Comparison of point-of-care versus laboratory-based CD4 cell enumeration in HIV-positive pregnant women

Landon Myer1, Kristen Daskilewicz2, James McIntyre1,2 and Linda-Gail Bekker3

Abstract

Introduction: Early initiation of antiretroviral therapy (ART) in eligible pregnant women is a key intervention for prevention of mother-to-child transmission (PMTCT) of HIV. However, in many settings in sub-Saharan Africa where ART-eligibility is determined by CD4 cell counts, limited access to laboratories presents a significant barrier to rapid ART initiation. Point-of-care (POC) CD4 cell count testing has been suggested as one approach to overcome this challenge, but there are few data on the agreement between POC CD4 cell enumeration and standard laboratory-based testing.

Methods: Working in a large antenatal clinic in Cape Town, South Africa, we compared POC CD4 cell enumeration (using the Alere Pima™ Analyzer) to laboratory-based flow cytometry in consecutive HIV-positive pregnant women. Bland–Altman methods were used to compare the two methods, including analyses by subgroups of participant gestational age.

Results: Among the 521 women participating, the median gestational age was 23 weeks, and the median CD4 cell count according to POC and laboratory-based methods was 388 and 402 cells/µL, respectively. On average, the Pima POC test underestimated CD4 cell count relative to flow cytometry: the mean difference (laboratory test minus Pima POC) was 22.7 cells/µL (95% CI, 16.1 to 29.2), and the limits of agreement were −129.2 to 174.6 cells/µL. When analysed by gestational age categories, there was a trend towards increasing differences between laboratory and POC testing with increasing gestational age; in women more than 36 weeks’ gestation, the mean difference was 45.0 cells/µL (p = 0.04).

Discussion: These data suggest reasonable overall agreement between Pima POC CD4 testing and laboratory-based flow cytometry among HIV-positive pregnant women. The finding for decreasing agreement with increasing gestational age requires further investigation, as does the operational role of POC CD4 testing to increase access to ART within PMTCT programmes.

Keywords: point-of-care test; CD4 cell count; reliability; pregnancy; HIV; antiretroviral therapy; South Africa.

Introduction

Initiation of lifelong antiretroviral therapy (ART) in eligible pregnant women is a critical intervention both to prevent the mother-to-child transmission (PMTCT) of HIV infection and to promote maternal health [1]. However, identification of ART-eligible pregnant women presents a significant barrier to PMTCT services across much of sub-Saharan Africa [2–4]. In settings where ART-eligibility is based on CD4 cell count, the limited availability of laboratory facilities for CD4 cell enumeration presents a fundamental concern [5]. Even in settings where laboratory services for CD4 testing are available, delays in the transport of specimens and return of results presents hurdles to initiation of ART in women identified as eligible [6].

Point-of-care (POC) CD4 cell count testing has been suggested as one approach to overcome the challenges related to limited laboratory access in many parts of Africa [7]. In adult HIV services, POC CD4 testing may increase the proportion of HIV-positive adults who are successfully referred to ART, with direct benefits in reducing attrition over time [8,9]. For pregnant women, POC CD4 testing in PMTCT services may allow identification of ART eligibility at the first antenatal visit in settings where ART initiation is based on CD4 cell count in pregnancy. This may help reduce important delays to ART initiation in the context of pregnancy where rapid ART initiation is a priority for preventing HIV transmission [10].

Although POC CD4 cell count testing has the potential to enhance the identification of ART-eligible women within PMTCT services, there are few data on the test reliability of POC CD4 cell count testing in pregnancy. Previous evaluations of POC CD4 technologies have suggested reasonable agreement with laboratory-based CD4 cell enumeration (usually based on flow cytometry), with some concerns raised around operational aspects of POC tests in real-world settings [11–14]. In addition, given the haematological changes observed during pregnancy, it is plausible that the reliability of POC testing may vary by gestation age, though data are sparse [15]. We compared POC versus laboratory CD4 cell enumeration in a cross-sectional study of HIV-positive pregnant women in Cape Town, South Africa.
Methods
The study took place in a single large antenatal clinic in Cape Town, South Africa, with a heavy burden of antenatal HIV infection (in 2012 the antenatal HIV seroprevalence was estimated to be 26%). At this clinic, all women making their first antenatal visit receive voluntary counselling and testing for HIV and undergo phlebotomy for routine antenatal screening. Venous specimens for laboratory CD4 testing were taken from HIV-positive women at this first antenatal visit. Routine CD4 cell count enumeration was conducted at the South African National Health Laboratory Services (NHLS) via Beckman-Coulter flow cytometry using panleucogated methodology, the standard of care in this setting and described in detail previously [16]. Results are returned to patients at the clinic 1–2 weeks later.

