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MAJOR ARTICLE

Development of a Clinical Prediction Score Including Monocyte-to-Lymphocyte Ratio to Inform Tuberculosis Treatment Among Children With HIV: A Multicountry Study

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Background. Clinical pediatric tuberculosis (TB) diagnosis may lead to overdiagnosis particularly among children with human immunodeficiency virus (CHIV). We assessed the performance of monocyte-lymphocyte ratio (MLR) as a diagnostic biomarker and constructed a clinical prediction score to improve specificity of TB diagnosis in CHIV with limited access to microbiologic testing.

Methods. We pooled data from cohorts of children aged ≤13 years from Vietnam, Cameroon, and South Africa to validate the use of MLR ≥0.378, previously found as a TB diagnostic marker among CHIV. Using multivariable logistic regression, we created an internally validated prediction score for diagnosis of TB disease in CHIV.

Results. The combined cohort had 601 children (median age, 1.9 [interquartile range, 0.9–5.3] years); 300 (50%) children were male, and 283 (47%) had HIV. Elevated MLR ≥0.378 had sensitivity of 36% (95% confidence interval [CI], 23%–51%) and specificity of 79% (95% CI, 71%–86%) among CHIV in the validation cohort. A model using MLR ≥0.28, age ≥4 years, tuberculin skin testing ≥5 mm, TB contact history, fever >2 weeks, and chest radiograph suggestive of TB predicted active TB disease in CHIV with an area under the receiver operating characteristic curve of 0.85. A prediction score of ≥5 points had a sensitivity of 94% and specificity of 48% to identify confirmed TB, and a sensitivity of 82% and specificity of 48% to identify confirmed and unconfirmed TB groups combined.

Conclusions. Our score has comparable sensitivity and specificity to algorithms including microbiological testing and should enable clinicians to rapidly initiate TB treatment among CHIV when microbiological testing is unavailable.

Keywords. biomarker; HIV; pediatric TB; prediction score; TB diagnosis.

In 2019 an estimated 1.2 million children developed tuberculosis (TB) disease, resulting in 230 000 deaths [1]. More than 96% of children who die from TB have not started treatment [2]. Diagnosis of active TB disease in children is complex, even with expansion of the Xpert MTB/RIF and Xpert Ultra platforms. Collection of induced sputum or gastric aspirate specimens from young children is challenging, and accessibility of Xpert MTB/RIF testing remains limited in low- and middle-income countries where most TB cases are diagnosed [3, 4]. When immediate microbiologic confirmation is not obtained, TB diagnosis in children relies on clinical presentation, contact history, diagnostics designed to detect *Mycobacterium tuberculosis* (*Mtb*) infection but not disease (ie, tuberculin skin testing [TST] and interferon-γ release assay), and chest radiography (CXR). This approach has many limitations and can lead to overdiagnosis, particularly among children with human immunodeficiency virus (CHIV) who have high incidence of non-TB pulmonary comorbidities [5–7]. Clinical diagnosis relies on clinical skills requiring support and standardization for those working at a low level of healthcare. Tools that maximize sensitivity with good specificity are required to provide this

Received 15 August 2022; editorial decision 10 October 2022; accepted 12 October 2022; published online 14 October 2022

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https://doi.org/10.1093/ofid/ofac548

support. Recently, 2 TB pediatric treatment decision algorithms published by Marcy et al and Gunasekera et al demonstrated high sensitivity (88.6%–90.1%) and moderate specificity (61.2%–52.1%) in children with and without HIV, respectively [8, 9]. However, the specificity of these models was enhanced by inclusion of Xpert MTB/RIF assay. In settings without access to microbiologic testing, there is a need for pediatric TB treatment decision algorithms that are easily deployable and highly sensitive, while maintaining moderate to high specificity.

The monocyte-to-lymphocyte ratio (MLR) obtained from routine complete blood counts (CBCs) has shown promise as a diagnostic biomarker for TB disease. *Mtb* preferentially infects cells of the myeloid lineage, including macrophages and monocytes, leading to proliferation and an increase in the absolute number of monocytes relative to the absolute number of lymphocytes in peripheral blood [10]. Elevated MLR predicted the risk of TB disease in infants with HIV infection or exposure [11–16] and MLR ≥0.285 achieved 91% sensitivity and 93% specificity to diagnose TB among adults without HIV [12]. The MLR can further be elevated in individuals with HIV, given their relative lymphocyte depletion [17]. Gatechompol et al found that MLR ≥0.23 had a sensitivity of 85% and specificity of 71% for predicting incident TB in people with HIV [18]. We previously found that MLR ≥0.378 was associated with active TB disease among hospitalized Kenyan CHIV, achieving a sensitivity of 79% and specificity of 77% [19]. MLR may be a proxy for *Mtb* bacteriologic burden, as we and others have found that MLR declines with TB treatment and Buttle et al found higher MLRs in smear-positive and cavitary pulmonary TB among adults [12, 19, 20]. Attractive features of MLR that can render it a feasible tool in low-resource settings include easy sample collection, availability of differential blood count in most laboratories globally, and easy calculation of the MLR ratio. Another option that has been explored is use of neutrophil-lymphocyte ratio.

The objective of this study was to validate the findings of MLR as a diagnostic biomarker in a multicountry cohort of children with and without HIV in both inpatient and outpatient settings. We then assessed the diagnostic performance of MLR when incorporated into a prediction score with demographic, clinical, and other laboratory features to inform TB treatment decisions among CHIV in settings where microbiologic testing is not readily available.

