Markers of poor adherence among adults with HIV attending Themba Lethu HIV Clinic, Helen Joseph Hospital, Johannesburg, South Africa

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Background: To date, there is no consensus on ideal ways to measure antiretroviral treatment (ART) adherence in resource limited settings. This study aimed to identify markers of poor adherence to ART.

Methods: Retrospective data of HIV-positive ART-naïve adults initiating standard first-line ART at Themba Lethu Clinic, Helen Joseph Hospital, Johannesburg, South Africa from April 2004 to December 2011 were analysed. Poisson regression models with robust error variance were used to assessed the following potential markers of poor adherence 'last self-reported adherence, missed clinic visits, mean corpuscular volume (MCV), CD4 count against definition of adherence, suppressed HIV viral load using traditional test metrics'.

Results: A total of 11 724 patients were eligible; 1712 (14.6%) had unsuppressed viral load within 6 months after initiating ART. The main marker of poor adherence was a combination of change in CD4 count and MCV; change in CD4 ≥expected and change in MCV <14.5 fL (RR 2.82, 95% CI 2.16–3.67), change in CD4 <expected and change in MCV <14.5 fL (RR 5.49, 95% CI 4.13–7.30) compared to change in CD4 ≥expected and change in MCV ≥14.5 fL.

Conclusions: A combination of less than expected increase in CD4 and MCV at 6 months after treatment initation was found to be a marker of poor adherence. This could help identify and monitor poor treatment adherence in the absence of viral load testing.

Keywords: ART, HIV, Low cost monitoring, Markers, South Africa, Themba Lethu Clinic

Introduction

The estimated prevalence of HIV infection in South Africa in 2012 was 12.2% which represents 6.4 million persons; 18.8% of these are persons aged 15 to 49 years.[1] The impact of the global HIV/AIDS pandemic has been mitigated by the expanding access to antiretroviral therapy (ART).[1,2] However, the gains made by widespread access to treatment have been impeded by poor adherence to ART.

There is no standard definition of good adherence; other scientists have defined it as taking at least 95% of the correct medication, in the correct quantities, at the correct time and following any associated recommendations such as those pertaining to food.[3,4] However, others have shown 80% adherence may be sufficient to maintain virological suppression[5–7] and others still define good adherence as taking at least 90% of prescribed medication.[8,9] A distinction has also been made between complete (100%) and incomplete adherence (<100%).[10,11] To be able to satisfactorily delay the development of antiretroviral drug resistant viral strains and poor clinical outcomes, a maximal level of treatment adherence needs to be achieved by each individual patient on ART. In addition, patients on ART who are adherent to treatment bear a lower likelihood of transmitting resistant viral strains to other patients. The public health challenges of drug resistant HIV strains circulating among patients on

ART include use of second line drugs which are more expensive and are not easily affordable to patients in resource limited settings where drug choice of ART are very limited.

Self-reporting is commonly used to measure adherence. However, the potential for errors from patient recall and dishonesty is of great concern.[12,13] A meta-analysis by Nieuwkerk et al.[14] found that self-reported adherence could distinguish between poor and good adherence as defined by viral loads of <400 vs ≥400 copies per ml respectively. Self-reporting is used to measure adherence at the Themba Lethu Clinic, along with viral load and the number of missed visits (defined as missing a scheduled medical or ARV drug pickup visit by at least 7 days[15] calculated from visit schedules.[16] On-time drug pick up does not, however, guarantee adherence.[17]

CD4+ count and mean corpuscular volume (MCV) have been used as surrogate clinical measures of adherence. Studies have shown that MCV is a reliable measure of adherence in patients on zidovudine (AZT) as most patients demonstrate an increase in MCV above the normal range within 4 weeks of starting AZT.[18–20] A drop in MCV of <14.5 fL (from baseline to 6 months) or at 6 months of <100 fL suggests poor adherence.[13,18,21] Mugisha and colleagues reported that 12 weekly MCV measurements may be useful in monitoring adherence to AZT-containing regimens in low-income countries, where HIV RNA may be unavailable.[22] Similarly, studies have also shown elevation of MCV in patients on stavudine (d4T), suggesting that it could also be used as a surrogate marker to monitor adherence in patients on d4T-containing regimens since MCV is an important initial test to assess presence of microcytic and macrocytic anaemia and is therefore used to diagnose anaemia among patients on nucleoside reverse-transcriptase inhibitor drugs, particularly AZT and d4T.[13,18,23] CD4 cell count has also been used to monitor ART adherence since an increase in CD4 cell count is closely correlated to viral load as such CD4 cell count increase could be used as a clinical proxy to viral load. While CD4 cell count may positively predict poor adherence, studies have shown that CD4 cell count poorly predicts treatment failure.[24,25]

Viral load is one of the most reliable measures of adherence or effectiveness of ART,[5,26] serving as a gold standard against which the efficacy of other routinely collected and more affordable measures could be assessed. However, the test is expensive costing approximately US$60/test.[14,15,22] Studies have classified participants as responders (HIV-RNA decrease ≥1 $\log_{10}$ or reaching <400 copies/ml) or non-responders (HIV-RNA decrease <1 $\log_{10}$ or reaching ≥400 copies/ml) following combination ART (cART) initiation.[22] Early identification of poor treatment adherence may trigger early intervention among patients on ART to improve compliance to ART which may ultimately result in better treatment outcomes. Such an undertaking might also maximize use of standard first line ART regimens in settings where therapeutic options are limited as second line regimens are expensive, hence not affordable. Surrogate measures such as self-reported adherence, missed visits, MCV and CD4 cell count have been used to assess adherence particularly in resource-limited settings since they are cheaper than viral load testing, but there is no consensus on the ranking of these measurements compared to the gold standard of viral load monitoring. It is this lack of consensus, together with the high cost of viral load testing that prompted us to conduct this study which was aimed at identifying suitable markers that could be used to routinely assess poor adherence to ART among patients with HIV.