We implemented the POC Alere Pima™ Analyzer (Alere Health Care, Waltham, Massachusetts, USA) into routine PMTCT services in this setting from June 2012. POC testing was conducted by either a nurse–midwife or a trained counsellor, both working in the PMTCT service, and both trained by the manufacturer. Testing used the same venous specimen that was sent for laboratory CD4 testing, with sampling using capillary tubes supplied by the manufacturer. Test procedures for the Alere Pima Analyzer followed manufacturer’s guidelines, including the use of daily quality control beads and routine machine maintenance. Results are returned to patients 20–30 minutes later.

Data on women’s age, prior ART use and gestation at the time of testing was abstracted from routine health care records, with approval by the Research Ethics Committee of the University of Cape Town. In analysis, we compared laboratory versus POC CD4 test results using Bland–Altman analysis for the mean difference and limits of agreement for laboratory versus POC testing [17]. Further analyses examined the test performance of the Alere Pima Analyzer where a laboratory-based test result < 350 cells/μL was treated as the definition of “true” ART eligibility, with a calculation of corresponding sensitivity, specificity and likelihood ratios. Fisher’s exact tests were used to compare proportions, and t-tests were used to compare means; all statistical tests are two-sided at α = 0.05. Results are presented with 95% confidence intervals (CI) throughout.

Results
A total of 546 women were tested using the POC Alere Pima Analyzer. During this period 629 test runs were conducted, with 61 women requiring multiple attempts at POC testing (four women did not receive POC CD4 testing due to repeated machine errors). The most common errors were related to inadequate or inappropriate specimen collection and/or cartridge use (e.g., air bubbles or debris in the sample; damage to cartridge during operation). Of these women, 521 women also had laboratory testing data available and are included in this analysis; there were no significant differences between those excluded and those included in the analysis. Three machines were used during this time, though the majority of tests (61%) were done with a single machine.

The median age of women participating was 27 years (IQR, 23 to 31), and 13% of women were on ART at the time of testing (Table 1). The median gestational age (among 442 women with data available) was 23 weeks (IQR, 17 to 29), and 44% and 42% of women were tested during the second and third trimesters, respectively. The median POC CD4 result was 388 cells/μL (IQR, 265 to 540; range, 6 to 1144), and the median laboratory CD4 result was 402 (IQR, 280 to 551; range, 21 to 1341). CD4 cell count values did not vary significantly with gestational age or participant demographic characteristics (not shown). Overall, 42% and 39% of women had CD4 cell counts < 350 cells/μL according to POC and laboratory methods, respectively.

The overall correlation between the Alere Pima Analyzer and laboratory-based CD4 cell counts was high (Pearson

Table 1. Characteristics of HIV-positive pregnant women undergoing CD4 cell count enumeration

| Pregnant women (n = 521) | Value or N (%) |
|--------------------------|----------------|
| Median age (IQR), years  | 27 (23–31)     |
| Age categories, years    |                |
| 15–24                  | 159 (31)       |
| 25–30                  | 201 (39)       |
| 31+                    | 150 (29)       |
| Median gestational age (IQR), weeks* | 23 (17–29) |
| Gestational age categories |                |
| <12 weeks               | 55 (12)        |
| 13–18 weeks             | 79 (18)        |
| 19–24 weeks             | 117 (26)       |
| 25–30 weeks             | 89 (20)        |
| 31–36 weeks             | 74 (17)        |
| 36+ weeks               | 27 (6)         |
| On ART at time of testing | 66 (13)   |
| Median laboratory CD4 cell count (cells/μL) | 402 (280–551) |
| Laboratory CD4 result ≤ 350 cells/μL | 203 (39) |
| Median Pima POC CD4 cell count (cells/μL) | 388 (265–540) |
| Pima result ≤ 350 cells/μL | 221 (42) |

*Data on gestation at the time of CD4 cell count enumeration available on 442 of 521 participants.

Figure 1. Scatterplot with best-fit line for relationship between laboratory versus Pima point-of-care CD4 cell enumeration, among HIV-positive pregnant women in Cape Town, South Africa.
testing with increasing gestational age; in women more than
10 weeks' gestation, the mean difference was 45.0 cells/μL
(95% CI, 11.1 to 78.7; p = 0.04).

