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Laboratory Reference Intervals in Africa

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1. Introduction

Over the last decade, there has been a significant increase in the number of clinical trials taking place in sub-Saharan Africa in a concerted effort to identify safe and effective prevention and treatment strategies to combat the heavy burden of infectious diseases in this region [1-3]. This is because numerous viral, parasitic and bacterial diseases are endemic in this region, including: 66% of the global HIV/AIDS infections, 31% of tuberculosis infections, and 86% of malaria cases [3, 4]. Routine capacity for clinical laboratory testing is also increasing in Africa. Clinical trials and clinical care in sub-Saharan Africa require accurate laboratory reference intervals for appropriate assessment of patients/participants, monitoring disease progression, and reporting of possible toxicity and adverse events.

This is particularly important in phase I and II clinical trials. Phase I trials often enroll a small group of healthy participants in order to determine the metabolic and pharmacologic actions of drugs, side effects associated with increasing doses and early evidence of efficacy. Phase II trials on the other hand are controlled clinical studies conducted to evaluate efficacy of drug/vaccine for a particular indication in a larger group of participants and to further evaluate its safety. Many HIV vaccine trials are slated for Phase I–III trials in Africa. The inception of the US President's Emergency Plan for AIDS Relief in 2004, with a mandate to treat 2 million HIV infections with anti-retroviral therapy by 2008 has accelerated the implementation of lymphocyte immunophenotyping in urban and rural areas in Africa as initiation of therapy is often predicated on absolute CD4 T- lymphocyte counts. Central to any HIV vaccine and/or care and treatment program is the capability to measure absolute CD4 counts. CD4 counts are important in the context of breakthrough infections during HIV vaccine trials and informing treatment. Correct diagnosis in patient management often involves accurate interpretation of results from laboratory testing [5]. Hence it is critical for medical professionals to have access to an accurate management resource such as reference intervals.

Historically, clinical studies as well as routine clinical patient management in most African countries have relied on European-generated automated instrument values, US established reference intervals or the U.S. NIH division of AIDS (DAIDS) toxicity grading tables in assessing clinical parameters in study participants. The US-established reference intervals are obtained from the Massachusetts General Hospital reference values and serve as the standard reference interval comparison for most studies [6]. The DAIDS toxicity tables, also derived from a Caucasian population, are used for grading the severity of adult and pediatric adverse events, whether or not they are considered to be related to the study intervention [7]. DAIDS provides guidelines for estimating severity of adverse events using specific reference intervals (Table 1) as criteria for determining what is 'normal' and among abnormal values, how to grade the severity of the abnormality.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
HEMOGLOBIN	10.0 – 10.9 g/dL	9.0 – 9.9 g/dL	7.0 – 8.9 g/dL	< 7.0 g/dL
NEUTROPHILS	1.0 – 1.3 x 10^9 cells/L	0.75 – 0.999 x 10^9 cells/L	0.5 – 0.749 x 10^9 cells/L	< 0.5 x 10^9 cells/L

Adult and pediatric values for age >57 days, HIV-negative from the DAIDS toxicity tables version 1.0, December 2004; clarification August 2009.

Table 1. Examples of DAIDS criteria of estimating severity grading based on laboratory parameters.

Reference values, in general, refer to the value or test result obtained by the observation or measurement of a particular type of quantity on an adequate number of persons (reference sample group) selected to represent the general population. Reference values are usually presented as reference intervals which refer to the interval between, and including two reference limits i.e., from the lower reference limit to the upper reference limit defined by a specific percentage (usually 95%). In certain parameters such as absolute counts of monocytes, eosinophils and basophils, only one reference limit (decision limit), more often the upper reference limit is of biological significance hence the lower reference limit assumes a value of zero.

Reference values go hand in hand with toxicity grading or decision limits, which can be defined as specific levels of the analyte that correspond to mild to life threatening clinical situations. Toxicity grading is particularly useful in the decision-making process of interpreting a measured value and assessing the health status of the subject being tested. For this reason reference values or toxicity grading are routinely used in clinical trials at enrollment to determine eligibility, establish baseline measures, and also during the course of the trial to monitor the participants' health. Moreover, several analytes are used either as markers for the possible presence of a disease or as direct evidence for that disease. Reference values, especially hematological and immunologic indices, are influenced by such factors as genetics, dietary patterns, pregnancy, gender, age, ethnic origin and prior exposure to environmental pathogens. Thus, it is important to consider these factors when

applying reference intervals in diagnostics as well as in recruitment in clinical trials. According to the Clinical Laboratory and Standards Institute (CLSI) guidelines [8], it is recommended that laboratories establish their own reference intervals from the local population or validate the use of those obtained from a different setting. Despite this, clinicians and researchers in Africa have continued to use reference values of European or North American populations. Our group in Kenya has recently published reference intervals based on the CLSI guidelines and are currently assisting regional laboratories to establish their own reference intervals[9].

