Reference Values for Clinical Laboratory Parameters in Young Adults in Maputo, Mozambique

Nelson Tembe1,2,3*, Orvalho Joaquim1, Eunice Alfai1, Nádia Sito1, Edna Viegas1,2,3, Eulalia Macovela3,4, Emilia Gonçalves3,4, Nafissa Osman3,4, Sören Andersson5, Ilesh Jani1, Charlotta Nilsson2,6,7

1 Instituto Nacional de Saúde, Maputo, Mozambique, 2 Department of Laboratory Medicine, Karolinska Institutet, Huddinge, Sweden, 3 Faculty of Medicine, Eduardo Mondlane University, Maputo, Mozambique, 4 Hospital Central de Maputo, Maputo, Mozambique, 5 Orebro University Hospital, Orebro, Sweden, 6 Public Health Agency of Sweden, Solna, Sweden, 7 Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

Abstract

Background: Clinical laboratory reference values from North American and European populations are currently used in most African countries due to the absence of locally derived reference ranges, despite previous studies reporting significant differences between populations. Our aim was to define reference ranges for both genders in 18 to 24 year-old Mozambicans in preparation for clinical vaccine trials.

Methods: A cross-sectional study including 257 volunteers (102 males and 155 females) between 18 and 24 years was performed at a youth clinic in Maputo, Mozambique. All volunteers were clinically healthy and human immunodeficiency virus, Hepatitis B virus and syphilis negative. Median and 95% reference ranges were calculated for immunological, hematological and chemistry parameters. Ranges were compared with those reported based on populations in other African countries and the US.

Results: The immunology ranges were comparable to those reported for the US and western Kenya. There were significant gender differences in CD4+ T cell values 713 cells/μL in males versus 824 cells/μL in females (p<0.0001). Hematologic values differed from the US values but were similar to reports of populations in western Kenya and Uganda. The lower and upper limits of the ranges for hemoglobin, hematocrit, red blood cells, white blood cells and lymphocytes were somewhat lower than those from these African countries. The chemistry values were comparable to US values, with few exceptions. The upper limits for ALT, AST, bilirubin, cholesterol and triglycerides were higher than those from the US. DAIDStables for adverse events predicted 297 adverse events and 159 (62%) of the volunteers would have been excluded.

Conclusion: This study is the first to determine normal laboratory parameters in Mozambique. Our results underscore the necessity of establishing region-specific clinical reference ranges for proper patient management and safe conduct of clinical trials.

Introduction

The number of clinical trials related to HIV/AIDS, tuberculosis and malaria conducted in Africa is increasing sharply and is expected to increase further in the coming years [1,2]. Routine health assessment and management of clinical trials relies on accurate laboratory references. Of primary importance in vaccine clinical trials is the evaluation of safety and tolerability in a clinically “normal” population. Furthermore, volunteers may be assessed for disease progression and evaluation of possible clinical trial-associated toxicity and adverse events.

Laboratory reference intervals for healthy populations have not been established in most African countries. Common practice in these countries, including Mozambique, is to use reference ranges derived from populations living in Europe or the United States (US). Studies have shown differences between clinical reference ranges in African populations compared to those established in industrialized countries [3–6]. Several studies have also reported that laboratory parameters vary geographically by ethnic origin, genetics, gender, altitude and environmental factors [3,4,7–9].

The use of improper clinical reference ranges to assess participant eligibility and safety for clinical trials may lead to unnecessary exclusion of eligible participants, contribute to over-reporting of adverse events (AEs) [10] and increase the number of referrals for clinical investigations. Laboratory abnormalities based on non-indigenous laboratory parameters and medical abnormalities were reported to be the main reasons volunteers were excluded from two Kenyan HIV vaccine clinical trials [7]. Moreover, studies have suggested that use of the US NIH Division...
of AIDS (DAIDS) toxicity tables may not be appropriate for African populations [10,11].

Prior to execution of a phase I/II HIV vaccine trial (TaMoVac 01) in Mozambique, we performed a study to define the prevalence and incidence of HIV and other sexually transmitted viruses in Maputo in a population of young adults. This was also an opportunity to establish clinical reference values in 18 to 24 year-old Mozambicans.

This study establishes reference ranges for immunological, hematological and chemistry parameters in healthy young adults in Mozambique. We determined gender differences and compared values established for Mozambican young adults with those previously reported for the same age group in other African countries and with established intervals from the US (Massachusetts General Hospital, MGH-USA) [12]. Additionally, we applied the division of AIDS (DAIDS) toxicity tables for grading of AEs [13] to evaluate their potential implications for vaccine trials.

Materials and Methods

Ethics Statement

This study was approved by the National Bioethics Committee for Health of Mozambique. Written informed consent was obtained from each participant prior to conducting any study procedures.

Study site and subjects

The study took place at the SAAJ clinic (SAAJ: ‘Serviço Amigo de Adolescentes e Jovens’ or Adolescent and Youth Friendly Service) at the Department of Obstetrics and Gynecology, Maputo Central Hospital. The SAAJ clinic provides services (free of charge) for young people seeking counseling and treatment for any health problem, but with particular attention to reproductive health and control of sexually transmitted infections (STIs). A low prevalence of STIs and HIV and a high level of awareness has previously been reported in a study performed at the SAAJ clinic [14].

Maputo is the capital of Mozambique and is situated in a coastal area adjacent to the Indian Ocean, between the coordinates 25° 50’ and 26° 10’ S and 32° 30’ and 32° 40’ E [15]. The city is situated at an average altitude of 47 meters, has an area of 346.77 km² and had a population of 1,094,315 in 2007 [16]. A total of 257 healthy individuals between 18 and 24 years old were recruited from a cohort of youths participating in a study of the prevalence and incidence of sexually transmitted viruses at the SAAJ clinic, Maputo Central Hospital. Medical staff performed physical examinations and collected clinical histories. Volunteers who were febrile, pregnant, or seropositive for HIV, syphilis or hepatitis B surface antigens were excluded from the study.

