Published in final edited form as:

Mol Biotechnol. 2016 January ; 58(1): 56–64. doi:10.1007/s12033-015-9903-0.

Low cost method to monitor patient adherence to HIV antiretroviral therapy using multiplex cathepsin zymography

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Abstract

Monitoring patient adherence to HIV antiretroviral therapy (ART) by patient survey is inherently error-prone, justifying a need for objective, biological measures affordable in low resource settings where HIV/AIDS epidemic is highest. In preliminary studies conducted in Ethiopia and South Africa, we observed loss of cysteine cathepsin activity in peripheral blood mononuclear cells (PBMCs) of HIV-positive patients on ART. We optimized a rapid protocol for multiplex cathepsin zymography to quantify cysteine cathepsins, and prospectively enrolled 350 HIV-positive, ART naïve adults attending the Themba Lethu Clinic, Johannesburg, South Africa, to test if suppressed cathepsin activity could be a biomarker of ART adherence (103 patients were included in final analysis). Poor adherence was defined as detectable viral load (>400 copies/ml)

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Authors' contributions

MOP and DE conceived of the project, conducted experiments, performed analysis, and wrote the paper. PMK, IKP, LMR, AWK, RLG conducted experiments, performed analysis, LM, DS, WA, and CP helped recruit patient populations and design low resource considerations to conduct these studies in these environments. All authors have edited and approved final manuscript.

Competing interests

The author(s) declare that they have no competing interests.

or simplified medication adherence questionnaire (SMAQ), 4–6 months after ART initiation. 86% of patients with undetectable viral loads after 6 months were cathepsin negative, and cathepsin positive patients were twice as likely to have detectable viral loads (RR 2.32 95% CI 1.26–4.29). Together, this demonstrates proof of concept that multiplex cathepsin zymography may be an inexpensive, objective method to monitor patient adherence to ART. Low cost of this electrophoresis based assay makes it a prime candidate for implementation in resource limited settings.

Keywords

monitoring; zymography; cysteine protease; infectious disease; AIDS; sub-Saharan Africa

Introduction

In 2013, the World Health Organization (WHO) reported 35 million people were living with the Human Immunodeficiency Virus (HIV) globally, with 1.5 million people dying of AIDS-related illnesses worldwide in 2013 (1). Combination antiretroviral therapy (cART) consists of three or more antiretroviral medications that reduces mortality, lowers viral load, results in immune reconstitution and decreases transmission of HIV to others (2). Adherence of between 80–90% of the prescribed drug regimen is required to keep plasma and intracellular concentrations of antiretrovirals (ARVs) high enough to sustain viral suppression, preventing generation and transmission of drug-resistant mutant viruses that rebound when patients are not adherent (3).

Assessing adherence to antiretroviral therapy (ART) at every patient contact is recommended by WHO, but adherence to therapy is notoriously difficult to measure accurately, and various definitions of adherence have been used. To date, there has been no clear consensus on the ideal way to measure adherence in resource-limited settings (4, 5). Viral load is perhaps the best indicator of poor adherence (through detection of circulating virus and treatment failure) but is relatively expensive and not easily accessible or available in many resource-limited settings (6). Self-report is the most commonly used method for measuring adherence in these settings; however, it tends to over-estimate adherence and typically only reflects short-term adherence (7). In the iPrEx study, pill counts and self-reporting were erroneous; self-reported pill use was always higher than what was measured by the scientifically objective method of liquid chromatography and tandem mass spectrometry (LC/MS) (8). LC/MS requires expensive pieces of equipment with expensive reagents and technical training for proper use, making its use prohibitive in low resource rural clinics in parts of South Africa where HIV infection incidence is as high as 15% of the population (9).