In this chapter, we give a brief background on the current status of participant recruitment in clinical trials and patient management in Africa. We will also describe how to select a reference population from which to derive the reference sample group. In addition, we describe various studies advocating the establishment of reference intervals performed in different regions of the African continent including our own. These studies show differences in hematological, biochemical and immunologic parameters between various African populations but these differences are statistically insignificant. However, most hematological, biochemical and immunologic parameters considered in the African studies are significantly different when compared to American and European derived values. This chapter will also discuss the proposed partitioning of adolescent males from adults given their increased recruitment into clinical trials. Adult males have significantly higher values for most hematological and biochemical parameters and we provide an explanation why this is so. While pregnant women and infants undergo physiological processes that alter their hematological and biochemical parameters, the partitioning of these cohorts has been slow. We discuss how pregnancy induces these changes and describe the particular parameters affected. We also highlight the dynamic changes in these parameters during infancy and how they differ from western-derived values. In this chapter, we also illustrate the downside of using inappropriate reference intervals in the recruitment of participants in clinical trials and patient management. We show how the use of such values results in exclusion of clinically healthy participants from clinical trials and may lead to inappropriate reporting of adverse events during the course of these studies. This potentially results in escalation of costs in the conduct of clinical trials. In this book chapter, we also propose the development of laboratory-derived African toxicity grades that, in addition to the already developed reference values, would be used for reporting adverse events in clinical trials and for determining critical values in routine health care.

2. Use of reference intervals, consequence of misclassification and selection of a reference population

2.1. The use of reference intervals

Reference intervals are useful both in the clinical and research environment. Medical laboratory reference intervals are primarily used for clinical purposes. They can be used as an indicator of good health. Alternatively, reference intervals/limits can be used to screen for physiological or pathological conditions hence important in routine health assessment,

particularly for screening of anemia, blood disorders and diseases of the immune system. Reference intervals are important for accurate interpretation of laboratory data and provide assistance to the clinician in creating a more comprehensive clinical perspective for diagnosis and management of patients [10]. Of particular importance is the use of reference values as surrogate markers for monitoring disease progression and response to antiretroviral therapy in HIV-infected individuals [11]. For example, decisions to initiate, continue, or change antiretroviral therapy regimens are determined using CD4+ T-lymphocyte cell (CD4) counts, while drug toxicity is monitored using liver function tests, renal function tests, and full blood counts (FBC) [12, 13]. The hemoglobin concentration is used as a marker of anemia. As part of the management of anemia, the clinician conducts additional tests to identify a reversible etiology for anemia (eg, iron deficiency, infection) and if present treats it appropriately. However, in the clinical environment, the statistical definition of reference intervals may not allow certain clinical uses. Because these reference intervals have been derived statistically from a healthy population, they may not be used to rule in or rule out specific medical conditions. The statistically derived 95% reference interval would mean that 5% of normal subjects would have abnormal laboratory values. This is erroneously interpreted that 95% of diseased individuals would fall outside the derived reference interval. It is recommended that the number of diseased individuals who fall outside the defined 95% reference intervals be determined through a study of the distribution of such persons with the target condition [14]. Thus, it is necessary to confirm the validity of the proposed reference intervals with clinicians using a particular test to manage patients.

In the research environment, however, the aim is to define a reference population that is as similar as possible to that for which a particular test will be applied with the exception of the presence of the disease. During clinical trials, reference intervals relevant to the study of interest are required to interpret normal values of standard laboratory test results from the target population [15]. This is particularly important during phase I/II safety trials where healthy individuals are assessed without a control group [15-17]. Moreover, clinical reference intervals are necessary in order to accurately assess potential adverse events observed during the course of clinical trials.

