The routine paediatric human immunodeficiency virus visit as an intervention opportunity for failed maternal care, and use of point-of-care CD4 testing as an adjunct in determining antiretroviral therapy eligibility

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South African women and children remain at the centre of the human immunodeficiency virus (HIV) pandemic, and maternal well-being plays a critical role in child health. In this study, we evaluated the impact of the paediatric visit on the determination of antiretroviral therapy (ART) eligibility in mothers using Alere Pima™ Analyser (Pima™) testing as an adjunct to routine care. Fifty-two mothers who had failed to obtain routine CD4 screening were enrolled during the paediatric visit at Tygerberg Children's Hospital between November 2011 and May 2012. Clinical staging assessments and parallel standard CD4 cell count testing were performed. Finger-prick samples for the Pima™, and simultaneous venous samples for reference flow cytometry, were obtained. The Pima™ identified 37% of mothers as meeting ART eligibility versus 35% using the reference flow cytometry method. An additional 4% of mothers were identified using World Health Organization clinical staging only. The sensitivity of the Pima™ was 89%, specificity 91%, and positive and negative predictive values 84% and 94%, respectively. These results indicate that the paediatric HIV care visit can provide a valuable additional intervention opportunity to identify mothers in need of ART, with point-of-care CD4 technologies being used as a meaningful adjunct in screening for ART eligibility.

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Introduction

An estimated 5.6 million South Africans are living with human immunodeficiency virus (HIV infection). With a national, prevalence of 29.5% and over 460 000 HIV-infected children in South Africa,[1] there is an urgent need to address the health of women and children who remain at the centre of the pandemic in terms of vulnerability, transmission and potential for intervention.

The mother is usually the primary or sole caregiver in Africa, and it is well documented that maternal well-being plays a critical role in the physical and mental health of children.[2,3] This is supported by studies in Uganda which suggest that maternal HIV infection increases the mortality risk of their children.[4,5] Hence the care of infants and children should incorporate the whole family unit, with particular emphasis on the mother. Apart from the impact of an estimated 1.2 million maternal acquired immune deficiency syndrome (AIDS) orphans in South Africa,[6] direct and indirect consequences of maternal ill health need to be considered. Importantly, the caregiver plays a critical role in achieving success with the child's HIV care by preventing virological failure and resistance through adherence to lifelong medication.

Early and accurate identification of mothers in need of antiretroviral therapy (ART) is one of the cornerstones of preserving maternal health. The CD4 test remains the gold standard surrogate marker of the immunological health of HIV-positive patients prior to and while on ART, and guides decisions on when to initiate and adjust treatment.[7-9]

Recently, point-of care (POC) CD4 analysers, such as the Alere Pima™ Analyser (Pima™), have been developed, with onsite finger-prick CD4 testing which produces rapid results within 20 minutes. Hence, POC testing may provide a valuable supplementary option to determine CD4, especially in rural settings where delays in CD4 count turnaround times or lack of availability of the test, result in failure to initiate ART and/or patient loss to follow-up.

We evaluated the paediatric HIV care visit as an opportunity to identify maternal ART eligibility in cases where mothers had missed their routine CD4 screening. To prevent further loss to follow-up, we used the Pima™ to obtain the result immediately, and compared and controlled this with a traditional laboratory CD4 test.

Method

This cross-sectional descriptive prospective study was conducted by researchers at the HOPE Cape Town Association and Trust, a non-governmental organisation (NGO) within Tygerberg Children's Hospital. Study participants were known to be the HIV-positive mothers of children who were either attending appointments at Tygerberg Hospital, or who were admitted to the Infectious Diseases Ward. The caretaker had to be an HIV-positive biological mother to be included in the study. Exclusion criteria were mothers who were already on ART, those already known to be eligible for ART, and those with a CD4 count of more than 500/μl within the preceding six months. The Western Cape Department of Health (DOH) ART guidelines, based on the South African national DOH

ART guidelines, were used to define the eligibility criteria for starting ART.[10] These criteria included a CD4 threshold of 350 cells/µl or less, the presence of tuberculosis, or a World Health Organization (WHO) clinical stage IV illness, regardless of CD4.