Methods

Study design

We used cohort data from patients attending care at Themba Lethu Clinic, an urban public sector treatment facilty in Johannesburg South Africa. Since 2004, Themba Lethu Clinic has enrolled approximately 30 000 patients with HIV into its HIV care and treatment programme, over 21 000 of whom have received ART.[16] Patients on treatment are typically seen at least every 3 months and routine blood tests for monitoring at the site including full blood count (FBC), CD4 count and viral load are performed according to the Department of Health ART guidelines.[27] The requirements for monitoring have changed over time with revisions to guidelines. Before 2010, an FBC was measured at treatment initiation then at 1, 2, 3 and 6 months. Thereafter monitoring was on a 6 monthly basis for patients who were taking AZT-based regimens. In addition, CD4 count and viral load measurement were both performed at baseline then 6 monthly thereafter.[28,29] The 2013 Department of Health ART treatment guidelines, however, stipulated that FBC should be done at 3 and 6 months only for patients on AZT-based regimens.[27] Based on the 2013 National Guidelines, CD4 count is measured at baseline and 1 year on ART. This means that subsequent monitoring of a patient's response to ART is done by viral load.[27] The viral load is still measured at 6 and 12 months after ART initiation and annually thereafter.[27]

Study population

The analysis included HIV-positive ART-naïve patients aged 18 years and above initiated on a standard first-line ART regimen between 1 April 2004 and 31 December 2011 who had been on ART for at least 6 months.[28] Patients that were lost to follow up (LTFU; defined as missing their last scheduled visit by more than 3 months),[30] transferred to another facility or died within the first 6 months on ART were excluded from the analysis (Figure 1).

Study variables

Self-reported adherence was defined as the last self-reported adherence level on or after 3-month visit, and not more than 3 months before the date of the viral load, but at least 28 days before the viral load was done. It was categorized as >90% (all pills), >60 to 90% (most pills), >30 to 60% (about half the pills), 10–30% (a few of the pills) and <10% (none of the pills).

The number of missed visits during the study period was defined as missing a scheduled visit date by 7 or more days[15] but less than 91 days (as patients would then be classified as LTFU).[30] Based on the type of visit, three additional variables were derived: total number of missed visits, number of missed ARV drug pick-up and number of missed medical visits. Each of these variables had three categories: 0, 1 or ≥2 missed visits. MCV at baseline or at 6 months was categorized as <80 fL, 80–100 fL and >100 fL. The change in MCV at 6 months was calculated as the difference between the MCV at 6 months and baseline (µ2-µ1). The variable was categorized as ≥14.5 fL and <14.5 fL.[21]

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26 278 patients initially

    → 125 patients below the age of 18 years.

    → 432 patients not initiated at Themba Lethu Clinic.

    → 3219 patients initiated before 1st April 2004 or after 1st January 2012.

    → 2034 patients started on a non-standard first-line regimen.

    → 983 patients that were not ART naïve (viral load <400 copies/ml at baseline or in the absence of viral load, a baseline CD4 count >1000 cells/mm³ with a baseline WHO stage that was I, II or missing).

    → 3363 patients lost to follow up, transferred out or dead before 6 months on ART.

    → 1738 patients with no documented scheduled visit in the first 6 months on ART

14 384 patients for the sensitivity analysis

    → 2660 patients with no viral load at 6 months.

11 724 patients eligible for the study
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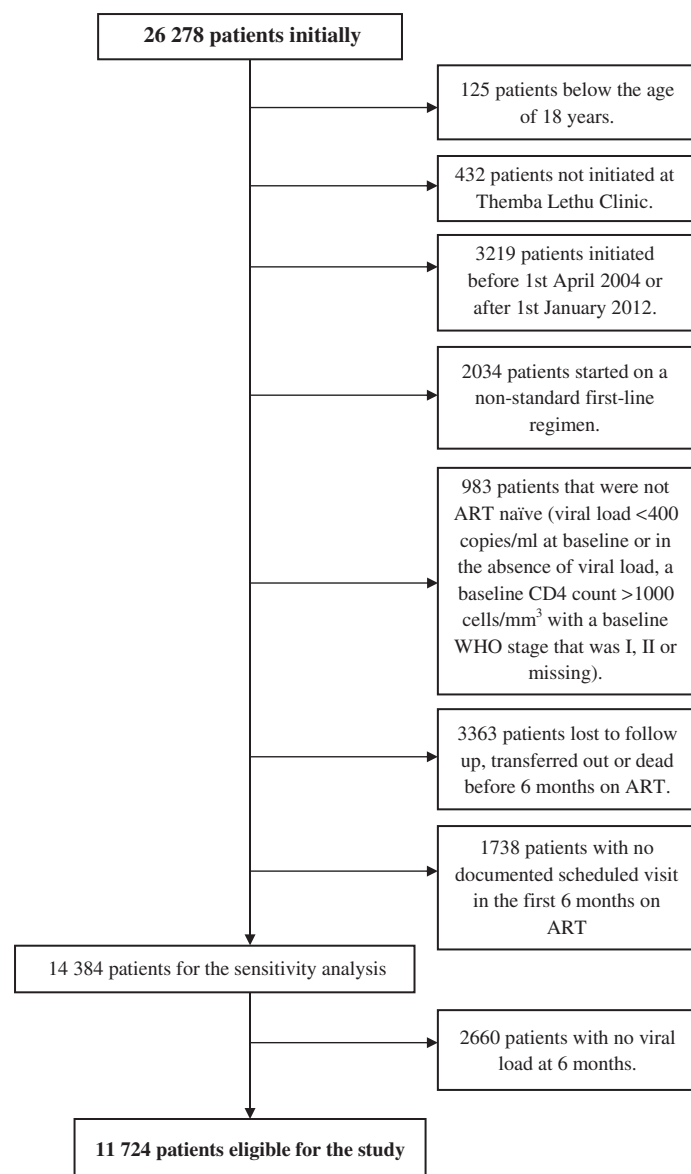
Figure 1. Patient flow chart for the selection of patients eligible for the study. ART: antiretroviral therapy.