HIV, syphilis and hepatitis B screening

The national algorithm for HIV testing was used to diagnose HIV. HIV testing was performed using two immunochromatographic assays, the Determine HIV-1/2 (Inverness Medical, Bedford, United Kingdom) followed by the UniGold HIV-1/2 (Trinity Biotech, Bray, Ireland). Syphilis testing was performed using SD Bioline Syphilis 3.0 (Standard Diagnostics, Suwon City, South Korea). Serum samples were tested for Hepatitis B virus (HBV) using the Hepatitis B Surface Antigen (HBsAg) ELISA Kit (Human, Wiesbaden, Germany).

Pregnancy testing

A urine pregnancy test was administered to all females prior to collection of blood samples, using the QuickVue One-Step HCG Urine Test (Quidel Corporation, San Diego, USA).

Blood collection

Sample collection took place between August 2009 and September 2012. Most samples (70%) were collected between November 2009 and August 2010, but the inclusion period was extended to recruit additional males to the study. Blood was collected in 4 ml EDTA Vacutainer tubes (Becton-Dickinson, Franklin Lakes, New Jersey, USA) in preparation for lymphocyte and hematological testing. Whole blood was collected in 10 ml serum Vacutainer tubes (Becton-Dickinson, USA) in preparation for testing chemical parameters and HIV status. Samples were collected in the morning between 8:00 AM and 12:00 noon, kept at room temperature and transferred to the laboratory of the National Institute of Heath in Maputo for analysis.

Flow cytometry analysis for Immunophenotyping

Immunophenotyping was performed using a FACS Calibur flow cytometer (Becton-Dickinson, Franklin Lakes, New Jersey, USA). Samples were analyzed within 24 h of specimen collection. In brief, 20 µl of CD3FITC/CD8PE/CD45perCP/CD4APC or CD3FITC/CD16+CD56PE/CD45perCP/CD19APC MultiTest reagents (Becton-Dickinson, USA) was mixed with 50 µl of whole blood and incubated in the dark at room temperature for 15 min. Red blood cells were then lysed by adding 450 µl of fluorescence-activated cell sorter lysing solution (Becton Dickinson, USA). The tubes were then incubated at room temperature for another 15 min. MultiSET software (Becton-Dickinson, USA) was used to perform the analysis.

Hematological analysis

A complete blood count and differential was performed using the Sysmex KX-21N Hematology Analyzer (Sysmex Corporation, Kobe, Japan) as recommended by the manufacturer. The samples were analyzed within 6 h of specimen collection. The machine automatically dilutes a whole-blood sample, lyses and counts the cells, and then gives a printout result. Seventeen parameters were analyzed; leukocytes (WBC), erythrocytes (RBC), platelets (PLT), lymphocytes (LYM), neutrophils (NEUT), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width measured by standard deviation (RDW-SD), red blood cell distribution width measured by coefficient of variation (RDW-CV), platelet distribution width (PDW), mean platelet volume (MPV), platelet larger cell ratio (P-LCR) and the percentages of lymphocytes (LYM), neutrophils (NEUT), and the mixed population of monocytes, basophiles and eosinophils (MXD). The absolute cell counts were expressed as number of cells × [10⁹] per liter.

Biochemistry analysis

Serum chemistry was performed using a Vitalab Selectra Junior (Vital Scientific, Dieren, Netherlands) per the manufacturers instructions. Serum was separated within 4 h of collection and analyzed within 7 h of blood draw. Each sample was analyzed for creatinine, total bilirubin (T-Bil), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, urea, uric acid, amylase, HDL cholesterol, triglycerides and alkaline phosphatase (ALP).
### Table 1. Lymphocytes subset reference ranges (median and 2.5th- 97.5th percentiles) derived from young adults in Maputo city, Mozambique.

| Parameter                  | Male          | Female         | Total          | p    |
|----------------------------|---------------|----------------|----------------|------|
|                            | N  | Range             | N  | Range             | N  | Range             | p    |
| CD45+ cells/μL             | 92 | 1958 (1185–3201) | 135 | 2085 (1134–3678) | 227 | 2046 (1151–3471) | 0.0788 |
| CD3+ cells/μL              | 91 | 1328 (716–1917)  | 129 | 1400 (756–2313)  | 220 | 1386 (729-2228)  | 0.2630 |
| CD3+ T cells/μL (%)        | 92 | 66.8 (50.2–79.5) | 133 | 67.5 (51.1–80.9) | 225 | 67.5 (51.7–80.7) | 0.3630 |
| CD4+ T cells/μL (%)        | 91 | 713 (357–1155)  | 135 | 824 (434–1479)  | 226 | 774 (381–1340)  | <0.0001 |
| CD4+ T cells/μL (%)        | 92 | 35.5 (23.2–49.1) | 133 | 40.4 (29.9–53.7) | 225 | 38.7 (25.8–52.2) | <0.0001 |
| CD8+ T cells/μL (%)        | 91 | 482 (214–902)   | 133 | 480 (234–965)   | 224 | 479 (218–952)   | 0.3954 |
| CD8+ T cells/μL (%)        | 92 | 25.5 (149–415)  | 132 | 23.5 (14.7–34.5) | 224 | 24.2 (14.7–37.6) | 0.0038 |
| CD4/CD8 ratio              | 91 | 1.5 (0.7–2.7)   | 133 | 1.7 (1.0–3.2)    | 224 | 1.6 (0.8–3.1)    | 0.0001 |
| CD16+56 cells/μL (%)       | 29 | 351 (151–761)   | 101 | 333 (95–845)    | 130 | 338 (101–820)    | 0.3978 |
| CD16+56 cells/μL (%)       | 29 | 17.5 (10.3–36.0) | 101 | 15.2 (4.1–31.1) | 130 | 15.9 (4.9–34.5) | 0.0390 |
| CD19+ cells/μL (%)         | 29 | 231 (97–475)    | 101 | 264 (85–548)    | 130 | 251 (86–545)    | 0.0751 |
| CD19+ cells/μL (%)         | 29 | 12.3 (6.8–17.0) | 100 | 12.3 (6.3–21.5) | 129 | 12.3 (6.3–20.9) | 0.2638 |