METHODS

Study Design

We performed a cross-sectional diagnostic accuracy study using combined data from 2 different pediatric cohort studies from Vietnam and Cameroon and South Africa to validate the performance of MLR as a diagnostic tool for pediatric TB, using a case-control approach with microbiologically confirmed TB and unlikely TB as cases and controls, respectively [21–23]. We secondarily constructed a diagnostic prediction score pooling data from these 2 studies and data from our previous Kenyan pediatric MLR study [19], using demographic, clinical, and laboratory features including MLR to increase the sensitivity and specificity of TB diagnosis in CHIV; we used a case-control approach with the same populations and secondarily tested our score in those with unconfirmed TB.

Study Population and Enrollment

Vietnam and Cameroon

As part of the French National Agency for Research on AIDS and Viral Hepatitis ANRS 12229 PAANTHER 01 study (NCT01331811), CHIV with presumptive TB from Vietnam and Cameroon aged ≤13 years were enrolled between April 2011 and August 2013 [21]. All children underwent a medical history and TB symptoms assessment, physical examination, CXR, CBC, TST, and microbiologic testing for TB.

South Africa

Children with and without HIV and presumptive TB aged ≤12 years were enrolled in a prospective TB diagnostic study between March 2012 and November 2017 [22, 23]. Children were evaluated with a complete physical examination, CXR, TST, and microbiologic TB testing. Children with a CBC collected at the time of enrollment were included in this analysis.

Kenya

As part of the Pediatric Urgent Start of HAART (PUSH) trial (NCT02063880), which compared impact on mortality of early antiretroviral therapy (ART) initiation within 48 hours versus 7–14 days after enrollment between April 2013 and May 2015 [24], children aged <12 years without central nervous system infection had complete medical history, physical examination, CBC, CXR, and microbiologic TB testing performed at enrollment.

All children in the above cohorts were classified as having confirmed, unconfirmed, or unlikely TB using the Clinical Case Definitions for Classification of Intra-thoracic TB in Children 2015 [25]. Further details of the cohorts are reported in the Supplementary Methods.

Sample Size

The post hoc sample size required to validate MLR ≥0.378 for diagnosis of confirmed TB versus unlikely TB was determined to be 403 children with an expected sensitivity and specificity of 70%, prevalence of confirmed TB of 20%, and precision/margin of error of 10% with 95% confidence level. The sample size required to estimate the diagnostic accuracy (area under the curve [AUC]) of prediction score for diagnosis of confirmed TB versus unlikely TB in children living with HIV was determined to be 58 confirmed TB cases and 58 non-TB cases (total n = 116) for AUC of 0.70 with precision/margin of error of 0.10 with 95% confidence level [26].

Statistical Analysis

To validate the findings from Kenya, data from the Vietnam/Cameroon and South Africa cohorts were pooled to create a

Table 1. Baseline Demographics of the Cohort Used for Validating Monocyte-Lymphocyte Ratio ≥0.378 for Diagnosis of Confirmed Tuberculosis

Baseline Characteristic	Total (N = 601)	Vietnam (n = 111)	Cameroon (n = 125)	South Africa (n = 365)
Age, y, median (IQR)	1.9 (0.9–5.3)	5.3 (1.6–8.8)	6.2 (1.8–9.4)	1.3 (0.7–2.5)
Male sex	300 (50)	59 (53)	55 (44)	186 (51)
Living with HIV	283 (47)	111 (100)	125 (100)	47 (13)
CD4%, median (IQR)	11.7 (2.9–21.3), n = 268	7.6 (1.2–18.7), n = 107	11.5 (3.4–20.3), n = 120	18.9 (13.0–27.0), n = 41
TB classification				
Confirmed	128 (21)	18 (16)	22 (18)	88 (24)
Unconfirmed	183 (31)	42 (38)	39 (31)	102 (28)
Unlikely	290 (48)	51 (46)	64 (51)	175 (48)
Cough >2 wks	343 (57)	89 (80)	105 (85)	149 (41)
Fever >2 wks	133 (22)	47 (42)	68 (55)	18 (4.9)
Failure to thrive	286 (48)	42 (38)	80 (64)	164 (45)
Reduced playfulness	193 (32)	21 (19)	94 (76)	78 (21)
TB contact history	147 (25)	10 (9)	5 (4)	132 (36)
TST >5 mm	99 (21), n = 475	13 (12), n = 105	9 (9), n = 95	77 (28), n = 275
CXR suggestive of TB [27]	283 (50), n = 568	70 (65), n = 107	82 (71), n = 116	131 (38), n = 345
WBC count, cells/μL, median (IQR)	10.9 (7.3–15.2), n = 592	7.6 (5.0–11.2), n = 107	7.3 (5.4–11.3), n = 120	12.9 (9.5–17.0), n = 365
Monocyte count, cells/μL, median (IQR)	7.4 (5.0–11.0), n = 592	5.0 (7.0–10.0), n = 107	10.0 (8.0–15.0), n = 120	6.5 (4.7–9.3), n = 365
Lymphocyte count, cells/μL, median (IQR)	38.6 (25.0–54.4), n = 592	40.0 (27.0–54.0), n = 107	37.0 (23.0–54.5), n = 120	38.4 (24.9–54.4), n = 365
MLR, median (IQR)	0.22 (0.14–0.38), n = 592	0.18 (0.12–0.30), n = 107	0.32 (0.20–0.43), n = 120	0.21 (0.13–0.37), n = 365
MLR if confirmed TB, median (IQR)	0.29 (0.17–0.53), n = 128	0.29 (0.19–0.38), n = 18	0.34 (0.30–0.60), n = 22	0.23 (0.16–0.52), n = 88
MLR if unconfirmed TB, median (IQR)	0.21 (0.12–0.36), n = 181	0.18 (0.12–0.33), n = 40	0.29 (0.20–0.40), n = 39	0.20 (0.11–0.34), n = 102
MLR if unlikely TB, median (IQR)	0.21 (0.13–0.35), n = 283	0.15 (0.11–0.24), n = 49	0.26 (0.17–0.44), n = 59	0.21 (0.12–0.36), n = 175

Abbreviations: CXR, chest radiography; HIV, human immunodeficiency virus; IQR, interquartile range; MLR, monocyte-lymphocyte ratio; TB, tuberculosis; TST, tuberculin skin test; WBC, white blood cell.