CD4 cell count at baseline and 6 months was categorized as a CD4 cell count of >200 cells/mm³, 101–200 cells/mm³, 51–100 cells/mm³ and ≤50 cells/mm³. The change in CD4 cell count at 6 months in cells/mm³ was calculated as the difference between the CD4 cell count at 6 months and baseline ($\mu2-\mu1$). CD4 cell count is expected to rise by 100 cells/mm³/year or 50 cells/mm³ in 6 months[20,31] as a sign of immune recovery among patients on ART. The change in CD4 cell count at 6 months was categorized as greater than or equal to the expected (≥100 cells/mm³/year × duration of treatment in years) and less than expected (<100 cells/mm³/year × duration of treatment in years).

TB was defined as: never on TB treatment (during the first 6 months on ART); on TB treatment at ART initiation (baseline); and TB treatment during the first 6 months on ART. Similarly,

pregnancy was defined as: never pregnant (during the first 6 months on ART); pregnant at baseline; and pregnant during the first 6 months on ART. The primary outcome of the analysis was defined as the first viral load done 90 to 270 days after ART initiation. The viral load result was then categorized as undetectable (<400 copies/ml, a proxy for good adherence) or detectable (≥400 copies/ml, a proxy for poor adherence).

Statistical analysis

Patient demographics and clinical characteristics at ART initiation were described using means with standard deviation for normally distributed variables, medians with interquartile range for variables that were not normally distributed and proportions for categorical variables. The diagnostic accuracy of each identified marker of adherence was assessed using sensitivity, specificity, positive predictive values (PPVs) and negative predictive values (NPVs) using the 6 month viral load result as the gold standard. Univariate and multivariate poisson regression models with robust error variance were also used to estimate incidence rate ratios (IRRs) and 95% CIs. IRRs were used to approximate the relative risk (RR) of poor adherence. Variables with $p<0.1$ on univariate analysis were included in the multivariate regression models. CD4 count at baseline, gender and age were included in the final multivariate model and variables with $p≤0.05$ were considered statistically significant factors. Data were analysed using STATA Release 13 (StataCorp, College Station, TX, USA).

The study was approved by the University of the Witwatersrand Human Research and Ethics Committee; Clearance Certificate number M120858.

Results

Description of the study population

In total 26 278 patients initiated ART at the Themba Lethu Clinic during the study period. We excluded patients <18 years, those who initiated outside the study period or at another facility or on a non-standard regimen and those who died, were LTFU or transferred out. The remaining, 11 724 patients were included in the final analysis.

Description of baseline characteristics

The baseline characteristics of enrolled patients is summarised in Table 1. Briefly, 63.2% (7409) were females; median age was 36.7 years (IQR 31.5–43.3). The median CD4 cell count at ART initiation was 99 cells/mm³ (IQR 39–171). At baseline the median MCV was 88.2 fL (IQR 83.6–92.2), the median body mass index (BMI) was 22 kg/m² (IQR 19–25) and the median haemoglobin was 11.7 g/dl (IQR 10.1–13.1).

Description of characteristics at 6 months

The median CD4 cell count was 243 cells/mm³ (IQR 163–346), with a median of 250 cells/mm³ (IQR 170–352) and 206 cells/mm³ (IQR 117.5–317.5) for patients with a viral load ≥400 and <400 copies/ml respectively. The median change in CD4 cell count was 128 cells/mm³ (IQR 69–204), with a median change of 133 cells/mm³ (IQR 74–207) and 99 cells/mm³ (IQR 34–188) for patients with a viral load <400 copies/ml and

Table 1. Distribution of baseline demographic and clinical characteristics by viral load and ART regimen