P-values indicate comparisons between males and females.
Immunophenotyping

Table 1 shows the median values and reference ranges for lymphocyte subsets generated from participants in the study. Analyses of lymphocyte subsets revealed statistically significant differences between genders with respect to the absolute number of CD4+ cells, percentage of CD4+ cells, percentage of CD8+ cells, and CD4:CD8 ratios. Reference ranges for the absolute number of CD4+ cells (p<0.0001) and percentage of CD4+ cells (p<0.0001), CD4:CD8 ratios (p=0.0001) as well as CD5+ cells (p=0.0420) were significantly higher in females than males; however, the percentage of CD8+ cells was significantly higher in males (p=0.0038). There were no significant gender differences in CD45+ cell counts (total lymphocytes), percentage of CD3+ and absolute CD8+ cell count. Gender comparisons were also performed for CD16+56+ (natural killer cells) and CD19+ cells (B-cells) despite the comparatively low number of male samples (n=29). A statistically significant gender difference was seen in percentage of CD16+56+ cells (p=0.0390). No significant gender difference was found with respect to absolute number of CD16+56+ cells or absolute number and percentage of CD19+ cells.

Our immune phenotype data were generally comparable to those derived from the present study. The reference ranges for CD4+ and CD8+ T cells derived in the present study were more comparable to values reported from the population in western Kenya than in Uganda (Table 2).

Hematology

Table 3 shows the medians and reference ranges for hematological parameters, grouped according to gender. We observed statistically significant differences between males and females in most hematological parameters, with the exception of PDW, MPV, absolute LYM, absolute MXD and percentage MXD. The males had higher values of RBC, Hb, HCT, MCV, MCH, MCHC and percentage LYM than females. The females had higher values of WBC, PLT, absolute NEUT and percentage NEUT, RDW-SD and RDW-CV than males.

Our reference ranges showed significant differences from the US population (Table 3). Close to 50% of our study participants had Hb values below the lower limits of the range determined in the US population; this was particularly true for females, 104 (69.3)% of whom had Hb values below the lower limit. We also found a considerable number of participants with platelet and WBC values outside the US reference ranges (47 (18%) and 64 (25%), respectively) (Table 4). Our reference ranges for young adults in Maputo were comparable to those derived from the western Kenyan and Ugandan populations. However, the lower

| Table 2. Lymphocyte reference ranges from youth in Maputo, Mozambique compared with sources from Africa and the United States of America. |
|-------------------------------------------------|-----------------|-----------------|--------------------|-----------------|
| Parameter                                      | Maputo-Moz.     | Western Kenya [11] | Uganda [4] | USA*               |
|                                                | (18–24 years old) | (18–34 years old) | (19–24 years old) |                |
| CD3 T-cells/μl                                | 729–2220        | NA               | NA                | 723–2737        |
| CD3 T-cells (%)                               | 51.7–80.7       | NA               | NA                | 56–86           |
| CD4 T-cells/μl                                |                 |                  |                   |                 |
| All                                           | 381–1340        | 444–1488         | NA                | 400–1612        |
| Male                                          | 357–1155        | 462–1306         | 504–1334          | NA              |
| Female                                        | 434–1479        | 440–1602         | 560–1961          | NA              |
| CD4 T-cells (%)                               |                 |                  |                   |                 |
| All                                           | 25.8–52.2       | NA               | NA                | 33–58           |
| Male                                          | 23.2–49.1       | 29–54            | 18.5–42.2         | NA              |
| Female                                        | 29.9–53.7       | 32–55            | 27.4–53.0         | NA              |
| CD8 T-cells/μl                                |                 |                  |                   |                 |
| All                                           | 218–952         | 211–1078         | NA                | 220–1129        |
| Male                                          | 214–902         | 201–1104         | 286–1579          | NA              |
| Female                                        | 228–965         | 262–1167         | 151–1226          | NA              |
| CD8 T-cells (%)                               |                 |                  |                   |                 |
| All                                           | 14.7–37.6       | NA               | NA                | 13–39           |
| Male                                          | 14.9–41.5       | 14.9–44.0        | 12.7–41.7         | NA              |
| Female                                        | 14.7–34.5       | 17.5–35.0        | 9.2–29.5          | NA              |
| CD4:CD8 ratio                                 | 0.8–3.1         | 0.8–2.8          | NA                | NA              |
| B-cells                                       | 86–545          | NA               | NA                | 80–616          |
| B-Cells (%)                                   | 6.3–20.9        | NA               | NA                | 5–22            |
| Nk Cells                                      | 101–820         | NA               | NA                | 84–724          |
| Nk Cells (%)                                  | 4.9–34.5        | NA               | NA                | 5–26            |

*Reference ranges provided by Becton-Dickinson with the MultiTEST IMK Kit Reagent package.
NA – Not available.
doi:10.1371/journal.pone.0097391.t002
Table 3. Hematology reference ranges (median and 2.5th-97.5th percentiles) derived from young adults in Maputo, Mozambique.

