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Benefit of viral load testing for confirmation of immunological failure in HIV patients treated in rural Malawi

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Summary

OBJECTIVE Viral load testing is used in the HIV programme of Chiradzulu, Malawi, to confirm the diagnosis of immunological failure to prevent unnecessary switching to second-line therapy. Our objective was to quantify the benefit of this strategy for management of treatment failure in a large decentralized HIV programme in Africa.

METHODS Retrospective analysis of monitoring data from adults treated with first-line antiretroviral regimens for >1 year and meeting the WHO immunological failure criteria in an HIV programme in rural Malawi. The positive predictive value of using immunological failure criteria to diagnose virological failure (viral load >5000 copies/ml) was estimated.

RESULTS Of the 227 patients with immunological failure (185 confirmed with a repeat CD4 measurement), 155 (68.2%) had confirmatory viral load testing. Forty-four (28.4%) had viral load >5000 copies/ml and 57 (36.8%) >1000 copies/ml. Positive predictive value was 28.4% (95% CI 21.4–36.2%). Repeat CD4 count testing showed that 41% of patients initially diagnosed with immunological failure did no longer meet failure criteria.

CONCLUSIONS Our results support the need for confirming all cases of immunological failure with viral load testing before switching to second-line ART to optimize the use of resources in developing countries.

keywords Africa, antiretroviral treatment, HIV, resource-limited setting, treatment failure

Introduction

In most resource-limited settings, access to routine viral load testing to monitor progression of HIV disease is limited or non-existent because of a combination of lack of laboratory capacity, prohibitive cost or logistic constraints (Calmy *et al.* 2007). This is especially true in sub-Saharan Africa, which bears the greatest burden of the HIV epidemic. As a result, evaluation of treatment outcomes and therapeutic decision-making are generally based on clinical and immunological findings. Discordance between recommended immunological and virological criteria for failure diagnosis is nevertheless well documented (Moore *et al.* 2006; Badri *et al.* 2008; Mee *et al.* 2008; Kantor *et al.* 2009; Keiser *et al.* 2009; Reynolds *et al.* 2009). In HIV programmes, lack of virological confirmation of failure may result in premature switching to second-line antiretroviral therapy (ART), ultimately leading to unnecessary exhaustion of available treatment options, and use of more complicated antiretroviral (ARV) regimens.

Following WHO recommendations (World Health Organization 2006), viral load testing is used in the HIV programme of Chiradzulu, Malawi, to confirm the diagnosis of immunological failure to prevent unnecessary switching to second-line therapy. The objective of this analysis was to quantify the benefit of implementing this strategy for management of treatment failure in a large decentralized HIV programme in Africa.

Methods

The Chiradzulu HIV programme

In collaboration with the Malawian Ministry of Health, Medecins Sans Frontières (MSF) has provided free ART to HIV-infected patients in the rural district of Chiradzulu since 2001 (Ferradini *et al.* 2006). HIV care and treatment are provided by clinical officers and nurses at the district hospital and in 10 peripheral primary health facilities. Criteria for starting ART are the diagnosis of a WHO clinical stage 3 or 4

event and/or a CD4 cell count of <250 cells/μl (Ministry of Health and Population 2008). The standard first-line ART regimen is a generic fixed-dose-combination of stavudine (d4T), lamivudine (3TC) and nevirapine (NVP).

Since 2007, routine CD4 cell testing is performed for all patients prior to ART initiation, at 6 and 12 months after the start of therapy, and annually thereafter. Since 2008, viral load testing is used for confirmation of immunological failure before switching to second line. Laboratory capacity limits the number of viral load tests to a maximum of 100 per month.

Study design and data collection

A longitudinal analysis of routine data from adults treated with first-line ART in the programme was performed. Individual clinical and immunological patient information, including age, sex, weight, height, WHO clinical stage, ART regimen prescriptions with dates of administration, and CD4 cell and viral load measurements, was routinely collected at each HIV consultation on standardized forms and entered into the FUCHIA software (Epicentre, Paris, France). All patient identifiers were removed from the database before analysis. The Malawian National Health Sciences Research Committee approved the use of data for this analysis.

