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A Plasma Metabolic Signature to Diagnose Pulmonary Tuberculosis and Monitor Treatment Response

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Background. High-resolution metabolomics has shown promise for identifying blood-based biomarkers of tuberculosis (TB). We sought to discover a metabolic signature to detect pulmonary TB disease and monitor treatment response.

Methods. Plasma from Ethiopian persons with pulmonary TB at diagnosis (n = 82) was compared to household contacts with TB symptoms (n = 104) and 2, 6, and 12 months after treatment initiation. Participants were divided into training and test sets for model building, with additional validation using independent cohorts from the countries of Georgia (n = 89) and South Africa (n = 85). Signatures were further evaluated in nonhuman primates infected with *Mycobacterium tuberculosis* (*Mtb*).

Results. Among the metabolites that most significantly differed in concentration, tryptophan (Trp) and retinol were significantly decreased in persons with TB disease (45.2 vs 62.5 μM and 4.1 vs 8.2 μM, respectively), while kynurenine (Kyn) was significantly increased (2.1 vs 1.6 μM; $q < .0001$ for all). A signature that included the Kyn/Trp ratio and retinol showed excellent classification for TB disease (area under the curve [AUC] = 0.97). The signature had an AUC of 0.97 in HIV-positive and 0.95 in HIV-negative persons with TB disease from South Africa and 0.93 in TB patients from Georgia. In Ethiopian participants, signature scores decreased after 2 months (0.85 to 0.42) and 6 months of TB treatment (0.42 to 0.18; $P < .0001$ for both) to similar levels as controls. Plasma retinol also declined in nonhuman primates infected with *Mtb* 15–16 weeks after infection (5.9 vs 3.6 μM; $P < .001$).

Conclusions. The plasma Kyn/Trp ratio and retinol represents a promising metabolic signature that could advance TB diagnostics.

Keywords. tuberculosis; diagnostics; biomarkers; metabolomics; treatment response.

In 2023, 25% of the estimated 10.8 million persons with incident tuberculosis (TB) disease, the leading global cause of infectious disease death, were never diagnosed [1]. Shortfalls in currently available TB diagnostics, which require laboratory testing of sputum specimens, remain a major barrier to prompt TB case identification [2, 3]. Many people with pulmonary TB do not produce sputum and most live in resource-limited countries [1], initially seeking care at clinics with little to no laboratory capacity [4, 5]. Further, currently available tests are not practicable for large-scale TB screening programs focused on identifying people with subclinical TB disease, many of whom have no symptoms at all [6]. Thus, TB diagnoses are often missed or delayed, increasing TB transmission and mortality. Further, clinicians lack reliable measures of treatment response, limiting their ability to individualize treatment regimens and duration. New TB disease biomarkers are a critical first step to develop point-of-care (POC) tests that can be performed on easily obtained clinical samples (eg, blood or urine) without the need of a laboratory [5, 7].

High-resolution plasma metabolomics has shown promise for identifying biomarker signatures of TB disease [8]. Unlike host RNA-based signatures, where the need for sample preservation and polymerase chain reaction (PCR) amplification limit translation to inexpensive, laboratory-free tests [9], detection of many metabolites is compatible with inexpensive POC platforms [10, 11]. Similar to other "-omics" studies, candidate

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metabolomics signatures of TB disease have varied, which may be due, in part, to variability in sample types, collection and extraction procedures, storage, and instrument protocols across studies [12]. However, regardless of the experimental approach used, the ratio of the plasma metabolites kynurenine to tryptophan (Kyn/Trp) has been shown to be a reproducible biomarker for TB disease in persons with human immunodeficiency virus (HIV) [13–16] and those without HIV [17–20]. However, it is not yet clear whether the Kyn/Trp ratio can reach a classification accuracy sufficient for a TB triage test (>90% sensitivity and 70% specificity) [21] or a clinical TB diagnostic (>65% sensitivity at 98% specificity for nonsputum POC tests) [22]. Additionally, while the Kyn/Trp ratio has shown promise as a marker of TB treatment response [17], this requires further validation.

To make progress toward a plasma metabolic signature that achieves the World Health Organization–recommended accuracy standards for either a TB triage test or POC, nonsputum TB diagnostic test, we performed a targeted metabolomics analysis on plasma from a cohort of persons with TB disease enrolled in Ethiopia. Our goal was to (1) evaluate the plasma Kyn/Trp ratio as a TB biomarker in a population of Ethiopian adults with TB symptoms, (2) determine whether combining the plasma Kyn/Trp ratio with other metabolites in a signature would enhance classification accuracy for TB disease, (3) validate the signature in independent cohorts including nonhuman primates (NHPs), and (4) determine whether such a signature can be used to monitor responses to TB treatment in humans.

