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Monocyte-to-lymphocyte ratio is associated with tuberculosis disease and declines with anti-TB treatment in HIV-infected children

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Abstract

Monocyte-to-lymphocyte ratio is associated with tuberculosis disease and declines with anti-TB treatment in HIV-infected children

By Rewa Choudhary

Objective: The blood monocyte-to-lymphocyte ratio (MLR) is associated with active tuberculosis (TB) in adults, but has not been evaluated as a TB diagnostic biomarker in HIV-infected children in whom respiratory sampling is difficult.

Design: In a cohort of HIV-infected hospitalized Kenyan children initiating antiretroviral therapy, absolute monocyte and lymphocyte counts were determined at enrollment and 4, 12, and 24 weeks thereafter.

Methods: Children were classified as confirmed, unconfirmed, or unlikely TB. ROC curves of MLR cutoff values were generated to distinguish children with confirmed TB from those with unconfirmed and unlikely TB. General estimating equations were used to estimate change in MLR over time by TB disease status.

Results: Of 171 children with median age 24 months, 14 (8.2%) had confirmed TB and 75 (43.9%) had unconfirmed TB. Median MLR among children with confirmed TB [0.428 (interquartile range (IQR) 0.378 - 0.675)] was higher than MLR in children with unconfirmed [0.217 (IQR 0.155 - 0.353), $p < 0.01$] or unlikely [0.213 (IQR 0.140 - 0.391), $p < 0.01$] TB. MLR above 0.378 identified children with confirmed TB with 79% sensitivity, 77% specificity, 24% positive predictive value, and 98% negative predictive value. After TB treatment, median MLR declined in children with confirmed TB and levels were similar to children with unlikely TB after 12 weeks.

Conclusions: Blood MLR distinguished HIV-infected children with confirmed TB from those with unlikely TB and declined with TB treatment. MLR may be a useful diagnostic tool for TB in settings where respiratory-based microbiologic confirmation is inaccessible.

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BACKGROUND

Mycobacterium tuberculosis disease (TB) is a bacterial infectious disease primarily affecting the lungs, which can also manifest in extrapulmonary sites of the body (1). The World Health Organization estimates that 1.7 billion people worldwide are infected with the bacteria and 5-15% of these individuals will develop TB disease (1). Globally TB is the leading infectious cause of mortality from a single agent, causing an estimated 1.7 million deaths in 2016 (1).

Estimating the number of TB cases and mortality in children is difficult as TB reporting data may not be disaggregated by age or the cause of death may be attributed to other diseases that clinically may appear similar to TB, such as pneumonia (2, 3). Furthermore, when a pediatric patient has a comorbidity such as human immunodeficiency virus (HIV) infection, the International Statistical Classification of Diseases and Related Health Problems-10 (ICD-10) indicates that tuberculosis should not be reported as the primary cause of death, which may lead to underestimation of TB prevalence among children (2). The World Health Organization estimates that among children under 15 years of age in 2016, there were over 1 million incident cases of TB and 253,000 TB-related deaths (1). The risk of developing TB increases with HIV infection due to loss of cell-mediated immunity (4), and autopsy analyses in sub-Saharan Africa have shown that tuberculosis is a leading cause of mortality among HIV-positive children (5). A mathematical modeling study found that in 2015 39,000 children who died of TB were also HIV-positive (2).

TB can be either bacteriologically confirmed (positive sputum smear microscopy, culture, or rapid molecular test) or clinically diagnosed (6). In children diagnosis is complicated by challenges in collecting adequate respiratory specimens for smear and culture, as the disease is often paucibacillary in this population (7). As smear and culture are often time-intensive, rapid molecular tests can offer diagnoses more promptly. At present the only rapid molecular test that the WHO recommends for children is the Xpert® MTB/RIF assay (1), a nucleic acid amplification test. If bacteriological confirmation cannot be obtained, the most current recommendations for diagnosis of TB in children are to use a combination of clinical signs and symptoms, chest radiograph, tuberculin skin test, interferon gamma release assay, TB contact history, and response to TB treatment (7).

Rapid molecular tests may not be available in low-resource settings, therefore host serum biomarkers for TB disease may provide an alternative to pathogen-based biomarkers for TB diagnosis (8). T cell activation markers and transcriptional profiling (9-13) have shown utility for diagnosis in children, but are costly and require specialized equipment and training. The blood monocyte-to-lymphocyte ratio (MLR), derived from blood counts that are routinely collected in resource-limited settings for the management of acute illness, has been shown to predict progression to TB disease in children and adults (14-16). In a cohort of HIV-infected and HIV-exposed uninfected South African and Botswanan children, elevated MLRs at 3-4 months of age were associated with increased risk of the onset of TB disease by 2 years of age (14). Naranbhai and colleagues have also shown that both low ($<5^{th}$ percentile) and high ($>95^{th}$ percentile)

values of the monocyte to lymphocyte ratio in HIV-infected South African adults starting combination antiretroviral therapy corresponded to higher incidence rates of TB over the follow-up time (16). MLR may also have a role as a diagnostic biomarker for TB disease, as demonstrated by gene expression profiling of monocytes, which showed that MLR was associated with mycobacterial growth *in vitro* and enrichment of ML-ratio-associated gene set (by 3-fold) in TB disease *in vivo* (17). Furthermore an Italian study of adults without HIV found that an MLR cutoff >0.285 had high sensitivity and specificity (91% and 94%, respectively) to identify patients with culture-confirmed TB (18).

To our knowledge, the performance of MLR as a diagnostic biomarker for TB has not been evaluated in children with or without HIV disease. In a cohort of hospitalized Kenyan HIV-infected children starting antiretroviral therapy with well-classified TB disease status, we investigated the association between MLR and active TB disease measured at enrollment.

References

1. World Health Organization. Global tuberculosis report 2017. http://www.who.int/tb/publications/global_report/en/0. Published 2017. Accessed November 30, 2017.

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(9):e898-e906.

3. Jenkins HE, Yuen CM, Rodriguez CA, Nathavitharana RR, McLaughlin MM, Donald P, et al. Mortality in children diagnosed with tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis 2017;17(3):285-295.

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 Suppl 1:S5.

5. Cox JA, Lukande RL, Lucas S, Nelson AM, Van Marck E, Colebunders R. Autopsy causes of death in HIV-positive individuals in sub-Saharan Africa and correlation with clinical diagnoses. AIDS Rev 2010;12(4):183-94.

6. World Health Organization. Definitions and reporting framework for tuberculosis - 2013 revision (updated December 2014). http://apps.who.int/iris/bitstream/handle/10665/79199/9789241505345_eng.pdf?sequence=1. Published 2013. Accessed March 23, 2018.

