Rapid CD4 cell count determination and *Cryptococcus* and *Histoplasma* antigen detection in people living with HIV: Implementation of a package of care strategy in a pilot study, Argentina

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ABSTRACT

Objectives: Despite advancements in HIV diagnosis and treatment, advanced HIV disease (AHD) is still a significant concern worldwide, especially in countries with a high percentage of undiagnosed cases and late-stage diagnoses.

Methods: A prospective pilot study was conducted in Buenos Aires, Argentina to assess the feasibility of implementing a package for people living with HIV, integrating a point-of-care clusters of differentiation (CD4) test, followed by rapid *Cryptococcus* and *Histoplasma* antigen (Ag) detection.

Results: A total of 105 people living with HIV were enrolled, during June 2021 to October 2021. The VISITECT CD4 Advanced Disease Lateral Flow Assay (CD4-LFA) (Accubio) classified 98 (93%) patients with AHD. Compared with flow cytometry, the CD4-LFA performed with a high sensitivity (100%) but low specificity (19%) and limited accuracy (47%). In the 98 patients classified with AHD using the CD4-LFA, 16 tested positive for any of the rapid Ag used, including 12 patients positive for the *Histoplasma* Ag test and four positive for *Cryptococcus* Ag; all four patients with positive *Cryptococcus* Ag in sera and were diagnosed with meningitis. In the 30-day follow-up, one death was recorded.

Conclusions: The CD4-LFA correctly classified all patients with CD4 ≤200 cells/µL by flow cytometry, but a high frequency of patients misclassified with AHD was recorded. We also observed a high prevalence of opportunistic fungal infections, as previously observed in the hospital where this pilot study was conducted; however, in contrast with those previous reports, mortality was lower. The study underscores the importance of scaling up comprehensive care strategies and collaborating with governmental and non-governmental partners to enhance access to essential diagnostic tools and treatments for people living with HIV. Further research with larger sample sizes is needed to validate these findings.

Introduction

Despite the accessibility of rapid assays for HIV diagnosis and substantial progress in the efficacy and availability of antiretroviral treatment, advanced HIV disease (AHD) remains a major global public health concern [1]. In Argentina, it is estimated that there are 140,000 people living with HIV (PLHIV), with approximately 13% unaware of their HIV status. Furthermore, in the province of Buenos Aires, 35% of newly diagnosed PLHIV are identified during an advanced stage of the disease [2]. According to the World Health Organization (WHO) definitions, AHD is defined as having a clusters of differentiation (CD) 4 cell counts below 200 cells/µL or with WHO clinical stages III/IV. Due to severe immunosuppression, people with AHD are at the highest risk of developing opportunistic infections (OIs), including cryptococcosis and histoplasmosis, infections highly endemic in the Americas, including Argentina [3–11].

Accurate and affordable CD4 testing is essential for assessing risk of developing OIs among PLHIV. Flow cytometry (FCM) testing is the gold-standard method for CD4 quantification; however, access is limited due to decentralization and the need for complex laboratory capacity; special conditions for specimen collection, transport, and testing; and highly trained laboratory professionals. Recently, Accubio developed the VISITECT CD4 Advanced Disease Lateral Flow Assay (CD4-LFA), a point-of-care test for semi-quantification of CD4 count, and rapid detection of AHD [12].

Cryptococcosis and histoplasmosis are associated with high mortality in PLHIV, especially in people living in low- and middle-income countries. Diagnosis of these infections has greatly improved in the last decade because of highly accurate and rapid assays for antigen detection [8,13–25]. These assays have increased case detection, reduced diagnosis time, and lowered mortality rates [8,13–20]. Some of these novel tests are based on LFA and enzyme immunoassay (EIA) and have the benefit of being performed using non-invasive specimens such as urine, serum, or fingerstick whole blood. In addition, these rapid tests have presented superior performance compared with conventional culture- and microscopy-based testing [22–24,26–31].

The aim of this study was to implement a comprehensive package of care for PLHIV, incorporating Point-of-Care CD4 screening for AHD, followed by rapid antigen testing for *Cryptococcus* and *Histoplasma* in people classified as having AHD using the CD4-LFA.

