
Victor Raúl Gómez Román,*,† Sanne Skov Jensen,‡ Christian Leo-Hansen,a Kristoffer Jarlov Jensen,*,# Christoph Mikkel Janitzek,a Candida Medina Rodrigues,b Sanne Jespersen,b Terese Lea Katzenstein,d David da Silva Té,b and Anders Fomsgaard*#

Department of Virology, Statens Serum Institut, Copenhagen, Denmark; Centro de Tratamento Ambulatório (CTA), Hospital Nacional Simão Mendes (HNSM), Bissau, Guinea-Bissau;‡ The Bandim Health Project, Bissau, Guinea-Bissau; and Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark#

Hematology and biochemistry reference intervals have been derived from healthy, HIV-negative populations to guide clinical trials worldwide. However, it is less clear how such values may be applied to clinical trials involving HIV-infected individuals. We show that contradictory interpretations about patient recruitability are reached when applying African versus North American reference intervals to an HIV-1 cohort in Guinea-Bissau. These observations underscore the need to question non-African guidelines in the context of HIV intervention clinical trials in Africa.

The Republic of Guinea-Bissau (RGB) is a West African country which borders the Atlantic Ocean, Senegal, and the Republic of Guinea. The highest point in the country is an elevation in the east, approximately 300 m above sea level (https://www.cia.gov/library/publications/the-world-factbook/geos/pu.html). RGB has an estimated HIV-1 prevalence ranging from 2% to 4.6% (2, 8). The World Health Organization estimates that 22,000 people are living with HIV in RGB and that less than 40% have access to antiretroviral therapy (ART) (8). Increasing the coverage of ART is therefore a high public health priority, and local clinical trials are being conducted to assess the safety, feasibility, and efficacy of various anti-HIV therapeutic interventions.

A major challenge in conducting clinical trials in RGB is the lack of relevant baseline hematology and biochemistry reference intervals. There is a general need for reference intervals to evaluate routine health assessments among HIV-1-positive individuals in RGB and to guide the rational interpretation of safety and toxicity studies of HIV interventions in the local population. In preparation for a therapeutic HIV vaccine phase 1 clinical trial, a small intention-to-treat cohort of 23 treatment-naïve, HIV-1-positive individuals was established. The objective of this paper is 2-fold: to report baseline biochemistry and hematological parameters in 23 HIV-1-positive individuals and to appraise the need for local intervals in asymptomatic, treatment-naïve, HIV-1-infected individuals in RGB.

As is the case with other studies in African countries, investigators in RGB are confronted with four choices: (i) to use unrelated African hematology and toxicity reference intervals in combination with the DAIDS (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health) toxicity grading scales to identify possible adverse events; (ii) to use reference intervals derived from diverse populations in Central, Eastern, and Southern Africa (5, 9); (iii) to conduct time-consuming baseline studies to assess local reference intervals in a large population; or (iv) to use an iterative "learning-by-doing" pilot study approach to ascertain which of the aforementioned three choices would be most feasible in larger clinical trials.

We have opted for the fourth approach. Approval for setting up this cohort was obtained from the Research Coordination Committee, Ministry of Health, in RGB. Informed consent was obtained from all individuals. HIV-1-seropositive and HIV-2-seronegative, treatment-naïve, clinically asymptomatic individuals with stable CD4+ T cell counts above 350 cells/μl were recruited from a pool of individuals being followed at the Centro de Tratamento Ambulatório (CTA), Hospital Nacional Simão Mendes (HNSM), RGB (Table 1). Although not powered statistically, the six major ethnic groups inhabiting the country were represented among the participants (Table 1). The female/male ratio reflects the population voluntarily attending the HIV clinic, as well as a stronger willingness to participate among the females. Potential candidates were excluded from the study if they were pregnant, had evidence of hepatitis C infection, tuberculosis, or other active chronic diseases, had severe medical conditions, or were in treatment with other experimental drugs. CD4+ T cell counts were determined by standard flow cytometry using a Partec CyFlow SL blue instrument. For biochemistry analyses, we tested sera using a Reflotron Plus chemical analyzer with Roche rapid tests for creatinine, bilirubin, and aspartate aminotransferase (AST) according to the manufacturer’s instructions. For hematology analyses, whole blood was collected in Vacuette K3EDTA tubes (Greiner bio-one) and the blood was analyzed using a Medonic CA530 Oden hematology analyzer (Clinical Diagnostic Solutions, Plantation, FL) and Medonic reagent sets according to the manufacturer’s instructions.

Received 24 March 2012 Returned for modification 27 April 2012 Accepted 29 May 2012 Published ahead of print 6 June 2012

Address correspondence to Anders Fomsgaard, afo@ssi.dk.


V.R.G.R. and S.S.J. contributed equally to this work.

Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/CVI.00170-12
A total of 12 parameters were analyzed (Table 2) during the rainy season from August to September 2010, and the 90% study reference intervals for asymptomatic, HIV-1-positive individuals are presented in Table 2. Limitations in the total number of study individuals (n = 23) and the numbers of females (n = 20) and males (n = 3) restricted us to calculating the 90% reference intervals for both sexes and females, whereas reference intervals could not be calculated for males (Table 2). The lower 90% reference limit was defined as the 5th percentile, while the upper limit was defined as the 95th percentile. Reference intervals for HIV-negative populations from North America (according to the American Medical Association [AMA]) and several African countries are likewise listed in Table 2. Gender-specific intervals are specified when available, and all reported intervals are for adults. In a study from Gambia (1), 90% reference intervals were calculated, whereas the other African studies calculated the 95% reference intervals (Table 2). The reference intervals listed in Table 2 are not directly comparable across studies, but to appraise the necessity of local reference intervals in asymptomatic, treatment-naïve, HIV-1-infected individuals in RGB, we list the number of study individuals (both sexes) with out-of-range (OOR) values for each interval. In our small cohort of HIV-1-positive individuals, bilirubin values were within the intervals observed in various large Eastern and Southern African HIV-negative cohorts (5, 9). All observed values for white blood cells (WBC) were within the reference intervals for Ethiopia (7), whereas 8.7 to 21.7% were outside the intervals reported for the Gambia (1), the Central African Republic (6), Western Kenya (9), and Eastern and Southern Africa (5). Not surprisingly, a high number of study individuals fell OOR for hemoglobin (65.2%) and hematocrit (47.8%) using intervals reported in populations living at high altitude in the Ethiopian highlands (7), whereas very few were OOR for reference intervals reported in other African studies (1, 5, 6, 9). Few of the enrolled individuals (0 to 26.1%) had OOR values for red blood cell count (RBC), absolute lymphocyte count, absolute monocyte count, and AST when using available reference intervals reported from previously published African studies. A frequency of 21.7% of the values for mean corpuscular volume (MCV) was OOR when using Gambian reference intervals (1). For absolute granulocyte counts and creatinine values, 26.1% and 21.7% of study individuals, respectively, were OOR when compared against Western Kenyan reference intervals (9).

When using AMA reference intervals, RBC, hemoglobin, hematocrit, and MCV values were in many cases lower, while AST was higher. Hematocrit and hemoglobin were the most disparate values, with most of the enrolled individuals (91.3%) falling outside the AMA intervals. This mismatch in hematocrit and hemoglobin values may be partially explained by a high proportion of females in our study (87%), as African females have lower hemoglobin and hematocrit levels than males (5, 9), a factor that is not contemplated in the AMA reference intervals (4). Furthermore, when we applied the DAIDS toxicity grading scales (3) to appraise HIV-1-infected individuals in our cohort, many of their values fell into the “adverse events” intervals. This is in agreement with the Eastern and Southern African studies mentioned earlier, in which a significant fraction of healthy, HIV-1-negative volunteers fell into the “adverse events” category due to their preexisting out-of-AMA-range values (5, 9). Misclassifying these individuals as “adverse events” based solely on AMA and DAIDS guidelines would result either in their exclusion from clinical trials or in the filing of a note-to-file to justify a special exemption for the patients to participate.

The disparity between what is “normal” and “abnormal” in terms of reference biochemistry and hematology ranges can be a subject of debate. The ramifications are not just intellectual but also practical and ethical, as this debate is likely to have an impact on how investigators should conduct clinical trials and interpret toxicity data in developing countries where safe interventions are most needed. Again, one view is that investigators should conduct local baseline studies in healthy, HIV-negative populations to be able to fully ascertain whether subsequent interventions are truly nontoxic in those specific populations. But the question of how to use reference intervals in HIV-1-positive populations deserves closer scrutiny. In our view, neither the North American nor the African reference intervals provide a gold standard to guide local studies in RGB. Our data would support the view that local baseline studies indeed need to be conducted in asymptomatic, HIV-positive populations to produce local reference intervals that may guide safety and toxicity studies more rationally. While undertaking such baseline studies can be time consuming and expensive in countries with little infrastructure and constrained resources, it is nonetheless a public health investment. Building capacity to publish baseline biochemistry and hematology studies for both HIV-negative and asymptomatic, HIV-positive populations can further generate country-specific information that local doctors can use as a reference in their habitual clinical practice.
TABLE 2 Clinical laboratory reference intervals and number of individuals from Guinea-Bissau HIV-1 cohort with out-of-range valuesa