MATERIALS AND METHODS

Setting and Study Participants

We prospectively enrolled participants with pulmonary TB disease from 7 outpatient TB treatment clinics in the Addis Ababa, Ethiopia, metropolitan area from December 2019 to July 2022. Patients aged ≥15 years who were seronegative for HIV and diagnosed with microbiologically confirmed pulmonary TB disease (positive Xpert MTB and *Mycobacterium tuberculosis* [*Mtb*] culture) were eligible for inclusion (see Supplementary Table 1 for full inclusion/exclusion criteria). Patients with TB disease received 2 months of rifampin/isoniazid/ethambutol/pyrazinamide, followed by 4 months of rifampin/isoniazid (2HRZE/4HR) administered via directly observed therapy. Plasma samples were collected within 7 days of TB treatment initiation (median 3 days) and serially at 2 months, 6 months, and 12 months after treatment start.

Ethiopian control participants were identified from a cohort study of household contacts (HHCs) of persons diagnosed with pulmonary TB. Participants were asked whether in the last month they had experienced cough, fever, night sweats, or weight loss. Only plasma samples from participants reporting at least 1 symptom were analyzed. Participants answering "yes" to any of these questions then underwent a detailed history and physical with a study nurse. Participants in whom TB disease was felt to be a diagnostic consideration underwent a chest X-ray and sputum *Mtb* culture and Xpert testing. All control participants were also tested for TB infection with a QuantiFERON Gold In-Tube Test (QFT). Participants with a positive QFT were followed for 2 years after exposure to monitor for progression to TB disease [23, 24]. Eight participants progressed to TB disease during study follow-up and were analyzed separately. For all cohorts, blood was collected in ethylenediaminetetraacetic acid–containing tubes and centrifuged within 2 hours of collection; isolated plasma was immediately frozen and stored at −80°C. Samples were subsequently shipped on dry ice to Emory University,

Metabolomics Analysis

Thawed plasma (50 µL) was treated with 130 µL acetonitrile (2:1, v/v) containing an internal isotopic standard mixture (3.5 µL/sample), as previously described [25]. Samples were analyzed in triplicate using an Orbitrap Q Exactive Mass Spectrometer (Thermo Scientific, San Jose, California, USA) with dual Hydrophilic Interaction Liquid Chromatography (HILIC) positive and c18 negative liquid chromatography (Higgins Analytical, Targa, Mountain View, California, USA, 2.1 × 10 cm) with a formic acid/acetonitrile gradient. Data were extracted and aligned using apLCMS [26] and xMSanalyzer [27]. Identities of targeted metabolites were confirmed and quantified by accurate mass, tandem mass spectrometry (MS/MS), and retention time relative to authentic standards [28, 29].

Human Validation Cohorts

Metabolic signatures were validated using previously collected high-resolution metabolomics data from persons with TB disease from the countries of Georgia and South Africa [17, 30–32]. All participants from Georgia had pulmonary drug-susceptible TB (DS-TB) confirmed by *Mtb* sputum culture, had a negative HIV antibody test, and were enrolled <7 days after treatment start (n = 89). Participants from South Africa had multidrug-resistant TB (MDR-TB) disease confirmed by *Mtb* culture and phenotypic drug susceptibility testing. Participants included persons with HIV (n = 64) and without HIV (n = 21) as indicated by HIV antibody testing and confirmed with viral load testing. Plasma samples were collected at the time MDR-TB was diagnosed, which generally occurred after 2–3 months of empiric DS-TB treatment while awaiting drug susceptibility testing results, as was standard of care at the time of the study [32]. Controls in the validation cohort included persons with latent TB infection (LTBI; QFT, tuberculin skin test [TST], and T-TSPOT.TB positive) enrolled from the Refugee Health Clinic of the DeKalb County Board of Health (n = 20; Georgia, USA) and uninfected (TST negative) healthcare workers in the United States (n = 37).

Rhesus Macaque Infections

Other data from the animals in this study were previously reported [33, 34]. Indian-origin rhesus macaques were housed

in biocontainment racks and maintained in accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and all applicable regulations, standards, and policies of an Animal Biosafety Level 3 vivarium fully accredited by the American Association for Accreditation of Laboratory Animal Care International. All procedures were performed utilizing appropriate anesthetics as listed in an animal study proposal approved by the Animal Care and Use Committee of the Division of Intramural Research, National Institute of Allergy and Infectious Diseases. In brief, rhesus macaques were infected with either 30–50 colony-forming units (CFU) of *Mtb* H37Rv (n = 12) or 120–150 CFU of *Mtb* H37Rv (n = 5) bronchoscopically instilled in 2 mL of saline. Serial plasma samples were collected before or shortly after *Mtb* infection (week 0–2) and again shortly prior to euthanasia (week 15–16). Six animals received anti-PD1 blocking monoclonal antibody (mAb) as described previously [33].