Materials and methods

Participants and study design

A prospective pilot study was conducted from June 2021 to October 2021 at Hospital Juan A. Fernández in Buenos Aires, Argentina. This hospital is a major public and academic institution affiliated with the University of Buenos Aires, providing free health care and education. The infectious diseases clinic serves over 4000 patients with HIV, offering antiretroviral treatment, clinical care, and approximately 36,200 scheduled appointments annually. The unit ensures quick access to specialized care, facilitates early hospital discharges, and supports patients until clinical stability. This study was conducted in the outpatient setting, consecutively enrolling patients with new HIV diagnoses, patients with known HIV status, and those returning to the HIV program.

We excluded individuals with undetectable viral load during the 3 months before enrollment, as well as those diagnosed with cryptococcosis or histoplasmosis within the previous year. In addition, we excluded individuals who had been on antifungal treatment in the last 14 months.

There were no rejections for participating in the pilot study. All enrolled individuals underwent a first testing using the VISITECT CD4 Advanced Disease Lateral Flow Assay (Accubio); this CD4-LFA testing was done using the same blood specimen used for FCM. Those patients with <200 CD4 cells/μL according to the CD4-LFA were further evaluated for the presence of *Cryptococcus* antigen (Ag) in sera using the IMMY's *Cryptococcus* Ag LFA (CrAg-LFA), and *Histoplasma* antigen in urine using the MiraVista Ag detection LFA (HistoAg-LFA), and the IMMY Clarus *Histoplasma* GM Ag detection EIA (HistoAg-EIA). Tests were performed following the manufacturer's instructions.

In addition, the following standard of care testing was performed: CD4 cells count by FCM (FACScalibur, Becton-Dickinson), HIV viral load (m2000 RealTime System, Abbott), histopathology, blood culture (BD, BACTEC Myco/F Lytic). Other laboratory testing, including special stains, cultures, and GeneXpert for tuberculosis were performed based on patient symptomatology and clinical suspicion of medical staff.

Patients with positive results by the *Cryptococcus* or the *Histoplasma* Ag assays, regardless of symptomatology, were clinically evaluated by medical staff before starting specific treatment. In patients with positive CrAg in sera, lumbar puncture was performed to rule out *Cryptococcus* meningitis, following WHO recommendations. Cerebrospinal fluid (CSF) was collected and tested by India ink, CrAg-LFA, and culture [32].

Statistical analysis

Based on data showing that AHD prevalence in the Provincia de Buenos Aires, Argentina is 35% [2] and using 2 × 2 tables, we calculated the analytical performance of the VISITECT CD4-LFA, including sensitivity, specificity, accuracy, positive predictive value, and negative predictive value, and calculated 95% confidence intervals [2]. For CD4-LFA validation, we used the FCM as a referent test. We calculated the absolute and relative frequencies of variables collected. All analyses were performed using STATA 11.0 or Microsoft Excel.

Results

A total of 106 patients were enrolled; one patient was excluded because the patient did not have a urine specimen (Figure 1). The mean
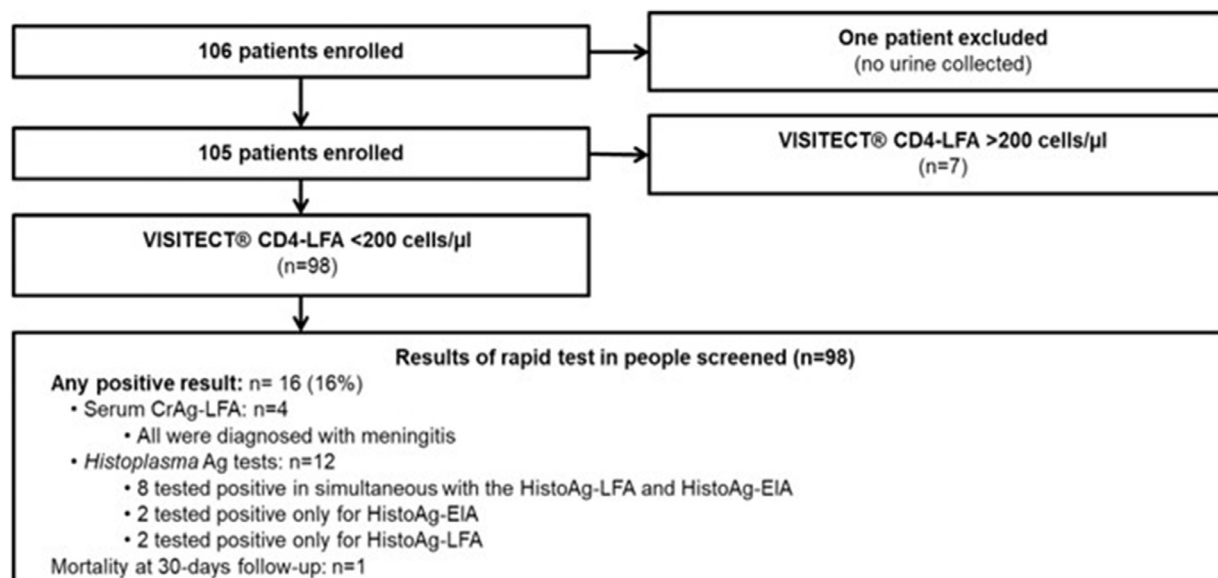