Clinicians working with patients taking ART could benefit greatly from a simple, inexpensive, reliable method for detecting the prevalence of poor adherence, to act quickly and counsel them back to adherence (4). As such, there is a need to identify new biological markers associated with adherence (10) that can be monitored affordably. We therefore aim to investigate whether cysteine cathepsins, obtained through minimally invasive methods,

may be an inexpensive surrogate biomarker of adherence to ART. Cysteine cathepsins are included in the papain family of proteases that comprises 11 members denoted by letters: cathepsins B, C, F, H, K, L, V, O, S, W, and Z (or X). In mammalian cells, cathepsins were first identified in lysosomes for their role in protein turnover, but are now known to play functional roles in other cellular compartments and even in the extracellular space after secretion (11–13). Cathepsins play critical roles in the development and progression of cardiovascular disease (14–20), and we have been studying these enzymes for their pathological role in HIV-mediated arterial wall remodeling that contributes to accelerated cardiovascular disease in HIV-positive individuals (21–23). Of particular importance, cathepsins K, S, and V are expressed in monocytes and monocyte derived macrophages (19, 24–27).

We previously developed a sensitive assay called multiplex cathepsin zymography that quantifies active cathepsins, K, L, S, and V in cells and tissue (28, 29) and then optimized it for time, and sensitivity to use it in low resource settings (30). Zymography has many benefits: 1) it is an SDS-PAGE-based assay using a technology platform used by laboratories across the globe, 2) it does not require antibodies making it relatively inexpensive and species-independent, 3) separation of proteins by molecular size and electrophoretic migration visually confirm enzyme identity, and 4) densitometry can be used for quantitative analysis. We began testing peripheral blood mononuclear cells (PBMCs) isolated from HIV-positive patients to investigate cardiovascular disease and found that HIV-positive patients on ART, particularly those on efavirenz (EFV) and tenofovir disoproxil fumarate (TDF), suppressed cathepsin activity compared to HIV-positive patients that had not yet started ART. This finding led us to test the hypothesis that loss of cathepsin activity, as measured by multiplex cathepsin zymography, could be a biomarker for adherence to ART. Cathepsin activity was measured by multiplex cathepsin zymography in HIV-positive ART naïve patients at ART initiation and then again after 4–6 months (when they also had a viral load test) to determine the feasibility of using multiplex cathepsin zymography and as a molecular assay to monitor patient adherence to ART.

Materials and Methods

Patient consent and approval by Ethics board

All protocols were reviewed and approved by the Institutional Review Board Committees at Addis Ababa University and Georgia Institute of Technology and the Human Ethics Committee of the University of the Witwatersrand, and informed consent was obtained from all individual participants included in the study.

Study sites and subjects

Assay development – preliminary findings—Eighteen- to 65-year-old HIV-negative, HIV-positive ART naïve, and HIV-positive subjects on EFV-, nevirapine (NVP)-, or lopinavir/ritonavir (LPV/r)-containing regimens for at least two months prior to the exam were recruited from Tikur Anbessa (Black Lion) Specialized Referral Hospital in Addis Ababa, Ethiopia to participate in this study.

Assay experiments in HIV-positive patients—Patients were enrolled from Themba Lethu Clinic, Johannesburg, South Africa. This cohort has been described elsewhere (31). Eligible subjects included HIV-positive ART naïve adults (>18 years) who were initiating standard first-line ART according to the South African Department of Health ART treatment guidelines (32). Patients returned to the clinic at 1, 3 and 6 months after ART initiation for a medical visit and each month to collect ART. A viral load test was performed at 4–6 months after ART initiation. Studies have classified adherence to ART by HIV-RNA decrease $1 \log_{10}$ or reaching <400 copies/ml or non-adherence by a HIV-RNA decrease <1 $\log_{10}$ or 400 copies/ml following cART (combination ART) initiation (33). We defined poor adherence by a detectable (>400 copies/ml) viral load and good adherence by an undetectable (<400 copies/ml) viral load at 4–6 months on treatment. A counselor/social worker administered a Simplified Medication Adherence Questionnaire (SMAQ) and adherence was defined as a dichotomous variable according to literature (33). Investigators were blinded to HIV and ART status until the analysis stage when patient data was matched.

