: 2. Exploration of sources of variation across the countries. *Clinica Chimica Acta, 467*, 83-97. doi:10.1016/j.cca.2016.09.015

Karita, E., Ketter, N., Price, M. A., Kayitenkore, K., Kaleebu, P., Nanvubya, A., . . . Kamali, A. (2009). CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. *PLOS ONE, 4*(2), e4401. doi:10.1371/journal.pone.0004401

Kibaya, R. S., Bautista, C. T., Sawe, F. K., Shaffer, D. N., Sateren, W. B., Scott, P. T., . . . de Souza, M. S. (2008). Reference ranges for the clinical laboratory derived from a rural population in Kericho, Kenya. *PLOS ONE, 3*(10), e3327-e3327. doi:10.1371/journal.pone.0003327

Omuse, G., Ichihara, K., Maina, D., Hoffman, M., Kagotho, E., Kanyua, A., . . . Erasmus, R. (2020). Determination of reference intervals for common chemistry and immunoassay tests for Kenyan adults based on an internationally harmonized protocol and up-to-date statistical methods. *PLOS ONE, 15*(7), e0235234. doi:10.1371/journal.pone.0235234

Omuse, G., Maina, D., Hoffman, M., Mwangi, J., Wambua, C., Kagotho, E., . . . Erasmus, R. (2017). Metabolic syndrome and its predictors in an urban population in Kenya: A cross sectional study. *BMC Endocrine Disorders, 17*(1). doi:10.1186/s12902-017-0188-0

Omuse, G., Maina, D., Mwangi, J., Wambua, C., Kanyua, A., Kagotho, E., . . . Erasmus, R. (2017). Comparison of equations for estimating glomerular filtration rate in screening for chronic kidney disease in asymptomatic black Africans: a cross sectional study. *BMC Nephrology, 18*(1), 369-369. doi:10.1186/s12882-017-0788-y

Omuse, G., Maina, D., Mwangi, J., Wambua, C., Radia, K., Kanyua, A., . . . Erasmus, R. (2018). Complete blood count reference intervals from a healthy adult urban population in Kenya. *PLOS ONE,* 1-19.

Ozarda, Y., Ichihara, K., Aslan, D., Aybek, H., Ari, Z., Taneli, F., . . . Degirmen, E. (2014). A multicenter nationwide reference intervals study for common biochemical analytes in Turkey using Abbott analyzers. *Clinical Chemistry and Laboratory Medicine (CCLM), 52*(12). doi:10.1515/cclm-2014-0228

Ozarda, Y., Ichihara, K., Bakan, E., Polat, H., Ozturk, N., Baygutalp, N. K., . . . Eker, P. (2017). A nationwide multicentre study in Turkey for establishing reference intervals of haematological parameters with novel use of a panel of whole blood. *Biochemia Medica, 27*(2), 350-377. doi:10.11613/BM.2017.038

Ozarda, Y., Ichihara, K., Barth, J. H., & Klee, G. (2013). Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. *Clinical Chemistry and Laboratory Medicine (CCLM), 51*(5), 1027-1040. doi:10.1515/cclm-2013-0249

Saathoff, E., Schneider, P., Kleinfeldt, V., Geis, S., Haule, D., Maboko, L., . . . Hoelscher, M. (2008). Laboratory reference values for healthy adults from southern Tanzania. *Tropical Medicine & International Health, 13*(5), 612-625. doi:10.1111/j.1365-3156.2008.02047.x

Zemlin, A. E., Matsha, T. E., Hassan, M. S., & Erasmus, R. T. (2011). HbA1c of 6.5% to diagnose diabetes mellitus -- does it work for us? -- the Bellville South Africa study. *PLOS ONE, 6*(8), e22558. doi:10.1371/journal.pone.0022558

Zemlin, A. E., Matsha, T. E., Kengne, A. P., & Erasmus, R. T. (2015). Derivation and validation of an HbA1c optimal cutoff for diagnosing prediabetes in a South African mixed ancestry population. *Clinica Chimica Acta, 448*, 215-219. doi:10.1016/j.cca.2015.06.019

