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Accuracy of immunological criteria for identifying virological failure in children on antiretroviral therapy – The IeDEA Southern Africa Collaboration

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Summary

OBJECTIVES To determine the diagnostic accuracy of World Health Organization (WHO) 2010 and 2006 as well as United States Department of Health and Human Services (DHHS) 2008 definitions of immunological failure for identifying virological failure (VF) in children on antiretroviral therapy (ART).

METHODS Analysis of data from children (<16 years at ART initiation) at South African ART sites at which CD4 count/per cent and HIV-RNA monitoring are performed 6-monthly. Incomplete virological suppression (IVS) was defined as failure to achieve ≥1 HIV-RNA ≤400 copies/ml between 6 and 15 months on ART and viral rebound (VR) as confirmed HIV-RNA ≥5000 copies/ml in a child on ART for ≥18 months who had achieved suppression during the first year on treatment.

RESULTS Among 3115 children [median (interquartile range) age 48 (20–84) months at ART initiation] on treatment for ≥1 year, sensitivity of immunological criteria for IVS was 10%, 6% and 26% for WHO 2006, WHO 2010 and DHHS 2008 criteria, respectively. The corresponding positive predictive values (PPV) were 31%, 20% and 20%. Diagnostic accuracy for VR was determined in 2513 children with ≥18 months of follow-up and virological suppression during the first year on ART with sensitivity of 5% (WHO 2006/2010) and 27% (DHHS 2008). PPV results were 42% (WHO 2010), 43% (WHO 2006) and 20% (DHHS 2008).

CONCLUSION Current immunological criteria are unable to correctly identify children failing ART virologically. Improved access to viral load testing is needed to reliably identify VF in children.

keywords children, antiretroviral therapy, immunological criteria, sensitivity, specificity, virological failure

Introduction

Poor access to viral load testing in resource-limited settings results in reliance on immunological criteria to identify treatment failure in patients on antiretroviral therapy (ART). Studies in adults have shown limited value of immunological criteria to detect virological failure (VF) (Badri *et al.* 2008; Mee *et al.* 2008; Keiser *et al.* 2009). While low sensitivity of immunological criteria for identifying VF could result in delayed switching to second-line treatment with accumulation of resistance mutations, low positive predictive value (PPV) may incorrectly identify patients as needing second-line treatment when they are virologically suppressed. The few paediatric studies of

diagnostic accuracy of immunological criteria for VF are limited by small cohort size and/or VF definition based on single elevated HIV-RNA measurements (Jittamala *et al.* 2009; Emmett *et al.* 2010; Ruel *et al.* 2010). We analysed data from routine paediatric ART clinics to determine the diagnostic accuracy of WHO (2006, 2010) and United States Department of Health and Human Services (DHHS) 2008 (National Institutes of Health 2008) criteria for immunological failure for identifying (i) incomplete virological suppression (IVS) during the first year on ART, and (ii) confirmed viral rebound (VR).

Methods

Data were collected prospectively from ART-naïve children (<16 years at ART start) initiating ≥3 antiretrovirals at South African sites participating in IeDEA–Southern Africa (IeDEA-SA, see http://www.iedea-sa.org), all of which had CD4 and HIV-RNA measurements performed 6-monthly. The characteristics of these sites 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 (bioMerieux), 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). We defined IVS as failure to achieve ≥1 HIV-RNA ≤400 copies/ml between 6 and 15 months on ART and VR as confirmed HIV-RNA ≥5000 copies/ml in a child on ART for ≥18 months whose HIV-RNA had suppressed during the first year on treatment. We examined the following immunological criteria: WHO 2010: No definition for children <2 years; CD4% <10% or CD4 <200 cells/mm^3 (age 2–4 years); CD4 <100 cells/mm^3 (age ≥5 years) (WHO 2010). WHO 2006: No definition for children <1 year; CD4% <15% (age 1–2 years); CD4% <10% (age 3–4 years); CD4 <100 cells/mm^3 (age ≥5 years) (WHO 2006). DHHS: During first year on ART: <5 percentage point CD4% increase from baseline (CD4% <15 and age <5 years at baseline) or <50 cells/mm^3 CD4 increase from baseline (CD4 <200 cells/mm^3 and age ≥5 years at baseline); decline of CD4% by 5 percentage points from previous value, confirmed at subsequent measurement (<365 days after first low value) (any age); return of CD4 count ≤baseline value (age ≥5 years at baseline) (National Institutes of Health 2008).