| Parameter      | Male                  | Female                | Total                 | p        |
|----------------|-----------------------|-----------------------|-----------------------|----------|
|                | N / Range             | N / Range             | N / Range             | p        |
| Hemoglobin (g/dL) | 101 14.1 (12.3–16.4)  | 151 11.2 (7.0–13.1)   | 252 12.2 (7.5–15.8)   | <0.0001  |
| Hematocrit (%)  | 97 42.8 (25.2–50.4)   | 150 33.8 (19.5–40.3)  | 247 36.4 (20.2–47.2)  | <0.0001  |
| MCV (fL)       | 102 85.8 (72.4–92.9)  | 150 81.1 (59.1–94.3)  | 252 83.1 (63.0–94.1)  | <0.0001  |
| MCH (pg)       | 102 28.4 (22.2–47.7)  | 151 26.9 (16.4–48.0)  | 253 27.5 (18.0–48.7)  | 0.0063   |
| MCHC (g/dL)    | 101 33.1 (30.4–54.2)  | 151 32.4 (25.8–56.1)  | 253 32.7 (28.1–55.9)  | 0.0143   |
| RDW-SD (fL)    | 95 43.6 (37.0–50.0)   | 150 44.6 (36.3–52.6)  | 245 44.2 (36.4–52.2)  | 0.0159   |
| RDW-CV (%)     | 95 14.1 (11.6–18.6)   | 149 14.8 (12.2–23.5)  | 244 14.4 (11.9–23.4)  | 0.0084   |
| PDW (fL)       | 97 13.5 (10.4–22.2)   | 147 13.5 (10.1–21.0)  | 244 13.5 (10.2–22.3)  | 0.8719   |
| MPV (fL)       | 97 10.6 (8.7–13.1)    | 147 10.5 (8.5–12.7)   | 244 10.5 (8.5–13.0)   | 0.9564   |
| Erythrocytes (10^6/µL) | 100 5.1 (2.7–6.1) | 150 4.2 (2.3–5.0) | 250 4.6 (2.4–5.9) | <0.0001 |
| Platelets (10^9/µL) | 102 231.1 (162.2–392.1) | 151 269.0 (128.8–503.0) | 253 252.0 (125.2–488.0) | <0.0001 |
| WBC (10^3/µL)   | 101 4.6 (2.9–7.7)     | 151 5.6 (3.2–9.1)     | 252 5.1 (3.0–8.7)     | <0.0001  |
| Neutrophils (10^3/µL) | 101 2.4 (1.1–5.1) | 149 3.2 (1.4–7.0) | 250 2.7 (1.2–6.1) | <0.0001 |
| Neutrophils (%) | 95 52.5 (34.4–70.8)   | 146 57.1 (37.0–76.7)  | 241 54.7 (34.9–74.9)  | 0.0006   |
| Lymphocytes (10^3/µL) | 97 1.8 (1.1–3.3) | 150 1.9 (1.0–3.1) | 247 1.9 (1.1–3.1) | 0.0535 |
| Lymphocytes (%) | 97 39.1 (15.5–57.1)   | 150 35.1 (17.8–53.6)  | 247 37.0 (16.6–56.2)  | 0.0103   |
| MXD (10^3/µL)   | 91 0.3 (0.0–0.9)      | 132 0.3 (0.0–0.8)     | 223 0.3 (0.0–0.8)     | 0.3807   |
| MXD (%)         | 85 6.8 (0.5–16.2)     | 131 6.3 (0.0–12.9)    | 216 6.3 (0.0–14.6)    | 0.1339   |

p-values indicate comparisons between males and females.
doi:10.1371/journal.pone.0097391.t003
and upper limits for some hematological parameters (Hb, HCT and lymphocytes, RBC and WBC) were lower than those derived from these African countries. The lower and upper limits of neutrophil and platelet reference ranges in our study were higher than those derived from populations in western Kenya and Uganda (Table 4).

### Biochemistry

Table 5 shows the median values and reference ranges for clinical chemistry parameters. There was a statistically significant difference between genders in all clinical chemistry analytes, with the exception of triglycerides and amylase. Males had significantly higher levels of T-Bil, glucose, uric acid, AST, ALT, albumin, urea, creatinine, and ALP than females. Females had significantly higher levels of cholesterol (p = 0.0196) and HDL cholesterol (p = 0.0086) than males.

The study reference ranges of clinical chemistry parameters were comparable to those derived from the North American population (MGH-USA), with a few exceptions. The upper limits of the reference ranges for ALT, AST, bilirubin and cholesterol derived in the present study were somewhat higher than those from the US. The Maputo values were lower compared to western Kenya in the same age group (Table 6). We also found a high proportion of study participants with glucose and T-Bil values outside of the US reference ranges, in 104 (41.1%) of 199 individuals and in 38 (15.0%) of 253 individuals, respectively (Table 7).

### Implications for clinical trials

Table 7 shows the frequency of potential adverse events by applying the DAIDS toxicity grading, which is commonly used in clinical trials, to the values obtained from the cohort of young adults in Maputo. Among the hematologic parameters, Hb and neutrophil counts accounted for the majority of the abnormal classifications. The low Hb levels among our study participants would have resulted in 32 reported AEs; 21 (8.3%) as grade 1, 7

---

**Table 4. Comparison of hematology reference ranges derived from young adults in Maputo, Mozambique with those from other countries.**