Data are presented as No. (%) unless otherwise indicated.

combined dataset and MLR was calculated using differential blood count for monocytes and lymphocytes at the initial diagnostic visit. A MLR value of ≥0.378 was used to evaluate TB diagnosis using the previously defined cutoff in the Kenyan cohort [19]. Microbiologically confirmed TB, either by mycobacterial culture or Xpert MTB/RIF (confirmed TB), versus unlikely TB was used as reference to calculate the sensitivity and specificity of MLR for TB diagnosis.

To construct a diagnostic prediction score, we combined data from CHIV in the Kenyan, South African, Vietnam, and Cameroon cohorts. We restricted analysis to children with at least 1 of the following symptoms: cough for >2 weeks, fever for >2 weeks, failure to thrive, or reduced playfulness and blood samples collected within 7 days of starting TB treatment. Multivariable logistic regression was performed with confirmed TB versus unlikely TB as the outcome. Predictor variables included TB-associated symptoms, TB contact history, CXR suggestive of TB (as a dichotomous outcome), TST ≥5 mm, MLR ≥0.28, age in years (dichotomized at 4 years), and sex. The candidate variables selected for model building were based on clinical data that are commonly collected while assessing a TB patient in high-incidence, low-resource settings. CXR was defined as suggestive of TB if there was a positive response for any one of the radiographic features: presence of hilar or paratracheal lymphadenopathy, tracheal or bronchial compression, pleural effusion, consolidation, cavitation, or miliary pattern [27]. For ease of use in clinical practice, we converted all continuous predictor variables into dichotomous variables using univariate receiver operating characteristic (ROC) curve analysis with the optimal cutoff based on the maximum value of Youden index, J (J = sensitivity + specificity – 1). Model selection using Akaike information criteria (AIC) was used to select the final, parsimonious model. We used bootstrap resampling (1000 samples) for internal validation and to obtain a value accounting for model optimism [28]. Log odds values from the final model were normalized by dividing them by their respective standard error and rounding off to the nearest integer. These integer values were considered the score items for these specific variables and a cumulative prediction score for each subject was calculated by summing these up. A ROC curve analysis was carried out to find the optimal cutoff for the prediction score using the maximum value of Youden index, J. We also assessed the performance of the derived prediction score against the composite reference standard of confirmed and unconfirmed TB as the TB case definition versus unlikely TB. Data were analyzed using Stata version 16 software (StataCorp, College Station, Texas). We follow the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) guidelines for reporting of results [29].

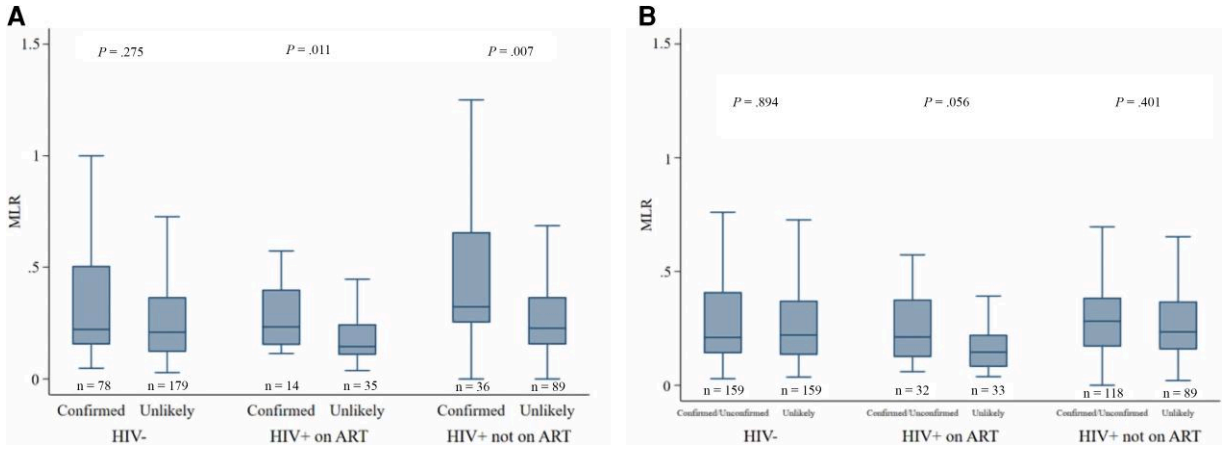

Figure 1. *A*, Monocyte-lymphocyte ratio (MLR) distribution by confirmed tuberculosis (TB) versus unlikely TB and human immunodeficiency virus (HIV) status (negative, positive with antiretroviral therapy [ART], or positive without ART) in children from Cameroon, Vietnam, and South Africa. *B*, MLR distribution by confirmed and unconfirmed TB versus unlikely TB and HIV status in children from Cameroon, Vietnam, and South Africa.