2.2. Consequences of misclassification

A majority of clinically healthy participants have been excluded from several clinical trials in Africa because laboratory hematological and biochemical parameters are classified as abnormal [18-20]. Unnecessary exclusion of potential participants generally results in increased cost for study recruitment to achieve the target sample size. Accurate reference intervals are required for monitoring adverse events during vaccine and drug trials to limit misclassification that might otherwise lead to discontinuation of such trials or erroneous conclusions that the trial interventions are associated with adverse events. A study documented that the expense of adverse event investigation and reporting accounted for at least one-third of the study cost, irrespective of the adverse event grade [18]. To overcome

these challenges, there is a need to establish accurate, locally derived reference intervals for the target population. Within the last decade, several studies in sub-Saharan Africa have attempted to establish hematological and biochemical reference intervals for use in clinical monitoring and patient management.

2.3. Selection of a reference population

The selection of a reference population is as per described in the Clinical Laboratory Standards Institute (CLSI, Wayne, PA, USA) guidelines [21]. The guidelines state that reference individuals selected for the determination of reference intervals should closely resemble the patient population undergoing medical examination and should be of similar age to be clinically significant [21]. The reference individuals should not be hospital or clinic patients unless absolutely necessary. The guidelines describe two selection methods for a reference population: *a priori* and *a posteriori*. *A priori* sampling method involves selection of reference individuals based on well-defined exclusion and partition criteria. The entire selection process takes place before any blood sample is drawn and a sufficient number of reference individuals are targeted to provide statistical validity. *A posteriori* sampling method involves selection of the reference population after the analyte has been tested. The CLSI guidelines recommend a minimum of 120 individuals to allow 90% confidence limits to be non-parametrically calculated for the reference limits [22]. Partitioning of reference intervals either by gender or age is recommended if clinically useful or physiologically well grounded. Even though 120 samples remains the recommended standard, an efficient laboratory, by considering the CLSI revised guideline strategies [8], can determine reference intervals using fewer samples [23]. Alternatively, a laboratory can adopt reference intervals established from another laboratory if the values are verified using the procedures set out in the guidelines.

In our study [9] of adolescents and adults living in rural western Kenya, all participants were screened by a review of medical history, a physical examination, tested for HIV and pregnancy (for females), and treated for any illnesses diagnosed. Participants were included if they were a permanent resident of the study area, between 13 and 34 years of age and able to provide informed consent or assent if a minor. Participants were excluded if they were HIV-seropositive, pregnant, exhibiting febrile symptoms or on any medication. Blood samples were therefore obtained from clinically healthy participants selected to generate hematologic and biochemical reference intervals. Data were partitioned by age (<18 years of age as adolescents and ≥18 as adults) and gender; median and 95th percentile intervals were calculated. The lower 95% reference limit was defined as the 2.5th percentile while the upper limit was defined as the 97.5th percentile. A Wilcoxon rank-sum test was used to test for age and gender differences. We compared our data against reference intervals from the Massachusetts General Hospital (MGH-USA) [6] and the U.S. NIH Division of AIDS (DAIDS) toxicity tables [7] to determine the number of study participants with values outside the MGH ranges or who had any adverse event as graded by the DAIDS criteria. However, while the CLSI guidelines recommend a description of the population from which reference intervals are derived, the DAIDS and Massachusetts General Hospital reference values do not provide such information.

3. Current status of reference values in Africa

Reference intervals for clinical laboratory parameters have traditionally been obtained from European and North American populations [2]. However, differences have been reported between these values when compared to healthy African population values [16]. These include lower hemoglobin, red blood cell counts, hematocrit, mean corpuscular volume, platelets and neutrophils, and higher monocyte and eosinophil levels for African population compared to their Western counterparts [16,24-26] and Africans of European decent [27, 28]. Moreover, variations in several indices have been reported between different African ethnic groups [26, 29-31]. These differences are postulated to occur due to factors such as genetics, dietary patterns, gender, age, ethnic origin and environmental pathogens which are known to influence hematological and immunologic indices [32-35].

While the differences observed in some laboratory parameters between African and Caucasian/Western populations may be attributed to nutritional differences, genetic polymorphisms, or more intense environmental exposure to endemic pathogens, it must be stressed that these reference values are being derived from population-based statistical analyses of norms among healthy persons. For example, healthy Africans tend to have lower white blood cell counts than Caucasians, but there is no evidence that they suffer any additional risk of developing severe infection or other sequelae. Also, African American populations, with environmental exposures more like their white American counterparts, tend to have lower 'normal values' in hematologic parameters than Caucasian Americans, suggesting a genetic basis for these population differences.

