Optimizing and validating a pediatric screening tool to more efficiently test and identify children living with HIV

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Objective: To develop and validate a screening tool to improve testing efficiency and increase case finding of children living with HIV.

Design: Cross-sectional study.

Methods: Between November 2020 and September 2021, children 18 months to 14 years presenting at outpatient departments in 30 health facilities in Zambia were administered a 14-question pediatric HIV screening tool and then tested for HIV. Data were analyzed using a randomly extracted 'validation' dataset and multivariable logistic regression to determine the highest performing and optimal number of screening questions. The final tool was then evaluated in the 'test' dataset. Sensitivity and specificity were calculated for both datasets. The final tool was then also implemented in 12 additional facilities to determine operational feasibility and uptake.

Results: A total of 9902 children were included in the final analysis. HIV prevalence was 1.3%. Six questions were significantly associated with HIV-positivity. The optimal screening cutoff score was to answer 'yes' to one or more of the six questions; using this cutoff sensitivity was 92.5% [95% confidence interval (CI) 85.7–96.7%] and specificity was 62.9% (95% CI 61.9–64%). In the test dataset, the same tool had a sensitivity of 84.6% (95% CI 65.1–95.6%) and specificity of 64.6% (95% CI 62.4–66.7%). Uptake was 89%.

Conclusion: The results of this study show sensitivity and acceptable specificity in a six-question validated HIV screening tool. Implementing this screening tool in settings where universal testing is not feasible should more efficiently accelerate identification of children living with HIV (CLHIV) and their timely initiation onto life-saving drugs.

AIDS 2023, **37**:000–000

Keywords: HIV testing, pediatrics, sensitivity and specificity, validation study, Zambia

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Received: 16 September 2022; revised: 24 February 2023; accepted: 4 March 2023.

DOI:10.1097/QAD.0000000000003583

Introduction

Globally, there is a large gap in HIV antiretroviral treatment (ART) coverage for children living with HIV (CLHIV). Despite substantial global gains in pediatric ART coverage, from 18% in 2010 to 52% in 2020, progress continues to lag noticeably behind adults, with adult treatment coverage reaching 73% in 2020 [1]. In Zambia, remarkable progress has been made over the past decade in increasing pediatric ART coverage from 24% in 2010 to an estimated 69% in 2020, but closing this gap is a persistent challenge, with the biggest barrier being finding the remaining undiagnosed CLHIV and linking them to care [1]. Identifying them is increasingly difficult, in part because of the lower HIV prevalence among children, which was estimated to be 1.1% in 2016, much lower in comparison with the 12% adult prevalence [2]. Zambia's achievements were realized through sustained efforts that went beyond testing at the most common pediatric entry points serving mothers living with HIV, activating and scaling provider-initiated testing and counselling in key wards such as tuberculosis, malnutrition, and immunization, following the 2015 WHO recommendations [3]. Zambia adopted a Universal Routine Testing policy in August 2017, which mandated universal HIV testing for all individuals presenting at health facilities [4]. Although testing was already increasing, this policy has contributed to a further increase in testing of children: data from the health management information system in Zambia shows increases in the number of children tested, with approximately 1.3 million tests conducted in 2018 compared with just over 355 000 in 2015 (over 300% increase). But the corresponding increase in the number of CLHIV identified is disproportionate, with only a 29% increase (approximately 17 000 children identified in 2018 compared with 13 200 identified in 2015) [5]. Decreasing HIV testing yields among children are not uncommon as vertical transmission decreases. Consequently, a more targeted approach may be better suited for the current circumstances.

Global progress towards the target that 95% of people with HIV should be aware of their status, and improvement of prevention of vertical transmission programs continue. At the same time, countries and donors are shifting effort and funding away from large-scale testing efforts, which makes identifying the remaining undiagnosed CLHIV even more difficult [6]. Achieving universal testing in high-volume wards in Zambia has not always been possible because of significant constraints in healthcare worker capacity and test supply. In 2018, after the adoption of the universal testing policy, in previous unpublished work, in a sample of 38 facilities, we found testing coverage in outpatient departments (OPD) to be only 26% and yield about 1%. As a result, many CLHIV may be missed through existing testing channels at health facilities. In the settings where universal testing is not being executed, question-based HIV screening tools are gaining evidence as a strategy proven effective in increasing identification of CLHIV attending typically low-yield, high-volume settings, such as OPD [7,8]. High-volume wards with low testing coverage present a critical opportunity to deploy a targeted screening approach to narrow the pool of at-risk children to be tested and accelerate identification of CLHIV. A 2020 meta-analysis highlighted a growing body of research on the use of HIV screening tools in both pediatric inpatient and outpatient populations and emphasized the need for rigorous investigation of the approach [9].