For IVS, we assessed the diagnostic accuracy of *never* achieving a CD4 above the immunological thresholds between 6 and 15 months on ART for identifying a child who *never* had HIV-RNA ≤400 copies/ml during the same period. Only children followed up for ≥1 year with ≥1 HIV-RNA measurement between 6 and 15 months were included. For VR, CD4 and HIV-RNA measurements were carried forward for up to 3 months where tests were performed asynchronously. For each unique paired CD4 and HIV-RNA measurement, we determined the diagnostic value of CD4-based criteria for identifying VR, using robust standard errors to account for multiple measures per patient. Immunological results for which there was still no concurrent virological diagnosis after carrying forward results were not evaluated. Further, the last immunological result before the end of follow-up/database closure was excluded to ensure that there was sufficient follow-up for a confirmatory low viral load measurement to have been performed. Separate sensitivity analyses were performed requiring either consecutive (within 365 days) CD4 measurements meeting immunological criteria or using an HIV-RNA threshold of 1000 copies/ml to define confirmed VR.

Results

Of 3640 children with ≥1 year of follow-up, 3115 (86%) had ≥1 HIV-RNA measurement between 6 and 15 months on ART. At ART initiation, median [interquartile range (IQR)] age was 48 (20–84) months. Most children were severely ill at ART initiation: WHO-defined severe immune suppression was present in 81% of 2911 children with baseline CD4 measures recorded, and 68% of 2294 children with WHO Clinical Stage data had Stage 3/4 disease (WHO 2006). In keeping with South African guidelines recommending protease inhibitor- (PI-) based first-line therapy in children <3 years old (National Department of Health South Africa 2005), 35% of children were on PI-based first-line treatment with non-nucleoside reverse transcriptase-based therapy in the remainder. IVS occurred in 12.6% of children and sensitivity of immunological criteria for identifying IVS ranged from 6% (WHO 2010) to 26% (DHHS) and PPV from 20% (WHO 2010 and DHHS) to 31% (WHO 2006) (Table 1a).

The accuracy of immunological criteria for identifying VR was assessed in 2513 children with at least 18 months follow-up on ART whose HIV-RNA had suppressed during the first year on treatment. The cumulative probability of VR in the following 2 years (by 42 months since ART start) was 5.5% [95% confidence interval (CI): 4.2–7.1]. Sensitivity of immunological criteria for identifying VR ranged from 5% (WHO 2006/2010) to 27% (DHHS) and PPV from 20% (DHHS) to 43% (WHO 2006) (Table 1b). For diagnosing both IVS and VR, requiring consecutive CD4 counts to meet immunological

Table 1 Diagnostic value of immunological criteria for identifying children (a) with incomplete virological suppression during first year on ART and (b) with viral rebound