7. Graham SM, Cuevas LE, Jean-Philippe P, Browning R, Casenghi M, Detjen AK, et al. Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children: An Update. Clin Infect Dis 2015;61Suppl 3:S179-87.

8. Perez-Velez CM, Roya-Pabon CL, Marais BJ. A systematic approach to diagnosing intra-thoracic tuberculosis in children. J Infect 2017;74 Suppl 1:S74-S83.

9. Rozot V, Patrizia A, Vigano S, Mazza-Stalder J, Idrizi E, Day CL, et al. Combined use of Mycobacterium tuberculosis-specific CD4 and CD8 T-cell responses is a powerful diagnostic tool of active tuberculosis. Clin Infect Dis 2015;60(3):432-7.

10. Portevin D, Moukambi F, Clowes P, Bauer A, Chachage M, Ntinginya NE, et al. Assessment of the novel T-cell activation marker-tuberculosis assay for diagnosis of active tuberculosis in children: a prospective proof-of-concept study. Lancet Infect Dis 2014;14(10):931-8.

11. Anderson ST, Kaforou M, Brent AJ, Wright VJ, Banwell CM, Chagaluka G, et al. Diagnosis of childhood tuberculosis and host RNA expression in Africa. N Engl J Med 2014;370(18):1712-1723.

12. Kaforou M, Wright VJ, Oni T, French N, Anderson ST, Bangani N, et al. Detection of tuberculosis in HIV-infected and -uninfected African adults using whole blood RNA expression signatures: a case-control study. PLoS Med 2013;10(10):e1001538.

13. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 2010;466(7309):973-7.

14. Naranbhai V, Kim S, Fletcher H, Cotton MF, Violari A, Mitchell C, 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, Stranix-Chibanda L, Nakabaiito C, Kamateeka M, et al. The association between the ratio of monocytes: lymphocytes and risk of tuberculosis among HIV-infected postpartum women. J Acquir Immune Defic Syndr 2014;67(5):573-5.

16. Naranbhai V, Hill AV, Abdool Karim SS, Naidoo K, Abdool Karim Q, Warimwe GM, 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 2014;209(4):500-9.

17. Naranbhai V, Fletcher HA, Tanner R, O'Shea MK, McShane H, Fairfax BP, et al. Distinct Transcriptional and Anti-Mycobacterial Profiles of Peripheral Blood Monocytes Dependent on the Ratio of Monocytes: Lymphocytes. EBioMedicine 2015;2(11):1619-26.

18. La Manna MP, Orlando V, Dieli F, Di Carlo P, Cascio A, Cuzzi G, et al. Quantitative and qualitative profiles of circulating monocytes may help identifying tuberculosis infection and disease stages. PLoS One 2017;12(2):e0171358.

MANUSCRIPT

Monocyte-to-lymphocyte ratio is associated with tuberculosis disease and declines with anti-TB treatment in HIV-infected children

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RUNNING TITLE: **MLR identifies TB in HIV+ children**

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INTRODUCTION

Mycobacterium tuberculosis disease (TB) is a leading cause of mortality in HIV-infected children (1). In 2016 there were over 1 million incident cases of TB and 253,000 TB-related deaths in children under 15 years of age (2). Microbiologic diagnosis of TB in children is difficult given paucibacillary disease and the operational challenges of obtaining respiratory specimens in young children who are unable to produce sputum (3). Host biomarkers for TB disease may provide an alternative to pathogen-based biomarkers for TB diagnosis (4). T cell activation markers and transcriptional profiling (5-9) have shown utility for diagnosis in children, but are costly and require specialized equipment and training.

The blood monocyte-to-lymphocyte ratio (MLR), derived from blood counts that are routinely collected in resource-limited settings for the management of acute illness, has been shown to predict progression to TB disease in children and adults (10-12). In a cohort of HIV-infected and HIV-exposed uninfected South African and Botswanan children, elevated MLRs at 3-4 months of age predicted onset of TB disease by 2 years of age (10). Among HIV-infected African women, elevated MLR during pregnancy was associated with increased risk for incident TB disease over 18 months of postpartum follow-up, even when controlling for $CD4^+$ count, antiretroviral treatment (ART), and World Health Organization HIV stage (11). Less is known about the diagnostic performance of MLR, but an Italian study of adults without HIV found that an MLR cutoff > 0.285 had high sensitivity and specificity (91% and 94%, respectively) to

identify patients with culture-confirmed TB (13). Furthermore, MLR may be useful as an indicator of treatment response, as demonstrated in a cohort of Chinese adults with TB in which MLRs normalized to ranges similar to those of healthy controls after a 6-month treatment course (14).

To our knowledge, the performance of MLR as a diagnostic biomarker for TB has not been evaluated in children with or without HIV disease. In addition, prior studies performed have not accounted for important co-factors that may alter MLR, such as immunosuppression (12), nutritional status (15) and malaria co-infection (12, 15-17). In a cohort of hospitalized Kenyan HIV-infected children starting antiretroviral therapy with well-classified TB disease status, we investigated the association between MLR and active TB disease measured at enrollment, and evaluated MLR changes over a 6-month time period as a potential indicator of treatment response. We evaluated $CD4^+$ count, nutritional status, and malaria infection as potential confounders of the association between MLR and TB.

METHODS

Study Population

We conducted a longitudinal cohort study nested within the Pediatric Urgent Start of HAART (PUSH) randomized clinical trial (NCT02063880) (18). Study subjects in the parent trial were HIV-infected, antiretroviral therapy-naïve children age 12 years old or

younger, hospitalized at four Kenyan hospitals (Kenyatta National Hospital and Mbagathi District Hospital in Nairobi; Kisumu County Hospital and Jaramogi Oginga Odinga Teaching and Referral Hospital in Western Kenya). Participants were randomized in the parent trial to urgent (less than 48 hours) or early (7-14 days) antiretroviral therapy and were excluded if they had a central nervous system infection at enrollment. Children were excluded from this sub-analysis if they received treatment for TB for more than 14 days prior to enrollment and if they did not complete at least one test for microbiologic confirmation of TB diagnosis.

The parent study was reviewed and approved by the Institutional Review Board (IRB) at the University of Washington, the University of Nairobi/Kenyatta National Hospital Ethical Review Committee (UoN/KNH ERC), and the Pharmacy and Poisons Board in Kenya. Written informed consent was obtained from all participants' caregivers in their preferred language (English, Kiswahili, or Dholuo).