Isolation of PBMCs from whole blood

Blood (5 ml) was collected in tubes containing EDTA, and then centrifuged at 800 g for 20 min to separate plasma, buffy coat, and red blood cells. 800 μ l of plasma was mixed with 200 μ l of dimethyl sulfoxide (DMSO) and this was added to the buffy coat sample to a final volume of 1 ml and stored at -80°C (34). After thawing, the isolated cells were then washed with a red blood cell lysis buffer (0.83% ammonium chloride, 0.1% potassium bicarbonate, and 0.0037% EDTA) for five minutes to remove any contaminating RBCs, then resuspended in PBS. This was repeated as necessary until the suspension was clear and any residual red/pink color was removed. Then cells were lysed in zymography lysis buffer, sonicated, and supernatant was assayed for total protein concentration with Micro-BCA kit (Pierce).

Cell culture and treatment

Human THP-1 acute monocytic leukemia cells (American Type Culture Collection) THP-1 monocytes were cultured in RPMI 1X 1640 medium (Corning Cell Gro) containing 10 % FBS, 0.05 % β -mercaptoethanol, 1 % L-glutamine, and 1 % penicillin/streptomycin and treated with 100 μ M efavirenz (EFV), tenofovir disoproxil fumarate (TDF), or lamivudine (3TC), (NIH AIDS Reagent Program) or vehicle controls (0.079% dimethyl sulfoxide [DMSO] (Sigma-Aldrich); 0.159% methanol (EMD) for 24 h. DMSO was the vehicle for TDF and 3TC, and methanol for EFV.

Multiplex cathepsin zymography

Multiplex cathepsin zymography (zymo) was performed as described previously (29, 35) for the Ethiopia cohort, but then later was modified to the mini-zymo fast protocol (30). Gels were imaged on a light box with a digital camera. Images were inverted in Adobe Photoshop and densitometry was performed using ImageJ.

Statistics and data analysis

Patient demographics and characteristics at ART initiation were summarized using means with standard deviation for normally distributed continuous variables, medians with

associated interquartile range for not normally distributed continuous variables and proportions for categorical variables. The primary outcome was proportion with poor adherence or a detectable (>400 copies/ml) viral load versus those with good adherence or undetectable (<400 copies/ml) viral load at 4–6 months. We tested the association between cathepsin activity in PBMCs (defined as negative or positive) and poor adherence using log-binomial regression to estimate risk ratios (RR) and 95% confidence intervals (CI). Age at ART initiation, gender and baseline CD4 count were included in an adjusted model with cathepsin activity as the main exposure. These results were presented as unadjusted and adjusted risk ratios with corresponding 95% confidence intervals. Zymo determination of adherence was then compared with self-reported simplified medication adherence questionnaires (SMAQ).

Results

Assay development - preliminary findings in South Africa and Ethiopia

In a pilot study, PBMCs isolated from whole blood of 30 patients from Themba Lethu Clinic in Johannesburg, South Africa were tested for cathepsin activity. Of these patients, 12 had been on ART for between 5 months to 7 years. The other 18 were ART naïve at the time of blood collection. One HIV-negative control patient was loaded in each zymogram for normalization across gels. The initial hypothesis was that HIV-positive individuals on ART would have higher cathepsin activity in support of the data that ART leads to increased cardiovascular disease (21, 22), and cathepsins are elevated in cardiovascular disease (14–20). However, the results clearly indicated that a majority of the patients on ART had suppressed cathepsin activity compared to the HIV-positive, ART naïve patients or the HIV-negative control, as shown in the representative zymogram by the cleared white bands that indicate active cathepsins (Figure 1A). Interestingly, the patient on d4T and nevirapine had a zymogram positive signal (zymo-positive, meaning there was a cleared white band indicating cathepsin activity), while patients on tenofovir and efavirenz had a negative signal (zymo-negative, meaning there was no cleared white band after Coomassie staining, indicating the absence of cathepsin activity). This suggested that the signal was related to the drugs themselves rather than the effect they have on viral load and thus warranted further investigation.