RESULTS

Cohort Characteristics

Of 663 children in the combined dataset from Vietnam, Cameroon, and South Africa, we excluded 71 children from the analysis because of missing MLR data. Sixty-two children from South Africa did not have blood samples collected within 7 days of starting TB treatment. These children did not differ demographically or clinically from the remaining 365 South African children who remained in the analytical dataset (Supplementary Table 1). Nine children from Vietnam and Cameroon did not have results for the blood counts recorded.

The median age of the combined cohorts was 1.9 (interquartile range [IQR], 0.9–5.3) years; 300 (50%) children were male. South African children were younger (median age, 1.3 [IQR, 0.7–2.5] years) than children from Vietnam and Cameroon (median age, 5.5 [IQR, 1.7–9.5] years). Overall, 283 (47%) children were living with HIV. The overall median white blood cell count was 10.9 (IQR, 7.3–15.2) cells/μL with South African children having a higher count (median, 12.9 [IQR, 9.5–17.0] cells/μL; median in CHIV: 11.0 [IQR, 8.2–14.8] cells/μL) compared with children from Vietnam and Cameroon (median, 7.3 [IQR, 5.1–11.2] cells/μL). Median lymphocyte and monocyte counts did not differ by country (Table 1).

Overall, 128 (21%) children met the criteria for confirmed TB, 183 (31%) had unconfirmed TB and 290 (48%) were unlikely to have TB. The overall median MLR was 0.22 (IQR, 0.14–0.38), ranging from 0.18 in Vietnam to 0.32 in Cameroon ($P < .001$). The median MLR among children with confirmed TB was 0.29 (IQR, 0.17–0.53). Children with unconfirmed TB had similar MLR (median, 0.21 [IQR, 0.12–0.36]) compared to children with unlikely TB (median, 0.21 [IQR, 0.12–0.35]) ($P = .77$). Median MLR was higher among CHIV with confirmed TB versus unlikely TB and more pronounced in the absence of ART (Figure 1*A*). MLR did not differ by TB disease classification among children without HIV (Figure 1*A*). MLR did not discriminate children with confirmed and unconfirmed TB grouped together compared to children with unlikely TB in either children with or without HIV (Figure 1*B*).

Diagnostic Utility of MLR ≥0.378

Using the MLR cutoff of ≥0.378, 145 (25%) children from the full cohort and 64 (23%) among CHIV were classified as having TB disease. Comparing confirmed TB versus unlikely TB, this MLR value had a sensitivity of 33% (95% CI, 25%–42%) and specificity of 77% (95% CI, 72%–82%) among the combined cohort of children with and without HIV (Table 2). Sensitivity and specificity values were similar when the analysis was repeated stratified by country (Table 2). Restricting the analysis to CHIV resulted in sensitivity of 36% (95% CI, 23%–51%) and specificity of 79% (95% CI, 71%–86%) (Table 2). Among CHIV with higher clinical TB severity (miliary pattern or cavitation on CXR or positive acid-fast bacilli smear), MLR value of ≥0.378 had a sensitivity of 46% (95% CI, 26%–67%) and specificity of 83% (95% CI, 36%–100%), comparing children with confirmed TB versus unlikely TB.

Deriving a Prediction Score for Diagnosis of TB in CHIV

One hundred thirty-one CHIV from the Kenya cohort were added to the overall dataset, resulting in a total of 414 CHIV of whom 63 (15%) had confirmed TB and 163 (39%) had unconfirmed TB. Baseline characteristics of the cohort are shown in Supplementary Table 2. Since MLR had better performance among CHIV, we developed a prediction score for CHIV with

Table 2. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value for Monocyte-Lymphocyte Ratio ≥0.378 (Confirmed vs Unlikely Tuberculosis)

Cohort and Characteristic	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Complete cohort	33 (25–42)	77 (72–82)	39 (30–49)	72 (66–77)
Vietnam	28 (10–54)	92 (80–98)	56 (21–86)	78 (65–88)
Cameroon	41 (21–64)	66 (53–78)	31 (15–51)	75 (61–86)
South Africa	32 (22–43)	77 (70–83)	41 (29–53)	69 (62–76)
HIV positive	36 (23–51)	79 (71–86)	41 (26–57)	75 (67–83)
HIV positive on ART	29 (8–58)	86 (70–95)	44 (14–79)	75 (59–87)
HIV positive not on ART	39 (23–57)	76 (66–89)	40 (24–58)	76 (65–84)
HIV negative	31 (21–42)	76 (68–82)	38 (26–51)	69 (62–76)
Male sex	36 (24–49)	76 (68–83)	42 (29–57)	71 (62–78)
Female sex	30 (20–43)	78 (70–84)	36 (24–50)	73 (65–79)

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; NPV, negative predictive value; PPV, positive predictive value.

confirmed and unlikely TB (n = 209). Using ROC curve analysis with Youden J, the best MLR cutoff was ≥0.28 with sensitivity of 67% (95% CI, 54%–78%) and specificity of 68% (95% CI, 61%–75%). We also used Youden index to determine the optimal age cutoff of ≥4 versus <4 years. Using AIC values, the best model to predict active TB disease had the following variables: MLR ≥0.28, age ≥4 years, TST ≥5 mm, contact history with a TB patient, fever for >2 weeks, and CXR suggestive of TB with an AUC of 0.85 (Figure 2). Model optimism was estimated to be 0.03%.