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit of measure</th>
<th>90% RI for Guinea-Bissau Gambia (1)</th>
<th>Central African Republic (6)</th>
<th>Ethiopia (7)</th>
<th>Western Kenya (9)</th>
<th>Eastern &amp; Southern Africa (5)</th>
<th>American Medical Association (4)</th>
<th>No. with DAIDS toxicity grade (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females and males (n = 23)</td>
<td>Females (n = 20)</td>
<td>90% RI (Y/Δ)</td>
<td>No. OOR</td>
<td>90% RI (Y/Δ)</td>
<td>No. OOR</td>
<td>90% RI (Y/Δ)</td>
<td>No. OOR</td>
</tr>
<tr>
<td>RBC count</td>
<td>×10¹²/liter</td>
<td>3.7–5.4</td>
<td>3.7–5</td>
<td>NR</td>
<td>NA</td>
<td>4.5–6.1 (Δ)</td>
<td>2</td>
<td>4.3–5.9 (Δ)</td>
</tr>
<tr>
<td>Hb</td>
<td>g/dl</td>
<td>9.5–14.7</td>
<td>9.7–13.2</td>
<td>11.1–16.6 (Δ)</td>
<td>1</td>
<td>3.4–5.4 (Δ)</td>
<td>1</td>
<td>3.7–5.2 (Δ)</td>
</tr>
<tr>
<td>Hct</td>
<td>%</td>
<td>29–44.7</td>
<td>29.6–40.5</td>
<td>NR</td>
<td>NA</td>
<td>39–52 (Δ)</td>
<td>1</td>
<td>41.6–55.1 (Δ)</td>
</tr>
<tr>
<td>MCV</td>
<td>fl</td>
<td>72–94.9</td>
<td>70.9–93.9</td>
<td>78–100 (Δ)</td>
<td>0</td>
<td>NR</td>
<td>NA</td>
<td>28–44 (Δ)</td>
</tr>
<tr>
<td>PLT</td>
<td>×10¹³/liter</td>
<td>153.3–325.8</td>
<td>169.5–327.7</td>
<td>124–397 (Δ)</td>
<td>0</td>
<td>NR</td>
<td>NA</td>
<td>117–382 (Δ)</td>
</tr>
<tr>
<td>WBC count</td>
<td>×10⁹/liter</td>
<td>4.2–9.7</td>
<td>4.1–9.3</td>
<td>124–367 (Δ)</td>
<td>1</td>
<td>2.9–8.3 (Δ)</td>
<td>1</td>
<td>3–9.8 (Δ)</td>
</tr>
<tr>
<td>Absolute lymphocyte count</td>
<td>×10⁹/liter</td>
<td>1.7–4.4</td>
<td>1.6–4.5</td>
<td>1.5–4.2 (Δ)</td>
<td>0</td>
<td>0.1–3.5 (Δ)</td>
<td>1</td>
<td>1–3.5 (Δ)</td>
</tr>
<tr>
<td>Absolute granulocyte count</td>
<td>×10⁹/liter</td>
<td>1.8–4.5</td>
<td>1.7–4.4</td>
<td>1.5–3.7 (Δ)</td>
<td>4</td>
<td>1.1–3.5 (Δ)</td>
<td>3</td>
<td>1.8 (Δ)</td>
</tr>
<tr>
<td>Absolute monocyte count</td>
<td>×10⁹/liter</td>
<td>0.3–0.7</td>
<td>0.3–0.7</td>
<td>0.2–0.7 (Δ)</td>
<td>0</td>
<td>0.2–0.9 (Δ)</td>
<td>0</td>
<td>0–0.6 (Δ)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>μmol/liter</td>
<td>56.8–114.9</td>
<td>56.5–116.2</td>
<td>56.8–114.9</td>
<td>0</td>
<td>0.2–0.7 (Δ)</td>
<td>0</td>
<td>0.2–0.7 (Δ)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>μmol/liter</td>
<td>9.3–21.3</td>
<td>9.6–21.3</td>
<td>9.3–21.3</td>
<td>0</td>
<td>5.3–30.7 (Δ)</td>
<td>0</td>
<td>5.8–36.1 (Δ)</td>
</tr>
</tbody>
</table>

RI, reference interval; OOR, out of range; DAIDS, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health (United States); RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; PLT, platelet or thrombocyte count; WBC, white blood cell; AST, aspartate aminotransferase; NR, not reported; NA, not applicable; NL, not listed as part of DAIDS toxicity grading scales; Δ, in females; Δ, in males; fl, femtoliter. The number of study individuals from Guinea-Bissau (both sexes) with out-of-range values is shown.

c Number of individuals with adverse events when using the upper limit number of AMA intervals to calculate the DAIDS toxicity grading intervals for grades 1, 2, 3, and 4.

c Segmented neutrophils.
ACKNOWLEDGMENTS

We thank the following: all the Bissau Guinean volunteers for participating in this study, Aly Jague and Lorenço Marciano Gomes for support in conducting hematology and biochemistry tests at the Céu E Terras non-government organization (NGO), Bente Østergaard for technical assistance, and Ingrid Karlsson and Lasse Vinner for critical revision of the manuscript.

This work was supported by grants from the Danish International Development Agency (DANIDA) and the European & Developing Countries Clinical Trials Partnership (EDCTP HIV-VAC-RGB) awarded to Anders Fomsgaard.

REFERENCES