Study Procedures

Blood specimens were obtained from each participant for full blood count and differential (including monocyte and lymphocytes) at enrollment and 4, 12, and 24-week follow-up visits. $CD4^{+}$ percentage was determined at enrollment, 4 and 24-week follow-up visits. Full blood counts were performed on an automated MS4 Haematology analyzer (Melet Schloesing Laboratoires, Osny, France) and $A^{C}\square T^{TM}$ 5diff Coulter® counter (Beckman Coulter, Inc., Brea, United States). A study nurse evaluated growth parameters

(height, weight, middle upper arm and head circumference) at every encounter. All children were evaluated at enrollment for intrathoracic tuberculosis by symptom screening, physical examination, tuberculin skin test (TST), chest radiograph, two sputum or gastric aspirate samples for direct Ziehl-Neelsen (ZN) smear microscopy and liquid culture using BACTEC Mycobacteria Growth Indicator Tube (MGIT)™ 960 system (Becton Dickinson, Sparks, MD, USA), one sputum or gastric aspirate specimen for PCR using Xpert MTB/RIF® (Cepheid, Sunnyvale, CA, USA), and one stool specimen for PCR using Xpert MTB/RIF®. For TST, 5 units (0.1 mL) of purified protein derivative (RT23 solution; Sanofi Pasteur, Lyon, France) were injected intradermally and a study nurse measured induration 48 to 72 hours later.

Participants were treated with combination antiretroviral therapy (cART) (abacavir and lamivudine with either nevirapine, efavirenz, or lopinavir/ritonavir) according to Kenyan Ministry of Health guidelines (19). Children with suspected TB as assessed clinically by hospital medical officers were treated with a six-month regimen of rifampin, isoniazid, pyrazinamide, and ethambutol per Kenyan National TB Program guidelines (20). ART regimens were adjusted as needed for children receiving concurrent TB treatment to avoid medication interactions (20).

Definitions

The monocyte-to-lymphocyte ratio (MLR) was determined by dividing absolute monocyte counts by absolute lymphocyte counts at each study time point. Weight-for-age

Z-scores (WAZ) and weight-for-height Z-scores (WHZ) were calculated based on WHO growth curves using WHO ANTHRO software (version 3.2.2 World Health Organization, Geneva, Switzerland) (21).

Children were classified as having microbiologically-confirmed TB, unconfirmed TB, or unlikely TB at enrollment based on international consensus clinical case definitions for intrathoracic TB (22). For the purposes of this analysis, failure to thrive was defined by underweight (WAZ $\leq$ -2) or wasting (WHZ $\leq$ -2 if under 5 years of age or MUAC < 12.5 for ages 5 to 12 years). Response to anti-TB treatment was defined as increase in weight and resolution of enrollment TB symptoms over 24 weeks of follow-up.

Statistical Analysis

Children were stratified by confirmed, unconfirmed, or unlikely TB classification. Descriptive measures of frequency (counts and percentages for categorical variables, medians and interquartile ranges [IQRs] for continuous variables) were calculated for all covariates. Fisher's exact tests were used to compare the distributions of categorical variables between confirmed versus unlikely TB groups and unconfirmed versus unlikely TB groups. Wilcoxon two-sample tests were used to compare the distributions of continuous variables between confirmed versus unlikely TB groups and unconfirmed versus unlikely TB groups. Study power was calculated using OpenEpi (Version 3.01, updated 04/06/2013) (23).

ROC curves of MLR cutoff values were used to distinguish children with confirmed TB from those with unconfirmed and unlikely TB participants. The optimal MLR diagnostic cutoff was determined based on the maximum value of Youden's index, *J*, where *J* = sensitivity + specificity – 1 (24).

General estimating equations (GEE) were used to estimate the association between TB status and changes in repeated MLR measures over the study period. We also estimated this association for absolute monocyte count and absolute lymphocyte counts individually. We evaluated baseline and time-varying $CD4^+$ percentage, WAZ and WHZ, and malaria infection as potential confounders of the association between MLR and TB. Time-varying $CD4^+$ percentage was considered *a priori* as a confounder of interest since $CD4^+$ percentage is associated with active TB and MLR, as was time since enrollment because it is a proxy for TB treatment over time. We assessed for multicollinearity and selected the most parsimonious model. Sensitivity analyses restricting participants to children who had completed the study and children who were treated for TB were also conducted.

Two-sided p-values < 0.05 were considered statistically significant. All analyses were conducted using SAS software (version 9.4, SAS Institute Inc, Cary, NC).

RESULTS

Cohort Characteristics

Of 183 randomized children from April 2013 to May 2015 in the parent trial, four were excluded due to TB treatment at time of enrollment and eight were excluded for failing to receive microbiologic confirmation testing, leaving 171 included in this analysis. Fourteen met criteria for confirmed TB (8.2%), 75 (43.9%) had unconfirmed TB, and 82 (48.0%) were unlikely to have TB (Figure, Supplemental Digital Content 1, patient inclusion flowchart). Overall, the median age at enrollment was 24.0 months (IQR 10.8 –60.9), 91 (53.2%) were male, 31 (18.1%) children died during the study period and 13 (7.6%) were lost-to-follow-up. The study power calculated to detect the difference in MLR between the confirmed TB (n=14) and unlikely TB (n=81) groups was 74.4% (assuming $\alpha = 0.05$ with two-tailed t-test).

The median enrollment $CD4^+$ percentages for children in the confirmed and unconfirmed groups were similar to the $CD4^+$ percentage in the unlikely TB group ($p = 0.19$ and $p = 0.11$, respectively); however, over all study intervals, the median $CD4^+$ percentage was significantly lower for participants in the confirmed and unconfirmed groups as compared to the unlikely TB group ($p = 0.03$ and $p < 0.01$, respectively). Over all study intervals, the median WAZ and WHZ were lower for the confirmed and unconfirmed TB children as compared to those in the unlikely TB group (WAZ: $p < 0.01$ and $p < 0.01$, respectively; WHZ: $p < 0.01$ and $p < 0.01$, respectively). Overall, 26 children were diagnosed with malaria during the study period, with 16 confirmed by positive blood smear and the remainder diagnosed clinically. Of 26 children with malaria,

none had confirmed TB (p = 0.23 compared to unlikely TB), 5 had unconfirmed TB (p = 0.01 compared to unlikely TB) and 21 were unlikely to have TB.

MLR Diagnostic Utility

At enrollment, the median MLR for children with confirmed TB [0.428 (IQR 0.378 – 0.675)] was higher than for children with unconfirmed [0.217 (IQR 0.155 – 0.353), p < 0.01] or unlikely [0.213 (IQR 0.140 – 0.391), p < 0.01] TB. Children with unconfirmed TB had similar MLRs compared to children with unlikely TB (p = 0.96) (Table 2).