Supplementary Table 3 summarizes the coefficients for the final logistic regression model and the corresponding prediction scores. The highest scoring variable in the prediction score was fever >2 weeks (4 points). The median cumulative prediction score was 5 (IQR, 3–8) with the AUC being 0.84 (Figure 2). Model optimism was estimated to be 0.01%. The optimal cutoff for diagnosis of active TB disease using Youden J was 7 points. This resulted in a sensitivity of 78% (95% CI, 63%–88%), specificity of 78% (95% CI, 70%–84%), positive predictive value of 51% (95% CI, 39%–63%), and negative predictive value of 92% (95% CI, 86%–96%).

Table 3 summarizes sensitivities and specificities associated with other potential cutoff values comparing children with confirmed versus unlikely TB. A cutoff of 5 points resulted in sensitivity of 94% and specificity of 48% meeting WHO-recommended target product profile for a triage test sensitivity of at least 90%, but below the specificity requirement of 70%. Using composite standard of confirmed/unconfirmed TB versus unlikely TB, the AUC for the prediction score was 0.74 (Figure 2). A cutoff of 5 points resulted in sensitivity of 82% and specificity of 48%.

Figure 3 shows how the prediction score can be operationalized using a simple clinical algorithm.

DISCUSSION

In a multicountry cohort of children with and without HIV from South Africa, Vietnam, and Cameroon, we found that MLR was associated with confirmed TB in CHIV. Elevated MLR ≥0.378 achieved lower sensitivity (36%) but similar high specificity (79%) among CHIV in our multicountry validation cohort compared to previous findings among Kenyan ART-naive hospitalized CHIV [19]. We found that MLR did not discriminate between confirmed and unlikely TB among children without HIV.

The lower sensitivity of MLR to detect TB in our multicenter study compared to previous findings in Kenya may be attributed to differences in the clinical cohorts. The Kenyan cohort included CHIV who were younger, ART naive, and who likely had more severe TB disease given severe immunosuppression, malnutrition, and hospitalized status compared to children in Vietnam and Cameroon who enrolled CHIV in the outpatient setting. South African CHIV were of similar age to those in the Kenyan cohort and also predominantly from a hospitalized setting, but importantly, almost 50% were on ART. The median monocyte count in South African CHIV was lower than that of Kenyan children, while median white count was similar, accounting for the lower median MLR. We found that elevated MLR ≥0.378 had similar sensitivity but lower specificity compared to other non-sputum-based microbiologic tests to diagnose active TB disease in children as reported in the literature (stool Xpert MTB/RIF: sensitivity 32%–81%, specificity 99%–100%; nasopharyngeal aspirate Xpert MTB/RIF: sensitivity 39%–65%, specificity 98%–99%) [30–33]. Elevated MLR ≥0.378 had higher specificity than clinical diagnosis for CHIV (previously reported between 25% and 62%) [34].

The relatively high specificity of MLR for a blood-based non-sputum-based diagnostic test offers potential for inclusion in pediatric TB treatment algorithms to limit overdiagnosis in settings without access to microbiologic testing [35]. Diagnosis of TB in CHIV in absence of microbiological testing is particularly difficult given nonspecific symptoms and CXR findings in the context of higher rates of other respiratory illnesses [6, 36]. We found that a score of 5 points resulted in a sensitivity of 94% and specificity of 48%, which is in line with the target product profile of WHO to achieve sensitivity of at least 90% for community-based triage tests but not the specificity requirements of at least 70% [8, 9, 37]. Yet our score had similar specificity compared to other treatment decision algorithms without reliance on the Xpert MTB/RIF assay [8, 9]. Our prediction score can easily be operationalized using a simple clinical algorithm (Figure 3) that should be externally validated before clinical use.

Limitations of our study include inability to stratify our analysis based on severity of the disease or type of automated hemocytometer used to measure MLR as this information was not