Participants were recruited by the HOPE Cape Town Association and Trust medical doctor from November 2011 to May 2012. A convenience sample of 52 patients was chosen. Each participant gave written consent to undergo a basic medical examination to clinically stage the HIV progression, according to the WHO clinical staging of HIV/AIDS for adults and adolescents,[11] and to have blood taken. Two blood samples were taken: a finger-prick capillary sample for the POC CD4, and a venous sample for the laboratory panleukogated (PLG) CD4, the CD4 reference standard for the study.[12]

The Pima™ was used to determine the finger-prick results. Prior to testing, three NGO staff members involved in operating the Pima™ were trained in finger-prick methodology and instrument use by the supplier, as prescribed by the manufacturer. Daily quality control assessments were performed on the Pima™ with the manufacturer-supplied reusable bead-filled cartridges (high and low controls). Finger-prick samples were obtained with Sarstedt lancets with a 1.5 mm blade. Self-contained disposable Pima™ test cartridges, with an integrated capillary to capture 5 *µl* of sample were used. The participants' CD4 results from the Pima™ were determined and interpreted within 20 minutes.

Venous blood was collected simultaneously by phlebotomy in a 4 ml ethylenediaminetetraacetic acid (EDTA) tube for the routine method of care (PLG) laboratory CD4 determination, and analysed using standardised protocols on a Beckman Coulter Flow Cytometer 500 MPL® (FC 500 MPL®) at the National Health Laboratory Services (NHLS), Tygerberg. Daily internal quality controls and bimonthly external quality control samples from the CD4 reference centre are run for this method. The results of external quality assurance results have to be within two standard deviations (SDs). The Tygerberg NHLS immunology laboratory is accredited with South African national accreditation systems. The laboratory staff was blinded to the Pima™ CD4 results. The FC 500 MPL® CD4 results were received via e-mail by the NGO doctor the following day, and then faxed to the participants' respective clinic. In addition, each participant received a letter addressed to her clinic, containing her WHO clinical staging and Pima™ CD4 result. This letter explained that the Pima™ results were not yet validated, and treatment decisions and direct comparisons to previous CD4 results should be based solely on the confirmatory laboratory CD4 result. This aimed to prevent the use of POC results to determine ART was necessary.

Clinical data were recorded in a secure Microsoft® Excel® database and Statistica® version 10 was used to analyse and display the data. The median values, together with the interquartile range (IQR), were calculated for the Pima™ and FC 500 MPL® CD4 results. To determine the limits of agreement between data obtained from the Pima™ CD4 analyser and those obtained from the FC 500 MPL® laboratory method, the Bland-Altman statistical bias analysis was performed by graphically plotting the difference between each data pair against the average of the pair methods. The average absolute difference between the two methods (the bias) and the limits of agreement (equivalent to the mean difference ± 1.96 SD) was then calculated.[13] Further supporting statistical analysis was performed using the percentage similarity (% SIM) model analysis, whereby the average between a new method and the gold standard is represented as a percentage of the gold standard. The representation of the percentage similarity values in a histogram format draws attention to the accuracy and precision of the compared method to a gold standard. The calculation of a coefficient of variation further defines agreement between methods.[14]

The Committee for Health Research Ethics at Stellenbosch University approved the study (N11/09/269) and the South African Medical Research Council and Declaration of Helsinki guidelines on the conduct of clinical research were followed.

Results

Fifty-two patients were recruited to the study. The results of two patients had to be excluded from the direct comparison analysis owing to a "no read/invalid" CD4 reading on the Pima™ analyser in one case, and the inability to perform analysis using the FC 500 MPL® because of a clotted venous blood sample in the other.