3.1 Variation in specific laboratory parameters

a. Hematologic parameters

The normal values of red cell counts and indices (i.e., hemoglobin concentration, hematocrit, mean corpuscular volume, red blood cell count), white cell counts and platelet counts are known to vary with age, sex and pregnancy [9, 16, 20, 31, 36]. In addition, genetic and environmental factors can also affect the reference intervals in certain populations [32-34, 37]. It is of particular importance that these differences in reference intervals be considered by clinicians in different settings.

i. Red blood cell (RBC) components

African RBC component values were significantly lower when compared to reference intervals obtained from the Massachusetts General Hospital [6] from a North American population, and thus a significant proportion are misclassified when the NIH DAIDS toxicity tables are applied [9, 34, 38]. Differences observed in the RBC components between African and Caucasian populations may be attributed to lower dietary iron intake, genetic polymorphisms such as thalassemia and sickle cell trait or chronic exposure to endemic parasites including helminths, malaria and schistosomiasis.

Statistically significant differences in median RBC, hemoglobin concentration (Hb) and hematocrit (Hct), mean corpuscular volume (MCV) and mean corpuscular hemoglobin

(MCH) by gender have been observed in several African studies, with adult males having higher values than adult females in East Africa [9, 16, 20, 31, 39, 40], Southern Africa [20, 36], West Africa [41] and Central Africa [42]. These gender differences in RBC parameters as illustrated in our findings (Table 2), are consistent with previously established evidence that males have higher values than females for these parameters and is partly attributed to the influence of the androgen hormone on erythropoiesis [43, 44] and to menstrual blood loss in women [16, 25, 39, 42, 45]. It has been reported that estrogens lower the Hb through hemodilution while testosterone increases the plasma volume but increases circulating RBC to an even greater extent [46].

| Parameter | Gender | Age 13-17 years | | | Age 18-34 years | | | |
		n	Median (95th percentile)	p-value (gender)	n	Median (95th percentile)	p-value (gender)	p-value (age)
Hemoglobin (g/dL)	Female	57	12.2 (8.1 - 14.2)	*<.0001*	83	12.1(8.0 – 14.2)	*<.0001*	*0.3243*
	Male	76	13.1 (10.6 - 15.6)			14.2 (11.4 - 16.9)		*<.0001*
Hematocrit (%)	Female	57	35.6 (24.8 - 43.1)	*<.0001*	83	35.8 (23.2 - 44.3)	*<.0001*	*0.8015*
	Male	76	38.8 (29.3 – 48.1)			41.7 (32.6- 51.5)		*<.0001*
RBC (x10^{12}/L)	Female	57	4.7 (3.3 - 5.4)	*0.0001*	83	4.5 (3.4 - 5.7)	*<.0001*	*0.2638*
	Male	76	4.9 (4.1 - 5.8)			5.3 (4.3 - 6.5)		*<.0001*
PLT(x10^9/L)	Female	57	233 (134 – 439)	*0.2958*	83	220 (88 – 439)	*0.0222*	*0.4034*
	Male	76	224 (103 – 386)			201 (102 -307)		*0.0094*
WBC (x10^9/L)	Female	57	5.2 (3.9-10.2)	*0.6359*	83	5.6 (3.3-9.7)	*0.0189*	*0.2038*
	Male	76	5.6 (3.3-8.3)			5.3 (2.5-7.4)		*0.6382*
Lymphocytes (x10^9/L)	Female	57	2.2 (1.1 - 3.1)	*0.9820*	83	2.2 (1.3 - 3.8)	*0.6901*	*0.9388*
	Male	76	2.2 (1.0 - 4.2)		77	2.2 (1.0 – 3.5)		*0.585*
Ab Neutrophils (x10^9/L)	Female	57	2.0 (1.0-6.2)	*0.4991*	83	2.3 (1.3-5.4)	*0.0004*	*0.0576*
	Male	76	1.9 (0.8-5.0)		77	2.0 (0.8-3.9)		*0.6575*
CD4: Absolute	Female	58	934 (465- 1553)	*0.4074*	83	866 (440-1602)	*0.0141*	*0.509*
	Male	76	874 (367-1571)		77	811 (462-1306)		*0.0209*
CD8: Absolute	Female	58	506 (195-1068)	*0.4506*	83	472 (262 - 1167)	*0.8706*	*0.9213*
	Male	76	468 (195-988)		77	468 (201-1104)		*0.4194*
CD4/CD8 ratio	Female	58	1.8 (0.9-3.2)	*0.9215*	83	1.8 (0.8-3.0)	*0.0728*	*0.4879*
	Male	76	1.8 (0.8-2.8)		77	1.6 (0.8-2.8)		*0.0543*