Figure 1. Flow chart of patients enrolled and rapid testing performed during the implementation of package of care strategy in people living with HIV in Argentina. CD4-LFA, VISITECT CD4 Advanced Disease Lateral Flow Assay; CrAg, *Cryptococcus* Ag.

Table 1
Analytical performance of the VISITECT CD4 Advanced Disease LFA. Comparison against the flow cytometry results obtained by the FACScalibur, Becton-Dickinson. Performance calculated using a prevalence of 35% of advanced HIV disease.

	FACScalibur, Becton-Dickinson	
Table 1a	≤200 CD4	>200 CD4
VISITECT CD4-Advanced disease LFA ≤200 **CD4**	63	30
>200 **CD4**	0	7
Sensitivity 100% (95%-CI: 94-100)		
Specificity 19% (95%-CI: 8-35)		
Accuracy 47% (95%-CI: 37-57)		
Positive predictive value 40% (95%-CI: 36-44)		
Negative predictive value 100% (95%-CI: 59%-100%)		

	FACScalibur, Becton-Dickinson	
Table 1b	≤350 CD4	>350 CD4
VISITECT CD4-Advanced disease LFA ≤200 **CD4**	84	9
>200 **CD4**	0	7
Sensitivity 100% (95%-CI: 96-100)		
Specificity 44% (95%-CI: 20-70)		
Accuracy 63% (95%-CI: 53-73)		
Positive predictive value 49% (95%-CI: 38-60)		
Negative predictive value 100% (95%-CI: 59-100)		

CD, cluster of differentiation; CI, confidence interval; LFA, lateral flow assay.
Advance HIV prevalence: 35% [2].

age was 41 years (SD 11.2); 61% (n = 64) were male. By FCM, the median CD4 count was 140 cells/μL (interquartile range [IQR]: 53-254). A total of 71% of patients were presented with symptoms compatible with OIs.

Performance of VISITECT CD4-LFA: According to the CD4-LFA, 98 of 105 (93%) enrolled patients were classified with AHD. FCM CD4 count was available for 100 of the 105 patients included. FCM classified 63 (63%) patients with <200 cells/μL and 37 (37%) with >200 CD4 cells/μL. Table 1a shows the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value with their 95% confidence interval ranges of the CD4-LFA compared with the gold standard, FCM. This assay performed with a sensitivity of 100% but a specificity of 19%. A total of 21 of the 30 patients classified by the CD4-LFA as <200 cells/μL had between 200 and 350 CD4 cells/μL according to the FCM. The remaining nine had over 350 cells/μL. Conversely, the FCM range of the seven specimens classified by the CD4-LFA as >200 cells/μL was 369 to 990 cells/μL.

Aiming to improve the analytical performance of the CD4-LFA, we recalculated the performance of the assay using the following cutoff of the FCM: 250 cells/μL, 300 cells/μL, and 350 cells/μL. We saw that by increasing the threshold of the CD4 cell count calculated by FCM, the analytical performance of the CD4-LFA improved, especially the specificity and accuracy. We observed that using a cutoff of 350 cells/μL by FCM, the CD4-LFA presented a sensitivity of 100% and a specificity of 44% (Table 1b).

OI screening

Among the 98 patients classified with AHD by the CD4-LFA, 16 (16%) had life-threatening infections; of those, 12 were diagnosed with histoplasmosis and four with *Cryptococcus* meningitis. All cases were symptomatic.