Table 3 shows the test characteristics of the Alere Pima
Analyzer in detecting laboratory CD4 values < 350 cells/μL,
overall and by participant characteristics. POC CD4 testing
was 92% sensitive and 89% specific in identifying women
with laboratory CD4 count ≤ 350 cells/μL; the overall
likelihood ratio for the test (LR-test) was 97.6. The overall
percent agreement was 90%, and the percent misclassified
was 10%. In the 16 women classified as CD4 ≤ 350 cells/μL
by laboratory testing, but CD4 > 350 cells/μL on the Pima
test, the mean difference (laboratory minus Pima) was 78
cells/μL. When the test characteristic analysis was restricted
to participants with CD4 count < 500 cells/μL according
to laboratory testing, the sensitivity remained constant
(92%), but the specificity of the POC test decreased to 79%
(LR-test, 43.0). The sensitivity observed in women tested
during the first trimester (88%) was slightly lower than
among women tested in the second or third trimesters
(92%), but this difference was not statistically significant
(p = 0.47).

Discussion
These data suggest that in the context of pregnancy, the
POC Alere Pima Analyzer appears to slightly underestimate
laboratory-based flow cytometry in CD4 cell enumeration. In
keeping with this, the overall sensitivity of this POC test in
detecting women who are ART-eligible based on laboratory
CD4 cell counts ≤ 350 cells/μL is high (92%). These data
point to the potential role that this POC CD4 test could play
in enhanced identification of ART-eligibility within PMTCT
services.

This is the largest evaluation to date of POC CD4 testing
in HIV-positive pregnant women, using venous blood speci-
mens in the Alere Pima Analyzer rather than capillary
blood. In smaller studies using capillary blood specimens
from pregnant women, similar mean biases were document-
ed (20.5 and 37.9 cells/μL for laboratory minus POC testing,
compared to 22.7 cells/μL in these data) [11,15]. Generally,

Table 2. Results of Bland–Altman analysis, overall and by participant subgroups, comparing laboratory versus Pima point-of-care CD4 cell enumeration, among HIV-positive pregnant women in Cape Town, South Africa

|                          | Mean difference | 95% CI     | Limits of agreement | p Value for variance |
|--------------------------|-----------------|------------|---------------------|----------------------|
| All patients             | 22.7            | 16.1 to 29.2 | −129.2 to 174.6     | <0.001               |
| Age categories, years    |                 |            |                     |                      |
| 15–24                    | 35.1            | 23.7 to 46.6 | −109.6 to 179.8     | 0.015                |
| 25–30                    | 14.9            | 3.9 to 25.9  | −140.9 to 170.7     | 0.001                |
| 31+                      | 23.0            | 10.1 to 35.8 | −132.6 to 178.5     | 0.654                |
| Trimester at time of testing |               |            |                     |                      |
| 1st                      | 6.5             | −16.0 to 29.0 | −159.9 to 172.9     | 0.786                |
| 2nd                      | 20.6            | 10.2 to 31.0 | −127.4 to 168.6     | 0.001                |
| 3rd                      | 32.4            | 21.4 to 43.4 | −121.8 to 186.6     | 0.005                |
| On ART at time of testing |                 |            |                     |                      |
| Yes                      | 21.8            | 5.6 to 38.0  | −108.0 to 151.5     | 0.265                |
| No                       | 23.1            | 15.7 to 30.4  | −133.2 to 179.3     | <0.001               |

Figure 2. Bland–Altman plot comparing laboratory versus Pima point-of-care CD4 cell enumeration, among HIV-positive pregnant women in Cape Town, South Africa (mean difference, 22.7 cells/μL; limits of agreement, −129.2 to 174.6).
All patients 92% (88%–95%) 89% (85%–93%) 8.6 0.09 97.6

Age categories, years

| Group       | Sensitivity  | Specificity  | LR + | LR − | LR test |
|-------------|--------------|--------------|------|------|---------|
| 15–24       | 98% (89%–99%) | 89% (82%–94%) | 9.2  | 0.02 | 379     |
| 25–30       | 91% (83%–96%) | 91% (84%–96%) | 10.2 | 0.10 | 103     |
| 31+         | 91% (81%–97%) | 87% (78%–93%) | 7.1  | 0.11 | 66      |