Table 2. Test of difference in hematologic and immunologic parameters between gender and age-groups from healthy 13-34 year olds in a rural western Kenya cohort (2003-2005).

Age-related differences in the RBC component have also been observed among male participants, with adults (≥18 years) having higher levels of Hb, Hct and RBC compared to male adolescents (13-17 years) as shown here (Table 2) [9]. This age variation is similar to that reported in a study of Caucasian adolescents [34]. This difference could be attributed to higher levels of androgen hormones among older males. This explanation is further strengthened by the absence of age-related hematological difference among female participants. It has also been postulated that an increase in the size and mass of muscle fibers as occurs in males is associated with an increase in the number of circulating red blood cells [47].

There are limited data comparing reference intervals for hematologic values among African children compared to Caucasian children and also few studies on relevant local reference values for African infants. However, these studies, similar to the adult studies, have highlighted differences in RBC components compared to values obtained from Caucasian children [48]. The lower RBC parameters, as in the adolescent and adult groups, may be attributed to impaired hematopoiesis as a result of lower dietary iron intake, chronic blood loss due to hookworm infestation or chronic malaria infection [25]. Endemic sickle cell trait (HbS) and α-thalassemia may also play an important role [49].

ii.   Platelets

In general, lower platelet counts are more common in African than in Western populations. While the lower platelet counts in African populations are consistent in several African studies [24, 25, 30, 31, 40], its etiology is unknown. Possibilities such as dietary, environmental and genetic factors have been proposed [24, 30, 31]. Nevertheless, the significant difference in the lower limit of the reference interval between African and Caucasian populations warrants consideration when interpreting platelet counts in patients or during clinical trial recruitment in African populations.

Among Africans males, significant age-related differences have been observed in platelet counts with adults having higher platelet counts compared to adolescents [9]. This variation is observed as a progressive increase with age from adolescence to young adulthood. In comparison, there is little age variation in platelet counts among females. However, females have higher platelet counts than males both in adolescence [9] and adulthood [9, 16]. These gender differences in platelet counts have been attributed to hormonal influences [50]. Platelet count have been observed to falls at the onset of menstruation while peak values are obtained in mid-cycle indicating that hormonal influences and/or menstrual blood loss may be involved [27]. While platelet levels remain stable during pregnancy, a decrease has been reported immediately after delivery, likely due to consumption during separation and delivery of the placenta.

iii. White Blood Cell (WBC) components

A high proportion of participants in the African studies have WBC counts below the lower range of the Massachusetts General Hospital US population-derived values [9, 20]. This phenomenon is consistent with a number of studies that have reported lower WBC counts in

African populations and those of African ancestry, including African Americans, than in Caucasian populations [24, 30, 51-53]. Because the reference interval for WBC counts is significantly different from that of Caucasian populations, it is advisable to use appropriate ethnic group intervals when interpreting blood counts [31].

Gender differences in the WBC counts exist in both African and Caucasian populations with females having higher values than males [9, 20, 54]. Age-related difference in WBC counts has been reported in several African studies [9, 25, 33]. Adolescents have higher WBC counts compared to adults as shown in our study (Table 2) [9].

1.   Neutrophils

Within the U.S., lower neutrophil counts are more common among blacks compared to Caucasians [28]. Thus, it is not unsurprising to observe a higher proportion of African study participants (22.5-35%) having neutrophil counts below the lower range of Massachusetts General Hospital's population-derived reference interval [9]. It is estimated that about 25% to 50% of Africans have "benign ethnic neutropenia," maintaining consistently low absolute neutrophil counts with no evidence of increased susceptibility to infection or other adverse events [28]. Possibile explanations for the lower neutrophil count include diet, genetic or environmental influences [53, 55].

In general, there are significant differences in neutrophil counts between male and female adults, with the females having higher neutrophil counts than males. This increase in neutrophil counts observed in women may be related to estrogen since a decrease in counts has been reported after menopause [39]. Oral contraceptives have also been implicated in neutrophilia [56].