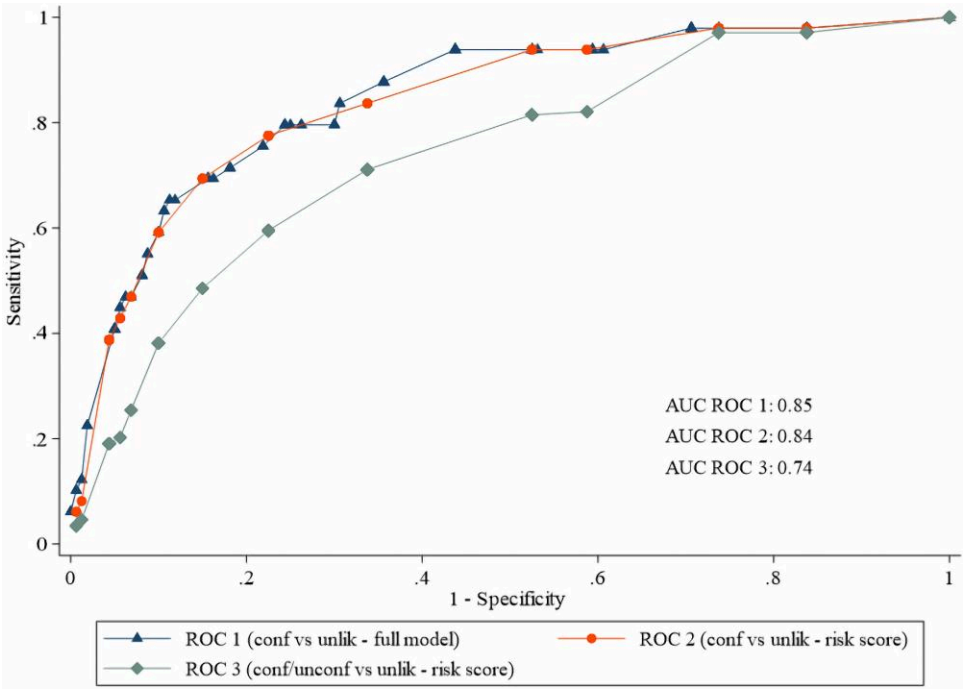

Figure 2. Receiver operating characteristic curves for confirmed tuberculosis (TB) versus unlikely TB and confirmed and unconfirmed TB versus unlikely TB. Full model: β0 + β1 monocyte-lymphocyte ratio ≥0.28 + β2 tuberculin skin test + β3 contact history + β4 fever >2 weeks + β5 age ≥4 years + β6 chest radiograph consistent with TB. Prediction score model: β0 + β1 prediction score. Abbreviations: AUC, area under the curve; conf, confirmed; ROC, receiver operating characteristic; unconf, unconfirmed; unlik, unlikely.

Table 3. Sensitivities and Specificities of Different Prediction Score Cutoffs

Prediction Score	Confirmed TB vs Unlikely TB		Confirmed/Unconfirmed TB vs Unlikely TB	
	Sensitivity, %	Specificity, %	Sensitivity, %	Specificity, %
4	93.9	41.3	82.1	41.3
5	93.9	47.5	81.5	47.5
6	83.7	66.3	71.1	66.3
7	77.6	77.5	59.5	77.5
8	69.4	85.0	48.6	85.0
9	59.2	90.0	38.2	90.0
10	46.9	93.1	25.4	93.1

Components of the prediction score include monocyte-lymphocyte ratio ≥0.28, tuberculin skin test, contact history, fever >2 weeks, age ≥4 years, and chest radiography consistent with TB.

Abbreviation: TB, tuberculosis.

available to us. The finding that MLR does not perform as well in children without HIV needs to be interpreted in the context that all children without HIV were from South Africa and there may be genetic and environmental influences on the relative abundance of monocyte counts [38]. Alternately, since MLR is associated with TB disease severity, improved MLR performance among CHIV may reflect a higher mycobacterial burden compared to children without HIV. MLR performed well as a TB diagnostic biomarker in prior studies of adults without HIV. Given the relative paucibacillary nature of pediatric TB, its use among children may be optimal in the context of more severe disease [12, 19, 20]. We developed the score using confirmed TB as the reference case definition for TB in comparison to unlikely TB. This can lead to misdiagnosis in children who do not have bacteriologically confirmed disease. However, our score performed similarly when the composite TB case definition included both confirmed and unconfirmed TB. Diagnosis in the youngest age groups continues to be a challenge, with our algorithm requiring more investigations to initiate treatment in those aged <4 years. This high-risk group requires urgent prioritization for improved diagnostic approaches. Last, we had missing predictor data on 17% of the cohort when creating the prediction score, mostly for CXR and TST.

Strengths of our prediction score include the use of prospective data from multicountry cohorts. Predictors in our score include parameters that are known to be associated with TB disease in children [25]. We used methods recommended for diagnostic prediction models to create our score and internally validate it using bootstrap resampling [9]. This method is in contrast to most previous pediatric TB diagnostic scores and algorithms, which have been based on expert opinions and have not been validated [34, 39]. Additionally, our score does not

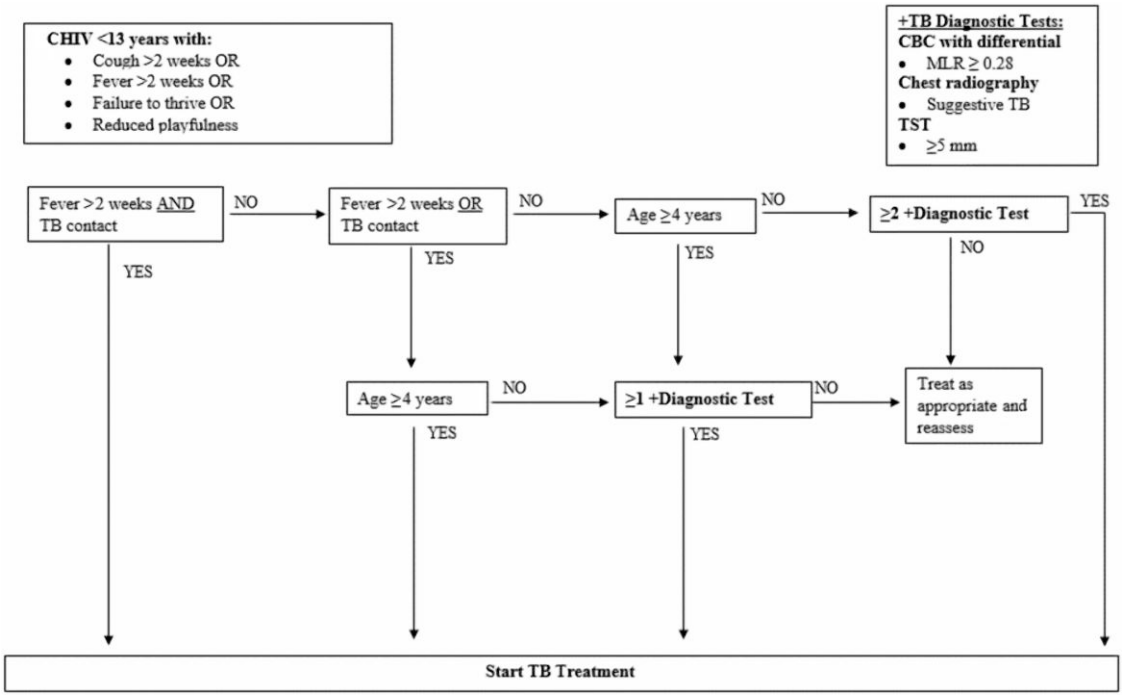

Figure 3. Treatment algorithm for children with human immunodeficiency virus. Abbreviations: +, positive; CBC, complete blood count; CHIV, children with human immunodeficiency virus; MLR, monocyte-lymphocyte ratio; TB, tuberculosis; TST, tuberculin skin test.

