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The role of targeted viral load testing in diagnosing virological failure in children on antiretroviral therapy with immunological failure

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Abstract

Objectives—To determine the improvement in positive predictive value of immunological failure criteria for identifying virological failure in HIV-infected children on antiretroviral therapy (ART) when a single targeted viral load measurement is performed in children identified as having immunological failure.

Methods—Analysis of data from children (<16 years at ART initiation) at South African ART sites at which CD4 count/percent and HIV-RNA monitoring are performed 6-monthly. Immunological failure was defined according to both WHO 2010 and United States DHHS 2008 criteria. Confirmed virological failure was defined as HIV-RNA >5000 copies/ml on 2 consecutive occasions <365 days apart in a child on ART for ≥18 months.

Results—Among 2798 children on ART for ≥18 months (median [IQR] age: 50 (21–84) months at ART initiation), the cumulative probability of confirmed virological failure by 42 months on ART was 6.3%. Using targeted viral load after meeting DHHS immunological failure criteria rather than DHHS IF criteria alone increased PPV from 28% to 82%. Targeted viral load improved the positive predictive value of WHO 2010 criteria for identifying confirmed virological failure from 49% to 82%.

Conclusion—The addition of a single viral load measurement in children identified as failing immunologically will prevent most switches to second-line treatment in virologically suppressed children.

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Keywords

HIV virological failure children; antiretroviral therapy; monitoring immunological failure

INTRODUCTION

In many resource-limited settings access to HIV-RNA measurement is limited and clinicians rely on clinical and immunological criteria to identify children failing first-line antiretroviral therapy (ART). These criteria have poor diagnostic accuracy for virological failure, with both low sensitivity and positive predictive value (PPV) (Davies et al. 2011, Ruel et al. 2010, Jittamala et al. 2009). Low PPV means that children may be inappropriately switched to limited and expensive second-line ART, when they are still virologically suppressed. Confirming treatment failure with a single elevated targeted HIV-RNA measurement once immunological failure criteria are met (targeted viral load [TVL] approach), together with a thorough assessment of adherence, may prevent switches to second-line when the virus is likely to still be sensitive to the first-line regimen. This approach is recommended in WHO 2010 pediatric treatment guidelines (WHO 2010, Rewari et al. 2010). For example, inappropriate switches to second-line of adults who had TVL performed were far less common than of those with CD4 monitoring only (12.4% vs 46.9%) (Sigaloff et al. 2011).

Different CD4 thresholds have been used to define immunological failure (IF). For example, the United States Department of Health and Human Services (DHHS) 2008 guidelines defined children as failing immunologically if they experienced any of: a confirmed decline of CD4% by 5 percentage points from the previous value or a return of CD4 count to below the baseline value (applicable to a child age ≥ 5 years at baseline) (National Institutes of Health 2008). The WHO 2010 guidelines consider a child to be failing immunologically if CD4% or CD4 count decline to $<10\%$ or 200 cells/mm^3 respectively (children aged 2–4 years) or CD4 count declines to $<100 \text{ cells/mm}^3$ (children aged ≥ 5 years). No definition of IF is provided for children <2 years of age (WHO 2010). We have previously shown that the sensitivity of the DHHS 2008 IF definition (27%; 95% confidence intervals [CI]: 19–35%) was greater than that of WHO 2010 definition (5%; 95% CI: 2–9%) for identifying children with confirmed virological rebound. (Davies et al. 2011) (Note: Confirmed virological rebound was defined as HIV-RNA >5000 copies/ml on 2 consecutive occasions <365 days apart in a child on ART for ≥ 18 months who achieved suppression during the first year on ART.) (Davies et al. 2011). However, PPV was low for both DHHS (20%; 95% CI 13–26%) and WHO 2010 (42%; 22–62%) criteria. While these results underline the value of routine HIV-RNA monitoring, access is likely to remain limited in the short term in many settings due to financial and logistical constraints. (Sigaloff et al. 2011) Therefore we aimed to use data from children receiving ART at South African IeDEA-Southern Africa (IeDEA-SA) sites, all of which had access to at least 6-monthly CD4 and HIV-RNA monitoring, to determine to what extent PPV for identifying virological failure improves when adding TVL to either DHHS and WHO 2010 IF criteria