	Comparison of immunological failure diagnosis (based on highest CD4 between 6 and 15 months on ART) with failure of viral suppression in same period			Comparison of immunological failure diagnosis (based on highest CD4 between 6 and 15 months on ART and confirmed on a subsequent measurement) with failure of viral suppression in same period		
(a)	WHO 2006*	WHO 2010†	USA	WHO 2006*	WHO 2010†	USA
Number of children (%)	90/2714 (3.3)	61/2380 (2.6)	355/2585 (13.7)	13/2502 (0.5)	9/2217 (0.4)	72/2277 (3.2)
Number of children with paired data‡	2581	2256	2470	2405	2126	2190
Sensitivity (%)	10 (7–14)	6 (3–10)	26 (21–31)	2 (0–4)	2 (0–4)	9 (5–12)
Specificity (%)	97 (97–98)	98 (97–98)	87 (86–89)	99 (99–100)	99 (99–100)	97 (97–98)
PPV (%)	31 (22–41)	20 (10–30)	20 (16–24)	38 (8–69)	33 (0–72)	27 (16–38)
NPV (%)	90 (89–91)	92 (91–93)	91 (89–92)	90 (89–91)	92 (91–93)	90 (89–92)
Number of true positives	28	12	69	5	3	19
Number of true negatives	2242	2017	1928	2151	1945	1916
Number of false negatives	250	179	197	241	172	204
Number of false positives	61	48	276	8	6	51
LR+	3.80	2.70	2.07	5.49	5.57	3.29
LR−	0.92	0.95	0.85	0.98	0.99	0.94
Area under ROC curve	0.537	0.520	0.567	0.508	0.507	0.530

ART, antiretroviral therapy; PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio of a positive test; LR−, likelihood ratio of a negative test; ROC, receiver operating characteristic.
*Only determined for children >12 months of age.
†Only determined for children >24 months of age.
‡Number of children with paired data (both CD4 and HIV-RNA measures available and CD4 criteria evaluable in terms of immunological criteria) differs for different definitions because of different age and data requirements for each definition.

	Comparison of immunological failure diagnosis (based on single CD4 measure meeting criteria) with confirmed HIV-RNA >5000 copies/ml			Comparison of immunological failure diagnosis (based on consecutive CD4 measures meeting criteria) with confirmed HIV-RNA >5000 copies/ml		
(b)	WHO 2006	WHO 2010*	USA	WHO 2006	WHO 2010*	USA
Cumulative probability by 2 years	2.2% (1.5–3.2)	2.3% (1.5–3.3)	28.1% (25.2–31.3)	0.4% (0.2–1.0)	0.3% (0.1–0.9)	10.4% (8.6–12.6)
Number of evaluable pairs of data†	2499	2593	2191	2395	2507	2110
Sensitivity (%)	5 (2–9)	5 (2–9)	27 (19–35)	2 (0–5)	2 (0–4)	13 (6–21)
Specificity (%)	99 (99–100)	99 (99–100)	88 (86–90)	100 (99–100)	100 (99–100)	95 (94–96)
PPV (%)	43 (23–63)	42 (22–61)	20 (13–26)	50 (19–81)	44 (14–75)	21 (10–32)
NPV (%)	91 (89–93)	91 (89–93)	92 (90–94)	91 (89–93)	91 (89–93)	91 (89–93)
Number of true positives	13	13	58	5	4	26
Number of true negatives	2245	2327	1740	2168	2269	1816
Number of false negatives	224	235	157	217	229	172
Number of false positives	17	18	236	5	5	96
LR+	7.30	6.83	2.26	9.79	7.81	2.62
LR−	0.95	0.95	0.83	0.98	0.98	0.91
Area under ROC curve	0.524	0.522	0.575	0.510	0.508	0.541

PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio of a positive test; LR−, likelihood ratio of a negative test; ROC, receiver operating characteristic.
*Only determined for children >24 months of age.
†Paired data created from carrying forward asynchronously measured CD4 and HIV-RNA results for up to 3 months; Number of children with paired data differs for different definitions because of different age and data requirements for each definition.

criteria increased PPV only slightly at the expense of sensitivity, without any improvement in the receiver operating characteristic curve (Table 1). For VR, lowering the VR threshold to 1000 copies/ml and using confirmed CD4 values to define immunological criteria yielded sensitivity (95% CI) of 2% (0–5%) (WHO 2006); 2% (0–4%) (WHO 2010) and 14% (8–19%) (DHHS); and PPV (95% CI) of 90% (72–100%) (WHO 2006); 89% (68–100%) (WHO 2010) and 38% (26–50%) (DHHS).