According to the Pima™ CD4 analyser results, 37% (19 of 51) of mothers were identified with a CD4 count of 350 cells/µl or less, meeting the eligibility criteria for the initiation of ART. This compared to 35% (18 of 51) of mothers similarly identified by the FC 500 MPL® CD4 method. Five sample results were discrepant, with three out of 19 POC samples giving false positive results for ART eligibility, and two of the 18 FC 500 MPL® CD4 samples being false negatives. Furthermore, an additional 4% (two of 51) of mothers were eligible for ART based on their clinical assessment alone [WHO stage III (pulmonary tuberculosis)], despite having CD4 > 350 cells/*µl* according to the FC 500 MPL® results. It is noteworthy that 100% of mothers who were eligible for ART (based on their laboratory CD4 counts) were WHO clinical stage I or II.

The median Pima™ CD4 result (n = 51) was 420 cells/*µl* (IQR 310-631), while the corresponding median FC 500 MPL® CD4 result (n = 51) was 394 cells/*µl* (IQR 264-535). The median difference was 11.5 cells/*µl* (IQR –29 to + 68).

As shown in Table I, the sensitivity of the Pima™ in detecting CD4 ≤ 350 cells/µl (as defined by the FC 500 MPL® CD4 result) was 89% [95% confidence interval (CI): 74-100], the specificity 91% (95% CI: 81-100), and the positive and negative predictive values 84% (95% CI: 68-100) and 94% (95% CI: 85-100).

Table I: Sensitivity and specificity based on CD4 threshold of 350 cells/µl

	FC 500 MPL® CD4		
	≤ 350 cells	> 350 cells	Total
Pima™ CD4			
≤ 350 cells	16 (89%)	3 (9%)	19
> 350 cells	2 (11%)	29 (91%)	31
Total	**18**	**32**	**50**

Beckman Coulter Flow Cytometer 500 MPL®, FC 500 MPL®: Pima™: Alere Pima™ Analyser

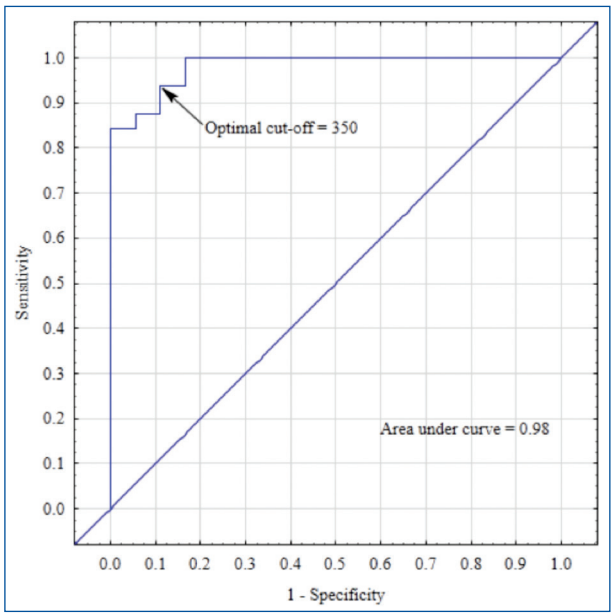


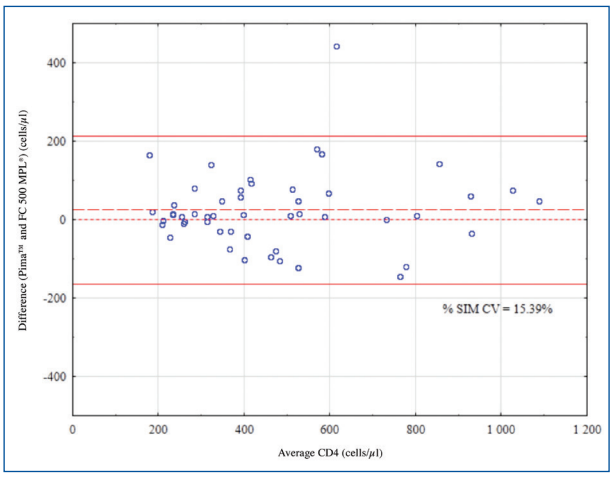
Figure 1: The results of the receiver operating curve analysis, showing the optimal Pima™ value to be 350 cells/µl

Receiver operating characteristic curve analysis was also performed, whereby potential values from the sample group were considered to be potential values that would best relate to a PLG cut-off of ≤ 350 cells/µl. The Pima™ value with the best combination of sensitivity and specificity in predicting a PLG cut-off of ≤ 350 cells/µl (ART eligibility) was fortuitously found to be exactly 350 cells/µl. Figure 1, showing an area under the curve of 0.98, shows how the results match those in Table I.