METHODS

Data were collected prospectively from ART-naïve children (<16 years at ART start) initiating ≥ 3 antiretroviral drugs at South African sites participating in IeDEA-SA (www.iedea-sa.org). Site characteristics have been described previously (Davies et al. 2009). Each site has institutional ethical approval to contribute data to IeDEA analyses. HIV-RNA was measured using Amplicor 1.5 (Roche Diagnostics) or NucliSens EasyQ assays (bioMérieux), with good comparability (Stevens et al. 2005). CD4 measurements were

performed using standard dual platform flow cytometry or the single platform PanLeucogated method (Glencross et al. 2008).

Confirmed virological failure (CVF) was defined as HIV-RNA >5000 copies/ml on 2 consecutive occasions <365 days apart in a child who had been on treatment for at least 18 months irrespective of whether suppression to <400 copies/ml was achieved in the first year on treatment. IF criteria were as follows: DHHS: a decline of CD4% by 5 percentage points from the previous value, confirmed at a subsequent measurement within 365 days after the first low value (applicable to a child of any age) or a return of CD4 count to less than the baseline value (applicable to a child age ≥ 5 years at baseline) (National Institutes of Health 2008). WHO 2010: No definition for children <2 years; CD4%<10% or CD4<200 cells/mm³ (age 2–4 years); CD4<100 cells/mm³ (age ≥ 5 years) (WHO 2010). For each of these IF criteria, children were further considered to meet TVL failure criteria if they met the respective IF criteria and the next HIV-RNA measurement (within 4 months of meeting IF criteria) was >5000 copies/ml. CD4 results for which there was no available HIV-RNA measurement within 4 months, which was required to assess TVL, were excluded from the analysis (1% and <1% of measurements for DHHS 2008 and WHO 2010 criteria respectively).

For all failure diagnoses (IF, TVL or CVF) measurements had to be taken during a period when the child was on treatment and not during a treatment interruption. Where tests were performed asynchronously, IF, TVL and CVF diagnoses were carried forward for up to 3 months. We compared each unique paired TVL and CVF diagnosis to determine diagnostic accuracy, using robust standard errors to account for multiple measures per patient. TVL results for which there were no concurrent CVF diagnosis after carrying forward results were not evaluated. The last TVL result before the end of follow-up was excluded to ensure that sufficient follow-up for a confirmatory low viral load measurement had been done. All analyses were performed using Stata 11 (Stata Corporation, College Station, Texas)

RESULTS

Data from 2798 children on ART for ≥ 18 months were included. Characteristics at ART initiation were as follows: median (interquartile range) age: 50 (21–84) months; 48% female; 64% WHO Clinical Stage 3 or 4 and 79% WHO-defined severe immune suppression. One third of children started a protease inhibitor-based first-line regimen. The cumulative probability of CVF by 42 months on ART was 6.3%. Using TVL after meeting DHHS IF criteria rather than DHHS IF criteria alone increased PPV from 28% to 82% and the likelihood ratio of a positive test from 2.08 to 23.89 respectively (Table 1). Among the 19 TVL-diagnosed false positive cases 8 (42%) had 2 HIV-RNA measurements >400 copies/ml but only 1 > 5000 copies/ml, and 11 (58%) had a single elevated HIV-RNA with resuppression at subsequent measurement.

Using TVL after meeting WHO IF criteria rather than WHO IF criteria alone increased PPV from 49% to 82% and the likelihood ratio of a positive test from 5.41 to 25.98 respectively (Table 1). Among the 4 TVL-diagnosed false positive cases 3 (75%) had 2 HIV-RNA measurements >400 copies/ml but only 1 >5000 copies/ml, and 1 (25%) had a single elevated HIV-RNA with resuppression at subsequent measurement.

DISCUSSION

These results illustrate that although even the most sensitive immunological criteria detect only a quarter of cases of virological failure, the addition of TVL to these criteria raises PPV and could reduce the number of switches to second-line treatment in virologically

suppressed children. More than 80% of children considered to be failing therapy according to either DHHS or WHO IF criteria together with TVL would also meet the criteria for CVF.