Among the 12 patients with positive *Histoplasma* antigen testing, eight tested positive by HistoAg-LFA and HistoAg-EIA, two tested positive only for HistoAg-EIA, and two tested positive for HistoAg-LFA only. The median CD4 count of these patients was 53 cells/μL (IQR 40-125). By the HistoAg-EIA, the antigen concentration ranged from 1.4 ng/ml to >25 ng/mL, the upper limit value of the test detection. Histoplasmosis was proven by culture in six (50%) of these 12 patients with a positive Ag test: three isolated from skin lesions, two from lymph nodes, and one from a biopsy of the thyroid (Table 2). Eight patients started treatment

with liposomal amphotericin B and three with oral itraconazole; one did not receive treatment because the patient left the hospital against medical advice. At 30 days of follow-up, one patient died; this patient was also diagnosed with tuberculosis (Table 2, patient 6).

The four patients with positive serum CrAg-LFA were symptomatic. The patients had a median CD4 cell count of 33 cells/μL (IQR 29-43). All of them had meningitis, with positive antigen and culture of CSF. Antigen titration in serum and CSF is detailed in Table 2. Three of four patients had a CSF positive India ink test; the negative CSF India ink test corresponded with the lower antigen titer in CSF (1:20). Patients were treated according to the national recommendations. All four patients were alive at the 30-day follow-up (Table 2).

The turn-around time for the results from the CD4-LFA, CrAg-LFA, and HistoAg-LFA was a maximum of 2 hours. The HistoAg-EIA was performed once a week, with results available within 1-7 days. Upon reporting a positive result for *Cryptococcus* or *Histoplasma* Ag, the infectious disease unit analyzed the case and implemented the specific antifungal treatment on the same day the result was reported.

Discussion

In this pilot study, a sequential rapid diagnostic package of care in PLHIV was successfully implemented. This package of care included rapid CD4 screening, followed by rapid antigen detection for *Cryptococcus* and *Histoplasma*. One of five people screened for OI tested positive for these two life-threatening fungal OIs.

The CD4-LFA succeeded in correctly identifying all PLHIV with <200 cells/μL; however, 30 of 37 patients with >200 cells/μL were misclassified as patients with AHD. However, it is important to highlight that 70% of those patients had CD4 counts between 200 and 350 cells/μL, meaning that these persons were at risk of developing OIs, as demonstrated in other studies [8,15–17]. We diagnosed a case of proved histoplasmosis in a patient with a CD4 count of 283 cells/μL. In this study, VISITECT CD4 Advanced Disease LFA identified patients with CD4 <350 cells/μL, with a sensitivity of 100% but a specificity of 44%, meaning that around one in two patients screened would have between 350 and 369 CD4 cells/μL according to our results. Future research could assess whether the automatic reading of VISITECT CD4 Advanced Disease LFA could improve interpretation and performance of this assay. Recent advancements were seen with the CrAg-LFA, where the use of a smartphone's

Table 2

Performance of the assays used in the diagnosis of 18 patients with positive results for *Cryptococcus* and *Histoplasma* Ag assays.