rely on sputum-based testing including Xpert MTB/RIF assay. Although availability of the Xpert MTB/RIF assay is increasing globally, access in resource-limited settings is still challenging, with clinical staff training, program guidelines, electrical supply, transportation, and cartridge availability as possible barriers [40, 41]. Even when the test is available, children may not be able to provide the requisite sputum sample [3]. Our score involves laboratory tests, including CBC, TST, and CXR. However, these are readily available in many high-incidence, low-resource settings.

CONCLUSIONS

With its discriminatory performance, our score would enable clinicians to rapidly initiate TB treatment among CHIV with presumed TB disease in absence of microbiological confirmation. Further studies to externally validate the prediction score and assess its clinical usefulness are needed before this can be used in clinical practice.

Supplementary Data

Supplementary materials are available at *Open Forum 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

Author contributions. A. A. M., N. R. G., and L. M. C. conceptualized the study and wrote the protocol. A. A. M., O. M., E. W., M. T., I. N. N., S. M. L., E. M. O., D. W., G. C. J.-S., and L. M. C. collected data. A. A. M., N. R. G., S. B. O., T. L. L., M. C. B., and L. M. C. performed and reviewed the analysis. A. A. M. and L. M. C. wrote the initial draft of the manuscript. All authors helped interpret the findings, read, and approved the final version of the manuscript.

Patient consent. All patients/guardians provided written consent at time of study enrollment. The design of the work has been approved by local ethical committees in respective countries (see details in the Supplementary Methods).

Financial support. The work was supported by the National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (award numbers K23AI143479 to L. M. C. and K24AI114444 to N. R. G.); TB Research Unit ASTRa (grant number U19AI111211); Emory University Center for AIDS Research (grant number P30AI050409); Fogarty International Center, NIH (award number K43TW011422 to I. N. N.); Program for Retaining, Supporting, and EleVating Early-career Researchers at Emory (PeRSEVERE) from the Emory School of Medicine (to L. M. C.); a gift from the Doris Duke Charitable Foundation (to L. M. C.); and Georgia Clinical and Translational Science Award (award number UL1-TR002378 to L. M. C.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. World Health Organization (WHO). Global tuberculosis control report 2020. Geneva, Switzerland: WHO; **2020**.
2. Dodd PJ, Yuen CM, Sismanidis C, Seddon JA, Jenkins HE. The global burden of tuberculosis mortality in children: a mathematical modelling study. Lancet Glob Health **2017**;5:e898–906.
3. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. Lancet **2005**; 365:130–4.
4. Venturini E, Turkova A, Chiappini E, Galli L, de Martino M, Thorne C. Tuberculosis and HIV co-infection in children. BMC Infect Dis **2014**; 14:S5.