| Parameter          | Maputo-Moz (18–24 years old) | Western Kenya [11] (18–34 years old) | Uganda [4] (19–24 years old) | USA [12] (18–24 years old) |
|--------------------|-------------------------------|-------------------------------------|-------------------------------|-----------------|
| Hemoglobin (g/dL)  |                               |                                     |                               |                  |
| Male               | 12.3–16.0                     | 11.4–16.9                           | 11.5–17.1                     | 13.5–17.5        |
| Female             | 7.3–13.2                      | 8.0–14.2                            | 9.9–13.7                      | 12.0–16.0        |
| Hematocrit (%)     |                               |                                     |                               |                  |
| Male               | 37.5–49.0                     | 32.6–51.5                           | 33.7–48.7                     | 41.0–53.0        |
| Female             | 20.9–40.2                     | 23.2–44.3                           | 28.9–40.0                     | 36.0–46.0        |
| RBC’s (10^6 cells/μl) |                               |                                     |                               |                  |
| Male               | 2.7–6.1                       | 4.3–6.5                             | 4.3–6.1                       | 4.5–5.9          |
| Female             | 2.3–5.0                       | 3.4–5.7                             | 3.6–5.4                       | 4.0–5.2          |
| MCV (fl)           |                               |                                     |                               |                  |
| All                | 63.0–94.1 b                   | 60–93 NA                            | 80.0–100.0                    |                  |
| Male               | 72.4–92.9                     | 102–307                             | 98–306                        |                  |
| Female             | 59.1–94.3                     | 88–439                              | 95–368                        |                  |
| Platelets (10^3 cells/μl) |                               |                                     |                               |                  |
| All                | 125.2–488.0                   | 103–390 NA                          | 150–350                       |                  |
| Male               | 116.2–392.1                   | 102–307                             | 98–306                        |                  |
| Female             | 128.8–503.0                   | 88–439                              | 95–368                        |                  |
| WBC (10^3 cells/μl) |                               |                                     |                               |                  |
| All                | 3.0–8.7                       | 3.3–9.3                             | 4.5–11.0                      |                  |
| Male               | 2.9–7.7                       | 2.5–7.4                             | NA                            |                  |
| Female             | 3.2–9.1                       | 3.3–9.7                             | NA                            |                  |
| Neutrophils (10^3 cells/μl) |                               |                                     |                               |                  |
| All                | 1.2–6.1                       | 0.9–5.2                             | 1.0–3.5                       | 1.8–7.7          |
| Male               | 1.1–5.1                       | 0.8–3.9                             | NA                            |                  |
| Female             | 1.4–7.0                       | 1.3–5.4                             | NA                            |                  |
| Lymphocytes (10^3 cells/μl) |                               |                                     |                               |                  |
| All                | 1.1–3.1                       | 1.1–3.5                             | 1.3–4.1                       | 1.0–4.8          |
| Male               | 1.1–3.3                       | 1.0–3.5                             | NA                            |                  |
| Female             | 1.0–3.1                       | 1.3–3.8                             | NA                            |                  |

b-values corresponding to individuals aged 13–34 years.

NA – Not available.

doi:10.1371/journal.pone.0097391.t004
Table 5. Clinical chemistry reference values (median and 2.5th-97.5th percentiles) derived from young adults in Maputo, Mozambique.

| Parameter   | Male          | Female        | Total         | p       |
|-------------|---------------|---------------|---------------|---------|
|             | N  | Range       | N   | Range       | N   | Range       |       |
| Metabolism  |     |             |     |             |     |             |       |
| Bilirubin, total (µmol/L) | 98  | 12.1 (5.8–36.0) | 154 | 7.5 (4.0–22.4) | 252 | 9.0 (4.4–27.9) | <0.0001 |
| Glucose (mmol/L) | 96  | 4.4 (3.1–5.7)  | 155 | 4.1 (3.2–5.3)  | 251 | 4.2 (3.1–5.5)  | 0.0082  |
| Triglycerides (mmol/L) | 80  | 0.6 (0.3–1.5)  | 154 | 0.6 (0.3–1.4)  | 234 | 0.6 (0.3–1.5)  | 0.3386  |
| Cholesterol (mmol/L) | 84  | 3.6 (2.7–5.6)  | 152 | 3.9 (2.6–5.8)  | 236 | 3.8 (2.6–5.8)  | 0.0196  |
| HDL Cholesterol (mmol/L) | 63  | 1.3 (0.8–1.9)  | 152 | 1.4 (0.9–2.3)  | 215 | 1.4 (0.9–2.2)  | 0.0066  |
| Uric Acid (mmol/L) | 83  | 3.4 (1.7–5.0)  | 155 | 2.1 (1.0–3.3)  | 238 | 2.4 (1.1–4.4)  | <0.0001 |
| Enzymes     |     |             |     |             |     |             |       |
| ALT (U/L)   | 97  | 15.9 (6.5–53.2) | 155 | 11.4 (4.8–38.5) | 252 | 12.9 (5.0–48.2) | <0.0001 |
| AST (U/L)   | 98  | 25.7 (16.8–45.5) | 155 | 20.4 (13.5–37.0) | 253 | 23.0 (13.7–42.8) | <0.0001 |
| ALP (U/L)   | 83  | 157.4 (97.7–266.1) | 148 | 135.3 (91.4–240.6) | 231 | 142.6 (91.1–258.9) | <0.0001 |
| Amylase (U/L) | 79  | 91.9 (51.0–167.0) | 154 | 89.2 (43.8–145.5) | 233 | 89.8 (45.1–190.0) | 0.3929  |
| Serum Proteins |     |             |     |             |     |             |       |
| Albumin (g/L) | 84  | 49.7 (43.4–55.2) | 151 | 47.7 (40.1–52.6) | 235 | 48.4 (40.7–54.1) | <0.0001 |
| Kidney Function |     |             |     |             |     |             |       |
| Creatinine (µmol/L) | 98  | 81.1 (58.2–109.0) | 155 | 65.4 (45.0–86.6) | 253 | 69.0 (47.1–103.2) | <0.0001 |
| Urea (mmol/L) | 84  | 3.8 (1.8–5.8)  | 153 | 2.8 (1.3–5.1)  | 237 | 3.1 (1.3–5.1)  | <0.0001 |

p-values indicate comparisons between males and females.
doi:10.1371/journal.pone.0097391.t005
(4.6%) as grade 2 and 4 (2.6%) as grade 3. The low neutrophil counts would have resulted in 16 AEs: 14 (5.5%) as grade 1 and 2 (0.8%) as grade 2. With respect to the clinical chemistry parameters, ALT would have resulted in 27 AEs, 22 (8.7%) grade 1 and 5 (1.9%) grade 2 and AST would have resulted in 197 AEs, 145 (57.3%) grade 1 and 52 (20.6%) grade 2 (Table 7). Additionally, T-Bil values would have resulted in 6 reported AEs, 5 (1.9%) grade 1 and 1 (0.4%) grade 2. In total, based on all hematological and clinical chemistry parameters, 292 AEs are predicted to have been reported. Notably, 159 (62.8%) of the healthy volunteers included into the present study would have been excluded due to abnormal laboratory values should they have been evaluated for a clinical trial using US based criteria.