Trimester at time of testing

| Group       | Sensitivity | Specificity | LR + | LR − | LR test |
|-------------|-------------|------------|------|------|---------|
| 1st         | 88% (68%–97%) | 87% (70%–96%) | 6.8  | 0.14 | 47      |
| 2nd         | 92% (84%–97%) | 90% (83%–95%) | 9.1  | 0.09 | 106     |
| 3rd         | 92% (83%–97%) | 88% (81%–93%) | 7.7  | 0.09 | 83      |

On ART at time of testing

| Group       | Sensitivity | Specificity | LR + | LR − | LR test |
|-------------|-------------|------------|------|------|---------|
| Yes         | 92% (79%–98%) | 63% (42%–81%) | 2.5  | 0.12 | 20.4    |
| No          | 92% (87%–96%) | 92% (88%–95%) | 11.2 | 0.09 | 128     |

LR + : positive likelihood ratio; LR − : negative likelihood ratio; LR test: likelihood ratio for the test (diagnostic odds ratio).
women (“Option A”), which remains the standard of care in many settings. POC CD4 testing may play an important role in PMTCT programmes in two different settings. First, POC CD4 testing provides a practical alternative in settings where there is no regular access to laboratory-based CD4 cell enumeration. Second, in settings that have access to laboratory CD4 cell enumeration but where delays in providing CD4 results hinder PMTCT services, POC CD4 testing may provide a rapid testing strategy: by determining eligibility on the same day as HIV testing, it may be possible to expedite substantially ART initiation. These data suggest sufficient agreement between the Pima POC CD4 test and laboratory-based testing for either of these roles, but additional research is required to understand the role that POC CD4 testing can play in each of these settings. In addition, from an operational perspective, we found that 83 additional tests were required on the Alere Pima Analyzer to provide CD4 results on 546 women, leading to an excess testing proportion of approximately 15%. This error rate is consistent with a previous evaluation of the Pima CD4 test in an antenatal setting (ranging from 10 to 20%) [11]. The error rate of POC CD4 testing is not widely reported but has implications in terms of both resource requirements and patient care, and this additional “hidden” cost should be included in future economic evaluations of POC CD4 testing [24].

In summary, these data suggest reasonable overall agreement between Pima POC CD4 testing and laboratory-based flow cytometry among HIV-positive pregnant women. The preliminary findings for decreasing agreement with increasing gestational age require further investigation, as does the operational role of POC CD4 testing to increase access to ART within PMTCT programmes.

Authors’ affiliations
1 School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa; 2 Anova Health Institute, Johannesburg, South Africa; 3 Desmond Tutu HIV Centre, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa

Competing interests
The authors declare no competing interests.

Authors’ contributions
LM and JM designed the study. KD and LM collected data and conducted data analysis. LM drafted the manuscript. All authors contributed to the writing of the manuscript and approved the final version.

Acknowledgements
LM is funded by an International Leadership Award from the Elizabeth Glaser Paediatric AIDS Foundation.