APPENDICES

Appendix 1: Reference Interval Study Approval-Kenya

THE AGA KHAN UNIVERSITY

Faculty of Health Sciences
Medical College

Ref: 2014/REC-46
22nd August 2014

Dr. Geoffrey Omuse
Principal Investigator and faculty member,
Aga Khan University-EA, Nairobi

Dear Dr. Omuse and team,

Re: ESTABLISHING ADULT REFERENCE INTERVALS FOR SELECTED ANALYSIS FROM SOUTH AFRICA, NIGERIA AND KENYA

The Aga Khan University, Nairobi Health Research Ethics Committee (REC)in a meeting held on 11th August 2014 reviewed your proposal, as submitted to the Research Support Unit (RSU) on 25th July 2014. The committee records that this is a well-articulated protocol. This proposal is also in compliance with the Aga Khan University Research Ethics Regulations

The committee has granted *conditional approval* for this project based on core ethical standards which have been fully instituted in the protocol. Prior to commencing the study, you will be expected to obtain approval from the Ministry of Health allowing transportation of samples to South Africa and a Research Permit from the National Commission for Science, Technology and Innovation (*NACOSTI).* Copies of the same should be filed with the RSU including the Material Transfer Agreement (MTA). Consequently, you are authorized to conduct this study from **15th August 2014**. This approval is valid until **14th August 2015.**

The study should be conducted in full accordance with all the applicable sections of the R&EC guidelines and you should notify the R&EC immediately of any changes that may affect your research project. You should report any unanticipated problems involving risks to the participants to the R&EC. You must provide an interim report before expiration of the validity of this approval and requested extension if additional time is required for study completion. As the principal investigator you must advise the R&EC when this study is finished or discontinued and a final report submitted to the RSU. Further approval from the hospital administration should be sought before publishing the results. If you have any questions, please contact Research Support Unit - kamanda.ciru@aku.edu or 020-366 2148.

Sincerely,

Dr. Amyn Lakhani
Chair, Health Research Ethics Committee, AKU (N)

Appendix 2: University Research Council grant award letter

آغا خان یونیورسٹی
THE AGA KHAN UNIVERSITY

February 6, 2015

Dr. Geoffrey Omuse
Instructor
Department of Pathology
Aga Khan University
Nairobi

URC Project ID: **142008EA**
Approved amount: KSh. 7,18,050

Project Title: **Establishing adult reference intervals for selected analytes from South Africa, Nigeria and Kenya**

Dear Dr. Omuse,

I am pleased to inform you that the University Research Council (URC) upon the recommendations of the Grant Review Committee (GRC) has approved funding of your proposal with some minor revisions and modifications to the budget (attached). This approval is subject to incorporation of the recommendations from the reviewers of your proposal and the Grant Review Committee.

Please contact Research Office for any information/clarification on financial and other matters related to the grant.

The URC congratulates you on the award of this grant, and looks forward to receive regular feedback from you on the progress of your research and on learning of its conclusion.

Yours sincerely,

Dr. El-Nasir Lalani
Chair
University Research Council

cc: Research Office – For record
Encl: Reviewers' and GRC comments

Appendix 3: Permission to ship biological samples

MINISTRY OF HEALTH
OFFICE OF DIRECTOR OF MEDICAL SERVICES

Telegrams:"MINHEALTH". Nairobi
Telephone; Nairobi 2717077 Fax: 2715239

OFFICE OF DIRECTOR OF
MEDICAL SERVICES
AFYA HOUSE
CATHEDRAL ROAD
P.O. BOX 30016
NAIROBI

MOH/ADM/1/1/81 VOL.1

27th October, 2014

Dr. Geoffrey Amuka Omuse
Clinical Pathologist and Senior Instructor
Department of Pathology
The Aga Khan University
P.O. Box 30270-00100
NAIROBI

Dear Dr. Omuse,

RE: AUTHORITY TO SHIP BIOLOGICAL SAMPLES

Your request for specimen export permit dated 20th October, 2014 refers.