The optimal MLR cutoff value of 0.378 identified 11/14 confirmed TB patients as having TB disease with sensitivity 79% (95% CI 52 – 92%), specificity 77% (95% CI 70 – 83%), positive predictive value (PPV) 24% (95% CI 14 – 38%), negative predictive value (NPV) 98% (95% CI 93 – 99%), positive likelihood ratio (LR) 3.5 (95% CI 3.1 – 3.9), and negative LR 0.3 (95% CI 0.1 – 0.5). The corresponding area under the ROC curve was 0.78 (95% CI 0.66 – 0.90) (Figure 2).

Longitudinal changes in MLR

The association between TB status and MLR over all study intervals was significant for the confirmed TB group compared to the unlikely TB group [β = 0.31, standard error (SE) 0.12, p = 0.01] on univariate analysis. There was no significant

difference between MLR among children with unconfirmed TB compared to children unlikely to have TB on univariate analysis (Table 3). In our multivariable general estimating equations model adjusting for time-varying $CD4^+$ percentage and visit week since enrollment, the association between confirmed TB diagnosis and MLR remained significant ($\beta = 0.26$, SE 0.11, $p = 0.02$; reference unlikely TB group), and the association between unconfirmed TB and MLR remained non-significant ($\beta = -0.01$, SE 0.02, $p = 0.76$; reference unlikely TB group). No other potential confounders were added to this model due to either lack of association with MLR (malaria status) or collinearity with time-varying CD4 percentage (time-varying WAZ and WHZ).

The two components of the MLR, absolute blood monocyte count and absolute blood lymphocyte count, were analyzed individually in unadjusted analyses for their association with TB status using GEE modeling (Table, Supplemental Digital Content 2, median monocyte and lymphocyte counts by visit week and TB group). Over all study intervals, only the unadjusted association between the absolute lymphocyte count and the confirmed TB group was statistically significant ($\beta = -2.01$, SE 0.97, $p = 0.04$; reference unlikely TB group) (Table 3).

Over 24 weeks of anti-TB treatment, median MLR declined by 0.319 among children with confirmed TB ($p = 0.01$) and was similar to MLR levels of children unlikely to have TB by week 12 of TB treatment ($p = 0.23$) (Figure 1, Table 2). In children with unconfirmed TB, median MLR was significantly higher than the unlikely TB group only at 4 weeks from enrollment ($p < 0.01$), but not at other time points.

In sensitivity analyses restricted to children with complete 6-month follow-up (excluding those who died or who were lost to follow-up), children with confirmed TB had a trend for higher median MLR over all study intervals as compared to children with unconfirmed or unlikely TB, but this did not reach statistical significance ($p = 0.09$ and $p = 0.08$, respectively). The trend of MLR among the three TB groups over the study period was similar to the longitudinal findings with all participants included (Table and Figure, Supplemental Digital Content 3A, median MLR by visit week and TB group among those patients who completed the study). When restricting participants to only those treated for TB, children with TB treatment response had lower enrollment median MLR [0.223 (IQR 0.152, 0.371)] compared with children who did not have treatment response or who died during the study period [(0.483 (IQR 0.282, 0.675); $p = 0.02$] (Table, Supplemental Digital Content 3B.1, comparing median MLR among those who had TB treatment response versus failure). The pattern of MLR longitudinal changes for the three TB groups when restricting to those patients with TB treatment response was also similar to the curves when all participants were included, although the differences between the TB groups did not reach statistical significance (Table and Figure, Supplemental Digital Content 3B.2 and 3B.3, median MLR by visit week and TB group among those patients who had TB treatment response).

DISCUSSION

Among HIV-infected children, blood MLR distinguished children with microbiologically-confirmed intrathoracic TB disease from those with unconfirmed or unlikely TB. An MLR value above 0.378 was associated with moderate sensitivity (79%) and specificity (77%) to identify confirmed TB cases at enrollment. By 12 weeks of anti-tuberculosis treatment the median MLR of the confirmed TB group declined to levels similar to the unconfirmed and unlikely TB groups. Furthermore, children who were treated for TB and died or did not respond to TB treatment had higher median enrollment MLRs compared to those who had treatment success.

The MLR cutoff value above 0.378 demonstrated good overall TB diagnostic performance in our cohort of HIV-infected Kenyan children. Our cutoff was higher and had lower sensitivity and specificity compared to a study of adults in Italy in which an MLR cutoff of 0.285 had sensitivity 91% and specificity 94% for identifying active TB in HIV negative adults compared to healthy controls (13). We hypothesize that the optimal cutoff value from our analysis was higher because we compared confirmed TB patients with pooled unconfirmed and unlikely TB patients who were all HIV-infected and hospitalized rather than healthy controls. When La Manna and colleagues compared participants with TB to those with latent TB infection, the optimal MLR cutoff increased to 0.305 with decreased sensitivity and specificity (13).

The performance of MLR is comparable to other rapid diagnostic methods for microbiologic confirmation that utilize non-sputum based sample collection in children. Studies evaluating stool Xpert MTB/RIF® reported test sensitivities between 32% and

81% and specificities between 99% and 100% as compared to the gold standard of culture-positive respiratory specimens (25-29). Sensitivity of stool Xpert MTB/RIF® improved when data were restricted to HIV-infected children (63% to 80%) (25, 27, 29). Nasopharyngeal aspirate Xpert MTB/RIF® assays had similar diagnostic performance to stool samples in children (sensitivity: 39% to 65% and specificity: 98% to 99%) (30, 31), while urinary lipoarabinomannan (LAM) assays performed less well (sensitivity from 0% to 70% and specificity from 60% to 97% depending on HIV infection status and type of assay) (29, 32-34). A meta-analysis of HIV-negative and positive adults found the sensitivity of serum C-reactive protein (CRP) > 1.0 mg/dL for TB diagnosis ranged between 56% to 96% and specificity between 0% to 67%; elevated CRP levels have been observed in children with active TB but diagnostic performance in children is not known (35, 36). While the sensitivities of these rapid diagnostic tests in children are similar to the sensitivity of MLR in our cohort, the specificity of MLR was lower. Overall, MLR may be an inexpensive and accessible option to inform clinical TB diagnosis when other testing methods are not available.

We hypothesize that elevation in MLR among children with confirmed TB disease could reflect higher mycobacterial burden. Monocytes proliferate in the presence of mycobacterial growth before migrating and differentiating into macrophages, while $CD4^+$ T-lymphocytes are the primary effectors of adaptive immune response to *Mycobacterium tuberculosis* infection (37, 38). Naranbhai et al. have shown *in vitro* that higher MLR was associated with mycobacterial growth and that an elevated ratio of gene expression transcripts of monocytes to lymphocytes was associated with TB disease *in*

vivo (39). In our cohort, higher MLR among children with treatment failure and decline in MLR with anti-TB treatment (consistent with previous studies in adults (13, 14)) support our hypothesis that MLR may be a useful biomarker for mycobacterial burden and increased risk of mortality. Future studies to evaluate MLR change over time in the first few weeks after TB treatment initiation will be useful to assess its role as a marker of early treatment response and mortality risk in this population.