The individual screening criteria that have the highest independent association with HIV positivity, or the number and type of screening questions required to maximize a tool's ability to identify CLHIV are not yet clear through robust evidence in Zambia. Although some implementing partners in Zambia are conducting targeted HIV screening with question-based tools in the pediatric population, the Ministry of Health does not currently have a harmonized or validated HIV screening tool. Current tools in use ask nonstandardized questions and have not been validated or assessed for efficacy. This study aimed to fill this gap in standardized screening and testing practices by first optimizing then validating an HIV screening tool for children that would accelerate identification of CLHIV, facilitate linking them to lifesaving treatment, and close the ART treatment gap. The results of the study will be used to inform national adoption and scale up of an optimized, validated pediatric HIV screening tool.

Methods

A cross-sectional study was conducted in 42 health facilities in Zambia. The study was carried out in three phases. Phase 1 was conducted from November 2020 to September 2021 in 30 facilities, with the objective of optimizing an HIV risk screening tool by determining how many and what questions should be asked, and what the optimal cut-off score for questions answered affirmatively was to maximize the sensitivity of the tool. Diagnostic accuracy measures of sensitivity, specificity, positive-predictive value (PPV), and negative-predictive value (NPV) were calculated on the optimized tool. Phase 2 was conducted concurrently with the aim of validating the performance of the final screening tool, calculating the same diagnostic accuracy measures again. In phase 3, the validated tool was implemented in March 2022 in 12 different facilities to generate operational evidence on the ability to administer the screening tool under routine conditions, assessed primarily through screening uptake.

Screening tool questions

In phase 1, we selected 14 screening questions for evaluation by reviewing questions from both

nonvalidated screening tools in Zambia and validated screening tools from other countries in sub-Saharan Africa, by adapting questions from research about CLHIV, and by considering contextual relevance in Zambia. Healthcare workers administered the tool to the child's caregiver/guardian and included questions that asked about the biological mother's HIV status, if biological parents were deceased, or if anyone in the household had HIV. Questions regarding the child's health status and history, such as if the child had any skin problems, ear discharge, tuberculosis (TB) symptoms, or general poor health were also asked. Some questions focused on the same topic but worded differently were purposefully included to assess whether phrasing performed differently (e.g. 'Is this child growing slowly?' versus 'Is this child short or light for age?').

Site selection

For phases 1 and 2 Copperbelt and Central provinces were first purposefully selected to target areas of high adult HIV prevalence and because neither province had similar studies planned there [2]. Next, six districts were selected by probability proportional to size sampling, restricted to districts that had more than five facilities that met the following criteria: facility was a hospital or health center, facility had an outpatient department that offered provider-initiated testing and counselling, and facility tested and identified at least 12 CLHIV in 2018. Finally, five facilities were randomly selected from each district. A total of 30 facilities, constituted of 24 health centers and six hospitals were selected. In phase 3, 12 sites were purposefully selected based on similar criteria.

Participant selection

Study participants included every child who attended OPD or maternal and child health wards during the study period. Children were eligible unless they were less than 18 months or 15 years of age and older, had previously tested HIV-positive, required immediate hospitalization, were not accompanied by a caregiver/guardian, or the caregiver/guardian was not aware of the child's health in the last six months. Trained healthcare workers obtained written informed consent from all participating caregivers/guardians, as well as obtained assent from children aged 7–14 years. The health worker then asked the caregivers/guardians all screening questions and subsequently tested all children for HIV using a rapid test, followed by a confirmatory test if the rapid test was positive.

Sample size

Sample size was calculated for phases 1 and 2 on the primary outcome of sensitivity, aiming for 80% sensitivity with a 10% margin of error. We used an equation for diagnostic accuracy to determine the total number of children that needed to test HIV-positive ($n = 123$), and then applied the Zambian pediatric HIV prevalence known at the time (1.1%) to calculate the approximate number of children that needed to be screened and accordingly, the number of sites ($n = 30$) [10]. Phase 3 did not require a sample size as we were not powering to any outcome. The number of sites ($n = 12$) was selected based on operational feasibility of implementation.

