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BRIEF REPORT

High Prevalence of Unconfirmed Positive HIV Polymerase Chain Reaction Test Results Among African Infants With HIV Exposure in the International Epidemiology Databases to Evaluate AIDS Consortium

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In a large, multiregional cohort of African infants with human immunodeficiency virus (HIV) exposure, 44% of those with a positive HIV polymerase chain reaction test lacked a confirmatory positive test. Efforts are needed to ensure high-fidelity implementation of HIV testing algorithms so that all positive results are confirmed.

Keywords. HIV; infant; Africa; polymerase chain reaction; confirmatory testing.

Vertical transmission prevention services have reduced incident infections among infants with human immunodeficiency virus (HIV) exposure (IHE); however, as vertical transmission declines, so does the positive predictive value of HIV diagnostic tests [1]. While nucleic acid amplification tests (NAATs) for HIV are very accurate [2–4], they fall short of 100% specificity in real-world settings [5–7]. Consequently, there is a small risk of false-positive results [2, 8], and diagnostic dilemmas can occur if antiretroviral therapy (ART) is initiated prior to confirmatory testing since virally suppressed and uninfected children can be difficult to distinguish [9–15]. Balancing the need for rapid ART initiation among infants with HIV against the risk of misdiagnosis based on false-positive results, the World Health Organization recommends that all infants with reactive HIV tests receive repeat testing from a new specimen concurrent with ART initiation [16]. Up to 50% of African IHE with reactive HIV tests might not receive confirmatory testing [6, 17, 18]; however, the extent to which this occurs in various regions and over time has not been well described. In this study, we determined the prevalence of unconfirmed positive HIV test results among IHE in the International epidemiology Databases to Evaluate AIDS (IeDEA) Consortium.

METHODS

Study Design and Setting

In this retrospective cohort study, we used data from IeDEA, which includes 7 regional data centers that collect, pool, and harmonize data from patients receiving routine HIV care at various clinical sites [19]. This analysis included paired mother–infant data from the following 4 IeDEA regions: Central Africa (CA), East Africa (EA), Southern Africa (SA), and West Africa (WA) [20]. CA included sites in Burundi, Democratic Republic of Congo, and Republic of Congo; EA included sites in Kenya; SA included 1 site in South Africa; and WA included sites in Benin, Côte d'Ivoire, Ghana, and Togo.

Participants and Data Sources

All IHE born from 2004–2021 and enrolled in care at an IeDEA-affiliated site at age <18 months were eligible for inclusion. ART cohort sites (ie, sites reporting data only for patients receiving ART) were excluded since these often lack initial and confirmatory testing data obtained prior to ART initiation. Only 1 SA site was included since all others were ART cohort sites.

Data were collected by local clinicians who provide routine care. Laboratories analyzed HIV NAATs following standard protocols and procedures. Clinical and laboratory data were recorded in medical records, then extracted, deidentified, and cleaned by regional data managers prior to being harmonized by IeDEA for analyses [21].

Variables and Definitions

IHE with only 1 positive NAAT (ie, qualitative or quantitative DNA or RNA polymerase chain reaction [PCR]) at age

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<18 months and no additional positive NAAT or antibody test at age ≥18 months were considered "unconfirmed positives." "Confirmed positives" were defined as IHE with any combination of 2 or more positive HIV tests. The primary outcomes of interest were prevalence of unconfirmed positives among all IHE and IHE with any positive NAAT.

Among unconfirmed positives, NAAT testing performed at any time after the initial positive result was described. In EA where ART data were most complete, the proportion of unconfirmed positives taking ART as well as the timing of ART initiation relative to test results were described. ART was defined as at least 3 antiretroviral medications taken for HIV treatment.

Statistical Analyses

Descriptive statistics were used to describe IHE and their testing and ART data. Results were stratified by region and calendar year. Analyses were completed in SAS version 9.4 (SAS Institute, Inc, Cary, NC).

Ethics Approvals

The study was approved by the University of North Carolina at Chapel Hill Institutional Review Board. Within IeDEA, each site and regional data center has ethical approval from local institutions to collect and share retrospective data. Individual written informed consent was not required since only routinely collected, deidentified data were used.

RESULTS

This analysis included 72 618 IHE with data from 2004–2020 in CA, 2004–2021 in EA, 2014–2017 in SA, and 2004–2018 in WA (Table 1). Overall, 3652 (5%) of IHE had at least 1 positive NAAT at age <18 months. Among those with any positive test, 2042 (56%) were confirmed positives, while 1610 (44%) lacked documentation of a confirmatory positive test and were considered unconfirmed positives. The proportion of unconfirmed positives among all IHE decreased over time (Supplementary Figure 1*A*); however, there was no secular trend when the proportion of unconfirmed positives among infants with any positive test was examined (Supplementary Figure 1*B*). Among the 1610 unconfirmed positives, 1393 (87%) lacked documentation of any testing after the initial positive result, while 217 (13%) had additional testing that was not positive (Supplementary Figure 2).

In EA, there were 1256 unconfirmed positives, of whom 403 (32%) received ART. Among those on ART, 32 (8%) initiated ART prior to their positive NAAT, and in 17 of these cases, the positive test was a high viral load (median, 292 080 copies/mL; interquartile range [IQR], 60 761–1 683 035), essentially confirming an HIV diagnosis.