Characteristics	All eligible patients			All eligible patients on AZT-based regimens[a]		
	Total n=11724	Viral load <400 copies/ml n=10011	Viral load ≥400 copies/ml n=1713	Total n=285	Viral load <400 copies/ml n=247	Viral load ≥400 copies/ml n=38
	n (%) and median (IQR)/mean±SD			n (%) and median (IQR)/mean±SD		
Gender						
Females	7409 (63.2%)	6391 (63.8%)	1018 (59.4%)	153 (53.7%)	138 (55.9%)	15 (39.5%)
Males	4315 (36.8%)	3620 (36.2%)	695 (40.6%)	132 (46.3%)	109 (44.1%)	23 (60.5%)
Age in years	36.7 (31.5–43.3)	36.8 (31.5–43.3)	36.3 (31.1–43.0)	39.3 (33.6–45.7)	39.3 (33.8–45.8)	39.9 (31.1–45.6)
≤median (36.7)	5893 (50.3%)	5002 (50.0%)	891 (52.0%)	104 (36.5%)	90 (36.4%)	14 (36.8%)
>median (36.7)	5831 (49.7%)	5009 (50.0%)	822 (48.0%)	181 (63.5%)	157 (63.6%)	24 (63.2%)
Education level						
≥Secondary school	7062 (60.2%)	5996 (59.9%)	1066 (62.2%)	148 (51.9%)	131 (53.0%)	17 (44.7%)
Primary school	1484 (12.7%)	1265 (12.6%)	219 (12.8%)	43 (15.1%)	35 (14.2%)	8 (21.1%)
<Primary school	343 (2.9%)	303 (3.0%)	40 (2.3%)	11 (3.9%)	10 (4.1%)	1 (2.6%)
Missing	2835 (24.2%)	2447 (24.5%)	388 (22.7%)	83 (29.1%)	71 (28.7%)	12 (31.6%)
Professional status						
Employed	5913 (50.4%)	5086 (50.8%)	827 (48.3%)	133 (46.7%)	116 (47.0%)	17 (44.7%)
Unemployed	5639 (48.1%)	4788 (47.8%)	851 (49.7%)	147 (51.6%)	127 (51.4%)	20 (52.6%)
Missing	172 (1.5%)	137 (1.4%)	35 (2.0%)	5 (1.7%)	4 (1.6%)	1 (2.7%)
CD4 count	99 (39–171)	100 (39–172)	94 (36–168)	124 (55–209)	120 (55–207)	168 (61–244)
>200 cells/mm^3	1473 (12.6%)	1281 (12.8%)	192 (11.2%)	65 (22.8%)	55 (22.3%)	10 (26.3%)
101–200 cells/mm^3	3638 (31.0%)	3152 (31.5%)	486 (28.4%)	64 (22.5%)	55 (22.3%)	9 (23.7%)
51–100 cells/mm^3	2069 (17.6%)	1766 (17.6%)	303 (17.7%)	42 (14.7 %)	39 (15.8%)	3 (7.9%)
≤50 cells/mm^3	3154 (26.9%)	2685 (26.8%)	469 (27.4%)	47 (16.5%)	42 (17%)	5 (13.2%)
Missing	1390 (11.9%)	1127 (11.3%)	263 (15.3%)	67 (23.5%)	56 (22.6%)	11 (28.9%)
BMI at initiation in kg/m^2	22 (19–25)	22 (19–25)	21.4 (19–25)	22.0 (19.5–26.3)	22.0 (19.5–28)	21.5 (19.8–24)
<18.5	1736 (14.8%)	1473 (14.7%)	263 (15.4%)	35 (12.3%)	33 (13.4%)	2 (5.3%)
18.5–24	5549 (47.3%)	4749 (47.4%)	800 (46.7%)	109 (38.2%)	90 (36.4%)	19 (50%)
25–29	1923 (16.4%)	1674 (16.7%)	249 (14.5%)	30 (10.5%)	28 (11.3%)	2 (5.3%)
≥30	900 (7.7%)	788 (7.9%)	112 (6.5%)	38 (13.3%)	37 (15.0%)	1 (2.6%)
Missing	1616 (13.8%)	1327 (13.3%)	289 (16.9%)	73 (25.7%)	59 (23.9%)	14 (36.8%)
Baseline haemoglobin	11.7 (10.1–13.1)	11.7 (10.2–13.1)	11.6 (10.1–13.0)	12.6 (11.6–14.0)	12.6 (11.2–14.0)	12.9 (12–14.2)
≤8 g/dl	579 (4.9%)	498 (5.0%)	81 (4.7%)	1 (0.4%)	1 (0.4%)	0 (0%)
>8 g/dl	10108 (86.2%)	8686 (86.8%)	1422 (83.0%)	227 (79.6%)	198 (80.2%)	29 (76.3%)
Missing	1037 (8.9%)	827 (8.2%)	210 (12.3%)	57 (20%)	48 (19.4%)	9 (23.7%)
WHO stage						
I	3850 (32.8%)	3397 (33.9%)	453 (26.4%)	111 (38.9%)	96 (38.9%)	15 (39.5%)
II	1926 (16.4%)	1659 (16.6%)	267 (15.6%)	20 (7.0%)	17 (6.9%)	3 (7.9%)
III	3066 (26.2%)	2610 (26.1%)	456 (26.6%)	59 (20.7%)	52 (21.1%)	7 (18.4%)
IV	1084 (9.2%)	918 (9.2%)	166 (9.7%)	20 (7.0%)	20 (8.1%)	0 (0%)
Missing	1798 (15.4%)	1427 (14.2%)	371 (21.7%)	75 (26.4%)	62 (25.1%)	13 (34.2%)
MCV	88.2 (83.6–92.2)	88.4 (83.9–92.3)	88.1 (83.5–92.1)	90.9 (86.6–99.0)	90.5 (86.6–99)	92.5 (86.9–97.5)
<80fL	1196 (10.2%)	1072 (10.7%)	124 (7.2%)	18 (6.3%)	16 (6.5%)	2 (5.3%)
80–100fL	8012 (68.3%)	6974 (69.7%)	1038 (60.6%)	147 (51.6%)	128 (51.8%)	19 (50%)
>100fL	376 (3.2%)	319 (3.2%)	57 (3.3%)	47 (16.5%)	41 (16.6%)	6 (15.8%)
Missing	2140 (18.3%)	1646 (16.4%)	494 (28.9%)	73 (25.6%)	62 (25.1%)	11 (28.9%)
First-line ART regimen						
d4T/3TC/EFV	3975 (55.5%)	3408 (58.7%)	567 (41.9%)	0 (0%)	0 (0%)	0 (0%)
d4T/3TC/NVP	440 (6.1%)	351 (6.0%)	89 (6.6%)	0 (0%)	0 (0%)	0 (0%)
TDF/3TC/NVP	157 (2.2%)	109 (1.9%)	48 (3.5%)	0 (0%)	0 (0%)	0 (0%)

Continued

Table 1. *Continued*

Characteristics	All eligible patients			All eligible patients on AZT-based regimens[a]		
	Total n=11724	Viral load <400 copies/ml n=10011	Viral load ≥400 copies/ml n=1713	Total n=285	Viral load <400 copies/ml n=247	Viral load ≥400 copies/ml n=38
	n (%) and median (IQR)/mean±SD			n (%) and median (IQR)/mean±SD		
TDF/FTC/NVP	2 (0%)	2 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
TDF/3TC/EFV	2318 (32.4%)	1711 (29.5%)	607 (44.8%)	0 (0%)	0 (0%)	0 (0%)
TDF/FTC/EFV	54 (0.8%)	51 (0.9%)	3 (0.2%)	0 (0%)	0 (0%)	0 (0%)
AZT/3TC/EFV	179 (2.5%)	150 (2.6%)	29 (2.1%)	150 (84.7%)	125 (87.4%)	25 (73.5%)
AZT/3TC/NVP	35 (0.5%)	24 (0.4%)	11 (0.9%)	27 (15.3%)	18 (12.6%)	9 (26.5%)

3TC: lamivudine; ART: antiretroviral therapy; AZT: zidovudine; d4T: stavudine; EFV: efavirenz; FTC: emtricitabine; MCV: mean cell volume; NVP: nevirapine; TDF: tenofovir.
[a] On AZT-based regimens throughout the study period.