In January 2009, all adults (≥15 years old) treated with first-line antiretroviral regimens for more than 12 months, who had more than one recorded CD4 cell measurement after ART start and met at least one of the immunological criteria of treatment failure, were identified. Results from virological testing confirmation were gathered over 1 year. Pregnant women were excluded because interpretation of absolute CD4 cell counts during pregnancy is problematic because of haemodilution related to the progressive rise of cortisol and plasma adenocorticotropine during the last stage of pregnancy (Ekouevi *et al.* 2007; Lebon *et al.* 2007).

Laboratory methods and study definitions

Patient blood specimens were collected daily and CD4 cell counts quantified at the Chiradzulu District Hospital using Partec© flow cytometry. Viral load testing was performed by the DREAM laboratory (Blantyre, Malawi) using bDNA for samples stored at −20 °C for a maximum of 7 days (minimal detection threshold of 50 copies/ml).

Immunological failure was defined as a decline in CD4 cell counts to or below treatment baseline values, decline ≥50% from on-treatment peak value or CD4 <100 cells/μl after 12 months of therapy (Murri *et al.* 2006; WHO 2006). Pre-therapy baseline CD4 cell count was the measurement closest in date and performed within 3 months of ART start. Virological failure was defined as a viral load >5000 copies/ml (>1000 in sensitivity analyses). Prior confirmation of immunological failure with a second CD4 cell measurement, although recommended, was inconsistently performed in the programme.

Statistical analysis

Standard summary statistics were used to describe the proportion of patients diagnosed with immunological and virological failure. The positive predictive value (PPV) of immunologic failure was estimated as the proportion of patients with >5000 copies/ml among those with available viral load results and failure diagnosis. Values were calculated for all patients and separately for patient with or without repeat CD4 count testing for immunological failure confirmation. Exact binomial 95% confidence intervals (CI) were calculated. Sensitivity analyses using the >1000 copies/ml threshold failure were finally performed. Data were analysed in STATA version 10 (StataCorp, College Station, TX, USA).

Results

In January 2009, a total of 7317 adults were receiving first-line ART for 1 year or more in the programme. Ninety-seven percent had more than one CD4 cell count measurement available during follow-up, and their initial CD4 cell count was 213 cells/μl [interquartile range, IQR 89–213] (Table 1). A total of 357 (4.9%) patients were diagnosed with immunological failure (Figure 1), and 66%

Table 1 Characteristics of patients receiving ART for >1 year included and excluded from the analyses

	Included *N* = 7092	Excluded (no CD4 cell count) *N* = 225
Men (%)	2108 (29.7)	71 (31.6)
Median age at ART start, years [IQR]	35.1 [29.9–42.6]	36.0 [29.1–43.6]
ART-naïve at therapy start (%)	6510 (91.8)	214 (95.1)
BMI <18.5 kg/m^2 (%)	2099 (29.6)	61 (27.1)
Tuberculosis treatment at ART start (%)	188 (2.7)	7 (3.1)
Median CD4 cell count at ART start [IQR]	213 [89–213]	182 [122–226.5]
3TC d4T NVP initial regimen (%)	6699 (94.5)	211 (93.8)
Months of ART use [IQR]	31.6 [19.8–48.8]	13.4 [12.5–15.9]

3TC, lamivudine; ART, antiretroviral therapy; BMI, body mass index; d4T, stavudine; IQR, interquartile range; NVP, nevirapine.