This study contributes to a growing body of research on MLR as a biomarker for TB diagnosis in children, which is vital as it is difficult to obtain respiratory samples for microbiologic diagnosis in this population. All children in our study were comprehensively evaluated for intrathoracic tuberculosis with two samples for smear microscopy and culture, Xpert MTB/RIF® and chest X-ray. Classifying children who had negative microbiologic testing but clinical signs of TB allowed us to analyze the utility of MLR for patients with unconfirmed TB. We also evaluated MLR longitudinally, allowing us to explore MLR changes over the TB treatment period. Moreover, we assessed for potential confounding by nutritional status and immunosuppression that may affect MLR. While our study was limited by a relatively small sample size, our power to detect differences in MLR between the confirmed and unlikely TB groups was robust.

Conclusion

In summary, the blood monocyte-to-lymphocyte ratio distinguished Kenyan hospitalized HIV-infected children with microbiologically-confirmed pulmonary TB

disease from children with unlikely TB. Blood MLR could be a useful diagnostic tool for TB disease in settings where bacteriological confirmation is difficult to obtain. MLR could also be evaluated as a component of future clinical diagnostic algorithms and/or biomarker for TB treatment response.

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LMC, RKC, and GJS designed the study. RKC and LMC analyzed clinical data. RKC and LMC wrote the manuscript. All authors read the manuscript draft, provided feedback and approved the final submitted manuscript.

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REFERENCES:

1. Cox JA, Lukande RL, Lucas S, Nelson AM, Van Marck E, Colebunders R. Autopsy causes of death in HIV-positive individuals in sub-Saharan Africa and correlation with clinical diagnoses. AIDS Rev 2010;12(4):183-94.

2. World Health Organization. Global tuberculosis report 2017. http://www.who.int/tb/publications/global_report/en/0. Published 2017. Accessed November 30, 2017.

3. 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 Suppl 1:S5.

4. Perez-Velez CM, Roya-Pabon CL, Marais BJ. A systematic approach to diagnosing intra-thoracic tuberculosis in children. J Infect 2017;74 Suppl 1:S74-S83.

5. Rozot V, Patrizia A, Vigano S, Mazza-Stalder J, Idrizi E, Day CL, et al. Combined use of Mycobacterium tuberculosis-specific CD4 and CD8 T-cell responses is a powerful diagnostic tool of active tuberculosis. Clin Infect Dis 2015;60(3):432-7.

6. Portevin D, Moukambi F, Clowes P, Bauer A, Chachage M, Ntinginya NE, et al. Assessment of the novel T-cell activation marker-tuberculosis assay for diagnosis of active tuberculosis in children: a prospective proof-of-concept study. Lancet Infect Dis 2014;14(10):931-8.

7. Anderson ST, Kaforou M, Brent AJ, Wright VJ, Banwell CM, Chagaluka G, et al. Diagnosis of childhood tuberculosis and host RNA expression in Africa. N Engl J Med 2014;370(18):1712-1723.

8. Kaforou M, Wright VJ, Oni T, French N, Anderson ST, Bangani N, et al. Detection of tuberculosis in HIV-infected and -uninfected African adults using whole

blood RNA expression signatures: a case-control study. PLoS Med 2013;10(10):e1001538.

9. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 2010;466(7309):973-7.

10. Naranbhai V, Kim S, Fletcher H, Cotton MF, Violari A, Mitchell C, 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.

11. Naranbhai V, Moodley D, Chipato T, Stranix-Chibanda L, Nakabaiito C, Kamateeka M, et al. The association between the ratio of monocytes: lymphocytes and risk of tuberculosis among HIV-infected postpartum women. J Acquir Immune Defic Syndr 2014;67(5):573-5.

12. Naranbhai V, Hill AV, Abdool Karim SS, Naidoo K, Abdool Karim Q, Warimwe GM, 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 2014;209(4):500-9.

13. La Manna MP, Orlando V, Dieli F, Di Carlo P, Cascio A, Cuzzi G, et al. Quantitative and qualitative profiles of circulating monocytes may help identifying tuberculosis infection and disease stages. PLoS One 2017;12(2):e0171358.

14. Wang J, Yin Y, Wang X, Pei H, Kuai S, Gu L, et al. Ratio of monocytes to lymphocytes in peripheral blood in patients diagnosed with active tuberculosis. Braz J Infect Dis 2015;19(2):125-31.

15. Cegielski JP, McMurray DN. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. Int J Tuberc Lung Dis 2004;8(3):286-98.

16. Berens-Riha N, Kroidl I, Schunk M, Alberer M, Beissner M, Pritsch M, et al. Evidence for significant influence of host immunity on changes in differential blood count during malaria. Malar J 2014;13:155.

17. Jelliffe DB, Chandra RK. Immunocompetence in undernutrition. The Journal of Pediatrics;81(6):1194-1200.

18. Njuguna IN, Cranmer LM, Otieno VO, Mugo C, Okinyi HM, Benki-Nugent S, et al. Urgent versus post-stabilisation antiretroviral treatment in hospitalised HIV-infected children in Kenya (PUSH): a randomised controlled trial. Lancet HIV 2017.

19. Kenya Ministry of Health. Guidelines for antiretroviral therapy in Kenya. In. 4th ed: Ministry of Medical Services, Republic of Kenya; 2011.

20. Kenya Ministry of Health. Guidelines for Management of Tuberculosis and Leprosy in Kenya. In: Division of Leprosy Tuberculosis and Lung Disease; 2013.

21. World Health Organization. WHO Anthro (version 3.2.2, January 2011) and macros. http://www.who.int/childgrowth/software/en/. Published 2011. Accessed November 30, 2017.

22. Graham SM, Cuevas LE, Jean-Philippe P, Browning R, Casenghi M, Detjen AK, et al. Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children: An Update. Clin Infect Dis 2015;61Suppl 3:S179-87.

23. Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 3.01. www.OpenEpi.com. Updated April 6, 2013. Accessed December 4, 2017.

24. Perkins NJ, Schisterman EF. The inconsistency of "optimal" cutpoints obtained using two criteria based on the receiver operating characteristic curve. Am J Epidemiol 2006;163(7):670-5.

25. Nicol MP, Spiers K, Workman L, Isaacs W, Munro J, Black F, et al. Xpert MTB/RIF testing of stool samples for the diagnosis of pulmonary tuberculosis in children. Clin Infect Dis 2013;57(3):e18-21.