≥400 copies/ml respectively. For MCV, the median change was 12.2 fL (IQR 5.7–18.3), with a median change of 13.2 fL (IQR 6.4–18.8) and 7.1 fL (IQR 2.7–12.6) for patients with a viral load <400 copies/ml and ≥400 copies/ml respectively. More than 70% of the patients did not miss a scheduled visit and 45.9% (5381) reported good adherence (self-reported) of more than 90% (Supplementary Table 1).

Self-reported adherence was measured at a median of 63 days before the viral load was done (IQR 56–84). The value for a variable at 6 months being defined as the first available value between 150 and 300 days after ART initiation, MCV at 6 months was measured at 202 days after ART initiation (IQR 170–268) and CD4 cell count at 6 months was measured at 204 days after ART initiation (IQR 171–272). For patients on an AZT-based regimen measurement was done 204 days after treatment initiation (IQR 175–261) for MCV and 211 days after ART initiation (IQR 176–267) for CD4 cell count. ART regimen was changed in 9.2% (1076) of the patients during the first 6 months (Supplementary Table 1).

Performance of the markers of adherence

We assessed the diagnostic accuracy (sensitivity, specificity, PPV and NPV) of each of the markers, compared to the first viral load result within 270 days of initiation as the gold standard, to correctly identify poor adherence during the first 6 months on ART. Change in MCV at 6 month had a sensitivity ranging from 70.2% for d4T and AZT-based regimens to 97.4% for tenofovir (TDF)-based regimens. Change in CD4 cell count at 6 months stratified by change in MCV for patients on TDF-based regimens had the highest sensitivity (98.2%) but for patients on d4T- or AZT-based regimens the sensitivity dropped to 76.8% and the specificity increased to 49.2% (Table 2).

Univariate and multivariate analysis of markers of poor adherence to ART

Poor adherence to treatment was observed in 1712 (14.6%) of the patients within 6 months of ART initiation. Univariate analysis for all eligible patients showed the following to be significantly associated with poor adherence: male gender, WHO stage of HIV disease at baseline and MCV at baseline (p<0.05). In addition, change in CD4 cell count at 6 months stratified by change in MCV at 6 months, number of missed medical visits, self-reported adherence, adverse events, low BMI indicative of rapid weight loss, change in CD4 cell count at 6 months, MCV at 6 months and change in MCV at 6 months were significantly associated with poor adherence (p<0.05). Number of missed ARV and other visits were not significantly associated with poor adherence at the univariate level. For all eligible patients on AZT-based regimens, the following factors were significantly associated with poor adherence: self-reported adherence, change in CD4 cell count at 6 months, number of missed visits, number of missed medical visits, MCV at 6 months, haemoglobin and pregnancy (p<0.05) (Table 3).

After adjusting for potential confounders in the multivariate regression analysis, change in CD4 cell count at 6 months stratified by change in MCV at 6 months, WHO stage at baseline and MCV at baseline were independently associated with poor adherence. For patients on AZT-based regimens, MCV at 6 months was an independent marker of poor adherence in the final multivariate model.

Discussion

Better patient adherence would help patients stay on first-line treatment for longer, hence reducing the need for more expensive second-line treatment. We identified several factors associated with adherence to ART, as measured by a suppressed viral load at 6 months. These include WHO stage and MCV at baseline and change in CD4 cell count stratified by change in MCV at 6 months, this being the strongest marker of poor adherence and the most relevant. The reason being that since baseline values of factors such as WHO stage, MCV and CD4 cell count are measured at treatment initiation stage, any change in

Table 2. Sensitivity, specificity, PPV and NPV of markers to identify poor adherence to ART using viral load as the gold standard

Variable	Sensitivity	Specificity	PPV	NPV
Change in MCV at 6 months for patients on d4T or AZT-based regimens[a]	70.2%	61.4%	18.2%	94.4%
Change in MCV at 6 months for patients on TDF-based regimens[b]	97.4%	3.1%	24.4%	78.4%
Change in CD4 count at 6 months	33.0%	81.4%	26.0%	86.0%
Change in CD4 count at 6 months for patients on AZT-based regimens[c]	64.7%	75.2%	29.7%	92.9%
Change in CD4 count stratified by change in MCV	86.5%	37.3%	18.8%	94.3%
Change in CD4 count stratified by change in MCV for patients on d4T or AZT-based regimens[a]	76.8%	49.2%	15.0%	94.8%
Change in CD4 count stratified by change in MCV for patients on TDF-based regimens[b]	98.2%	2.4%	24.2%	80.8%
Pregnant during the first 6 months on ART for patients on AZT-based regimens[b]	20.0%	97.6%	60.0%	87.0%

ART: antiretroviral therapy; AZT: zidovudine; MCV: mean cell volume; NPV: negative predictive value; PPV: positive predictive value; TDF: tenofovir.
[a] On d4T or AZT-based regimens throughout the study period; [b] On TDF-based regimens throughout the study period; [c] On AZT-based regimens throughout the study period.

such measure afterwards could be attributed to change in the level of adherence.

During the study period, 63% (3651) of the patients in this study were on a d4T or AZT-based regimen. A change in MCV at 6 months after initiation was significantly associated with poor adherence in the univariate analysis, this finding was not unusual. While change in MCV at 6 months is a marker of poor adherence, for patients on TDF based regimens there is a low specificity. Likewise, the specificity for the change in CD4 cell count stratified by change in MCV at 6 months was also low for patients on TDF-based regimens. Nevertheless, regardless of the regimen type, patients who had a change in CD4 cell count less than expected at 6 months and a change in MCV that is <14.5 fL, had a significantly higher risk of having a detectable viral load at 6 months. MCV is known to increase, particularly in adherent patients on AZT-based regimens.[18,20] For patients on AZT-based regimens, however, change in MCV at 6 months was not significantly associated with adherence. This disparity may also explain why there was no interaction between change in MCV and change in CD4 cell count at 6 months for patients on AZT-based regimens. In these patients, change in CD4 cell count at 6 months was independently associated with poor adherence.