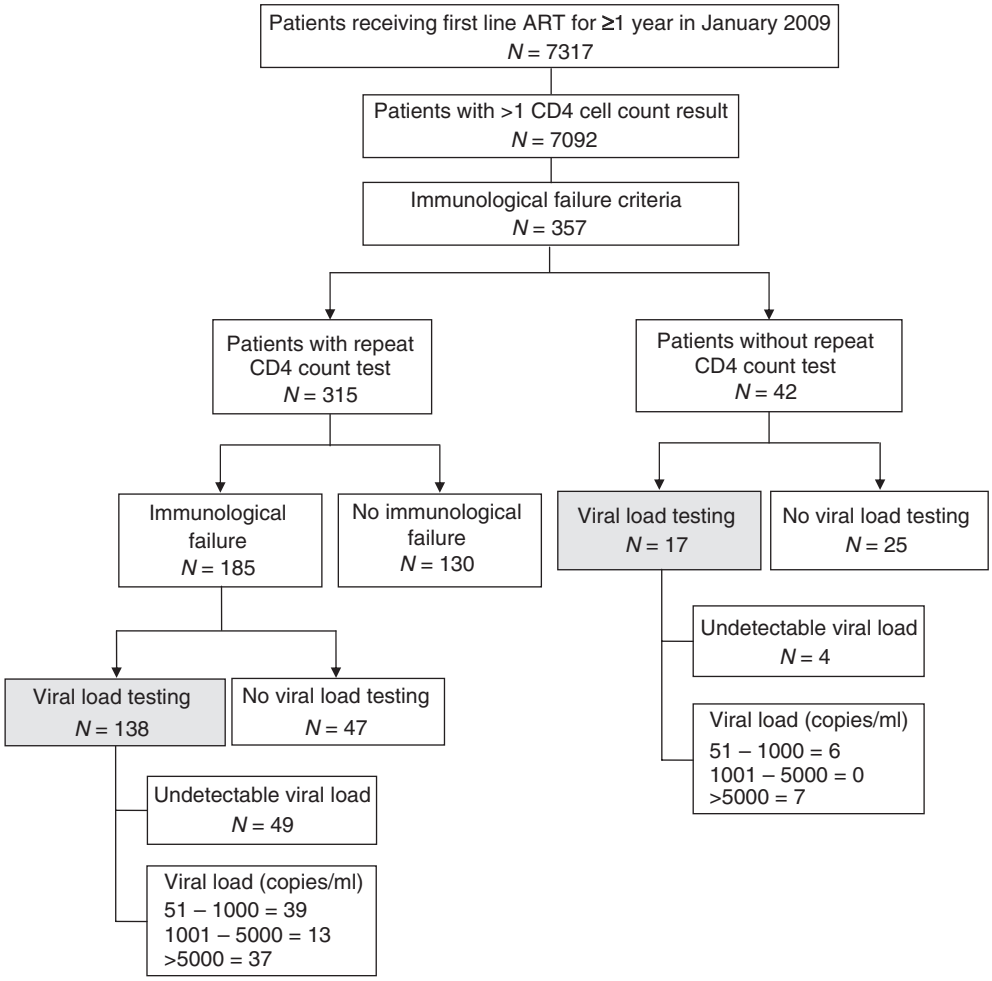

Figure 1 Study profile.

were women. At ART initiation, median age was 34 years, 330 (92.4%) patients were ART naïve, and 331 (92.7%) received 3TC-d4T-NVP. Median CD4 cell count at ART start was 182 cells/μl [IQR 87–260, n = 259]. At immunological failure diagnosis, median time on ART was 25.2 months [IQR 12.5–47.7], median CD4 cell count 175 cells/μl [IQR 91–259] and 25 (7.0%) patients received tuberculosis treatment.

CD4 cell testing was repeated for 315 (88.2%) patients, 59 within 3 months of the first CD4 measurement. The age, sex, time on ART and initial CD4 counts were similar in patients with or without repeat CD4 cell counts (data not shown). Although 130 (41.3%) patients with repeat CD4 results no longer had immunological failure, five had been already switched to second-line therapy without viral load confirmation. Of 185 patients with confirmed immunological failure, two died and one was lost to follow up before viral load testing could be performed. Ninety-one percent of patients had declines of CD4 count levels below initial values, 88% a fall of 50% from on-treatment peak values and 86% persistent levels below 100 cells/μl. Viral load testing was performed for 138 (74.6%) patients. Forty-nine patients (35.5% of the 138 tested) were virologically suppressed, and 37 (26.8%) had >5000 copies/ml. Of patients with detectable viral load, 29 (32.6%) were switched to a second-line regimen, eight of whom had viral loads ≤5000 copies/ml. At the time of analysis, however, 16 patients with viral loads >5000 had not been switched to second line (two had defaulted and the others were actively followed).