Data collection

Data were collected by healthcare workers at each facility who recorded all responses to the screening questions and HIV test results onto a paper-based study-specific form, which was later entered into an electronic study database. In phase 3, similar data on numbers screened and tested were collected, as well as OPD attendance numbers. A short questionnaire was also administered to key health professionals at each facility.

Data analysis

Complete case analysis was conducted, meaning any record that had incomplete responses (e.g. 'missing', 'don't know', or 'refused') to any of the screening questions was excluded from the analysis. Collinearity was examined between all screening questions; if any two questions were highly correlated, the question that performed worse was removed from further analysis.

To analyze phase 1 and 2 separately, a random generator was used to split the dataset into the two mutually exclusive groups, ensuring an equal number of CLHIV in each dataset: 80% of the data was put into a 'validation' dataset for phase 1 and the remaining 20% became the 'test' dataset for phase 2. In the validation dataset, univariate logistic regression was run on the screening questions. Any variable with a P value less than 0.1 was kept and put into a multivariate model, where we then used a stepwise backward selection until only variables with a P value less than 0.05 remained in the model. Next, using a Receiver Operating Characteristic (ROC) curve, we calculated the area under the ROC curve (AUC) for different cut-off points of the screening tool (e.g. number and combination of questions that should be asked) to determine the optimal sensitivity and specificity of the screening tool.

To ensure that the final model was robust and not dependent on the particular sample of 80% that we generated, we ran 1000 Monte Carlo simulations, which creates 1000 different iterations of the 80% random sample, and repeated the regression analyses. Once the final set of questions for the screening tool were identified, we calculated sensitivity, specificity, PPV, NPV, and the number needed to test to determine how many tests were needed to identify one HIV-positive child. The final screening tool was validated in the test dataset by calculating these same indicators.

This study received ethics approval from the ERES Institutional Review Board, reference number 2019-Sep-088.

Results

A total of 11 018 children were screened and tested, among which 1116 (10%) were excluded from analysis: 22 were age-ineligible, three were ineligible based on other criteria, and 1091 did not respond yes or no to all 14 screening questions. Among the remaining 9902, 132 CLHIV were identified, resulting in an HIV prevalence of 1.3%. The median age [interquartile range (IQR)] of HIV-negative children was 4 years (IQR: 2–8) whereas the median age of CLHIV was older at 7 years (IQR: 4–10) (Table 1). Only 16% of children screened had previously tested HIV-negative, with no difference seen in previous testing between children who tested HIV-positive or children who tested HIV-negative in the study. Ten (8%) CLHIV did not answer 'yes' to any of the 14 original screening questions and 122 (92%) answered yes to one or more. Two screening questions were found to be highly collinear with other screening questions and, therefore, were excluded from further analyses.

In the validation dataset, a child having a biological mother living with HIV or with unknown HIV status had the highest adjusted odds ratio (aOR) for HIV positivity at 8.34 [95% confidence interval (CI) 5.26–13.22], followed by a child being short or light for age (aOR: 7.71; 95% CI 4.37–13.61) (Table 2). After completing the stepwise backward regression, six of the 14 original questions were included in the final model. These same six questions were confirmed in the Monte Carlo simulations, with three questions remaining significant in 100% of the simulations and the final six questions remaining significant in over 70% of the simulations. Different cut-off scores were examined and plotted as an ROC curve (Fig. 1). A score of one or more was found to perform best with a sensitivity of 92.5% (95% CI 85.7–96.7%), specificity of 62.9% (95% CI 61.9–64.0%), and an AUC of 0.78 (95% CI 0.75–0.80) (Table 3). Sensitivity analyses also confirmed that had we kept all children in the analysis, treating a missing or 'don't know' response as a 'yes' or 'no' answer would not have changed results significantly.

In the test dataset, performance was similar with a sensitivity of 84.6% (95% CI 65.1–95.6%) and specificity of 64.6% (95% CI 62.4–66.7%). The PPV was 3% (95% CI 2–5%) while the NPV was 100% (95% CI 99–100%). The number needed to test to identify one HIV-positive child would be reduced from 76 to 32 if the six-question screening tool with a cut-off score of one was implemented. However, for every 317 tests conducted, the tool would also misclassify one HIV-positive child as not at risk of HIV and, therefore, that child would not be tested, and a diagnosis would be missed.