Additionally, several studies in southern Africa have documented high rates of neutropenia in infants of women receiving Prevention of Mother to Child Transmission interventions [57-591]. Evaluation of neutropenia in infants receiving antiretroviral prophylaxis or treatment (directly or indirectly through maternal exposure in utero or through breastfeeding) remains a challenge. Neutropenia is a known side effect of zidovudine [60] and trimethoprim/sulfamethoxazole, which is often prescribed for prevention of opportunistic infections in HIV-infected and/or HIV-exposed infants/children. This problem is further compounded by the paucity of normative data for hematologic values in African infants.

2.   Basophils and eosinophils

Basophil and eosinophil counts in African populations are significantly elevated in both genders when compared to the US-based reference intervals [9, 20]. This may be due to a high prevalence of parasitic infections in the environment including schistosomiasis, helminthic infections, perennial malaria and exposure to a broader range of environmental antigens [25, 39]. However, the eosinophil counts do not vary significantly by gender or by age, as assessed between adolescent and adult African participants [9, 34].

3.   Monocytes

Generally, no ethnic or age differences are observed between Caucasian and African populations [46]. Monocyte counts in Eastern and Southern Africa are comparable to the US derived valuesand thus there is no need for separate reference intervals [9, 20].

In the African studies, no differences are observed in absolute monocyte counts between adolescents and adults or by gender [9, 20]. Previous studies from Eastern and Southern African populations indicate an increase in monocyte counts in males compared with females but the difference is not significant [3, 24, 26, 29, 30].

4.   Lymphocytes

Among healthy, HIV-uninfected persons, there are no significant differences in lymphocyte counts between Caucasian and African populations but females generally have higher lymphocyte counts than males [54]. This is corroborated by studies within Africa that indicated higher CD4 cell percentage and absolute CD4 counts in females compared to males [9, 26, 61].

However, geographical variation exists in lymphocyte counts with some populations in Southern African showing significantly lower reference values than other parts of Africa [62]. In assessing age-related variability, younger age is associated with higher CD4 cell counts and a higher CD4:CD8 ratio. However, the differences are not significantly except for CD4 cell counts between male adolescent and male adults [9]. These age and geographical variations need to be considered when interpreting lymphocyte counts.

b.   Clinical chemistry parameters

Most African studies [9, 15, 16, 20] report reference intervals for most parameters (creatinine, direct bilirubin, amylase and albumin) that are in agreement with reference intervals published in the United States [6]. However, certain parameters such as Creatine Kinase (CK) and Lactose Dehydrogenase (LDH) have upper intervals that are substantially higher than those published in the Massachusetts General Hospital intervals [16, 20]. Other parameters with a similar trend include total bilirubin (T-bil) and blood urea nitrogen (BUN). The upper range for T-bil is about twice as high as that of the US-derived upper reference limit while the lower range for BUN is a about a third of the US-derived lower reference limit [9, 15, 20]. The etiology of high T-bil in the African population may arise from a number of factors including RBC hemolysis caused by malaria infection or sickle cell disease, malnutrition or physical exertion. Moreover, the presence of similar trends among other African populations is suggestive of a common environmental or genetic factor.

Our findings indicated gender and age variations in blood chemistry analytes of liver and renal function among African adolescents and adults. Male adolescents and adults had higher values for alanine aminotransferase (ALT), aspartate aminotransferase (AST), T-bil and creatinine than females adolescents and adults (Table 3). These gender differences were significantly greater for T-bil and creatinine in both adolescents and adults while for AST, the difference was significant only among the adolescents. However, these differences were

not clinically significant. There were no gender differences in BUN and glucose levels for all age groups and no significant differences in T-bil, AST, ALT and glucose between the two age groups for both males and females. However adult men and women had higher values for creatinine and BUN compared to adolescent males and females, respectively.