In a separate experiment, 32 samples from males and females were collected and tested in Addis Ababa, Ethiopia at Black Lion Hospital. Of these, 4 were HIV-positive ART naïve, 3 were HIV-negative, and 25 were HIV-positive ART experienced patients. These patients were on different combinations of ART including nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PI), and there was a significant reduction in cathepsin activity by more than 50% if the patient was prescribed either tenofovir or efavirenz ($n=7$, $p<0.05$) (Figure 1B).

Tenofovir and efavirenz cause reduced active cathepsins in monocytes

To confirm that tenofovir or efavirenz had suppressive effects on cathepsin activity in the cells even without effects of active HIV, we tested their effects directly on monocytes from the Thp-1 cell line. Monocytes were incubated with 100 μ M of either efavirenz (EFV),

tenofovir disoproxil fumarate (TDF), or lamivudine (3TC) for 24 hours, and we confirmed that TDF and EFV significantly reduced the cathepsin zymo signal compared to vehicle controls (Figure 2) ($n=3$, $*p<0.05$ or 0.001). Lamivudine (3TC), the third most commonly used drug in first-line ART cocktails, had no statistical difference in intracellular cathepsin activity after 24 hours pre-treatment.

Patient characteristics

Results from the preliminary experiments above showed that HIV-positive patients on ART suppressed cathepsin activity in PBMCs compared to HIV-positive ART naïve patients – although there was no normalization for independent patient variability and on ART for different lengths of time. To test for differences, patients were recruited at baseline (day of ART initiation) and tested for cathepsin zymo activity and then again at 4–6 months after ART initiation. A new cohort of 350 patients were recruited from Themba Lethu Clinic, and followed from ART initiation until 24 months. Thirteen patients were excluded from the analysis either because they had previously received ART or because they never initiated ART, leaving 337 that were ART naïve and eligible for the study. Of these, 261 were tested for cathepsin activity at baseline and 258 had a viral load result at baseline. 133 returned for cathepsin zymo activity test at 4–6 months, and of these only 103 had a viral load result available (116/219; 53% missing) at 4–6 months. Viral load is monitored routinely (between 4 – 7 months after initiation of a new regimen, then 6 months after ART initiation and yearly thereafter) in this setting according to the South African ART treatment guidelines whereas patients provided consent for an additional blood sample to be collected for the cathepsin activity (36).

Patient demographics and baseline characteristics of the 337 patients, stratified by cathepsin activity at baseline, are presented in Table 1. Patients that were zymo-positive ($n=94$) were similar to those who were zymo-negative ($n=167$) at ART initiation in terms of gender, age, education, employment, WHO stage III/IV, prevalent tuberculosis, CD4+ cell count, body mass index, hemoglobin, viral load, or ART regimen ($p>0.05$). By the end of follow-up, 84% of zymo-positive patients were alive and in care compared to 86% of zymo-negative patients.

Undetectable viral loads correlate with cathepsin negative zymography signals

While a number of patients had a zymo-positive signal at baseline, there were more zymo-negative signals after 4–6 months after ART initiation. Representative sets of zymograms from baseline and after 4–6 months ART are shown in Figure 3. Of those with an undetectable viral load at 4–6 months ($n=71$), 86% were zymo-negative while 14% were zymo-positive. A positive cathepsin zymo signal was associated more with a detectable viral load than with CD4 count. Of those with a detectable viral load at 4–6 months ($n=32$), 63% were zymo-negative while 37% were zymo-positive. Compared to zymo-negative patients, zymo-positive patients were twice as likely to have a detectable viral load at 6 months (RR 2.21 95% CI 1.29 3.79).

When age (< 40 years), gender and baseline CD4 count were included in the model, only cathepsin activity was strongly associated with a detectable viral load at 4–6 months (RR

2.32 95% CI 1.26 – 4.29). Positive cathepsin activity had a poor sensitivity (38%) but good specificity (86%) to identify patients with poor adherence, here specificity being defined as if a patient had an undetectable viral load, then there was an 86% likelihood that patient would also have a zymo-negative signal detected by multiplex cathepsin zymography. The positive likelihood ratio of 2.66 (95% CI 1.29 – 5.51) indicated an increased probability that patients with a zymo-positive test would have poor adherence. At 6 months self-reported adherence using SMAQ had a sensitivity of 41% (95% CI 28 – 56) and specificity of 63% (95% CI 55 – 72) to correctly identify poor adherence.