The mean bias of the Pima™ CD4 analyser was +23.86 cells/*µl* (95% CI: –3.6 to +51.4) relative to the FC 500 MPL®, with 95% limits of agreement from –166.1 cells/*µl* to +213.8 cells/*µl* (*Figure* 2). Additional % SIM analysis (Figure 2) showed similar results (n = 50, mean % SIM 104.8% ± SD = 16.1%). % SIM coefficient of variation (CV) showing precision of Pima™ to FC 500 MPL® was 15.39% (Figure 3).

Discussion

The findings in this study document the value of the routine paediatric HIV visit as an intervention opportunity to assess maternal ART eligibility. More than one third of mothers who had previously missed their routine CD4 screening opportunities were identified as requiring ART. This represents a significant number of mothers who would have been lost within the healthcare system. At the paediatric visit, the focus of the healthcare worker and the mother is on the child's health, with the mother's own health often being overlooked until symptoms of illness occur. A study that was conducted in KwaZulu-Natal showed that children of women who were HIV-infected and not initiated on ART were four times more likely to die than children of uninfected mothers.[15] Expanding the focus of the paediatric visit to include the preventative health care of the child's mother, with an emphasis on ART eligibility, ensures that an important gap in HIV care is addressed. Moreover, the Joint United Nations Programme on HIV/AIDS has recognised the importance of integrating maternal HIV interventions into child health services.[16]



% SIM CV: percentage similarity coefficient of variation
Mean difference: 23.86
Mean - 1.96 standard deviation = -166.09
Mean + 1.96 standard deviation = 213.82

Figure 2: Bland-Altman plots were analysed by plotting the difference between the absolute CD4 T-lymphocyte values generated using the Pima™ CD4 analyser and those from the FC 500 MPL® CD4 system, against the mean absolute values of the paired systems (n = 50)

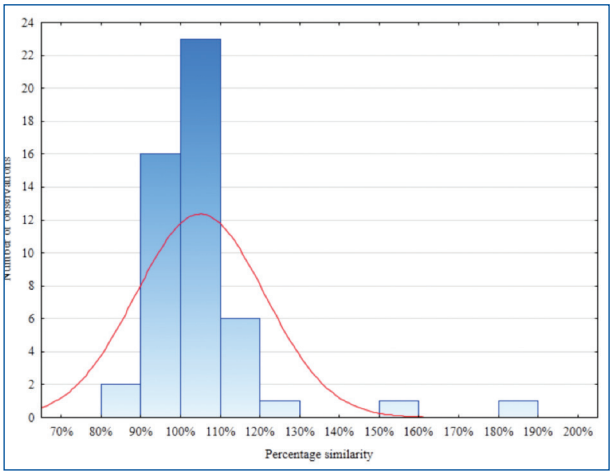


Figure 3: Percentage similarity plot of absolute CD4 T-lymphocyte count deriving from the Pima™ CD4 analyser and from the FC 500 MPL® CD4 system

Our results show that 100% of the mothers who were eligible for ART, based on their laboratory CD4 counts, were clinical stage I or II. These results concur with those of other studies which demonstrated that clinical staging alone was not sufficient or reliable in ascertaining ART eligibility, and that it frequently correlated poorly with immunological markers.[7-9] However, clinical assessment and staging should not be completely discounted. In our study, two mothers whose laboratory CD4 counts were above the initiation threshold of 350 cells/ul (508 cells/µl and 452 cells/µl) were still in need of ART based on their clinical staging [WHO clinical stage III (tuberculosis]). Interestingly, in both cases, the Pima™ CD4 analyser reported CD4 results lower than the laboratory results (414 cells/ul and 350 cells/µl, respectively), thereby classifying one of these patients as requiring ART. This overall finding re-emphasises the importance of a routine clinical assessment and staging, as an adjunct to CD4 count staging.