The title of your study is noted to be "***Establishing adult reference intervals for selected analysis from south Africa for analysis as part of a study".***

Authority is hereby granted for shipment of biological samples related to this research.

- 2500 cryovials (5 aliquots of plasma/serum in 2ml cryvials for a maximum of 500 participants).

The shipment contact details are as follows:

Pathcare Reference Lab
Neels Bothma Street
N1 City – Goodwood
Pathcare park
Cape Town N/A 7480
South Africa
Te: 27215963830

DR. Charles K. Kandie
FOR: DIRECTOR OF MEDICAL SERVICES

Appendix 4: Material Transfer Agreement

Material Transfer Agreement Form

AGREEMENT FOR THE TRANSFER OF MATERIAL AND/OR RELATED INFORMATION

THIS MEMORANDUM OF AGREEMENT IS MADE THIS 24th, day of Septemebr

2015 between the Aga Khan University Hospital Nairobi (hereinafter referred to as

'AKUHN') of the one part and Stellenbosch University acting through its Department of Pathology (hereinafter referred to as 'the Receiving Party'), of the other part.

WHEREAS AKUHN desires to provide the material and/or related information detailed hereunder on the terms and conditions hereinafter set forth, and

WHEREAS the Receiving Party is ready and willing to accept the material and/or related information on the said terms and conditions,

NOW THEREFORE the Parties hereby agree as follows:

1. ***Scope of Agreement***

(a) This Agreement covers the following biological material, related information and/or activities as these may be supplemented by any annex to this Agreement that is duly signed by the Parties hereto:

i) Brief description of the material, name, amount, type,etc:

A maximum of 2500 cryovials of approximately 2 mls each of human plasma and/or serum obtained from 400-500 volunteers (5 cryovials from each participant)

ii) Intended use(s) of material

To test the following analytes for each study participant as spelt out in the approved study protocol:

Lipids: Triglyceride, Total Cholesterol HDL-cholesterol and LDL-cholesterol
Electrolyte: Sodium, potassium, chloride, calcium, inorganic phosphate, iron and magnesium
Enzymes: AST, ALT, ALP, LD, GGT, CK, amylase and lipase
Miscellaneous: Glucose, Total Protein, Albumin, Creatinine, Urea, Uric Acid,
Immunoturbidimetry/immunoassays: CRP, IGG, IgA, IgM, Transferrin, ferritin, TSH, T3, T4 and thyroid auto-antibodies, Troponin T and I,

(b) Any activities involving the material and/or related information that are not expressly authorised by the provisions of this Agreement and any annexes hereto shall be considered as expressly prohibited. This shall be understood so as to include, but not be limited to, any activities involving transfer to third parties,

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activities aimed at commercialisation or the claiming of rights of any kind over material and/or related information not specifically addressed by this Agreement.

2. ***Maintenance of Ownership and Rights by*** **AKUHN**

(a) AKUHN maintains ownership and all rights to the material covered by this Agreement, understood so as to include ownership and rights to any unmodified derivatives thereof and any material contained or incorporated in modifications. Recipient maintians ownership of all information developed as a direct result of the provision of material and/or related information. AKUHN shall have a perpetual and royalty free right to use the information developed for academic research and teaching purposes.

(b) The terms and conditions of this Article shall be subject to any express written agreement to the contrary that shall be attached as an annex hereto.

(c) The terms and conditions of this Article shall be subject to any third party ownership or possession of rights to the material and/or related information that is the subject of this Agreement. Where such third party rights exist, they shall be detailed in the annex to this Agreement along with evidence of AKUHNs legal authority to execute this Agreement.

(d) The material is of an experimental nature and is provided without warranty of fitness for any purpose, or any other warranty or representation, whether express or implied.