In a sub-analysis which was restricted to patients that were zymo-positive at baseline, the sensitivity and specificity of a positive zymo signal to correctly identify poor adherence increased to 53% and 90%, respectively. Conversely, where the analysis was restricted to patients that were zymo-negative at baseline, the sensitivity and specificity dropped to 23% and 70%, respectively.

Discussion

In conclusion, we report that multiplex cathepsin zymography can be used in low resource settings to monitor cathepsin activity in PBMCs from HIV-positive patients on ART as a surrogate marker for patient adherence. This would be especially applicable when TDF or EFV are in the ARV cocktail. The 86% specificity for this assay indicates that if a patient has an undetectable viral load, then there was an 86% likelihood that patient would also have a zymo-negative signal detected by multiplex cathepsin zymography, strengthening support for the utility of this inexpensive assay performed with standard polyacrylamide electrophoresis equipment and reagents. Having already employed this assay in a laboratory in Johannesburg, South Africa and Addis Ababa, Ethiopia to collect the data and run the zymograms presented and analyzed here, its application in resource limited settings is already established. With the rapid mini-zymo protocol(30), we were able to process 400 blood samples in only two days with just two researchers.

The Ethiopia data, though a small sample size (n=7), suggested that the cathepsin activity was susceptible to suppression by TDF and EFV, and this was corroborated with *in vitro* studies of THP1 monocytes (Figure 2) showing that TDF or EFV were sufficient to reduce active cathepsins in monocytes, but that 3TC was not. The implications for patients on regimens other than TDF or EFV are still to be determined, but since up to 85% of the patients in the larger Themba Lethu cohort were prescribed a regimen that included TDF or EFV, we are encouraged that this will be applicable to a high majority of HIV-positive patients on first line regimens. Further investigations are needed to ascertain if cathepsin activity is related to ART drug themselves, the effect they have on viral load, and poor response to ART or drug resistance, but the data from Figure 2 indicating that incubation with ARVs alone was sufficient to induce a significant reduction in cathepsin activity in uninfected monocytes is encouraging. This suggests that the response seen in the patient PBMCs may simply reflect mechanisms of the ARVs alone.

The biological mechanisms underlying ART suppression of cathepsin activity in PBMCs must still be determined to improve the sensitivity/specificity of zymography and its

applicability to all patients. From this cohort, a number of patients were zymo-negative at baseline, and this was not associated with CD4 count or viral load. It may be that this subgroup of patients had other opportunistic infections or co-morbidities such as tuberculosis or hepatitis which complicates the immune cell landscape and perhaps the cathepsin expression/activity of those cells. This is still to be determined. Some patients may also need a longer time to reach the zymo-negative point on ART depending on the underlying viral and immunologic dynamics of their particular disease.

Finally, this quantitative, objective measurement of adherence had a similar sensitivity but better specificity (86% vs. 63%) than self-reported adherence when both were compared to viral load as the gold standard. Of course, the cost of those methods is substantially lower than mass spectrometry, which justifies their inclusion despite concerns of error, but objective measures of adherence are needed (37). Data from several studies show that possibly as much as 30% of subjects who experience virological failure on a first- or second-line ART have no HIV drug resistance mutations present (38–40). This is important because two other South African studies showed that 40–50% of patients undergoing virological failure were able to resuppress their viral load after adherence counseling (41, 42). These patients, and possibly others, could benefit from adherence interventions if poor adherence was identified earlier. Early identification of poor adherence may not only result in better treatment outcomes, and could also conserve and maximize ART regimens in settings where therapeutic options are limited (10).