The South African Department of Health ART treatment guidelines released in 2013, stipulate that FBC, from which MCV is derived, should be assessed at initiation of ART, 3 and 6 months for patients on AZT-based regimens to detect AZT toxicity.[27] However, the findings of this study make the case for MCV monitoring for patients on d4T as well, in order to monitor adherence in these patients. After 6 months of ART, MCV monitoring to assess adherence may still be necessary for patients on AZT and d4T-based regimens. The interaction between changes in MCV and CD4 cell count at 6 months might also indicate that CD4 cell count may need to be tested at 6 months and beyond the currently stipulated schedule of 1 year on ART.[27]

Both MCV and CD4 cell count at 6 months could thus help health workers identify patients with poor adherence to treatment in the absence of viral load testing. These patients could then be offered further interventions to improve adherence. One such intervention would be more intensive adherence counselling, which would include identifying and seeking solutions to the reasons for poor adherence. Additionally, pill count could be added to quantify adherence. Pill count, however, is prone to error as medication may be removed and discarded, thus, unannounced pill counts may be more accurate.[7] Other interventions such as electronic or therapeutic drug monitoring could also be used in addition to intensified counselling, but are too expensive for resource-limited settings (at least US$115 and US$38 respectively). In addition, electronic drug monitoring tells you the time the pill bottle was opened, but not whether medication was actually taken. Therapeutic drug monitoring on the other hand is vulnerable to white coat adherence.[12,32] This is full adherence for a short interval prior to a clinic visit, making serum drug monitoring unreliable.[32] Furthermore, serum drug levels cannot routinely measure nucleoside reverse transcriptase inhibitor levels because active moieties are intracellular.[12]

There was no observed association between CD4 cell count at baseline and poor adherence. WHO stage ≥2 at baseline was significantly associated with a detectable viral load at 6 months and hence poor adherence. These results are contrary to the findings of Maqutu and colleagues in South Africa, where adherence of <95%, in the first month and measured by pill count, was found to be associated with a higher CD4 cell count at baseline.[33] Similarly, Heckman and colleagues in the USA found good adherence measured by self-report to be associated with lower progression to AIDS.[34] The DART trial showed that complete (100%) adherence, measured by pill count, was associated with a higher baseline CD4 cell count.[10] The fact that the risk of a detectable viral load at 6 months increases with higher WHO stage at baseline emphasises the need for people to know their HIV status and start ART early if eligible.

In a meta-analysis by Nieuwkerk et al.,[14] self-reported adherence was found to distinguish between poor and good adherence, as evidenced by detectable and undetectable viral loads. The absence of self-reported adherence from the final multivariate model in this study may be due to the large proportion of patients (>50%) without self-reported adherence, making the variable or marker unreliable. It may also reflect the fact that

Table 3. Univariate and multivariate analysis of markers of poor adherence to ART among HIV-infected patients

Variable	All eligible patients				All eligible patients on AZT-based regimens[a]			
	Univariate analyses		Multivariate analysis		Univariate analyses		Multivariate analysis	
	Crude RR (95% CI)	p	p	p	Crude RR (95% CI)	p	Adjusted RR (95% CI)	p
Gender								
Females	1		1		1		1	
Males	1.17 (1.07–1.28)	<0.001	1.07 (0.90–1.28)	NS	1.78 (0.97–3.27)	NS	1.80 (0.53–6.11)	NS
Age								
≤36.7 years	1		1		1		1	
>36.7 years	0.93 (0.85–1.02)	NS	0.86 (0.71–1.02)	NS	0.99 (0.53–5.82)	NS	1.10 (0.30–4.00)	NS
Baseline CD4								
>200	1		1		1		1	
101–200	1.02 (0.88–1.20)	NS	1.12 (0.83–1.51)	NS	0.91 (0.40–2.10)	NS	1.07 (0.35–3.27)	NS
51–100	1.12 (0.95–1.33)	NS	1.07 (0.77–1.49)	NS	0.46 (0.14–1.59)	NS	0.28 (0.04–1.79)	NS
≤50	1.14 (0.98–1.33)	NS	1.28 (0.95–1.72)	NS	0.69 (0.25–1.89)	NS	0.40 (0.04–3.86)	NS
Baseline WHO stage							NA	NA
I	1		1		1			
II	1.18 (1.02–1.36)	0.023	1.18 (0.90–1.54)	NS	1.11 (0.35–3.50)	NS		
III	1.26 (1.12–1.43)	<0.001	1.41 (1.13–1.76)	0.002	1.88 (0.38–2.04)	NS		
IV	1.30 (1.10–1.53)	0.002	1.44 (1.10–1.90)	0.009				
MCV at baseline							NA	NA
<80 fL	1		1		1			
80–100 fL	1.25 (1.05–1.49)	0.013	1.46 (1.07–1.99)	0.016	1.01 (0.43–2.39)	NS		
>100 fl	1.46 (1.09–2.00)	0.011	1.03 (0.61–1.75)	NS	0.87 (0.19–3.94)	NS		
CD4 at 6/12			NA	NA			NA	NA
≥expected	1				1			
<expected	1.72 (1.51–1.96)	<0.001			3.41 (1.16–10.01)	0.025		
MCV at 6/12							NA	NA
<80 fL	1				1		1	
80–100 fL	1.35 (1.00–1.84)	0.051			3.58 (1.56–8.59)	0.003	5.54 (1.70–18.02)	0.004
>100 fL	0.65 (0.48–0.90)	0.009						
MCV at 6/12			NA	NA			NA	NA
≥14.5 fL	1				1			
<14.5 fL	2.82 (2.34–3.40)	<0.001			1.94 (0.60–6.24)	NS		
CD4 & MCV 6/12							NA	NA
CD4 ≥ & MCV ≥14.5 fL	1		1		1			
CD4 ≥ & MCV <14.5 fL	2.55 (2.01–3.23)	<0.001	2.82 (2.16–3.67)	<0.001	3.67 (0.40–33.70)	NS		
CD4 < & MCV ≥14.5 fL	0.96 (0.60–1.54)	NS	1.20 (0.73–1.97)	NS	6.60 (0.66–66.31)	NS		
CD4 < & MCV <14.5 fL	4.83 (3.77–6.20)	<0.001	5.49 (4.13–7.30)	<0.001	6.2 (0.69–55.61)	NS		