3. ***Rights and Obligations***

(a) The rights and obligations of the Parties are strictly limited to the terms and conditions of the Agreement. Accordingly, the Parties shall not be entitled to any benefit, payment, subsidy, compensation or entitlement except as expressly provided in this Agreement.

(b) The Receiving Party shall be solely liable for claims by third parties arising from the Receiving Party's own willful or negligent acts or omissions in the course of performing this Agreement, and under no circumstances shall AKUHN be held responsible for any such claims by third parties.

4. ***Consideration***

(a) The Receiving Party's use of the material and/or related information for the purposes stipulated in this Agreement shall constitute consideration provided by AKUHN.

(b) The Receiving Party shall be considered to have provided adequate consideration by either of the following actions, unless expressly stated to the contrary in this Agreement or any annexes hereto:

(i) Providing AKUHN with rights to or rights of access to the results of any research involving the material and/or related information undertaken by the Receiving Party, subject to Article 1 and any annexes hereto; or,

(ii) AKUHN recognises the desire of the Recipient to publish details of academic research in scientific journals or theses and AKUHN agrees that the Recipient will be free to publish results , providing that AKHUN is provided with a copy of any such manuscript or abstract at least thirty (30) days prior to submitting such publication to the scientific journal or to examiners, to give AKHUN the opportunity of requesting the removal of any proprietary confidential information pertaining to the material. Recipient must comply with AKHUN's reasonable removal requirement to the reasonable satisfaction of AKHUN prior to submitting such publication.

5. ***The AKUHN Intellectual Property Policy***

(a) Except as may be explicitly provided for to the contrary, in this Agreement or any annexes hereto, this Agreement shall be subject to the terms and conditions as spelt out in the Aga Khan University Policy on Intellectual Property Rights.

(b) The Parties hereby certify that they have read and understood the provisions of the Aga Khan University Policy on Intellectual Property Rights.

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6. Duration

(a) Where relating to material, this Agreement shall remain in force until the said biological material, and any derivatives and/or related information thereof, is returned to the satisfaction of AKUHN.

(b) Where relating to information related to biological material, this Agreement shall be subject to any associated rights, such as copyright or trade secrets, which might be attached thereto.

(c) This Agreement may be replaced at any time by a subsequent agreement between the Parties.

7. Amendment or Variation of Agreement

Notwithstanding anything to the contrary contained in this Agreement, or any annexes hereto, this Agreement may be amended or varied to the extent mutually agreed by and between the parties hereto. Such agreement shall be stated expressly in writing and attached as an annex hereto.

8. Termination of Agreement

(a) This Agreement may be terminated by either Party at any time subject to the terms and conditions of Article 6 herein.

(b) In the event of the termination of this Agreement by either Party, such Party shall notify the other Party in writing, including details for such termination as are required to fulfill the terms and conditions of Article 6 herein.

9. Dispute Resolution

(a) The Parties agree to make good faith attempts to negotiate the resolution of disputes arising pursuant to this Agreement.

(b) In the event of any dispute arising between the Parties in relation to the terms of this Agreement, the Parties shall use their best endeavours to resolve the matter on an amicable basis. This Agreement shall be governed by and shall be construed in accordance with the laws of Kenya without regard to any conflicts of laws provisions. The parties consent to the appropriate court of competent jurisdiction for the resolution of all disputes or controversies between the parties hereto that parties are unable to settle amicably

IN WITNESS WHEREOF, the Parties thereto have executed this Agreement.