Analyses by age group showed some variation, although results were not consistent between the validation and test datasets, probably because of small sample sizes, especially among the CLHIV. The oldest children (10–14 years) had the highest sensitivity at 97.5% (95% CI 86.8–99.9%) in the validation dataset but the lowest in the test dataset at 80% (95% CI 28.4–99.5%) (Table 4). Children

Table 1. Characteristics of 9902 children screened and tested by HIV status.

Characteristics	HIV-negative median (IQR)/*n* (%)	HIV-positive median (IQR)/*n* (%)	All median (IQR)/*n* (%)
Total screened and tested	9770	132	9902
Age (years): median (IQR)	4 (2–8)	7 (4–10)	4 (2–8)
Has tested HIV-negative more than 1 year ago	1567 (16%)	20 (15%)	1587 (16%)
Screening questions (yes response)			
Biological mother is HIV-positive or unknown status	1797 (18%)	96 (73%)	1893 (19%)
Father, siblings, or other household members are HIV-positive[a]	1543 (16%)	81 (61%)	1624 (16%)
One or more biological parents deceased	459 (5%)	31 (23%)	490 (5%)
Child or anyone in the family went hungry because there was not enough money for food	767 (8%)	33 (25%)	800 (8%)
Has been in poor health or admitted to the hospital in the last 3 months	513 (5%)	45 (34%)	558 (6%)
Admitted to hospital in the last 6 months	329 (3%)	20 (15%)	349 (4%)
Lives with someone who has been diagnosed with TB	369 (4%)	16 (12%)	385 (4%)
Has any of the following symptoms of TB: cough fever, poor weight gain, or night sweats	1334 (14%)	50 (38%)	1384 (14%)
Had recurring skin problems	521 (5%)	33 (25%)	554 (6%)
Had frequent ear discharge	127 (1%)	13 (10%)	140 (1%)
Has symptoms of pneumonia	93 (1%)	8 (6%)	101 (1%)
Is short or light for age	171 (2%)	35 (27%)	206 (2%)
Is growing slowly[a]	156 (2%)	34 (26%)	190 (2%)
Has symptoms of an STI such as vaginal or urethral discharge or genital sores[b]	42 (0%)	3 (2%)	45 (0%)
Screening tool score (max of 14): median (IQR)	0 (0–1)	3 (2–5.5)	0 (0–1)
0	5485 (56%)	10 (8%)	5495 (55%)
1 or more	4285 (44%)	122 (92%)	4407 (45%)
Screening positive (score of ≥1 to 6 final questions)	3589 (37%)	120 (91%)	3709 (37%)

IQR, interquartile range; STI, sexually transmitted infection; TB, tuberculosis.
[a]Question found to be collinear with previous question and subsequently removed from further analysis.
[b]Asked only to children aged 10 and older.

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AIDS-D-22-00538

Table 2. Odds ratios of screening questions on HIV-positivity for validation dataset.

	n	%	Odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Screening questions (yes response)	7922			
Biological mother is HIV-positive or unknown status	1517	19%	12.35 (7.99–19.08)	8.34 (5.26–13.22)
One or more biological parents deceased	1307	16%	8.74 (5.88–13.01)	1.97 (1.13–3.40)
Child or anyone in the family went hungry because there was not enough money for food	652	8%	4.14 (2.67–6.42)	
Has been in poor health or admitted to the hospital in the last 3 months	459	6%	9.82 (6.52–14.78)	3.79 (2.33–6.17)
Admitted to hospital in the last 6 months	280	4%	5.09 (2.95–8.78)	
Lives with someone who has been diagnosed with TB	306	4%	3.92 (2.21–6.96)	
Has any of the following symptoms of TB: cough fever, poor weight gain, or night sweats	1118	14%	4.28 (2.89–6.34)	2.37 (1.50–3.73)
Had recurring skin problems	449	6%	5.99 (3.83–9.37)	1.97 (1.11–3.49)
Had frequent ear discharge	108	1%	8.20 (4.15–16.21)	
Has symptoms of pneumonia	78	1%	6.45 (2.74–15.19)	
Is short or light for age	166	2%	21.11 (13.34–33.41)	7.71 (4.37–13.61)
Has symptoms of an STI such as vaginal or urethral discharge or genital sores	38	0%	6.48 (1.96–21.39)	

OR, odds ratio; STI, sexually transmitted infection; TB, tuberculosis.

18 months to 4 years had similar sensitivities in both datasets. Specificity did not vary as much by age, with the oldest children having the highest in both datasets.