26. Walters E, van der Zalm MM, Palmer M, Bosch C, Demers AM, Draper H, 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.

27. 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(2):161-166.

28. 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(2):198-203.

29. LaCourse SM, Pavlinac PB, Cranmer LM, Njuguna IN, Mugo C, Gatimu J, et al. Stool Xpert MTB/RIF and urine lipoarabinomannan (LAM) for diagnosing tuberculosis in hospitalized HIV-infected children. Aids 2017.

30. Zar HJ, Workman L, Isaacs W, Dheda K, Zemanay W, Nicol MP. Rapid diagnosis of pulmonary tuberculosis in African children in a primary care setting by use

of Xpert MTB/RIF on respiratory specimens: a prospective study. Lancet Glob Health 2013;1(2):e97-104.

31. Zar HJ, Workman L, Isaacs W, Munro J, Black F, Eley B, et al. Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. Clin Infect Dis 2012;55(8):1088-95.

32. Kroidl I, Clowes P, Reither K, Mtafya B, Rojas-Ponce G, Ntinginya EN, et al. Performance of urine lipoarabinomannan assays for paediatric tuberculosis in Tanzania. Eur Respir J 2015;46(3):761-70.

33. Nicol MP, Allen V, Workman L, Isaacs W, Munro J, Pienaar S, et al. Urine lipoarabinomannan testing for diagnosis of pulmonary tuberculosis in children: a prospective study. Lancet Glob Health 2014;2(5):e278-84.

34. Iskandar A, Nursiloningrum E, Arthamin MZ, Olivianto E, Chandrakusuma MS. The Diagnostic Value of Urine Lipoarabinomannan (LAM) Antigen in Childhood Tuberculosis. J Clin Diagn Res 2017;11(3):EC32-EC35.

35. Yoon C, Chaisson LH, Patel SM, Allen IE, Drain PK, Wilson D, et al. Diagnostic accuracy of C-reactive protein for active pulmonary tuberculosis: a meta-analysis. Int J Tuberc Lung Dis 2017;21(9):1013-1019.

36. Pavan Kumar N, Anuradha R, Andrade BB, Suresh N, Ganesh R, Shankar J, et al. Circulating biomarkers of pulmonary and extrapulmonary tuberculosis in children. Clin Vaccine Immunol 2013;20(5):704-11.

37. Schluger NW, Rom WN. The host immune response to tuberculosis. Am J Respir Crit Care Med 1998;157(3 Pt 1):679-91.

38. Scriba TJ, Coussens AK, Fletcher HA. Human Immunology of Tuberculosis. Microbiol Spectr 2017;5(1).

39. Naranbhai V, Fletcher HA, Tanner R, O'Shea MK, McShane H, Fairfax BP, et al. Distinct Transcriptional and Anti-Mycobacterial Profiles of Peripheral Blood Monocytes Dependent on the Ratio of Monocytes: Lymphocytes. EBioMedicine 2015;2(11):1619-26.

Table 1: Baseline and time-varying characteristics of study participants in TB Confirmed versus TB Unlikely and TB Unconfirmed versus TB Unlikely diagnostic classification groups.

Baseline Characteristics	Total	TB Confirmed	TB Unconfirmed	TB Unlikely	TB Confirmed	TB Unconfirmed
	N = 171	N = 14* (8.2%)	N = 75[§] (43.9%)	N = 82[‡] (48.0%)	p-value[a]	p-value[a]
Median age at enrollment in months (IQR[b])	24.0 (10.8 - 60.9)	51.2 (15.6 - 86.3)	24.0 (14.6 - 57.6)	23.2 (8.9 - 52.7)	0.21	0.42
No. of subjects in age categories (n, %)						
0 to < 12 months	43 (25.1%)	2 (14.3%)	15 (20.0%)	26 (31.7%)		
12 months to < 24 months	44 (25.7%)	4 (28.6%)	23 (30.7%)	17 (20.7%)	0.29	0.31
24 months to < 60 months	41 (24.0%)	2 (14.3%)	19 (25.3%)	20 (24.4%)		
> or equal to 60 months	43 (25.1%)	6 (42.9%)	18 (24.0%)	19 (23.2%)		
Gender (n, %)						
Male	91 (53.2%)	9 (64.3%)	38 (50.7%)	44 (53.7%)	0.57	0.75
Female	80 (46.8%)	5 (35.7%)	37 (49.3%)	38 (46.3%)		

Malaria status (n, %)[c]						
Positive	26 (N = 68, 38.2%)	0 / 3 (0%)	5 / 25 (20.0%)	21 / 40 (52.5%)	0.23	0.01
Negative	42 (N = 68, 61.8%)	3 / 3 (100%)	20 / 25 (80.0%)	19 / 40 (47.5%)		
Laboratory-based malaria diagnosis (n, %)						
Positive	16 (N = 63, 25.4%)	0 / 3 (0%)	2 / 23 (8.7%)	14 / 37 (37.8%)	0.54	0.02
Negative	47 (N = 63, 74.6%)	3 / 3 (100%)	21 / 23 (91.3%)	23 / 37 (62.2%)		
Baseline Median CD4 count (IQR), n	699 (273 - 1262), 170	370 (104 - 799), 14	680 (266 - 1262), 74	757 (369 - 1445), 82	0.04	0.56
Baseline Median CD4 percentage (IQR), n	15.0 (9.0 - 22.2), 170	11.4 (6.0 - 24.0), 14	14.0 (8.0 - 19.8), 74	16.6 (9.5 - 24.0), 82	0.19	0.11
Baseline Median WAZ[d] (IQR), n	-2.68 (-3.9 - -1.6), 165	-2.64 (-4.7 - -1.6), 13	-3.47 (-4.6 - -2.4), 73	-1.99 (-3.1 - -1.0), 79	0.07	<0.01
Baseline Median WHZ[e] (IQR), n	-1.81 (-3.2 - -0.3), 128	-2.46 (-4.0 - -1.9), 8	-2.65 (-3.8 - -1.2), 57	-0.96 (-2.3 - 0.3), 63	0.01	<0.01

Time-varying Characteristics	Total	TB Confirmed	TB Unconfirmed	TB Unlikely	TB Confirmed p-value[a]	TB Unconfirmed p-value[a]
Median CD4 count over study period (IQR), n	988 (473 - 1628), 517	442 (104 - 934), 36	868 (428 - 1550), 229	1114 (606 - 1840), 252	<0.01	<0.01
Median CD4 percentage over study period (IQR), n	18.9 (12.0 - 25.7), 516	15.9 (6.4 - 25.3), 36	17.0 (11.4 - 24.0), 229	20.1 (13.5 - 27.9), 251	0.03	<0.01
Median WAZ[d] over study period (IQR), n	-1.88 (-3.1 - -1.0), 1202	-2.58 (-4.4 - -1.3), 74	-2.44 (-3.7 - -1.4), 560	-1.45 (-2.3 - -0.7), 568	<0.01	<0.01
Median WHZ[e] over study period (IQR), n	-0.99 (-2.3 - 0.2), 887	-2.24 (-3.5 - -0.8), 46	-1.40 (-3.0 - -0.3), 405	-0.45 (-1.5 - 0.5), 436	<0.01	<0.01

[a]p-values from Fisher's exact test reported for categorical variables. Wilcoxon two-sample test t approximation or student t-test p-values reported for continuous variables. Reference group is TB Unlikely.