Parameter	Gender	n	Age 13-17 years Median (95th percentile)		n	Age 18-34 years Median (95th percentile)	p-value (gender)	p-value (age)
AST/SGOT (µ/L)	Female	62	22.6 (12.0 – 43.1)	*0.0102*	82	22.2 (13.5 - 48.5)	*0.0822*	*0.5905*
	Male	77	26.9 (17.0 – 59.2)		77	26.7 (12.5-69.3)		*0.9147*
ALT/SGPT (µ/L)	Female	62	17.4 (4.2-65.3)	*0.6289*	82	18.9 (10.7-61.3)	*0.2247*	*0.1305*
	Male	77	20.5 (4.9-42.4)		77	22.4 (12.0-80.6)		*0.0901*
Total Bilirubin (µmol/L)	Female	62	9.7 (3.7-38.5)	*0.0331*	82	11.5 (5.8-36.1)	*0.0368*	*0.7132*
	Male	77	13.9 (5.7 – 62.6)		77	13.8 (5.3 - 50.7)		*0.6662*
Creatinine (µmol/L)	Female	62	64.5 (48.0-87.6)	*0.0229*	82	70.7 (52.4-96.8)	*<.0001*	*0.0013*
	Male	77	66.3 (49.6-103.7)		77	83.1(54.2-137.8)		*<.0001*

Table 3. Test of difference in clinical chemistry parameters between gender and age-groups from healthy 13-34 year olds in a rural western Kenya cohort (2003-2005).

4. Should establishing separate normal ranges for African adolescents and pregnant women be considered?

A number of studies similar to our published data [9], have reported age-related variation between male adolescents as compared to adults for Hb, Hct and RBC levels [25, 34, 45]. This observation is physiologically grounded on hormonal influence and as per the CLSI guidelines, partitioning reference intervals by age (or other subgroup considerations) may be appropriate. While these observations may not be of any medical significance, it should be taken into consideration whenever clinical trials target this population. To satisfy the statistical requirement for partitioning, there is need for further research on reference values among adolescents, as their participation in clinical trials increases.

Other than the RBC components mentioned above, no significant age differences have been observed in other laboratory parameters measured among males or in any parameters measured among females except for creatinine and BUN. Thus, for such parameters for which no differences are reported, adult values can be used in clinical trials involving adolescents.

With the advent of antiretroviral therapy for HIV and other interventions to improve maternal and child health, pregnant women and infants have become the focus of many health programs. However, few data exist regarding these important populations, despite increased clinical trials aimed at reducing mother-to-child HIV transmission. Although pregnancy-induced changes occur in hematological values including Hb, Hct and RBC count, very few laboratories provide specific reference ranges for pregnant women [63, 64].

In pregnancy, blood volume increases resulting in hemodilution. While the red cell mass increases during pregnancy, the plasma volume increases more resulting in a relative anemia. This leads to a lower Hb level, Hct and RBC. Hb is known to vary with gestational age with the highest values within the first and last trimesters and lowest during the second trimester. Similarly, the Hct and RBC decreases with gestational age. A stable higher upper reference limit for WBC count during pregnancy has been reported [65, 66]. WBC count is known to peak at delivery, thus limiting the use of this parameter as a marker for infection during delivery. This increase in WBC count results primarily from an increase in neutrophil counts and a slight increase in lymphocyte counts. Currently, there exists no African study designed to establish reference intervals during pregnancy and most laboratory information systems report reference values based on samples obtained from non–pregnant women which may not be useful for clinical decisions during pregnancy. Thus, there is an increased risk of overlooking important physiologic alterations resulting from pathological conditions and of misinterpreting normal changes as pathological events [64]. It is therefore important to develop reference intervals for women during pregnancy and the postpartum period for use in patient monitoring and management.

5. A case for African/ Region specific toxicity tables?

Under a research-based approach, applying the US Massachusetts General Hospital derived reference intervals to our reference population from western Kenya during screening for a clinical trial (Table 4), over 58% of the volunteers would have been excluded from the trial despite having laboratory results consistent with the general population from which they were derived. This erroneous screening out of otherwise healthy volunteers would have important implications on study costs, work load and time, as more volunteers would be need to be screened in order to meet the required target [15].

Similarly, applying the DAIDS toxicity tables to our population, some of our calculated reference intervals fall between the normal, and grade 1–2 toxicity grading in the DAIDS system (Table 4). Using the clinic based approach, 40% of our otherwise healthy study participants would have erroneously been considered to have at least one laboratory-based grade 1–4 toxicity adverse event. The lower range for Hb, neutrophil counts, as well as the upper range for eosinophil counts and bilirubin would be considered as grade 2 adverse events, for example. Even though studies have documented these findings, this information is not widely known and as a result, DAIDS has issued only 1 set of "standard" toxicity tables without considering racial or ethnic differences [57]. Thus, during international clinical trials, these tables are used as guidelines in the conduct of such trials. This may result in a situation where the results of a clinical trial cannot be generalized to the population in question since a majority of otherwise healthy participants are screened out. Moreover, given that the investigational product is intended for use within the same population being sampled, this may complicate post-market analysis or application of the product for the general population. Unfortunately, there are no comparable tables from Africa on which such clinical decisions can be based. It is therefore important that African countries carry out large studies in different regions of Africa for such parameters to establish African toxicity tables.