In phase 3, 4050 children were screened out of 4574 children presenting in OPD, resulting in a screening uptake of 89%. Fifty-one percent of the children screened were female, with a median age of 7 years (IQR: 4–11). The most common reasons for presenting at OPD was a respiratory tract infection, malaria, and common cold. Just under half (1857 or 46%) of children screened positive, meaning they answered yes to one or more of the six questions of the validated tool. Ninety-one percent of those screened positive went on to be tested, with nine children testing HIV-positive, a yield of 0.5%. Seven of the nine children who tested HIV-positive (78%) were linked to care and initiated on ART within 2 weeks. There were no statistical differences seen in age or sex between those that screened positive versus negative, nor those that tested positive versus negative.

Questionnaire responses revealed that staff believed the screening questions were appropriate to ask and easy to administer, and overall felt the tool very simple to use. The biggest challenge health workers faced was children who did not present with a caregiver/guardian, and despite asking them to return with them, rarely did. Keeping a consistent supply of HIV test kits was not an

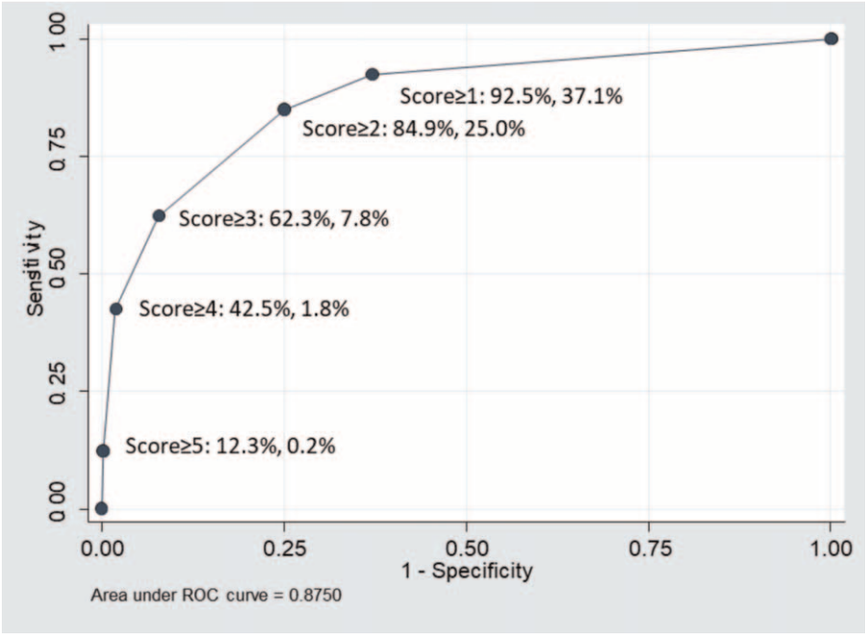

Fig. 1. Receiver operative characteristic curve for the validation dataset and cut-off scores of 1 to 5.

Table 3. Sensitivity and specificity for validation and test datasets among 9902 children screened and tested for HIV in 30 public health facilities in Zambia.

	Validation dataset ($N = 7922$)		Test dataset ($N = 1980$)	
	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Screening question				
Biological mother HIV+ or unknown status	73.6% (64.1–81.7%)	81.6% (80.7–82.4%)	69.2% (48.2–85.7%)	81.7% (79.9–83.4%)
One or more biological parents deceased	23.6% (15.9–32.8%)	95.3% (94.8–95.8%)	23.1% (9.0–43.6%)	95.3% (94.3–96.2%)
Child in poor health in last 3 months	35.8% (26.8–45.7%)	94.6% (94.1–95.1%)	26.9% (11.6–47.8%)	95.3% (94.3–96.2%)
Child has TB symptoms	40.6% (31.1–50.5%)	86.2% (85.5–87.0%)	26.9% (11.6–47.8%)	86.7% (85.2–88.2%)
Child has skin problems	25.5% (17.5–34.9%)	94.6% (94.1–95.1%)	23.1% (9.0–43.6%)	94.9% (93.9–95.9%)
Child is short or light for age	27.4% (19.1–36.9%)	98.2% (97.9–98.5%)	23.1% (9.0–43.6%)	98.3% (97.6–98.8%)
Screening tool score ≥ 1	92.5% (85.7–96.7%)	62.9% (61.9–64.0%)	84.6% (65.1–95.6%)	64.6% (62.4–66.7%)

CI, confidence interval; TB, tuberculosis.

issue. The most common recommendation was to ensure a dedicated room to provide privacy during the screening.