Name: Robert W. Armstrong MD PhD FRCPC (Signature)
Date: 04/09/15

Position in Organization: Foundation Dean, AKUHN
Address of Organization: Aga Khan University Hospital, Nairobi
3rd Parklands Avenue, Off Limuru Road, P.O. Box 30270,GPO 00100 Nairobi Kenya

Name of key scientist: Dr. Geoffrey Omuse
Study Ref:2014/REC-46
Tel: +254 724932640
Email: geoffrey.omuse geoffrey.omuse@aku.edu

(Signature)
Address: Department of Pathology, Aga Khan University Hospital, Nairobi
3rd Parklands Avenue, Off Limuru Road, P.O. Box 30270,GPO 00100 Nairobi Kenya

For and on behalf of the Recipient Organization (Signature)
Name: Prof NC Gey Van Pittius
Date: 30/09/2015

Organization: Stellenbsoch University

Position in Organization: Deputy Dean Research:
Faculty of Medicine & Health Sciences
Address of Organization: RW Wilcocks Building, room 2037, Victoria street, Stellenbosch, 7602, South Africa
Recipient Scientist: Dr M Hoffmann
Tel: 021 938 4165
E-mail: mariza@sun.ac.za
M.Hoffmann
(Signature)
Address: Department of Chemical Pathology,
Faculty of Medicine & Health Sciences, Francl Van Zijl drive, Parow, 7505 ,South Africa
Date: 17/09/2015

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Appendix 5: Reference Interval Study Approval-South Africa

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Ethics Letter

23-Sep-2013

Ethics Reference #: S12/05/147
Title: ESTABLISHING ADULT REFERENCE INTERVALS FOR SELECTED ANALYTES FROM SOUTH AFRICA, NIGERIA AND KENYA

Dear Professor Rajiv Erasmus,

At a meeting of the Health Research Ethics Committee that was held on 18 September 2013, the progress report for the abovementioned project has been approved and the study has been granted an extension for a period of one year from this date.

Please remember to submit progress reports in good time for annual renewal in the standard HREC format.

Approval Date: 18 September 2013 Expiry Date: 18 September 2014

If you have any queries or need further help, please contact the REC Office 0219389207.

Sincerely,

pp.
REC Coordinator
Mertrude Davids
Health Research Ethics Committee 2

Appendix 6: Informed consent

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Project Name:

ESTABLISHING ADULT REFERENCE VALUES FOR SELECTED ANALYTES IN KENYA NIGERIA AND SOUTH AFRICA

Project leaders: Dr Geoffrey Omuse/Dr Daniel Maina

Address: Department of Pathology, Aga Khan University Hospital, 3rd Parklands Ave, Nairobi, Kenya

Contact numbers: Dr Geoffrey Omuse +254724932640 **OR** +254-20-3662226

Dr Daniel Maina +254720927338 **OR** +254-20-3662226

You are being invited to take part in a research project that is taking place in 18 countries globally which includes 3 from Africa. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you initially agree to take part. This study has been approved by the Scientific and Ethical committees of Aga Khan University Hospital Nairobi and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

The investigators are in the process of reviewing "normal values" for selected blood tests. Doctors will use these normal values to compare against values of patients in order to asses the health of an individual e.g. comparing the blood sugar level of a diabetic patient with that of normal individuals. You will be asked about your health status and your activity levels. It is **extremely important** that you answer these questions honestly and to the best of your ability. This information will not be shared with anyone outside of this study, but it is very important for us to know so that we can correctly determine what is normal for our population.

All Kenyan born healthy individuals of African Ethnic origin will be considered for the study. Individuals with the following conditions will not be included in the study:

- Under regular medications for illnesses (e.g. high blood sugar, high blood pressure, allergies and depression)
- Pregnant females or within 1 year after child birth
- Alcohol: more than 5 drinks a day OR 1400ml beer OR 580ml wine OR 175ml whiskey/brandy etc
- Individuals who had an operation or had to be admitted to hospital for an illness in the past 2 weeks
- Smoking more than 20 cigarettes per day
- BMI > 35 kg/m^2 (very obese)

Why have you been invited to participate?

We are interested in enrolling "healthy individuals" and we expect that all your test results will be "normal". Therefore we will be able to use your test results to calculate "normal values".

What will your responsibilities be?