Parameter	n	MGH USA reference intervals (25th percentile)[6] out of range Comparison 95th percentile			Division of AIDS (DAIDS) toxicity grading Grade 1		Grade 2		Grade 3		Grade 4	
		95th percentile	n	%	n	%	n	%	n	%	n	%
Hemoglobin Males (g/dl)	140	13.5-17.5	65	46	2	1.3	0	0	2	1.3	0	0
Hemoglobin Females (g/dl)	153	12-16	61	40	11	7.9	8	5.7	14	10	0	0
Hct (females) (%)	140	36-46	74	53								
Hct (males) (%)	153	41-53	88	58								
RBC (males) (10^{12} cells/L)	140	4.5-5.9	29	19								
RBC (females) (10^{12} cells/L)	153	4.0-5.2	32	23								
MCV (fL)	293	80-100	157	54								
Platelets (10^9 cells/L)	293	150-350	53	18	6	2	6	2	0	0	0	0
WBC (10^9 cells/L)	293	4.5-11.0	66	23	2	0.7	0	0	0	0	0	0
Lymphocyte count (10^9 cells/L)	293	1.0-4.8	6	2	0	0	0	0	0	0	0	0
Neutrophil count (10^9 cells/L)	293	1.8-7.7	110	38	25	8.5	9	3.1	1	0.3	0	0
Eosinophil (10^9 cells/L)	293	0-0.5	130	44	60	20.5	12	4.1	0	0	0	0
Basophil count (10^9 cells/L)	293	0-0.2	5	2								
Monocyte count (10^9 cells/L)	293	0-0.8	0	0								
ALT (SGPT) (U/ L)	293	0-35	30	10	12	4.1	1	0.3	0	0	0	0
AST (SGOT) (U/ L)	293	0-35	40	13	9	3.1	3	1	0	0	0	0
Total Bilirubin (μmol/L)	293	5.1-17.0	90	30	37	12.7	27	9.2	4	1.4	1	0.3
Creatinine (μmol/L)	293	0-133	4	1	4	1.4	0	0	0	0	0	0
Glucose mmol/L	293	4.2-6.4	210	71								
BUN (mmol/L)	293	3.6-7.1	246	84								
*CD4 (Cells/ μl)	293	404-1612	6	2	3	1	1	0.3	0	0	0	0
*CD8 (Cells/ μl)	293	220-1129	13	4								

*Reference ranges provided by Becton-Dickinson with the MultiTEST IMK Kit Reagent package (12/2000;23-3602-02) - DAIDS- Division of AIDS tables for grading the severity of adult and pediatric adverse events [26] - MGH- Massachusetts General Hospital weekly case records [25]

Table 4. Frequency of adverse events and out of range values comparing western Kenyan cohort to DAIDS and North American derived MGH values

6. Conclusion

While it is desirable to generate reference intervals for different populations, the procedure remains a challenge due to the prohibitive cost involved in performing these studies and the limitation in identifying suitable healthy reference individuals. Thus, the CLSI recommendation that all diagnostic laboratories should determine and maintain their own reference interval for each laboratory parameter is impractical. The revised CLSI guidelines

have recommended that if it is not possible to establish detailed reference studies, then validation of published reference intervals can be performed using methodology tailored for the population served by the laboratory. As few as 20 specimens can be used to validate reference values within each laboratory by performing a formal outlier test.

Given the number of clinical trials and persons receiving clinical services is expected to increase substantially in sub-Saharan Africa, there is a need for the establishment of locally derived clinical laboratory reference values to ensure appropriate general health assessment, treatment monitoring, and efficient implementation of clinical trials. Even more important is the need for the establishment of toxicity grading tables for application in clinical care among Africans based on the documented differences between laboratory reference values from African populations and Caucasians or Western populations of mixed ethnic origin.

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