Of the 42 patients with no repeat CD4 count measurement, four (9.5%) died and eight (19.0%) defaulted before viral load was tested, and 17 (40.5%) had viral load results. Twenty-nine (69.0%) patients had

Table 2 Patient characteristics at ART initiation and at immunological failure diagnosis

	Viral load testing *N* = 155		No viral load testing *N* = 72		Total *N* = 227	
	ART start	Immunological failure	ART start	Immunological failure	ART start	Immunological failure
Men	50 (32.3)		29 (40.3)		79 (34.8)	
Median age, years [IQR]	34.0 [27.1–40.5]	37.6 [30.3–45.2]	35.5 [29.1–46.0]	39.1 [32.1–49.9]	34.1 [28.2–41.0]	37.7 [31.4–45.8]
Clinical stage (%)						
1 or 2	21 (13.5)	–	15 (20.8)	–	36 (15.9)	–
3	72 (46.5)	–	27 (37.5)	–	99 (43.6)	–
4	62 (40.0)	–	30 (41.7)	–	92 (40.5)	–
ART-naïve (%)	140 (90.3)	–	66 (91.7)	–	206 (90.7)	–
BMI group, kg/m^2 (%)						
<18.5	59 (38.0)	33 (21.3)	20 (27.7)	12 (16.7)	79 (34.8)	45 (19.8)
18.5–24.9	71 (45.8)	93 (60.0)	47 (65.3)	48 (66.7)	118 (52.0)	141 (62.2)
≥25	10 (6.5)	13 (8.4)	3 (4.2)	5 (6.9)	13 (5.7)	18 (7.9)
Missing	15 (9.7)	16 (10.3)	2 (2.8)	7 (9.7)	17 (7.5)	23 (10.1)
3TC d4T NVP regimen (%)	143 (91.6)	101 (65.2)	69 (95.8)	54 (75.0)	212 (93.4)	155 (68.3)
Median time receiving ART, months [IQR]	–	47.4 [29.2–60.7]	–	28.2 [19.8–47.7]		37.5 [24.5–59.9]
Receiving tuberculosis treatment (%)	2 (1.3)	8 (5.2)	1 (1.4)	3 (4.2)	3 (1.3)	11 (4.8)
Median CD4 cell count, cells/μl [IQR]	126 [51–223]	119 [69–210]	234 [112–458]	238 [140–360]	154 [56–269]	159 [77–257]
CD4 group, cells/μl (%)						
<50	25 (16.1)	22 (14.2)	13 (18.1)	3 (4.2)	38 (16.7)	25 (11.0)
50–250	58 (37.5)	106 (68.4)	19 (26.4)	36 (50.0)	77 (33.9)	142 (62.6)
>250	17 (10.9)	27 (17.4)	30 (41.6)	33 (45.8)	47 (20.8)	60 (26.4)
Missing	55 (35.5)	–	10 (13.9)	–	65 (28.6)	–

3TC, lamivudine; ART, antiretroviral therapy; BMI, body mass index; d4T, stavudine; IQR, interquartile range; NVP, nevirapine.
Initial clinical stage data were missing for 330 patients and initial CD4 cell count for 98.

Table 3 Positive predictive values (%) of immunological failure to identify virological failure

	Positive predictive value, % Based on virological failure threshold	
	>5000 copies/ml	>1000 copies/ml
Repeat CD4 cell count (n = 138)	26.8 (19.6–35.0)	36.2 (28.2–44.8)
No repeat CD4 cell count (n = 17)	41.2 (18.4–67.1)	41.2 (18.4–67.1)
All (n = 155)	28.4 (21.4–36.2)	41.3 (33.0–50.0)

declines of CD4 count levels below initial values, 19 (45.2%) a 50% fall from on-treatment peak values and 15 (35.7%) persistent levels <100 cells/μl. A total of four patients (23.5%) were virologically suppressed, and 7 (41.2%) had viral load >5000 copies/ml. Six patients were switched to second-line regimens, four with viral load >5000 copies/ml.