Multiplex cathepsin zymography may provide this simple biological test to monitor adherence, and would be much cheaper than the liquid chromatography/mass spectrometry required to accurately quantify plasma and intracellular levels of antiretroviral drugs in patients. This method may be an affordable and accessible laboratory marker to identify patients at risk of virologic failure and those that require targeted interventions such as intensified adherence counselling. The *in vitro* cell data showing that uninfected monocytes are still susceptible to ART mediated cathepsin suppression further suggests that this assay could have applications for pre-exposure prophylaxis (PrEP) clinics that are providing ARVs to HIV-negative individuals at high risk of contracting HIV. In the Partners PrEP and iPrEx PrEP clinical trials, the data overwhelmingly supported high efficacy when patients were adherent as detected by intracellular and plasma measurements of ARVs using mass spectrometry (8, 43), but FEM-PrEP, was ended early due to lack of protection and low adherence was reported (44). Here, we propose that multiplex cathepsin zymography measurement could be the assay that fills that gap by providing a minimally invasive, reliable, and inexpensive method to monitor patient adherence for HIV-positive individuals on ART, and perhaps even for HIV-negative individuals on PrEP.

Acknowledgments

This work was completed partially with funding from a Creative and Novel Ideas in HIV Research (CNIHR) grant sponsored by the National Institutes of Health and The University of Alabama at Birmingham Center for AIDS Research (CFAR) program (5P30A1027767) (MOP and DE), funding from NIH Award Number DP2OD007433 from the Office of the Director, National Institutes of Health (MOP). American Heart Association (RLG), and United States Agency for International Development (USAID) (DE). This study is made possible by the generous support of the American people through Cooperative Agreement AID 674-A-12-00029 from the United States Agency for International Development (USAID). The contents are the responsibility of the authors and do not

necessarily reflect the views of USAID or the United States Government and does not necessarily represent the official views of the Office of the Director, National Institutes of Health or the National Institutes of Health. We would like to extend our gratitude to Hazel Molefe and Anna Segoneco from CHRU and Keshendre Moodley and Lindi Coetzee from the University of the Witwatersrand. We would like to thank Hannah Song from University of Toronto for assistance. We would like to acknowledge the directors and staff of Themba Lethu Clinic (TLC), CHRU, HE²RO and Right to Care (RTC) - a PEPFAR (US President's Emergency Plan for AIDS Relief) funded NGO. We would like to acknowledge the Gauteng Provincial and National Department of Health for providing for the care of the patients at TLC as part of the National Comprehensive Care, Management and Treatment (CCMT) of HIV and AIDS program. Lastly, we would like to sincerely thank the patients attending the Themba Lethu Clinic for their continued trust in the treatment and care provided at the clinic.

Abbreviations

3TC	lamivudine
ART	Antiretroviral therapy
ARV	antiretrovirals
CI	confidence interval
EFV	efavirenz
HIV	Human Immunodeficiency Virus
cART	combination active antiretroviral therapy
LC/MS	liquid chromatography tandem mass spectroscopy
LPV/r	lopinavir boosted with ritonavir
NNRTI	non-nucleoside reverse transcriptase inhibitors
NRTI	nucleoside reverse transcriptase inhibitors
NVP	nevirapine
PBMC	peripheral blood mononuclear cells
PI	protease inhibitors
PrEP	pre-exposure prophylaxis
RR	risk ratio
SMAQ	simplified medication adherence questionnaire
TDF	tenofovir disoproxil fumarate
WHO	World Health Organization

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Figure 1. Patients on antiretroviral therapy have reduced cathepsin activity in their peripheral blood mononuclear cells in cohorts from Johannesburg and Addis Ababa

A) PBMCs isolated from whole blood of HIV-positive patients attending the Helen Joseph Clinic in Johannesburg, South Africa were lysed and equal amounts of protein (10 µg) were loaded for multiplex cathepsin zymography and quantified by densitometry. Patients on ART for different lengths of time did not have cathepsin activity. **B)** HIV-positive patients attending Black Lion Hospital in Addis Ababa, Ethiopia multiplex cathepsin zymography. Data normalized to the HIV-negative controls for each gel. There was statistically significant reduction in cathepsin activity in patients on either tenofovir (TDF) or efavirenz (EFV) (n=7, **p<.05). Neg- HIV-negative, NVP-nevirapine, AZT-zidovudine, d4T-didanosine, PI-protease inhibitor, ART Naïve- HIV-positive patients.