POC diagnostics present a further opportunity, particularly in resource-limited settings, to provide early access to screening and testing, and may have the potential of improving ART initiation rates, reducing the number of clinic visits and preventing possible loss to follow-up. However, the uptake of such technology has been slow. This is because of a current lack of policy in the public health sector for POC CD4 testing, combined with limited guidance on optimal implementation strategies and the challenges of ensuring test quality, as testing is decentralised to more rural settings. The African Society for Laboratory Medicine is in the process of addressing and overcoming these difficulties, and it is likely that in the near future, POC systems will be incorporated, in some form, into our healthcare systems.[17] In the South African context, difficulties with screening, testing, initiating ART and retaining patients in care are multifactorial, and it is important to acknowledge that POC technologies alone may have an important, but limited, role in providing solutions to these.

Larger studies that were conducted in Thailand, Senegal and South Africa, where the primary focus was on Pima™ CD4 analyser validation, showed the Pima™ system to be a simple and reliable method of screening HIV-positive adults in resource limited-settings.[18-20] Unlike conditions in these studies whereby different variables were controlled, such as using EDTA venous, instead of finger-prick, blood samples, and ensuring use of the same machines and operators, our study was designed to reflect a realistic "add-on to routine care" situation in which the Pima™ is likely to be used in South Africa. It is important to note that other studies in this realistic operation framework have shown that formal practical training on capillary blood sampling and rigid adherence to operating protocol is imperative in ensuring the accuracy of the Pima™ CD4 results.[20,21] This is a critical step, required to ensure the success of POC services, particularly in rural settings where skills, support and monitoring may be limited. It would be necessary to identify and nominate specific personnel in clinics to undergo specialised, preferably onsite, capillary blood sampling training. This would need to be sustained with ongoing training, and reinforced with strict monitoring protocols. However, it is encouraging to see that under well supported conditions, as a screening test for ART eligibility, the Pima™ has repeatedly shown good sensitivity and predictive values in studies in Uganda, South Africa and Zimbabwe.[22-24]

Validation of the Pima™ CD4 analyser was a secondary objective in our study, and it showed promising results, with a reasonable sensitivity, specificity and a clinically acceptable mean bias of +23.86 cells/*µl*, compared to the gold standard. Even though the limits of agreement seemed to be relatively wide (a total of 379 cells/µl), they were able to contend reasonably well with similar local field studies, where limits of agreement ranged from an acceptable total of 272 cells/µl to an unacceptable total of 635 cells/µl.[21] Unlike other studies that have shown increasing bias with increasing CD4 count results, our study did not reveal a noticeable change in Pima™ CD4 analyser accuracy at a higher or lower CD4 count range.[18,20,25] This may be problematic in lower, clinically relevant CD4 ranges (300-400 cells/µl) where a variation in results may result in the misclassification of ART eligibility. This may need to be considered when making recommendations.

Further analysis of Pima™ precision showed a % SIM CV of 15.39%, which fell within acceptable levels in similar local studies (varying from 17.6-28.8% CV), but neither attained optimal precision levels (10-11.3% CV).[21] Despite this relative imprecision when compared to laboratory based testing, it is important to highlight that with the sensitivity of 89% and specificity of 91% obtained in our study, very few patients were missed at the 350 cells/ul-threshold for ART initiation. Thus, overall, Pima™ fulfilled an essential role in making patients aware of their ART eligibility status at the time of their consultation visit, with minimal delay.