Nevertheless, TVL results in a small but important number of false positive diagnoses as a proportion of children resuppress after a single elevated HIV-RNA measurement. Although children were not considered to be failing therapy according to any definition if measurements were taken during a documented treatment interruption, we did not have detailed adherence data. It is possible that these measurements may have been taken during adherence lapses. Since these data come from programs with routine HIV-RNA monitoring, the elevated HIV-RNA measurement may have actually facilitated identification of poor adherence, prompting counseling and intervention with subsequent resuppression (Wilson et al. 2009). This adds to the evidence in adults supporting HIV-RNA measurement as a tool to discriminate between poor adherence and therapeutic failure (Orrell et al. 2007, Calmy et al. 2007). Routine HIV-RNA monitoring also allows early accurate identification of virological failure and consequent switching to second-line, thus preventing accumulation of resistance mutations, which is not possible with a more limited TVL approach (Sigaloff et al. 2011, Wilson et al. 2009). Indeed modeling studies in children suggest that annual HIV-RNA monitoring after an initial screen 6 months after ART start would result in a 77% reduction in time spent with virological failure compared with no HIV-RNA monitoring (Schneider et al. 2011).

Strengths of this study include the large cohort across many sites in South Africa with routine access to both CD4 and HIV-RNA measurement, hence the large number of TVL and CVF results available for comparison. Limitations of these routinely collected data include missing baseline CD4 values in some children, which limited evaluation of DHHS criteria, work-up bias that may occur if either the reference (HIV-RNA) or index (CD4 with single HIV-RNA) tests are not applied consistently (Whiting et al. 2004) or when people switch to a second-line regimen before immunological failure occurs. The study was further limited by lack of data with respect to intercurrent illnesses that should be excluded before considering a low CD4 count to be indicative of immunological failure (WHO 2010).

We conclude that the addition of targeted viral load to CD4 monitoring will prevent most switches to second-line therapy in virologically suppressed children. This is particularly important to avoid exhausting the limited drug options for children facing lifelong therapy. Our previous study demonstrated the low sensitivity of immunological criteria for identifying virological failure (Davies et al. 2011), and TVL should not replace routine HIV-RNA monitoring in settings such as South Africa where it is available or easily achievable. Rather, the introduction of TVL may be a first step towards increasing access to routine viral load monitoring where this is currently unavailable.

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Table 1

Diagnostic accuracy of DHHS and WHO immunological failure (IF) criteria alone and DHHS and WHO IF criteria with targeted viral load monitoring (TVL) for identifying children with confirmed virological failure (CVF).

	DHHS IF criteria met	DHHS IF criteria met AND next HIV-RNA \geq 5000 copies/ml	WHO IF criteria met	WHO IF criteria met AND next HIV-RNA \geq 5000 copies/ml
Cumulative probability by 2 years (95% CI)	28.8% (26.0 – 31.8)	5.8% (4.6 – 7.3)	2.4% (1.7 – 3.4)	1.2% (0.8 – 2.0)
Number of evaluable pairs of data*	2524	2480	2945	2906
Sensitivity (%) (95% CI)	24 (19 – 29)	22 (17 – 27)	4 (2–6)	4 (2 – 6)
Specificity (%) (95% CI)	88 (87 – 90)	99 (99–100)	99 (99 – 100)	100 (100 – 100)
PPV (%) (95% CI)	28 (22 – 35)	82 (74 – 90)	49 (31 – 66)	82 (65 – 99)
NPV (%) (95% CI)	86 (84–88)	87 (85 – 89)	86 (83 – 88)	86 (84 – 88)
Number true positives	98	86	18	18
Number true negatives	1873	2066	2487	2473
Number false negatives	306	309	421	411
Number false positives	247	19	19	4
LR +	2.08	23.89	5.41	25.98
LR –	0.85	0.79	0.97	0.96
Area under ROC curve	0.563	0.604	0.517	0.520

* Evaluable pairs refers to each unique occasion where a diagnosis according to either IF or IF+TVL criteria can be compared with the CVF diagnosis. Number of evaluable pairs differs for different definitions because of different data requirements for each definition.