Discussion

Clinical laboratory reference ranges have not previously been established in Mozambique. In this study we established reference ranges using samples derived from healthy young adults aged 18–24 years who attended a youth clinic at Maputo Central Hospital. The number of males was disproportionate to the number of females (40.3% vs 59.7%), mainly because a lower proportion of males attended the SAAJ clinic. This affected the recruitment of males into the study, and the target of 120 subjects tested per analyte as recommended by CLSI [18] was not reached despite extending the enrollment period. However, a robust bootstrap analysis was used to eliminate bias due to the small sample size, as recommended by the Canadian laboratory initiative on pediatric reference intervals (CALIPER) [19].

Our immunophenotyping data showed significant gender differences between total lymphocyte counts, absolute T cell counts, absolute count of and percentage of CD4+ T cells and CD8+ T cell percentage, with females having higher values than males in each case. These findings are consistent with those reported for young adults in Kericho, Kenya [22]. Several other studies have also reported that females have higher CD4+ T cell counts than males [4,23–26]. Overall, the reference ranges reported here for lymphocyte subsets were comparable to ranges reported for young adults in the US (Becton-Dickinson, USA) and western Kenya [11].

Due to insufficient access to reagents for natural killer cells and B cells determinations, only 34 males and 104 females were tested for these parameters. Although the number of participants was fewer than the 120 recommended by CLSI, a significant gender difference was seen for percentage of CD16+56+ natural killer cells and absolute count of CD19+ B cells. Lower percentages of CD16+56+ natural killer cells were seen in females compared to males, while absolute CD19+ B cells were lower in males than

| Table 6. Comparison of chemistry reference ranges derived from young adults in Maputo, Mozambique compared with those from western Kenya and the United States of America. |
|---|---|---|
| Analyte | Maputo-Moz | Western Kenya [11] | USA [12] |
| | (18–24 years old) | (18–34 years old) | |
| METABOLISM | | | |
| Bilirubin, total (µmol/L) | | | |
| All | 4.4–27.9 | 5.1–40.7 | 5.1–17.0 |
| Male | 5.8–36.0 | 5.3–50.7 | NA |
| Female | 4.0–22.5 | 5.8–36.1 | NA |
| Glucose (mmol/L) | | | |
| All | 3.1–5.5 | 2.1–6.6 | 4.2–6.4 |
| Cholesterol (mmol/L) | | | |
| All | 2.6–5.8 | NA | <5.17 |
| Triglycerides (mmol/L) | | | |
| All | 0.3–1.5 | NA | <1.8 |
| ENZYMES | | | |
| ALT (U/L) | | | |
| All | 5.0–48.2 | 7.2–61.3 | 0–35 |
| Male | 6.5–53.2 | 12.0–80.6 | NA |
| Female | 4.8–38.5 | 10.7–61.3 | NA |
| AST (U/L) | | | |
| All | 13.7–42.8 | 13.8–50.4 | 0–35 |
| Male | 16.8–45.5 | 12.5–69.3 | NA |
| Female | 13.5–37.0 | 13.5–48.5 | NA |
| Amylase (U/L) | | | |
| All | 43.5–160.4 | NA | 60–180 |
| SERUM PROTEIN | | | |
| Albumin (g/L) | | | |
| All | 40.7–54.1 | NA | 35–55 |
| KIDNEY FUNCTION | | | |
| Creatinine (µmol/L) | | | |
| All | 47.1–103.2 | 50–113 | 0–133 |
| Male | 58.2–109.0 | 54.2–137.8 | NA |
| Female | 45.0–86.6 | 52.4–96.8 | NA |

| NA – Not available. |
| doi:10.1371/journal.pone.0097391.t006 |
Table 7. Frequency of predicted adverse events in the Maputo youth cohort based on a comparison with values from DAIDS.

| Parameter                  | N  | n   | %   | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|----------------------------|----|-----|-----|---------|---------|---------|---------|
| Hemoglobin (g/dL)          |    |     |     |         |         |         |         |
| Male                       | 100| 14  | 14  | 1       | 0.9     | 0       | 0       |
| Female                     | 150| 104 | 69.3| 20      | 13.2    | 7       | 46      |
| Platelets (10^6 cells/l)   | 253| 47  | 18.6| 3       | 1.2     | 4       | 16      |
| WBC (10^6 cells/l)         | 252| 64  | 25.4| 1       | 0.4     | 0       | 0       |
| Neutrophils (10^3 cells/l) | 254| 24  | 94  | 14      | 5.5     | 2       | 0.8     |
| Lymphocytes (10^3 cells/l) | 247| 3   | 12  | 0       | 0       | 0       | 0       |
| T CD4 (Cells/l)            | 226| 4   | 1.8 | 6       | 2.7     | 1       | 0.4     |
| ALT (U/L)                  | 253| 15  | 59  | 22      | 8.7     | 5       | 19      |
| AST (U/L)                  | 253| 14  | 5.5 | 145     | 57.3    | 52      | 20.6    |
| T-Bil (µmol/L)             | 253| 38  | 15.0| 5       | 1.9     | 1       | 0.4     |
| Creatinine (µmol/L)        | 253| 0   | 0   | 0       | 0       | 0       | 0       |
| Glucose (mmol/L)           | 251| 104 | 41.1| 0       | 0       | 0       | 0       |

Division of AIDS (DAIDS) toxicity grading

Number Ineligible

per US Comparison

Parameter N n % n % n % n % n % n %

Hemoglobin (g/dL)

| Parameter                  | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|----------------------------|---------|---------|---------|---------|
| Male                       |         |         |         |         |
| Female                     |         |         |         |         |
| Platelets (10^6 cells/l)   |         |         |         |         |
| WBC (10^6 cells/l)         |         |         |         |         |
| Neutrophils (10^3 cells/l) |         |         |         |         |
| Lymphocytes (10^3 cells/l) |         |         |         |         |
| T CD4 (Cells/l)            |         |         |         |         |
| ALT (U/L)                  |         |         |         |         |
| AST (U/L)                  |         |         |         |         |
| T-Bil (µmol/L)             |         |         |         |         |
| Creatinine (µmol/L)        |         |         |         |         |
| Glucose (mmol/L)           |         |         |         |         |
females. These findings were in general comparable to those reported in two studies performed in Tanzanian adults, which reported significant gender differences in percentage of natural killer cells and both the absolute count and percentage of CD19\(^+\) B cells [6,25]. In both Tanzanian studies, females had a significantly lower percentage of natural killer cells than males, and males had a significantly lower absolute count and percentage of CD19\(^+\) B cells than females. These findings are consistent with a study of US adolescents (13–19 y) in which CD19\(^+\) B cells were reported to significantly decrease in males as age increased [24].