Notably, the high negative predictive value of 94% highlights the potential usefulness of the Pima™ CD4 as a "rule out" test. Based on this, it could be recommended that if the Pima™ returned a result of greater than 350 cells/µl, then the patient would need only be followed-up for a repeat CD4 test in six months' time. Whereas, with a positive predictive value of only 84%, if the Pima™ result was less than 350 cells/µl, additional confirmatory laboratory CD4 testing would be required. In addition to this, it could be advocated that the Pima™ result be given as an "above" or "below" current ART eligibility threshold, rather than a numerical value. This would ensure that the Pima™ result was used in the correct context of assessing ART eligibility, rather than the potentially misleading situation of direct comparison with a prior or subsequent laboratory CD4 result. This study did not evaluate the Pima™ CD4 analyser as a tool to monitor response to treatment. In this regard, greater emphasis is now being placed on the use of viral loads, rather than the CD4 count.[26] Further research is vital to evaluate performance in the field of this and other emerging POC tests.

Importantly, the perception by healthcare workers and attending doctor involved in this study was that the immediate, tangible CD4 result at the time of the clinical visit significantly aided patient education, and understanding and motivation with respect to access to clinic care. However, our study was not designed to comment on the impact of subsequent ART uptake, or on the successful referral and ART initiation pattern. Positive impact from studies in Mozambique and South Africa was documented which showed that instantaneous CD4 results at the time of HIV testing improved ART initiation rates, and could have assisted with retention in care.[27-29] It is worth noting that in current circumstances, even with POC CD4 testing, patients who qualify for ART will still be required to pay additional visits to the clinic for a review of other necessary test results, such as tuberculosis screening and renal function tests. For this reason, it may be worthwhile researching additional innovative methods that focus on behavioural interventions, such as SMS communications, which could potentially reduce time spent visiting the clinic.

Although the Pima™ CD4 analyser produces results relatively rapidly, it would still be regarded as a time-consuming procedure in the context of a busy paediatric clinic. Depending on the prevalence of HIV-infected mothers who miss their routine CD4 screening, the CD4 testing could become rate limiting in terms of how many patients could be seen. In such a case, it would be more practical to implement a nurse-driven service to ensure that maternal Pima™ CD4 testing is performed prior to the doctor's consultation. In turn, many factors, such as excessive patient load, lack of adequately trained staff, and quality and stock control issues,

might negatively influence the feasibility of such a practice. The clinical setting is likely to influence whether or not the benefits of integrating this POC testing would outweigh implementation difficulties. The ideal would be to provide a "one-stop shop" in rural areas where patients are required to travel vast distances to visit the clinic. Mothers could have instant access to their CD4 results at the same time as attending their child's appointment, negating the need for them to return on a separate occasion to obtain their CD4 results. Careful thought and planning around efficient patient flow could mitigate problems surrounding the time required to complete a POC CD4 test.

A technical limitation of the Pima™ device is that it does not provide values for a CD4 T-lymphocyte count percentage. Conventionally, this percentage value is used more in paediatric patients, because it varies less with age than the absolute count, and this restriction would have to be a consideration before the incorporation of POC systems into paediatric clinics. However, it is noted that in recent studies, more emphasis is being placed on the absolute CD4 cell count as a prognostic indicator of short-term disease progression in children.[30,31]

This study did not address the cost-effectiveness of the Pima™ POC system. Further controlled, cost-benefit field studies, preferably in the community in a primary or secondary setting, are needed to evaluate the Pima™ POC device, as well as other POC devices, in a comparison with current delivery methods. This would ensure the most efficient use of resources.

Conclusion

Our study showed that the routine paediatric HIV care visit provides a valuable, additional intervention opportunity to identify a break in maternal HIV care. Point-of-care CD4 technology can meaningfully supplement screening for ART eligibility, particularly with reference to the exclusion of patients who are not eligible for ART. With good quality control of the POC deployment, the Pima™ CD4 could have a valid place as a low-cost, first-line screening tool, requiring a confirmatory laboratory test if found to be in the CD4 range in which treatment is indicated. The exact role that this early intervention would play, in terms of improving ART uptake, cost-effectiveness and best implementation of the POC systems in HIV care needs to be established with regard to relevant local circumstances.

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