[b]Interquartile range.

[c]Positive malaria status defined as positive clinical or laboratory smear diagnosis of malaria at enrollment.

[d]Weight-for-age Z scores (WAZ) were based on WHO child growth standards.

[e]Weight-for-height Z scores (WHZ) were based on WHO child growth standards for children 5 years of age and under.

*In the confirmed TB group, 7 died and 1 was lost to follow-up.

§In the unconfirmed TB group, 9 died and 8 were lost to follow-up.

‡In the unlikely TB group, 15 died and 4 were lost to follow-up.

Table 2: Median blood monocyte to lymphocyte ratio (MLR) by visit week and TB classification.

Time from Enrollment (weeks)	Overall *median (IQR[a]), n*	TB Confirmed *median (IQR), n*	TB Unconfirmed *median (IQR), n*	TB Unlikely *median (IQR), n*	TB Confirmed p-value[b]	TB Unconfirmed p-value[b]
0	0.223 (0.151 - 0.397), 169	0.428 (0.378 - 0.675), 14	0.217 (0.155 - 0.353), 74	0.213 (0.140 - 0.391), 81	<0.01	0.96
4	0.120 (0.080 - 0.214), 135	0.356 (0.212 - 0.581), 10	0.151 (0.097 - 0.223), 62	0.095 (0.064 - 0.161), 63	< 0.01	< 0.01
12	0.097 (0.065 - 0.143), 128	0.119 (0.115 - 0.532), 5	0.100 (0.069 - 0.142), 60	0.095 (0.052 - 0.145), 63	0.23	0.46
24	0.081 (0.055 - 0.121), 126	0.109 (0.048 - 0.348), 6	0.083 (0.055 - 0.121), 58	0.080 (0.058 - 0.118), 62	0.53	0.91

[a]Interquartile range.

[b]Wilcoxon two-sample test t approximation reported. Reference group is TB Unlikely.

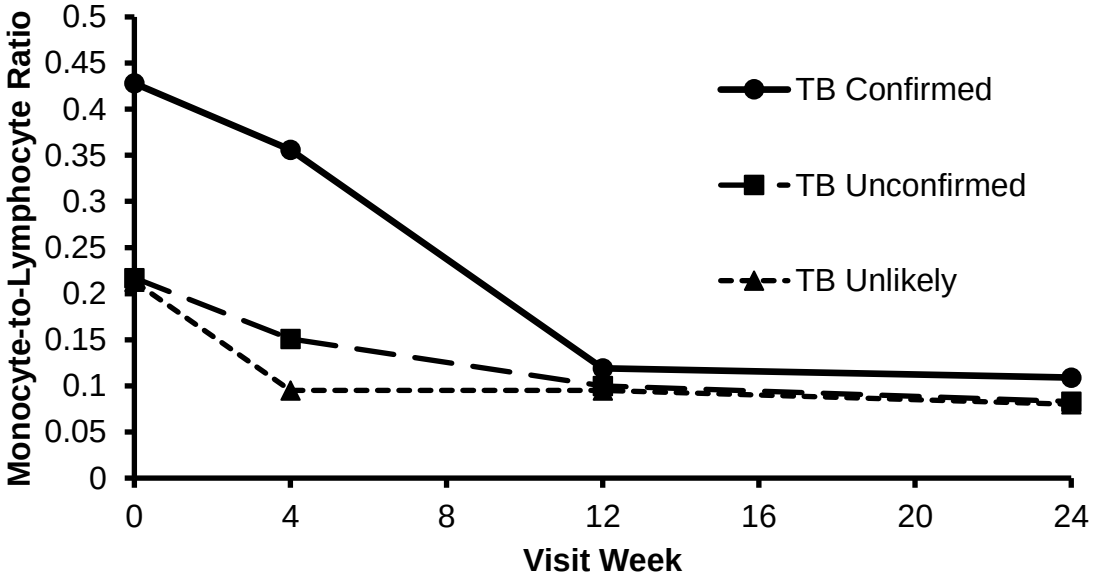

Monocyte-to-Lymphocyte Ratio
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
4
8
12
16
20
24
Visit Week
TB Confirmed
TB Unconfirmed
TB Unlikely

Figure 1. Median blood monocyte-to-lymphocyte ratio over visit weeks from enrollment by TB classification (TB confirmed, unconfirmed or unlikely). The median MLR in the TB confirmed group declined to levels similar to the TB unconfirmed and TB unlikely groups by 12 weeks of anti-TB treatment.

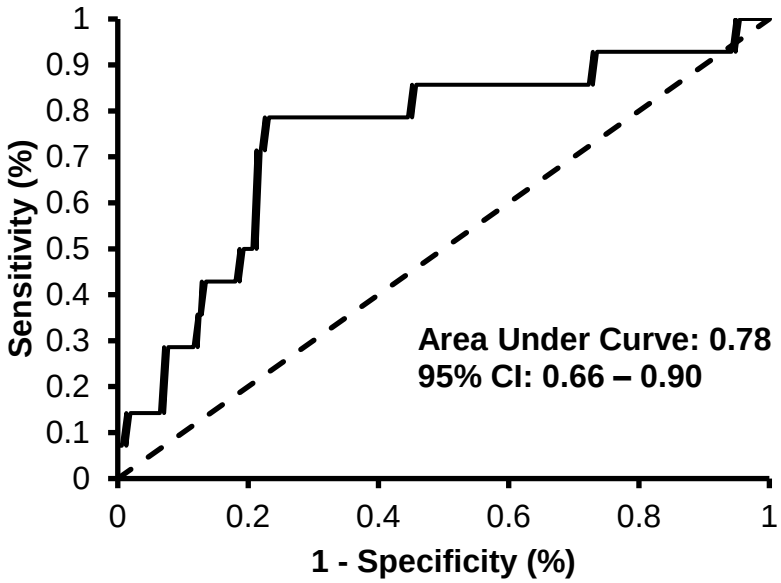
Table 3: Association of TB confirmed and TB unconfirmed groups with absolute monocyte count, absolute lymphocyte count, and MLR over all study intervals.