In the present study, we found significant gender differences in red blood parameters (RBC, HB, HCT and MCV), with males having higher values than females; these results are consistent with previous reports [3,4,5,6,11,12,27,28,29]. The reason for this gender difference may be due to the influence of androgen on erythropoiesis and menstrual blood loss in females [4,11,22]. Consistent with previous reports of African populations, a gender difference in platelet counts was seen, with females having higher values than males [11,27,30]. We also noted significant gender differences in total WBC count, again with females having higher values than males. This result is consistent with reports from Uganda [10], Ghana [24] and Kericho, Kenya [22] but differs from reports from Ethiopia [3], Central African Republic [5], and western Kenya [11], where total white blood cell counts did not vary between genders. In a UK study that included women of different ethnic origins (Caucasian, Afro-Caribbean and African) gender differences in total WBC and platelet counts were reported for all ethnic groups; women had higher values than men [31].

Most of the hematological values derived from this study were lower than those derived from the US population, which is consistent with studies of similar age groups conducted in western Kenya and Uganda [4,11]. The lower limits for many parameters in the present study were lower than those derived from those two African countries. Notably, close to 50% of our study participants had Hb values that were outside the lower limits of the range derived from a US population, with 69.3% of females showing Hb values outside of the lower limit. Factors such as poor nutritional status, genetic red blood cell disorders or parasitic infections have been suggested to account for low Hb values. While a fairly low frequency of sickle cell trait (5.6%) was reported in an early study of pregnant women in Mozambique [32], a recent national survey of schistosomiasis and soil-transmitted helminthes showed that parasitic infections are common [33].

Clinical chemistry reference ranges derived in the present study were slightly higher than those from the US, which is consistent with results from Uganda [11]. The Maputo reference values were lower than those reported in western Kenya for the same age group [11].

The clinical reference ranges derived from US MGH have been used in most clinical research studies due to the absence of local ranges. A comparison between values derived from the present study and US MGH values [17] reveals higher variations in most values, especially in hematology. If US MGH ranges were used as inclusion and/or exclusion criteria in potential clinical studies, 159 (62%) of the participants of the present study would be excluded due to abnormal laboratory parameters. However, if the local reference ranges were used, only 40 (16%) of the participants would be excluded. The use of improper reference ranges has been reported to affect the time period of trial enrollment due to the large number of participants that must be screened to reach the requested target sample size. The long enrollment period impacts both workload and study cost [10].

The Division of AIDS (DAIDS) toxicity table for grading adverse events is used in many clinical trials but may not be appropriate for the young adults included in the present study. For example, the lower limit ranges of neutrophil (1200 /μL), Hb (7.7 d/L) and AST (13.7 U/L) would qualify as grade 1, 3 and 1 AEs, respectively. The upper limits for ALT (48.2 U/L) and T-Bilirubin (27.9 μmol/L or 1.63 mg/dl) would be qualified as grade 1 AEs. This is consistent with results found in Uganda [10] and western Kenya [11].

Several limitations are apparent in the design of the present study. Importantly, we did not screen for all medical conditions that might have influenced the laboratory findings. Participants with parasitic infections or with other subclinical conditions may have been included, which may have influenced the results. Secondly, the participants were recruited at a youth health center in Maputo and the findings may therefore not be generalizable to young adults in the rest of Mozambique or other African countries. Despite these limitations, the reference values established here are being used in ongoing HIV-vaccine trials in Maputo that recruit healthy volunteers from the cohort of young adults attending the Maputo Central Hospital Youth Clinic. Additionally, given that indigenous reference values have not been previously defined for any Mozambican population, the ranges defined in this study are suitable for use in assessment of young adults in a more generalized setting.

In conclusion, this study is the first to assess clinical laboratory reference ranges in Mozambique. The hematological and biochemistry reference values from this African population differ from those derived from a North American population. This study also highlights the need for region-specific clinical reference ranges for patient management and clinical research.

Acknowledgments

We extend special thanks to the volunteers who agreed to participate in this study.

Author Contributions

Conceived and designed the experiments: NT IJ CN SA NO EM NS. Performed the experiments: NT EA NS. Analyzed the data: OJ NT. Wrote the paper: NT CN IJ SA. Served as study physicians: EV EG EM. Revised and approved the final version of the manuscript: NT CN IJ SA OJ EV NO EA EM EG NS.