In order for this project to be a success, healthy individuals are expected to complete a questionnaire and allow for height, weight and blood pressure measurement. A small amount of blood will be collected from selected individuals and this blood will be analysed in Pathcare laboratories in South Africa and Kenya. We are only interested in routine tests including tests for normal functioning of liver, kidney and thyroid.

Will you benefit from taking part in this research?

Though it will take a few weeks to get results out, we will get these results to you via email. If results are severely abnormal, a referral letter will be written to your local clinic or general practitioner. There will be no direct financial benefits to individuals involved in the study. The establishment of normal values for our local population will help doctors to better diagnose and treat patients.

Are there any risks involved in your taking part in this research?

Apart from slight discomfort at the site of where blood is drawn, there are no risks involved.

If you do not agree to take part, what alternatives do you have?

Your participation in this study is completely voluntary and you can withdraw at any stage of the study. Your participation will not influence any treatment you are currently on. You can withdraw from the study at any stage, in which event your stored samples will be discarded.

Who will have access to your data?

Your information will not be made public. Only the principal and co-investigators in the study will have access to your result. In addition, once we have drawn your blood, your samples and information will be identified with a number only. If the results of the study are published, your identity will not be made known.

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled:

ESTABLISHING ADULT REFERENCE VALUES FOR SELECTED ANALYTES

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurized to take part.
- I may choose to leave the study at any time and will not be penalized or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) .....…..…….. on (*date*) ……...……..

Signature of participant……

Signature of witness……

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a translator. (*If a translator is used then the translator must sign the declaration below.*

Signed at (*place*) .....…..…….. on (*date*) ……...…….. .

Signature of investigator……

Appendix 7: Questionnaire

INTERNATIONAL REFERENCE RANGE STUDY QUESTIONAIRRE

Study number (Ichihara Barcode) _____ Laboratory number (Pathcare Barcode) _____

Date of sampling _____ Year/month/Day Time of sampling _____ hour : minutes

CONTACT DETAILS OF PARTICIPANT

Name and surname:
ID number: Date of Birth:
Postal address:

Contact numbers: Cell: Home/Office: Fax:

E-mail address:

INCLUSION CRITERIA

1. Do you consider yourself healthy? NO YES
2. Are you at least 20 years of age? NO YES

EXCLUSION CRITERIA

1. Is your BMI > 35 kg/m2? NO YES
2. Do you drink > 5 glasses of wine/5 beers/5 shots per day? NO YES
3. Do you smoke > 20 cigarettes/day? NO YES
4. Do you have/had chronic disease (including diabetes, hypertension, depression etc) requiring regular medication? NO YES
5. Do you have results from a blood test that point to a severe disease? NO YES
6. Have you been hospitalized or seriously ill in the past 2 weeks? NO YES
7. Have you given blood as a donor in the previous 3 months? NO YES
8. Are you a known carrier of Hepatitis B/C or HIV? NO YES
9. Are you pregnant or within one year after childbirth? NO YES
10. Have you participated in a research study involving an investigational drug in the past 12 weeks? NO YES

DEMOGRAPHICS

Are you fasting ? NO YES
Last food intake: Time since last meal _____ (AM / PM) (hours)

Age: _____ years

Gender: Male Female

Ethnicity: Caucasian African Mixed Other (specify)

Occupation: _____

Measurements: Height _____ cm BMI
Weight _____ kg Waist circumference _____ cm

Blood pressure: _____ mmHg

ABO blood type: A+ B+ AB+ O+ not known
A- B- AB- O-

PERSONAL HABITS

Do you smoke? NO YES

If yes, how many per day for how many years? nr/day total nr of years

Do you drink alcohol? NO YES

If yes, how often? Rarely 1x/week 2 - 3x/w 4 - x/w every day

If yes, how much do you drink in the typical week.