5. Marais BJ, Gie RP, Hesseling AC, et al. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. Pediatrics **2006**; 118:e1350–9.
6. Roya-Pabon CL, Perez-Velez CM. Tuberculosis exposure, infection and disease in children: a systematic diagnostic approach. Pneumonia **2016**; 8:23.
7. Frigati LJ, Ameyan W, Cotton MF, et al. Chronic comorbidities in children and adolescents with perinatally acquired HIV infection in sub-Saharan Africa in the era of antiretroviral therapy. Lancet Child Adolesc Health **2020**; 4:688–98.
8. Gunasekera KS, Walters E, van der Zalm MM, et al. Development of a treatment-decision algorithm for HIV-uninfected children evaluated for pulmonary tuberculosis. Clin Infect Dis **2021**; 73:e904–12.
9. Marcy O, Borand L, Ung V, et al. A treatment-decision score for HIV-infected children with suspected tuberculosis. Pediatrics **2019**; 144:10.
10. Sia JK, Georgieva M, Rengarajan J. Innate immune defenses in human tuberculosis: an overview of the interactions between *Mycobacterium tuberculosis* and innate immune cells. J Immunol Res **2015**; 2015:747543.
11. Agarwal A, Bhat MS, Kumar A, Shaharyar A, Mishra M, Yadav R. Lymphocyte/monocyte ratio in osteoarticular tuberculosis in children: a haematological biomarker revisited. Trop Doct **2016**; 46:73–7.
12. La Manna MP, Orlando V, Dieli F, et al. Quantitative and qualitative profiles of circulating monocytes may help identifying tuberculosis infection and disease stages. PLoS One **2017**; 12:e0171358.
13. Naranbhai V, Hill AV, Abdool Karim SS, et al. Ratio of monocytes to lymphocytes in peripheral blood identifies adults at risk of incident tuberculosis among HIV-infected adults initiating antiretroviral therapy. J Infect Dis **2013**; 209:500–9.
14. Naranbhai V, Kim S, Fletcher H, et al. The association between the ratio of monocytes: lymphocytes at age 3 months and risk of tuberculosis (TB) in the first two years of life. BMC Med **2014**; 12:120.
15. Naranbhai V, Moodley D, Chipato T, et al. The association between the ratio of monocytes: lymphocytes and risk of tuberculosis (TB) amongst HIV infected postpartum women. J Acquir Immune Defic Syndr **2014**; 67:573–5.
16. Wang J, Yin Y, Wang X, et al. Ratio of monocytes to lymphocytes in peripheral blood in patients diagnosed with active tuberculosis. Braz J Infect Dis **2015**; 19:125–31.
17. Doitsh G, Greene WC. Dissecting how CD4 T cells are lost during HIV infection. Cell Host Microbe **2016**; 19:280–91.
18. Gatechompol S, Sophonphan J, Kerr SJ, et al. Monocyte-to-lymphocyte ratio as a predictor of TB among people living with HIV. Int J Tuberc Lung Dis **2021**; 25:933–8.
19. Choudhary RK, Wall KM, Njuguna I, et al. Monocyte-to-lymphocyte ratio is associated with tuberculosis disease and declines with anti-TB treatment in HIV-infected children. J Acquir Immune Defic Syndr **2019**; 80:174–81.
20. Buttle TS, Hummerstone CY, Billahalli T, et al. The monocyte-to-lymphocyte ratio: sex-specific differences in the tuberculosis disease spectrum, diagnostic indices and defining Normal ranges. PLoS One **2021**; 16:e0247745.
21. Marcy O, Ung V, Goyet S, et al. Performance of Xpert MTB/RIF and alternative specimen collection methods for the diagnosis of tuberculosis in HIV-infected children. Clin Infect Dis **2016**; 62:1161–8.
22. Walters E, Demers A-M, van der Zalm MM, et al. Stool culture for diagnosis of pulmonary tuberculosis in children. J Clin Microbiol **2017**;55:3355–65.
23. Walters E, van der Zalm MM, Palmer M, et al. Xpert MTB/RIF on stool is useful for the rapid diagnosis of tuberculosis in young children with severe pulmonary disease. Pediatr Infect Dis J **2017**; 36:837–43.
24. Njuguna IN, Cranmer LM, Otieno VO, et al. Urgent versus post-stabilisation antiretroviral treatment in hospitalised HIV-infected children in Kenya (PUSH): a randomised controlled trial. Lancet HIV **2018**; 5:e12–22.
25. Graham SM, Cuevas LE, Jean-Philippe P, et al. Clinical case definitions for classification of intrathoracic tuberculosis in children: an update. Clin Infect Dis **2015**; 61:S179–S87.
26. Hajian-Tilaki K. Sample size estimation in diagnostic test studies of biomedical informatics. J Biomed Inform **2014**; 48:193–204.
27. Graham SM, Ahmed T, Amanullah F, et al. Evaluation of tuberculosis diagnostics in children: 1. Proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. J Infect Dis **2012**; 205:S199–208.
28. Smith GC, Seaman SR, Wood AM, Royston P, White IR. Correcting for optimistic prediction in small data sets. Am J Epidemiol **2014**; 180:318–24.
29. Collins GS, Reitsma JB, Altman DG, Moons KGM. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. BMC Med **2015**; 13:1.
30. Zar HJ, Workman L, Isaacs W, et al. Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. Clin Infect Dis **2012**; 55:1088–95.
31. Chipinduro M, Mateveke K, Makamure B, Ferrand RA, Gomo E. Stool Xpert((R)) MTB/RIF test for the diagnosis of childhood pulmonary tuberculosis at primary clinics in Zimbabwe. Int J Tuberc Lung Dis **2017**; 21:161–6.
32. LaCourse SM, Pavlinac PB, Cranmer LM, et al. Stool Xpert MTB/RIF and urine lipoarabinomannan for the diagnosis of tuberculosis in hospitalized HIV-infected children. AIDS **2018**; 32:69–78.
33. Moussa H, Bayoumi FS, Mohamed AM. Gene Xpert for direct detection of *Mycobacterium tuberculosis* in stool specimens from children with presumptive pulmonary tuberculosis. Ann Clin Lab Sci **2016**; 46:198–203.
34. Pearce EC, Woodward JF, Nyandiko WM, Vreeman RC, Ayaya SO. A systematic review of clinical diagnostic systems used in the diagnosis of tuberculosis in children. AIDS Res Treat **2012**; 2012:401896.
35. Houben R, Lalli M, Kranzer K, Menzies NA, Schumacher SG, Dowdy DW. What if they don't have tuberculosis? The consequences and trade-offs involved in false-positive diagnoses of tuberculosis. Clin Infect Dis **2019**; 68:150–6.
36. Rabie H, Goussard P. Tuberculosis and pneumonia in HIV-infected children: an overview. Pneumonia **2016**; 8:19.
37. World Health Organization (WHO). High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva, Switzerland: WHO; **2014**.
38. Patel AA, Yona S. Inherited and environmental factors influence human monocyte heterogeneity. Front Immunol **2019**; 10:2581.
39. Hesseling A, Schaaf H, Gie R, Starke J, Beyers N. A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis. Int J Tuberc Lung Dis **2002**; 6:1038–45.
40. Cazabon D, Pande T, Kik S, et al. Market penetration of Xpert MTB/RIF in high tuberculosis burden countries: a trend analysis from 2014–2016. Gates Open Res **2018**; 2:10.
41. Clouse K, Blevins M, Lindegren ML, et al. Low implementation of Xpert MTB/RIF among HIV/TB co-infected adults in the international epidemiologic databases to evaluate AIDS (IeDEA) program. PLoS One **2017**; 12:e0171384.