References

1. Mgone CS, Makanga M (2010) Fighting HIV/AIDS, tuberculosis and malaria: one world, one partnership. Tropical Medicine and International Health 15: 973–974.
2. Mgone CS, Salami W (2009) EDCTP: a genuine north–south partnership. Tropical Medicine and International Health 14: 1327–1328.
3. Tsegaye A, Messele T, Tilahun T, Hailu E, Sahlu T, et al. (1999) Immunohematological reference ranges for adult Ethiopians. ClinDiagn Lab Immunol 6: 410–414.
4. Lugada ES, Mermin J, Kaharuza F, Ulvestad E, Were W, et al. (2004) Population-based hematologic and immunologic reference values for a healthy Ugandan population. ClinDiagn Lab Immunol 11: 29–34.
5. Menard D, Mandeng MJ, Tothy MB, Kelembho EK, Gesenguet G, et al. (2003) Immunohematological reference ranges for adults from the Central African Republic. ClinDiagn Lab Immunol 10: 483–485.
6. Saathoff E, Schneider P, Kleinfeldt V, Geis S, Hasle D, et al. (2008) Laboratory reference values for healthy adults from southern Tanzania. Trop Med Int Health 13: 612–625.
7. Omose-Yankeyi G, Jaoko W, Anzala O, Ogutu H, Wakisaka S, et al. (2011) Reasons for inequality in Phase 1 and 2A HIV vaccine clinical trials at Kenya Aids Vaccine Initiative (KAVI), Kenya. PLoS ONE 6: e14580.
8. Clerici M, Butto S, Lukovia M, Sarrealla M, Declich S, et al. (2000) Immune activation in Africa is environmentally-driven and is associated with upregulation of CCR5. Italian-Ugandan AIDS Project. Aids 14: 2083–2092.
9. Stevens W, Kamali A, Karita E, Anzala O, Sanders EJ, et al. (2008) Baseline morbidity in 2,990 adult African volunteers recruited to characterize laboratory reference intervals for future HIV vaccine clinical trials. PLoS ONE 3: e2043.
10. Eller LA, Eller MA, Ouma B, Kataa H, Kyabaggu D, et al. (2008) Reference intervals in healthy adult Ugandan blood donors and their impact on conducting international vaccine trials. PLoS ONE 3: e3919.
11. Zeh C, Anzala O, Osindo P, Osoyo O, at al. (2011). Population-based biochemistry, immunologic and hematological reference values for adolescents and young adults in a rural population in western Kenya. PLoS ONE 6: e21040.
12. Krato A, Ferraro M, Shuss PM, Lewandrowski KB (2004) Case records of the Massachusetts General Hospital. Laboratory reference values. N Engl J Med 351:1548–1563.
13. DAIDS (2004) Division of AIDS table for grading the severity of adult and pediatric adverse events. Bethesda, MD, USA. DAIDS.
14. Melo J, Folgesa E, Manjate D, Osman N, Francois I, et al. (2008) Low prevalence of HIV and other sexually transmitted infections in young women attending a youth counselling service in Maputo, Mozambique. Tropical Medicine and International Health 13: 17–20.
15. Vicente EM, Jermy CA, Schreiner HD (2009) Urban geology of Maputo, Mocambique. Engineering geology of tomorrow’s cities. Geological Society, London, Engineering Geology Special Publication, 22.
16. CMM (2010) Perfil Estatico do Municı´pio de Maputo. In: Socio Economas. Conselho Municipal de Maputo.
17. R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/
18. Clinical and Laboratory Standards Institute (2008) Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline—Third Edition.National Committee for Clinical Laboratory Standards, Wayne, PA, USA C28-A3, Vol.28 No 30.
19. Schnabl K, Chen MK, Gong Y, Adeli K (2008) Closings the Gaps in Paediatric Reference Intervals: The CALIFER Initiative. ClinBiochem Rev 29: 69–96.
20. Coskun A, Ceyhan E, Isal TC, Serteser M, Unsal I (2013) The comparison of parametric and nonparametric bootstrap methods for reference interval computation in small sample size groups. Accreditation and Quality Assurance, 18(1), 51–60.
21. Cherno MB, LaBude R A (2011) An Introduction to Bootstrapping Methods with Application to R, John Wiley & Sons.
22. Kibaya RS, Bautista CT, Sowe FK, Shaffer DN, Sateren WB, et al. (2008) Reference ranges for the clinical laboratory derived from a rural population in Kericho, Kenya. PLoS ONE 3: e3327.
23. Maini MK, Gilson RJ, Chavula N, Gill S, Faloyra A, et al. (1996) Reference ranges and sources of variability of CD4 counts in HIV-seronegative women and men. Genitourin Med 72:27–31.
24. Rudy BJ, Wilson CM, Durako S, Moscicki AB, Muenz L, et al. (2002) Peripheral blood lymphocyte subsets in adolescents: a longitudinal analysis from the REACH project. ClinDiag Lab Immunol 9: 959–965.
25. Utassa WK, Mbena EM, Swei AB, Gaines H, Mhalu FS, et al. (2003) Lymphocyte subset enumeration in HIV seropositive and HIV-1 seropositive adults in Dar es Salaam, Tanzania: determination of reference values in males and females and comparison of two flow cytometric methods. J Immunol Methods 277: 65–74.
26. Oladepo DK, Idigbe EO, Adu RA, Iyang US, Imade GE, et al. (2009) Establishment of reference values of CD4 and CD8 lymphocyte subsets in healthy Nigerian adults. Clin Vaccine Immunol 16: 1374–1377.
27. Dsouw DK, Kayan K, Ahs-Gyasi D, Kraaj E, Ocrau J, et al. (2012) Haematological and biochemical reference values for healthy adults in middle belt Ghana. PLoS ONE 7:e36308.
28. Subhashree AR, Parmaceawari PJ, Shanti B, Revathy C, Parijatham BO (2012) The reference intervals for the haematological parameters in healthy adult population of Chennai, southern India. J ClinDiag Research 6:1675–1680.
29. Wakenan I, Al-Ismail S, Benton A, Beddall A, Gibbs A, et al. (2007) Robust, routine haematology reference ranges for healthy adults. InJnl Lab Hem 29:227–233.
30. Bain B, Seed M, Goodland I (1984) Normal values for peripheral blood white cell counts in women of four different ethnic origins. J ClinPathol 37:108–193.
31. Bain BJ (1996) Ethnic and sex differences in the total and differential white cell count and platelet count. J ClinPathol 49:664–666.
32. Wilcox MG, Liljestrand J, Bergstrom S (1980) Abnormal haemoglobin among pregnant women from Mozambique. J Med Genet 17:151–152.
33. Augusto G, Nalé R, Cosmì V, Sabonete A, Mapaco L, et al. (2009) Geographic distribution and prevalence of Schistosomiasis and soil-transmitted helminthes among schoolchildren in Mozambique. Am J Trop Med Hyg 81:799–803.