ART: antiretroviral therapy. ARV: antiretroviral; AZT: zidovudine; BMI: body mass index; IRR: incidence rate ratio; MCV: mean cell volume; NA: not applicable; NS: not signifiant.

The reference group for patients on AZT-based regimens is >100 fL, the following group is 80–100 fL and the last group is <80 fL. This is because <80 fL had no observations for MCV at 6 months in patients on AZT-based regimens.

[a] On AZT-based regimens throughout the study period.

the variable is not reliable as it is vulnerable to errors from patient recall and dishonesty[12,13]; separating these two was found to be difficult. It should be noted that self-reported adherence is a measure of how ART is taken but does not encompass the full definition of adherence, which includes following other recommendations pertaining to food.[35]

Limitations

Missing data might have introduced information bias if patients with missing data were systematically different from patients with available data and the variable is somehow related to the viral load at 6 months. In addition, professional status and education level are recorded only at initiation and it was, therefore,

not possible to adjust for any changes in the two variables during the study period. Furthermore, as viral load testing was not routinely done at baseline, it was not possible to adjust for this variable in the multivariate model.

Selection bias was another limitation as patients excluded due to transferring out, loss to follow up or death during the study period may have introduced systematic error in the study results. There is a possibility the patients who were lost to follow up or died during the first 6 months on ART might have have adhered poorly to treatment. This early poor adherence may be a strong predictor of loss to follow up and ultimate progression to AIDS and mortality.[36,37]

An additional limitation was that the outcome variable, viral load at end-point (6 months) is itself a surrogate marker of adherence, as poor adherence is the most common cause of a detectable viral load at 6 months on ART. Other factors such as malabsorption, drug interactions, diabetes mellitus and the acquisition of a drug-resistant HIV strain may contribute to a detectable viral load at 6 months, despite good adherence. It was also noted that there was no known surrogate clinical marker for the detection of adherence levels for tenofovir disoproxil fumarate (TDF), lamivudine (3TC), emtricitabine (FTC) nevirapine (NVP), efavirenz (EFV). Change in MCV at 6 months was a marker only for patients on d4T or AZT-based regimens, who were in the majority in this analysis.

Conclusions

In conclusion, the strongest marker of poor ART adherence among adults with HIV at Themba Lethu Clinic was change in CD4 cell count stratified by change in MCV at 6 months. MCV and WHO stage at baseline, were also predictors of poor adherence. For adult patients on AZT-based regimens, the markers of adherence to ART were change in CD4 cell count at 6 months and pregnancy during the first 6 months on ART. Therefore, special attention should be paid to the above-mentioned markers of poor adherence. These markers could help health workers identify poor adherence to treatment in the absence of viral load testing and target patients for interventions to prevent virological failure. Further studies are needed to verify whether the markers of adherence remain consistent over longer periods of time on ART. Further studies are also recommended in order to confirm whether the markers of adherence remain consistent as accessibility to and number of patients on TDF-based regimens increases, i.e., if these markers are useful for other ART regimens besides AZT and d4T.

Supplementary data

Supplementary data are available at *Transactions of The Royal Society of Tropical Medicine and Hygiene* online.

References

1 Shisana, O, Rehle, T, Simbayi LC et al. South African National HIV Prevalence, Incidence and Behaviour Survey, 2012. Cape Town: HSRC Press; 2014.

2 UNAIDS. Global AIDS response progress reporting 2015. http://www.unaids.org/sites/default/files/media_asset/JC2702_GARPR2015guidelines_en.pdf [accessed 30 September 2016].

3 Hill S, Kavookjian J. Motivational interviewing as a behavioral intervention to increase HAART adherence in patients who are HIV-positive: a systematic review of the literature. AIDS Care 2012;24:583–92.

4 Maqutu D, Zewotir T, North D et al. Determinants of optimal adherence over time to antiretroviral therapy amongst HIV-positive adults in South Africa: a longitudinal study. AIDS Behav 2011;15:1465–74.

5 El-Khatib Z, Ekstrom A, Coovadia A et al. Adherence and virologic suppression during the first 24 weeks on antiretroviral therapy among women in Johannesburg, South Africa – a prospective cohort study. BMC Public Health 2011;11:1–13.

6 Hofer CB, Schechter M, Harrison LH. Effectiveness of antiretroviral therapy among patients who attend public HIV Clinics in Rio de Janeiro, Brazil. J AIDS 2004;36:967–71.

7 Bangsberg DR. Less than 95% adherence to non-nucleoside reverse transcriptase inhibitor therapy can lead to viral suppression. Clin Infect Dis 2006;43:939–41.

8 Levine AJ, Hinkin CH, Castellon SA et al. Variations in patterns of highly active antiretroviral therapy (HAART) adherence. AIDS Behav 2005;9:355–62.

9 Johnson MO, Charlebois E, Morin SF et al. Perceived adverse effects of antiretroviral therapy. J Pain Symptom Manage 2005;29:193–205.

10 Muyingo SK, Walker AS, Reid A et al. Patterns of individual and population-level adherence to antiretroviral therapy and risk factors

for poor adherence in the first year of the DART trial in Uganda and Zimbabwe. J AIDS 2008;48:468–75.