		Absolute Monocyte Count (10^3 cells/uL)			**Absolute Lymphocyte Count (10^3 cells/uL)**			**Monocyte-Lymphocyte Ratio**		
		β[a]	SE[b]	Model p-value	β	SE	Model p-value	β	SE	Model p-value
TB Confirmed	*Unadjusted model*	0.57	0.36	0.11	-2.01	0.97	0.04	0.31	0.12	0.01
	Adjusted model[c]							0.26	0.11	0.02
TB Unconfirmed	*Unadjusted model*	0.15	0.13	0.26	-0.04	0.57	0.95	0.01	0.03	0.83
	Adjusted model[c]							-0.01	0.02	0.76
TB Unlikely		ref			ref			ref		

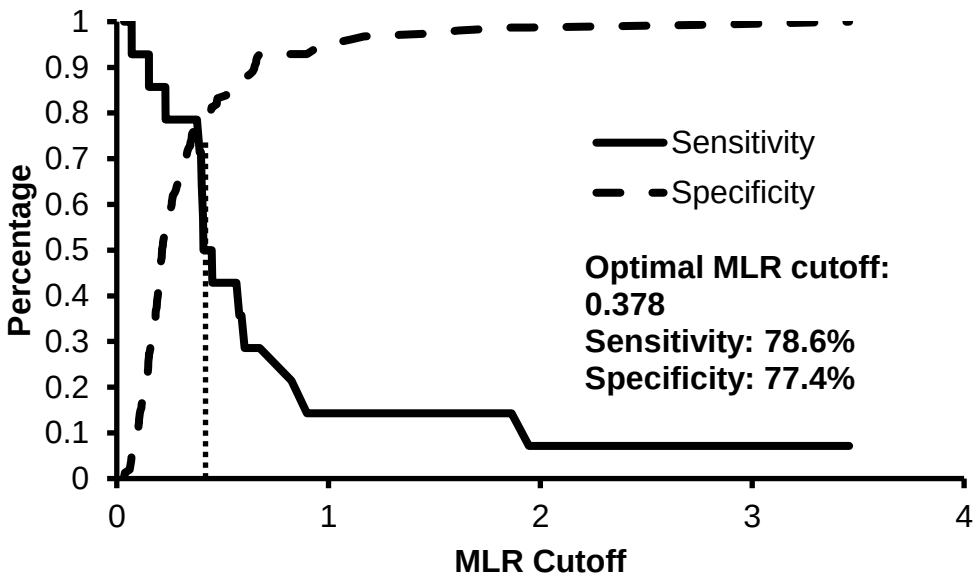
[a]Parameter estimate.

[b]Standard error.

[c]Model adjusted for time-varying CD4 percentage and time from enrollment.


Sensitivity (%)
1 - Specificity (%)
Area Under Curve: 0.78
95% CI: 0.66 – 0.90

A


Percentage
MLR Cutoff
Sensitivity
Specificity
Optimal MLR cutoff: 0.378
Sensitivity: 78.6%
Specificity: 77.4%

B

C

MLR Distribution Percentile	MLR Cutoff Value	Sensitivity (%), 95% CI	Specificity (%), 95% CI	Positive Predictive Value (%), 95% CI	Negative Predictive Value (%), 95% CI	Area Under Curve, 95% CI
5th	0.070	100 (78.5, 100)	5.2 (2.6, 9.9)	8.7 (5.3, 14.1)	100 (67.6, 100)	0.53 (0.51, 0.54)
10th	0.095	92.9 (68.5, 98.7)	10.3 (6.5, 16.1)	8.6 (5.1, 14.1)	94.1 (73.0, 99.0)	0.52 (0.44, 0.59)
25th	0.151	92.9 (68.5, 98.7)	26.5 (20.1, 33.9)	10.2 (6.1, 16.7)	97.6 (87.7, 99.6)	0.60 (0.52, 0.67)
50th	0.223	85.7 (60.1, 96.0)	52.9 (45.1, 60.6)	14.1 (8.3, 23.1)	97.6 (91.7, 99.3)	0.69 (0.59, 0.80)
73.4th	0.378*	78.6 (52.4, 92.4)	77.4 (70.2, 83.3)	23.9 (13.9, 37.9)	97.6 (93.1, 99.2)	0.78 (0.66, 0.90)
95th	1.043	14.3 (4.0, 39.9)	95.5 (91.0, 97.8)	22.2 (6.3, 54.7)	92.5 (87.4, 95.7)	0.55 (0.45, 0.65)
< 5th and > 95th	-	14.3 (4.0, 39.9)	90.3 (84.7, 94.1)	11.8 (3.3, 34.3)	92.1 (86.7, 95.4)	0.52 (0.43, 0.62)

*Optimal MLR cut-off value defined by the maximum value of Youden's index, J, where J = sensitivity + specificity – 1.

Figure 2. Receiver operating characteristic curve (A), sensitivity and specificity curve (B) and corresponding values (C) for MLR cutoffs identifying TB confirmed patients. The optimal MLR cutoff above 0.378 had sensitivity 78.6%, specificity 77.4%, positive predictive value 23.9%, and negative predictive value 97.6%.

PUBLIC HEALTH IMPACT

This study contributes to the growing body of research on rapid and alternative diagnostic methods for tuberculosis disease in children. The study results showed that the blood monocyte-to-lymphocyte ratio distinguished Kenyan hospitalized HIV-infected children with microbiologically-confirmed pulmonary TB disease from children with unlikely TB. This indicates that the blood MLR could be a useful adjunct diagnostic tool for children with TB disease in settings where bacteriological confirmation may be difficult to obtain. In future studies, MLR could also be evaluated as a component of clinical diagnostic algorithms and as a biomarker for early TB treatment response.

Controlling the spread of tuberculosis and preventing mortality from the disease are integral to the success of the United Nation's Sustainable Development Goals (SDG), specifically for Target 3.3 "By 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, waterborne diseases and other communicable diseases" (1). The World Health Organization's vision of a "World Free of TB" is delineated into the following indicators: the percentage reduction in the absolute number of TB deaths, percentage reduction in the TB incidence rate, and the percentage of TB-affected households experiencing catastrophic costs due to TB (1). Facilitating the diagnosis of childhood TB through the use of host serum biomarkers, such as MLR, could contribute to a decline in the absolute number of TB deaths, especially among the most vulnerable of TB patients.

References

1. World Health Organization. Global tuberculosis report 2017. http://www.who.int/tb/publications/global_report/en/0. Published 2017. Accessed November 30, 2017.