Discussion

This study validated an HIV screening tool for use among children aged 18 months to 14 years in high-volume facility settings in a country with high HIV prevalence. We identified a set of six questions which, when validated, showed a sensitivity of 84.6% and acceptable specificity of 64.6% for identifying CLHIV. If implemented appropriately, this tool would reduce testing volumes by almost two-thirds and more efficiently identify CLHIV in settings where universal HIV testing may not be possible or consistently implemented.

Our results are comparable to a meta-analysis of four outpatient studies conducted in Zimbabwe and South Africa, where use of HIV screening tools showed a pooled sensitivity of 81.4% (95% CI 70.5–88.9) and a specificity of 69.4% (95% CI 46.7–85.5) [9]. Since then, two additional studies have also demonstrated similar results: in Tanzania, an optimized five-question screen tool yielded a sensitivity of 85.3% (95% CI 74.6–92.7%) and specificity of 44.2% (95% CI 43.5–44.9%) while a two-step algorithm in Uganda found a sensitivity of 88.1% (95% CI 80.8–92.8%) and specificity of 69% (95% CI 61.9–75.3%) [11,12].

We were able to optimize the screening tool and reduce the number of questions asked from 14 to six, while sacrificing very small losses in sensitivity. Other researchers have also found that asking approximately five questions can yield optimal results [5,7]. In sensitivity analyses, we examined different variations of the final model, including models that only had five final questions, instead of six, but did not find better performance. There is a difficult balance between asking all the possible questions that could predict HIV-positivity and acknowledging time constraints of already-burdened healthcare workers. Operational feasibility must be considered, as a short, efficient tool is more likely to be administered to every child, which is needed to see the full impact of the screening tool. Experts agree that risk screening tools need to be short and easily administered while screening positive as many undiagnosed CLHIV as possible [7].

Although there is some variation in the questions asked in all these tools, there is also considerable overlap. For example, most tools include questions about a child being orphaned, having recurrent skin problems, or being in general poor health. One difference we found was that asking older children if they had a sexually transmitted infection did not predict being at risk for HIV infection;

Table 4. Sensitivity and specificity, by age, for validation and test datasets among 9902 children screened and tested for HIV in 30 public health facilities in Zambia.

	Validation dataset ($N = 7922$)			Test dataset ($N = 1980$)		
	N	Sensitivity (95% CI)	Specificity (95% CI)	*N*	Sensitivity (95% CI)	Specificity (95% CI)
Age group						
18 months to 4 years	4027	90.9% (75.7–98.1%)	62.4% (60.9–63.9%)	1010	88.9% (51.8–99.7%)	64.2% (61.2–67.2%)
5–9 years	2378	87.9% (71.8–96.6%)	62.9% (60.9–64.9%)	607	83.3% (51.6–97.9%)	63.4% (59.3–67.2%)
10–14 years	1488	97.5% (86.8–99.9%)	64.8% (62.3–67.2%)	350	80.0% (28.4–99.5%)	67.2% (62.0–72.2%)

CI, confidence interval.

perhaps this question is best asked only to older adolescents. Among the 20 children who previously tested HIV-negative, 19 caregivers responded 'yes' to the biological mother being HIV-positive or of unknown status, so it is likely most of these children seroconverted during the breastfeeding period. A 'no' response to previous HIV testing also included if the result was not available, which could explain why no association was found.

As 10 CLHIV in the study responded no to all 14 screening questions included in the original tool, regardless of the selection of the final set of screening questions some children will be missed in the absence of universal testing. However, ensuring that universal testing is consistently implemented and has a low opt-out rate is critical. This chance of missed diagnoses is an acknowledged drawback to question-based HIV screening tools [13]. Continuous monitoring of design, implementation, and outcomes of screening is thus critical to ensure tools are implemented with fidelity and have the intended impact on both yields and case identification in real-world settings. Considerations should be made to implement screening in conjunction with other effective pediatric case finding modalities, such as index testing, to maximize case identification in facilities and communities.