Discussion

In this large longitudinal study, we found that sensitivity and PPV were low for identifying VF using WHO and DHHS immunological criteria. For example, using confirmed HIV-RNA ≥5000 copies/ml after initial virological suppression to define VR, sensitivity was only 4% using either WHO 2006 or 2010 criteria. Fewer than half of children meeting WHO 2006/2010 criteria would have HIV-RNA ≥5000 copies/ml.

These results concur with those of previous small studies from resource-limited settings. Among 116 children in Uganda, 20 (17%) had sustained viraemia (≥400 copies/ml) beyond 24 weeks on ART (Ruel *et al.* 2010). Only two of these ever met WHO 2006 immunological criteria and did so after >550 days of viraemia, while none met WHO 2010 criteria (Ruel *et al.* 2010). Similarly, in a cross-sectional study of 206 children in Tanzania on ART for a median duration of 2.4 years, 32% had a single HIV-RNA ≥400 copies/ml (Emmett *et al.* 2010). WHO 2006 clinical and immunological failure criteria combined had a PPV of 100% but identified only 3.5% of children with HIV-RNA ≥400 copies/ml (Emmett *et al.* 2010). In Thailand, the sensitivity and PPV of DHHS immunological criteria for identifying children with a single HIV-RNA >1000 copies/ml were 15% and 16%, respectively (Jittamala *et al.* 2009).

The poor sensitivity of immunological criteria for identifying VF is disappointing; however, perhaps, an even greater concern is preventing the incorrect diagnosis of treatment failure in a virologically suppressed child, with unnecessary switch to second-line treatment, hence the importance of PPV. While PPV reached 89% for identifying VR ≥1000 copies/ml using a confirmed CD4 value meeting WHO 2010 criteria, given that the WHO 2010 guidelines HIV-RNA threshold for switching to second-line is 5000 copies/ml, in most instances the number of false positives using immunological criteria is unacceptably high. Further, absence of immunological failure on ART provides no assurance that a child is virologically suppressed and not accumulating resistance mutations, and even high CD4 thresholds (e.g. DHHS criteria) have low sensitivity for VF (PENPACT1 Study Team 2011). Improved access to HIV-RNA monitoring both to assess adherence and identify VF is therefore needed (Wilson *et al.* 2009; Ford & Calmy 2010).

There are several limitations to this analysis of routinely collected data. Missing baseline CD4 values limited the evaluation of DHHS criteria. Baseline CD4 values are often unavailable to clinicians if children initiate ART on clinical criteria, records are lost or a child changes treatment site after ART initiation, highlighting the value of simple criteria using current or recent measurements. The accuracy of WHO 2006 and 2010 criteria could not be evaluated in children <1 and <2 years old, respectively. Work-up bias may occur if either the reference (HIV-RNA) or index (CD4) tests are not applied consistently (Whiting *et al.* 2004). In particular, rigorous confirmation of low CD4 values is seldom performed in South Africa, owing to access to HIV-RNA measurements to diagnose treatment failure. Exclusion of intercurrent illness as a cause of low CD4, as advised in WHO guidelines (WHO 2010), was not possible because of limited data on episodes of clinical illness. Immunological criteria may have performed better if only low CD4 counts not explained by intercurrent illness had been considered. PPV is dependent on the VF incidence in different programs; however, the incidence in this cohort was similar to that of other studies (Kamya *et al.* 2007; Jittamala *et al.* 2009; Davies *et al.* 2011).

In summary, our results suggest that current immunological criteria are unable to correctly identify children failing ART virologically. Improved access to viral load testing appears to be the only feasible approach at this stage for reliably identifying VF in children on ART. There are several existing technologies for the measurement of viral load (Stevens & Marshall 2010), and expanded access should be supported by governments and donors.

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