References
1. Mofenson LM. Prevention in neglected subpopulations: prevention of mother-to-child transmission of HIV infection. Clin Infect Dis. 2010; 50(Suppl 3):S120–48.
2. Sinnot K, Boulel A, Coetzee D, Abrams EJ, Myer L. Initiation of highly active antiretroviral therapy among pregnant women in Cape Town, South Africa. Trop Med Int Health. 2010;15(7):825–32.
3. Weinig R, Hosseinipour MC, Feldacker C, Gareta D, Twwey H, Chiwoko J, et al. Ensuring HIV-infected pregnant women start antiretroviral treatment: an operational cohort study from Lilongwe, Malawi. Trop Med Int Health. 2012; 17(6):751–9.
4. Ferguson L, Lewis J, Grant AD, Watson-Jones D, Vusha S, Ong’ech JO, et al. Patient attrition between diagnosis with HIV in pregnancy-related services and long-term HIV care and treatment services in Kenya: a retrospective study. J Acquir Immune Defic Syndr. 2012;60(3):e90–7.
5. World Health Organization. Global HIV/AIDS response: epidemic update and health sector progress towards universal access: progress report 2011. Geneva: WHO; 2011.
6. Myer L. Initiating antiretroviral therapy in pregnancy: the importance of timing. J Acquir Immune Defic Syndr. 2011;58(2):125–6.
7. Zachariah R, Reid SD, Challet P, Massaquoi M, Schouten EJ, Harris JF. Viewpoint: why do we need a point-of-care CD4 test for low-income countries? Trop Med Int Health. 2011;16(1):37–41.
8. Jani IV, Siteo NE, Alafi ER, Chongo PL, Quevedo J, Rocha BM, et al. Effect of point-of-care CD4 cell count tests on retention of patients and rates of antiretroviral therapy initiation in primary health clinics: an observational cohort study. Lancet. 2011;378(9802):1572–9.
9. Larson BA, Schnippel K, Ndibongo B, Xulu T, Brennan A, Long L, et al. Rapid point-of-care CD4 testing at mobile HIV testing sites to increase linkage to care: an evaluation of a pilot program in South Africa. J Acquir Immune Defic Syndr. 2012;61(2):e83–7.
10. Myer L, Zulliger R, Black S, Pienaar D, Bekker LG. Pilot programme for the rapid initiation of antiretroviral therapy in pregnancy in Cape Town, South Africa. AIDS Care. 2012;24(8):896–92.
11. Glenncross DK, Coetzee LM, Faal M, Massango M, Stevens WS, Venter WF, et al. Performance evaluation of the Pima™ point-of-care CD4 analyser using capillary blood sampling in field tests in South Africa. J Int AIDS Soc. 2012;15(1):3.
12. Faal M, Naidoo N, Glenncross DK, Venter WD, Osh R. Providing immediate CD4 count results at HIV testing improves ART initiation. J Acquir Immune Defic Syndr. 2011;58(3):e54–9.
13. Sukapirom K, Onnamoon N, Thepthai C, Polisila K, Tassaneenthibhop B, Pattanapanayat K. Performance evaluation of the Alere PIMA CD4 test for monitoring HIV-infected individuals in resource-constrained settings. J Acquir Immune Defic Syndr. 2011;58(2):141–7.
14. Matipuri Zinyawera S, Chideme M, Mangwanya D, Mugurungi O, Guduleya S, Katzold K, et al. Evaluation of the PIMA point-of-care CD4 analyzer in VCT clinics in Zimbabwe. J Acquir Immune Defic Syndr. 2010;55(1):1–7.
15. Mnyani CN, McIntyre JA, Myer L. The reliability of point-of-care CD4 testing in identifying HIV-infected pregnant women eligible for antiretroviral therapy. J Acquir Immune Defic Syndr. 2012;60(9):260–4.
16. Glenncross DK, Janossy G, Coetzee LM, Lawrie D, Aggett HM, Scott LE, et al. Large-scale affordable PassiveLigated CD4+ testing with proactive internal and external quality assessment: in support of the South African national comprehensive care, treatment and management programme for HIV and AIDS. Cytomtery B Clin Cytom. 2008;74(Suppl 1):S40–51.
17. Altmann DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. Statistician. 1983;32:307–17.
18. Van Schaik N, Kranzer K, Myer L, Radithalo E, Thebus E, Davies N, et al. Field validation of the PIIMA analyzer in a mobile clinic setting in South Africa. Poster 9673 presented at the 18th Conference on Retroviruses and Opportunistic Infections; 2012 Feb 27–Mar 2; Boston, MA.
19. Schouten EJ, Jahn A, Midiani D, Makombe SD, Mthambala A, Chirwa Z, et al. Prevention of mother-to-child transmission of HIV and the health-related millennium development goals: time for a public health approach. Lancet. 2011;378(9767):282–4.
20. Elouevi DK, Inwoley A, Torwe-Gold B, Danel C, Becquet R, Viho I, et al. Variation of CD4 count and percentage during pregnancy and after delivery: implications for HAART initiation in resource-limited settings. AIDS Res Hum Retroviruses. 2007;23(12):1469–74.
21. Gelson E, Oguche O, Johnson M. Cardiovascular Changes in Normal Pregnancy. London: RCOG Press; 2006.
22. Blackburn ST, Loper DL, Maternal, fetal, and neonatal physiology: a clinical perspective. Philadelphia: WB Saunders; 1992.
23. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommenda- tions for a public health approach. June 2013. Geneva: WHO; 2013.
24. Larson B, Schnippel K, Ndibongo B, Long L, Fox MP, Rosen S. How to estimate the cost of point-of-care CD4 testing in program settings: an example using the Alere Pima Analyzer in South Africa. PLoS One. 2012;7(4):e35444.