Our results from phase 3 show that implementing a validated tool is very feasible. Although uptake was not perfect, at 89%, we felt this was acceptable. The linkage to care rate (78%) was lower than desired but with more time, it is possible the remaining children will engage in care. Qualitative surveys revealed concern that during the month of implementation there was an outbreak of influenza, so many more children were presenting with fever than usual. Consequently, the number screening positive was probably higher than it would have been over a longer period of time – and indeed, we did see a higher proportion in phase 3 than phases 1 and 2 (46 versus 38%). This could also partially explain why we saw lower HIV testing yields in phase 3.

Challenges

One of the main challenges early on in this study was ensuring a sufficient and consistent supply of HIV testing kits. The study did not procure any testing kits for the facilities; instead, a written checklist from the Ministry of Health was distributed to study sites that provided operational guidance and troubleshooting procedures for managing and addressing low-stock and stockouts of test kits during study implementation. However, when stockouts did become an issue, a series of coordination meetings between the national pediatric HIV program, Medical Stores Ltd, the study team and key contacts at study sites were conducted to provide study sites with further guidance on ordering and networking, which successfully resolved the stockouts. In settings with persistent challenges with under resourcing, including for testing, testing coverage has been low in high-volume settings despite a universal testing policy. The challenge around securing enough HIV rapid tests underscored the need for an acceptable alternative strategy to universal testing, and a strategy that can make best use of available resources.

Limitations

This study had several limitations. Because finding undiagnosed CLHIV was rare, the study team made efforts to keep every child newly identified as HIV-positive in the analysis, which included following up on 22 caregivers who had originally responded 'don't know' to one or more of the screening questions and determining a yes or no response. However, in a real-world setting, some caregivers will answer 'don't know' or 'refused', so these response options need to be included in guidelines. Sensitivity analyses showed that treating any non-yes response as a 'no' did not alter results. Seasonality could also have impacted responses, especially in phase 3, as implementation only occurred for one month during the rainy season. Finally, we did not examine implementation fidelity, ensuring healthcare workers administered the tool with conformity (not skipping or re-wording questions). A process evaluation in Tanzania found that conformity in screening tool implementation was a key factor in ensuring that the tool performs as intended under routine settings [14].

In conclusion, given the success of the HIV program in Zambia, including strong prevention of vertical transmission, infections are increasingly being prevented and CLHIV are increasingly being identified and linked to care. Finding the remaining undiagnosed CLHIV not on ART is, therefore, increasingly difficult. Globally, the majority of undiagnosed children are estimated to be between 5 and 14 years of age, meaning early infant diagnosis programs will not find these children and they are less likely to have frequent contact with the health system [15]. Although universal testing should still be prioritized in high-yield entry points, in high-volume settings, such as OPD or routine immunization, it is often not feasible to test every child in low-income and middle-income countries because of human resource constraints and supply stock. There are also trade-offs to be considered – utilizing a screening tool versus suboptimally implementing universal testing will both result in missing CLHIV, with the hope being that a screening approach would be more targeted and effective. The screening tool assessed in this study can help to improve efficiency and effectiveness of pediatric HIV case finding in high-volume settings, offering a promising approach to help close the ART treatment gap among children.

In 2019, the Zambian Ministry of Health issued a memo stating that all public health facilities should begin using screening tools to help determine high-risk individuals, including children, in need of HIV testing; however, this

decision was overturned in 2021 pending evidence of a validated tool. Now, with this evidence of a validated HIV screening tool for children, the Ministry of Health can consider revising their position again, and conversations have begun to discuss implementing the tool in last-mile settings. A validated tool such as this can provide a standardized way to screen all children and test with confidence those deemed to be at higher risk of HIV infection, and ultimately create efficiencies and improve pediatric HIV case finding.

Acknowledgements

We gratefully acknowledge the collaborating efforts from the Ministry of Health, staff from participating health facilities, and the women and children who enrolled in the study. We also thank Marta Prescott, PhD for her review of our statistical methodology.

Data availability: the datasets generated from this study are available from the corresponding author on reasonable request and with the proper data sharing agreements in place.

Author contributions: this project was conceived of and planned by J.J., F.M., S.D., K.S., H.S., and G.M. Implementation and data collection were led by F.M., P.H., P.H., F.M., and M.S.. J.J. conducted all statistical analyses. The initial manuscript draft was written by J.J.. All authors reviewed the manuscript, helped to critically interpret the results, approved the final version of the manuscript for submission, and meet ICMJE authorship requirements.

Funding: this study was made possible from a grant awarded to the Clinton Health Access Initiative from the ELMA Foundation.

Conflicts of interest

There are no conflicts of interest.

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