Beer litres
Cider litres
Wine glasses (6 glasses/bottle)
Spirits bottles

✻Calculation by the investigator:
Amount of alcohol consumption in gram of ethanol per day and week
grams per day
grams per week

PHYSICAL ACTIVITIES AND EXERCISE

Daily activities

Time spent standing/walking hours/day
Time spent sitting hours/day
Time spent sleeping hours/day
Time spent outdoors hours/day

Physical activity at work

Does your work involve vigorous activity? NO YES
If yes, how much time do you spend on the activity (e.g. digging)? hours min/typical day

Does your work involve moderate intensity activity (e.g. walking)? NO YES
If yes, how much time do you spend on this activity? hours min/typical day

Physical activity (sports, fitness, recreation) on a regular basis (at least once per week for > 1 year)

Do you do any vigorous exercise regularly (e.g. running, aerobics)? NO YES
If yes, what type?
If yes, frequency and duration days/wk hrs + min on a typical day

Do you do any moderate-intensity exercise regularly (e.g. brisk walking)? NO YES
If yes, what type?
If yes, frequency and duration days/wk hrs + min on a typical day

MEAL PATTERN (frequency and amount per week)

Red Meat	None	1 - 2 x/W	3-4x/W	5-6 x/W	every day
Fish	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day
Vegetables	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day
Pulses (Beans, Peas, Lentils)	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day
Milk/diary	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day
Fruit	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day
Sweets	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day
Fried/sauteed	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day
Salty foods	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day
Rice	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day
Ugali (maize flour)	None	1 - 2 x/W	3-4 x/W	5-6 x/W	every day

CURRENT HEALTH STATUS

Have you suffered from any of the following infections which required treatment in the past year?

Bacterial infection (e.g. pharingitis, pneumonia)	No	once/y	twice/y	three/more
Viral infection (e.g. influenza, viral gastroenteritis)	No	once/y	twice/y	three/more
Parasite infection	No	treated <2yrs	currently treated	untreated

Do you have air pollution in your living environment?

Polution	None	Mild	Moderate	Fairly	Heavy
Source	Car	Factory	Both	Other_____	
Time spent outside in air polution	hrs/day				

Are you taking any nutritional supplements regularly? NO YES
If yes, specify type

Are you taking any medication regularly? ☐ NO ☐ YES

If yes, specify ALL DRUGS

Have you had any of the following allergic disorders in recent years?

☐ Allergic rhinitis ☐ Bronchial asthma
☐ Food allergy ☐ Other (Specify):_____
☐ Atopic dermatitis ☐ I have had no allergic disorder

Have you had chest pain in the past year for which you sought medical attention or was hospitalized?

☐ NO ☐ YES

VITAMIN D STATUS

Have you eaten oily fish (e.g. tuna, sardines, snoek, mackerel, salmon, including canned, fresh or smoked) in the last week?

☐ NO ☐ YES

If yes, how many times? ☐ times/week

On an average day, how much time do you spend outdoors between 10 am and 4 pm?

☐ < 15 min ☐ 15 min ☐ 30min ☐ 1 hr ☐ 2 hrs
☐ 3 hrs ☐ 4 hrs ☐ 5 hrs ☐ 6 hrs

When you are outdoors, what parts of your body are exposed and NOT covered?

☐ Face ☐ Arms ☐ Hands ☐ Legs ☐ Torso

WOMEN (If you are post menopausal or have had a hysterectomy you can skip this section)

When was the first day of your last period? ☐ yyyy/mm/dd

How is your menstrual cycle? ☐ regular ☐ irregular ☐ menopause
☐ under hormone therapy or taking contraceptive pills

If "regular", what is the average length of the cycle? ☐ days

Do you suffer from hypermenorrhoea (heavy/lasting > 7 days) or hypomenorrhoea (extremely light)?

☐ I suffer from NONE of the above ☐ hypermenorrhoea ☐ hypomenorrhoea

FOR MEN ONLY

Have you had any urinary problems in the past 3 months (frequent urge to urinate, burning or painful urination or difficulty urinating)?

☐ NO ☐ YES

THANK YOU FOR YOUR COOPERATION. YOUR INFORMATION WILL BE USED SOLELY FOR THIS STUDY