Overall, patients with viral load testing had lower median CD4 cell counts (119 *vs.* 238 cells/μl) and were receiving ART for longer time periods (median of 47.4 *vs.* 28.2 months) than patients with viral load measurements (Table 2). The positive predictive value of immunological failure to identify virological failure among the 155 patients with viral load results, 138 with repeat and 17 without repeat CD4 count testing was 28.4% (95% CI 21.4–36.2%) (Table 3). It was 41.3% (95% CI 33.0–50.0%) when the ≥1000 copies/ml viral load threshold were used. Positive predictive values were higher among patients with no repeat CD4 count measurement (41.2% compared to 26.8%), but 95% confidence intervals overlapped, and this analysis was based on small numbers of patients.

Discussion

This field evaluation in rural Malawi provided evidence of the benefit of routinely using viral load testing for confirmation of immunological failure before switching to second-line therapy in HIV programmes of resource-limited settings. In our analysis, only 28% of patients diagnosed with immunological failure actually experienced virological failure as defined in the new WHO guidelines (viral load >5000 copies/ml) (World Health Organization 2010).

In resource-limited settings where the rapid expansion and scale-up of HIV programmes has necessitated a simplified approach to ARV use, diagnosis and management of treatment failure remain challenging. WHO recommends using immunological failure criteria in settings without access to viral load testing (World Health Organization 2010). However, the use of the current algorithm is likely to result in unnecessary early switching of regimens. In our analysis, 34% of patients diagnosed with immunological failure actually were virologically suppressed. In the absence of virological confirmation, these patients might have been switched unnecessarily to second line. Switching to a more costly second-line regimen, currently not available in fixed-dose combinations (Médecins Sans Frontières 2010), is likely to compromise long-term adherence because of increased pill burden and consume limited financial resources allocated to ART programmes. Confirmatory viral load testing, although more costly than CD4 testing in the short term, can prevent the unnecessary switching of many patients to second-line therapy. This is particularly important in the current global financial climate with threatened 'flat-lining' of HIV funding. Furthermore, as HIV programmes age and expand accurate diagnosis of patients, failing therapies will become increasingly important to optimize patient management and drug supply. Implementing the most cost-effective strategies of patient management is therefore essential.

Findings of this analysis where virological failure was defined according to new WHO criteria (viral load >5000 copies/ml) are consistent with results from evaluations reporting poor performance of WHO immunological failure criteria (Bisson *et al.* 2006; Moore *et al.* 2006; Badri *et al.* 2008; Mee *et al.* 2008; van Oosterhout *et al.* 2009) based on the previous WHO definition of virological failure (>10 000 threshold) (World Health Organization 2006), although in areas where access to viral load testing is limited because of cost or logistic constraints, repeat CD4 count testing could allow improved targeting of patients who are more likely to experience virological failure. Indeed, three of seven patients initially thought to meet immunological failure criteria in our study did not require viral load testing and were easily identified with this diagnostic algorithm.

This study was based on the analysis of routine monitoring data, and not all patients identified with immunological failure were tested for viral load in a timely manner (68% of the individuals diagnosed during the study period). Furthermore, patients without viral load confirmatory results had higher CD4 cell counts (median of 238 *vs.* 119 cells/μl) and were receiving ART for shorter time periods (median of 28.2 *vs.* 47.4 months) than those with viral load measurements. Because of these differences, we might have under- or overestimated the positive predictive values of immunological failure diagnosis in our setting. The large volume of patients treated in the programme of Chiradzulu and the high patient/clinician ratio frequently leave clinicians with limited time to consider alternative

aetiologies for observed decreasing CD4 measurements [e.g. presence of bacterial or parasitic infection or use of corticosteroids (van Oosterhout *et al.* 2009)], and virological failure might actually not reflect treatment failure in some of the patients. Although this issue could contribute to explain the poor performance of immunological and virological results, it is unlikely to be the only explanation; our study primarily reflects the reality and difficulties of clinical decision-making in resource-poor settings.

Conclusions

Our findings support the need to confirm all cases of immunological failure with viral load testing before switching to second-line therapy. Developing simple, reliable and affordable point-of-care tools for viral load testing is essential to improve patient management and optimize the use of resources in developing countries.

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