11 Ramadhani HO, Thielman NM, Landman KZ et al. Predictors of incomplete adherence, virologic failure, and antiviral drug resistance among HIV-infected adults receiving antiretroviral therapy in Tanzania. Clin Infect Dis 2007;45:1492–8.

12 Berg KM, Arnsten JH. Practical and conceptual challenges in measuring antiretroviral adherence. J AIDS 2006;43:S79–87.

13 Segeral O, Madec Y, Ban B et al. Simplified assessment of antiretroviral adherence and prediction of virological efficacy in HIV-infected patients in Cambodia. AIDS Res Treat 2010;2010:1–6.

14 Nieuwkerk PT, Oort FJ. Self-reported adherence to antiretroviral therapy for HIV-1 infection and virologic treatment response: a meta-analysis. J AIDS 2005;38:445–8.

15 Brennan AT, Maskew M, Sanne I et al. The importance of clinic attendance in the first six months on antiretroviral treatment: a retrospective analysis at a large public sector HIV clinic in South Africa. J Int AIDS Soc 2010;13:1–10.

16 Fox MP, Maskew M, MacPhail AP et al. Cohort profile: the Themba Lethu Clinical Cohort, Johannesburg, South Africa. Int J Epidemiol 2013;42:430–9.

17 Harrigan PR, Hogg RS, Dong WWY et al. Predictors of HIV drug-resistance mutations in a large antiretroviral-naive cohort initiating triple antiretroviral therapy. J Infect Dis 2005;191:339–47.

18 Romanelli F, Empey K, Pomeroy C. Macrocytosis as an indicator of medication (zidovudine) adherence in patients with HIV infection. AIDS Patient Care STDs 2002;16:405–11.

19 Rivas P, Gorgolas M, Fernandez-Guerrero ML. Zidovudine and red-cell distribution width. NEJM 2005;352:2141–2.

20 Bartlett JG, Gallant JE, Pham PA. 2009-2010 Medical Management of HIV Infection. 15th ed. Durham, North Carolina: Knowledge Source Solutions LLC; 2009.

21 Kamya MR, Mayanja-Kizza H, Kambugu A et al. Predictors of long-term viral failure among Ugandan children and adults treated with antiretroviral therapy. J AIDS 2007;46:187–93.

22 Mugisha JO, Donegan K, Fidler S et al. Mean corpuscular volume as a marker for adherence to zidovudine-containing therapy in HIV-infected adults. Open AIDS J 2012;6:45–52.

23 Steele RH, Keogh GL, Quin J et al. Mean cell volume (MCV) changes in HIV-positive patients taking nucleoside reverse transcriptase inhibitors (NRTIs): a surrogate marker for adherence. Int J STD AIDS 2002;13:748–54.

24 Kantor R, Diero L, DeLong A et al. Misclassification of first-line antiretroviral treatment failure based on immunological monitoring of HIV infection in resource-limited settings. Clin Infect Dis 2009;49:454–62.

25 Reynolds SJ, Nakigozi G, Newell K et al. Failure of immunologic criteria to appropriately identify antiretroviral treatment failure in Uganda. AIDS 2009;23:697–700.

26 Oyugi JH, Byakika-Tusiime J, Charlebois ED et al. Multiple validated measures of adherence indicate high levels of adherence to generic HIV antiretroviral therapy in a resource-limited setting. J AIDS 2004;36:1100–2.

27 National Department of Health. The South African Antiretroviral Treatment Guidelines 2013, Pretoria. http://www.kznhealth.gov.za/medicine/2013_art_guidelines.pdf [accessed 30 September 2016].

28 National Department of Health, South Africa. National Antiretroviral Treatment Guidelines. 1st ed. Pretoria: Jacana; 2004. http://apps.who.int/medicinedocs/documents/s17758en/s17758en.pdf [accessed 30 January 2016].

29 National Department of Health, South Africa 2010. Clinical Guidelines for the Management of HIV and AIDS in Adults and Adolescents. http://www.who.int/hiv/pub/guidelines/south_africa_art.pdf [accessed 10 August 2016].

30 Rosen S, Fox MP, Gill CJ. Patient retention in antiretroviral therapy programs in Sub-Saharan Africa: a systematic review. PLoS Med 2007;4:1691–700.

31 Mocroft A, Phillips AN, Gatell J et al. Normalisation of CD4 counts in patients with HIV-1 infection and maximum virological suppression who are taking combination antiretroviral therapy: an observational cohort study. Lancet 2007;370:407–13.

32 Podsadecki T, Vrijens B, Tousset E et al. 'White coat compliance' limits the reliability of therapeutic drug monitoring in HIV-1-infected patients. HIV Clin Trials 2008;9:238–46.

33 Maqutu D, Zewotir T, North D et al. Factors affecting first-month adherence to antiretroviral therapy among HIV-positive adults in South Africa. Afr J AIDS Res 2010;9:117–24.

34 Heckman BD, Catz SL, Heckman TG et al. Adherence to antiretroviral therapy in rural persons living with HIV disease in the United States. AIDS Care 2004;16:219–30.

35 Tiyou A, Belachew T, Alemseged F et al. Predictors of adherence to antiretroviral therapy among people living with HIV/AIDS in resource-limited setting of southwest Ethiopia. AIDS Res Ther 2010;7:1–10.

36 Tran DA, Ngo AD, Shakeshaft A et al. Trends in and determinants of loss to follow up and early mortality in a rapid expansion of the antiretroviral treatment program in Vietnam: findings from 13 outpatient clinics. PLoS One 2013;8:1–8.

37 Hambisa MT, Ali A, Dessie Y. Determinants of mortality among HIV-positives after initiating antiretroviral therapy in Western Ethiopia: A hospital-based retrospective cohort Study. ISRN AIDS. 2013;2013:1–7. DOI:http://dx.doi.org/10.1155/2013/491601.