#	CD4 count	Result of Ag testing					Case definition based on rapid test
		Histoplasma Ag lateral flow assay	*Histoplasma* Ag enzyme immunoassay (concentration)	Serum CrAg (titer)	Cerebrospinal fluid CrAg (titer)	Culture	
1	53	Positive	Positive (>25 ng/ml)	Negative	Negative	*Histoplasma capsulatum*	Proven histoplasmosis[a]
2	94	Positive	Positive (>25 ng/ml)	Negative	Negative	*Histoplasma capsulatum*	Proven histoplasmosis
3	52	Positive	Positive (>25 ng/ml)	Negative	Negative	*Histoplasma capsulatum*	Proven histoplasmosis
4	42	Positive	Positive (2.6 ng/ml)	Negative	Negative	*Histoplasma capsulatum*	Proven histoplasmosis
5	283	Negative	Positive (1.4 ng/ml)	Negative	Negative	*Histoplasma capsulatum*	Proven histoplasmosis
6	12	Positive	Positive (>25 ng/ml)	Negative	Negative	*Histoplasma capsulatum*	Proven histoplasmosis / tuberculosis[b]
7	156	Negative	Positive (2.7 ng/ml)	Negative	Negative	Negative	Probable histoplasmosis
8	54	Positive	Positive (5.3 ng/ml)	Negative	Negative	Negative	Probable histoplasmosis
9	30	Positive	Positive (12.0 ng/ml)	Negative	Negative	Negative	Probable histoplasmosis
10	46	Positive	Positive (1.5 ng/ml)	Negative	Negative	Negative	Probable histoplasmosis
11	194	Positive	Negative	Negative	Negative	Negative	Probable histoplasmosis
12	39	Positive	Negative	Negative	Negative	Negative	Probable histoplasmosis
13	53	Negative	Negative	P (>1:2560)	P (>1:2560)	*Cryptococcus neoformans*	Cryptococcal meningitis
14	36	Negative	Negative	P (>1:2560)	P (1:80)	*Cryptococcus neoformans*	Cryptococcal meningitis
15	8	Negative	Negative	P (>1:2560)	P (1:20)	*Cryptococcus neoformans*	Cryptococcal meningitis
16	41	Negative	Negative	P (1:320)	P (>1:2560)	*Cryptococcus neoformans*	Cryptococcal meningitis

Ag, antigen; CrAg, *Cryptococcus* Ag.

[a] Acid-fast bacilli in urine smear.

[b] Died during the 30-day follow-up.

application "TIRASPOT" improved the capacity of testing interpretation [33].

We observed that one of 10 patients screened was positive for HistoAg and all of them had symptoms compatible with disseminated histoplasmosis. This finding is consistent with data from previous studies where histoplasmosis prevalence ranges from 2% to 59% (median 20%) [5,21]. Previous studies performed in the Hospital Fernández showed that prevalence differs depending on the diagnostic assay used, as this study has also shown [34,35]. In that study, conventional laboratory assays such as microscopy and culture were used to diagnose eight histoplasmosis cases (0.6 cases per month), whereas the antigen test was positive in 12 cases, meaning 2.4 cases per month [35]. Subsequently, another study with 123 PLHIV showed similar results: 12 cases of histoplasmosis were diagnosed (incidence 10%; 1.2 cases per month); all had a positive Ag test but only eight had a positive antibody test [34]. This reinforces the idea that antigen detection is much more sensitive than conventional diagnostic tools and, thus, may be beneficial in any settings where PLHIV receive care.

Hospital Fernandez has routinely implemented *Cryptococcus* antigen testing since 1992. During the early implementation of CrAg testing, a total of 10 cases were detected in 123 PLHIV evaluated (8% prevalence). Six (60%) of these patients had meningitis, and two died during hospitalization [35]. In addition, we routinely titrate the CrAg because it is well-known that CrAg titers in sera higher than 1:160 are associated with meningitis [36]. This finding could be an alternative for ruling out the suspicion of meningitis in patients where testing of CSF is not possible but further studies are needed.

The mortality in this study was lower than our historical data. In a previous study, 33% of patients with *Cryptococcus* meningitis died; here, all patients survived at the 30-day follow-up [35]. For histoplasmosis, our previous data showed 20% mortality; here, we observed one death [34]. Due to the small sample size and the pilot design of this study, we are not able to draw conclusions regarding whether the testing package improved patient outcomes. However, an acceptable and quick assessment of OIs risk can be done with the VISITECT CD4 test, allowing the screening patients within the same day and facilitating the rapid initiation of effective treatment for life-threatening fungal diseases. Studies are ongoing to confirm these findings. Our group is working with government and non-governmental partners in the implementation of this package of care strategy on a large scale.

Conclusion

The implementation of a comprehensive care package for PLHIV, which includes point-of-care CD4 testing and rapid turn-around diagnosis of OIs, has the potential to reduce PLHIV mortality rates. It is well-documented that rapid diagnosis and targeted treatment have a positive impact on patient survival. There are ongoing studies with larger sample sizes aiming to confirm these findings, with the goal of scaling up the implementation of comprehensive care strategies. Collaboration with governmental and non-governmental partners is essential to improve access to essential diagnostic tools and treatments for PLHIV.

Institutional review board statement

This study was approved by the ethical committee of the Hospital Juan A. Fernández, Buenos Aires, Argentina, approval #3370, on November 9, 2020.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

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