Of the 161 who were unconfirmed positives in EA and had additional testing that was not positive, 50 (31%) received ART. Among those on ART, 4 (8%) initiated ART prior to their positive NAAT; in 3 of these cases, their positive test was a high viral load (7 671, 186 804, and 687 923 copies/mL). The remaining 46 started ART after their positive NAAT; 10 had a high viral load (median, 107 354 copies/mL; IQR, 3659–275 419), 2 had viral loads at the lower limit of detection (≤40 copies/mL), and 34 had DNA PCR tests that were positive. The 34 infants who started ART after a single positive DNA PCR test were a median age of 6.2 months (IQR, 1.9–11.7) at the time of their positive test, 13 of them had a subsequent negative NAAT on the same day or before starting ART, and 21 had their subsequent negative NAAT after starting ART. In this latter group, negative tests could be due to viral suppression on ART or an initial false-positive result.

DISCUSSION

In a large cohort of African IHE at IeDEA-affiliated sites from 2004–2021, almost half of IHE who had a positive HIV NAAT before age 18 months lacked documentation of a confirmatory test, consistent with previous studies in South Africa that reported confirmatory testing rates of 50%–75% [6, 17]. We also found that the proportion of unconfirmed positives among IHE with any positive NAAT did not decrease over time, suggesting that the decrease in unconfirmed positives among all

Table 1. Infants With Human Immunodeficiency Virus (HIV) Exposure and Their HIV Testing Data Stratified by International epidemiology Databases to Evaluate AIDS Region

	Central Africa, 2004–2020	East Africa, 2004–2021	Southern Africa, 2014–2017	West Africa, 2004–2018	All Regions
1. Infants with human immunodeficiency virus exposure, n	10 520	47 015	8600	6483	72 618
2. Any positive virologic test at age <18 m among no. 1, n (%)	415 (4)	2980 (6)	154 (2)	103 (2)	3652 (5)
3. Only 1 positive test (ie, "unconfirmed positives") among no. 2, n (%)	240 (58)	1256 (42)	20 (13)	94 (91)	1610 (44)
4. No additional virologic test performed after the initial positive test among no. 3, n (%)	192 (80)	1095 (87)	19 (95)	87 (93)	1393 (87)
5. Additional (≥1) virologic test performed and not positive among no. 3, n (%)	48 (20)	161 (13)	1 (5)	7 (7)	217 (13)

IHE was a function of fewer positives over time rather than improvement in confirmatory testing rates. A small proportion of IHE with unconfirmed positive HIV tests later had testing that was not positive, raising the possibility that initial results were falsely positive, perhaps leading to misdiagnoses of HIV.

A review reported that 5.4% of initial reactive HIV NAATs were followed by negative repeat tests, and up to 12.5% of all non-negative infant HIV PCR tests could be false-positives [22]. Barriers to confirmatory testing include preanalytical (eg, sample collection, labeling, and transport issues) and analytical (eg, cross-contamination) errors that lead to missed diagnostic opportunities or invalid results [18]. Performing confirmatory testing from a new specimen reduces the risk of contamination [7], and point-of-care testing [23] and other interventions to shorten turnaround time between sample collection and result reporting [6] can improve confirmatory testing rates. Confirmatory testing is cost-effective and reduces the risk of HIV misdiagnosis, protecting patients from potential medical, social, psychological, and economic burdens that come with an HIV diagnosis, associated stigma, and lifelong ART [2].

Because infants with HIV and taking ART can have negative HIV test results, diagnostic dilemmas can occur if confirmatory testing is not performed prior to starting ART [1, 5, 9–11, 24]. In a study from 3 African countries, retesting patients for HIV following suspected misdiagnoses did not decrease patients' confidence in the care programs and actually increased faith in testing outcomes [25]. Further work is needed to carefully address possible diagnostic dilemma cases among IHE.

Inferences about the marked regional variability in confirmatory testing rates are limited by the relatively small number of children in some regions, disparate observation periods, and the inclusion of only 1 site in the SA region where hospital-based birth testing is more common than elsewhere. However, at least part of the observed regional heterogeneity could be due to differing country- or program-level guidelines and practices, access to healthcare resources, or data quality. It is also difficult to discern whether confirmatory testing was simply not done or rather missing due to lack of documentation, death, or care disengagement prior to repeat testing. While it is a strength that our analysis included data from a large consortium of sites in 4 African regions over a long period of time, IeDEA-affiliated sites may not be representative of others in these regions.

In conclusion, nearly half of IHE with an initial positive HIV NAAT did not receive confirmatory testing, and there was no evidence of improvement in confirmatory testing rates over time. There is an ongoing need for initiatives to ensure higher-fidelity implementation of infant HIV testing algorithms, and future research should investigate the contexts in which unconfirmed positive tests occur and whether these have caused diagnostic dilemmas that might need to be resolved.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Disclaimer. This work is solely the responsibility of the authors and does not necessarily represent the views of any of the institutions listed here.

Data availability. Complete data for this study cannot be posted in a supplemental file or a public repository because of legal and ethical restrictions. For more information about access to data, please contact the IeDEA Executive Committee at: https://www.iedea.org/working-groups/executive-committee/.

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