Figure 2. Efavirenz (EFV) and tenofovir (TDF) suppress cathepsin activity in monocytes, but lamivudine (3TC) does not

THP-1 monocytes were incubated with 100 μ M of either EFV, TDF, or 3TC for 24 hours or with appropriate vehicle control (veh); DMSO for TDF and 3TC, methanol for EFV. Then cells were lysed, and equal protein amounts were examined by multiplex cathepsin zymography and quantified by densitometry (n=3, * p<0.05 or 0.001).

Figure 3. Six months of ART reduces cathepsin activity in PBMCs as measured by multiplex cathepsin zymography

A) Representative zymograms (of more than 30 individual gels run and imaged) are shown from baseline when patients were ART naïve and B) after 6 months of ART. Arbitrary numbering of wells in the gels between baseline and 6 months, not patient matched. Zymo-negative signal indicated by bold, underlined number, and zymo-positive signal is not).

Table 1

Baseline Characteristics of the Subjects.

		All (n=337)	Cathepsin –ve (n=167)	Cathepsin +ve (n=94)*
Gender, Male	n, %	118/337 (35%)	63/167 (38%)	27/94 (29%)
Age, years	Median (IQR)	37.2 (32.0 – 44.1)	36.9 (31.7 – 43.7)	37.6 (32.0 – 44.6)
Education primary school	n, %	261/299 (87.3%)	130/149 (87.2%)	69/83 (83.1%)
Employed	n, %	204/337 (60.5%)	105/167 (62.9%)	51/94 (54.3%)
WHO stage III/IV	n, %	23/132 (17.4%)	16/75 (21.4%)	5/31 (16.1%)
TB at ART initiation	n, %	9/337 (2.7%)	4/167 (2.4%)	3/94 (3.2%)
CD4	Median (IQR)	184 (94 – 265)	169 (83 – 265)	177 (91 – 256)
50 cells/mm ³	n, %	42/298 (14.1%)	31/167 (18.6%)	11/94 (11.7%)
51 – 100 cells/mm ³	n, %	38/298 (12.7%)	23/167 (13.8%)	13/94 (13.8%)
101 – 200 cells/mm ³	n, %	132/298 (44.3%)	66/167 (39.5%)	45/94 (47.9%)
>200 cells/mm ³	n, %	86/298 (28.9%)	47/167 (28.1%)	25/94 (26.6%)
Body mass index	Median (IQR)	23.4 (20.8 – 27.4)	24.2 (21.0 – 28.6)	22.9 (20.7 – 26.7)
<18.5kg/m ²	n, %	24/268 (9.0%)	9/118 (7.6%)	10/83 (12.1%)
Haemoglobin	Median (IQR)	12.7 (11.2 – 13.8)	12.7 (10.9 – 14.0)	12.3 (10.9 – 13.2)
<10g/dL	n, %	35/318 (11.0%)	21/159 (13.2%)	9/86 (10.5%)
Viral load, log₁₀	Median (IQR)	5.1 (4.6 – 5.5)	5.1 (4.5 – 5.5)	5.1 (4.7 – 5.4)
ART regimen (3TC_TDF_EFV)	n, %	271/337 (80.4%)	142/167 (85.0%)	69/94 (73.4%)
Outcomes				
Alive in care	n, %	281/337 (83.4%)	143/167 (85.6%)	79/94 (84.0%)
Dead	n, %	5/337 (1.5%)	1/167 (0.6%)	4/94 (4.3%)
LTFU	n, %	33/337 (9.8%)	11/167 (6.6%)	8/94 (8.5%)
Transferred out	n, %	18/337 (5.3%)	12/167 (7.2%)	3/94 (3.2%)

* no significant differences between cathepsin +ve and cathepsin – ve at ART initiation