Human Subjects Approval

Written informed consent was obtained from all study participants, and study approval was obtained from the Institutional Review Board (IRB) of Emory University (Atlanta, Georgia, USA) and by the individual IRBs associated with the original cohort studies at the Armauer Hansen Research Institute (Addis Ababa, Ethiopia), the National Center for Tuberculosis and Lung Diseases of Georgia (Tbilisi, Georgia), the University of KwaZulu-Natal (Durban, South Africa), and the Georgia Department of Public Health (Atlanta, Georgia, USA), respectively, depending on the site of participant enrollment.

Statistical Analysis

Statistical comparisons of metabolite concentrations were performed in R version 4.4.1. For comparisons between persons with pulmonary TB at diagnosis and control participants, metabolite concentrations were $\log_2$ transformed and compared between groups using linear regression, controlling for age and sex [35]. We used a Benjamini-Hochberg false discovery rate (FDR) to account for multiple comparisons. A *q* value (FDR-adjusted *P* value) of <.05 was considered statistically significant. Changes in metabolite concentrations over time were assessed using a Wilcoxon signed-rank test, with a *P* value <.05 considered statistically significant. AUC values of the receiver operating characteristic curves were calculated using logistic regression.

RESULTS

Study Participants

We enrolled 82 adults with pulmonary TB in Ethiopia. Among this cohort, 54 (66%) were male with a median age of 27 years (interquartile range, 23–34 years) (Table 1). Sixty (74%) had ≥1+ acid-fast bacilli (AFB) on sputum smear microscopy. Sixty-eight participants had at least 1 follow-up visit after starting TB treatment. Only 6% remained culture positive after 2 months of treatment and none were known to have treatment failure or relapse. Control participants (n = 104) were HHCs of persons with pulmonary TB, of whom 79% were QFT positive. All HHCs answering yes to the symptom questionnaire underwent a detailed clinical evaluation by a study nurse and 6 (6%) were felt to have a syndrome compatible with pulmonary TB (Figure 1*A* and 1*B*). Of these 6, 3 had an abnormal chest X-ray, while all had a negative sputum Xpert and *Mtb* culture. Eight QFT-positive HHCs progressed to TB disease during the 2-year follow-up including 6 participants with pulmonary TB, 1 participant with pleural TB, and 1 participant with peritoneal TB. The 6 participants progressing to pulmonary TB were confirmed by *Mtb* culture and Xpert MTB while the 2 participants with extrapulmonary TB were diagnosed clinically.

Table 1. Demographic Data of Ethiopian Study Participants

Variable	TB Disease (n = 82)	Controls (n = 104)	TB Progressors (n = 8)
Female sex	28 (34)	58 (56)	4 (50)
Age, y, median (IQR)	27 (23–34)	31 (23–41)	22 (20–26)
Never smoker	72 (88)	94 (90)	8 (100)
No alcohol consumption	56 (69)	50 (48)	5 (62.5)
BMI, kg/m^2, median (IQR)[a]	18.5 (17.3–20.4)	20.3 (18.0–23.1)	19.0 (17.8–21)
History of diabetes	4 (5)	5 (5)	0 (0)
TB symptoms[b]			
Cough	80 (98)	60 (58)	0 (0)
Fever	58 (71)	24 (23)	0 (0)
Weight loss	67 (82)	10 (10)	0 (0)
Night sweats	65 (79)	19 (18)	0 (0)
Positive QFT result	…	82 (79)	8 (100)
AFB sputum smear		…	…
Negative/Scanty	21 (26)		
1–2+	41 (51)		
3–4+	19 (23)		
Positive Xpert MTB at TB diagnosis[c]	100 (100)	…	6 (75)
Positive *Mtb* culture at TB diagnosis[c]	100 (100)	…	6 (75)
Mtb culture positive after 2 mo[d]	3 (6)	…	…
Cavity on chest X-ray	34 (41)	…	…

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

Abbreviations: AFB, acid-fast bacilli; BMI, body mass index; IQR, interquartile range; *Mtb*, *Mycobacterium tuberculosis*; QFT, QuantiFERON Gold In-Tube Test; TB, tuberculosis.

[a]BMI was not measured for 40 control participants.

[b]Symptom assessment in the TB progressor cohort was performed at the visit prior to TB diagnosis.

[c]Microbiologic testing in the TB progressor cohort was performed at the time of TB diagnosis.

[d]Seventeen participants were missing a culture result at 2 months.

Plasma Kyn/Trp Ratio Is a Reproducible Biomarker of TB Disease

We first sought to confirm prior findings that the plasma Kyn/Trp ratio mirrors disease activity in persons with pulmonary TB

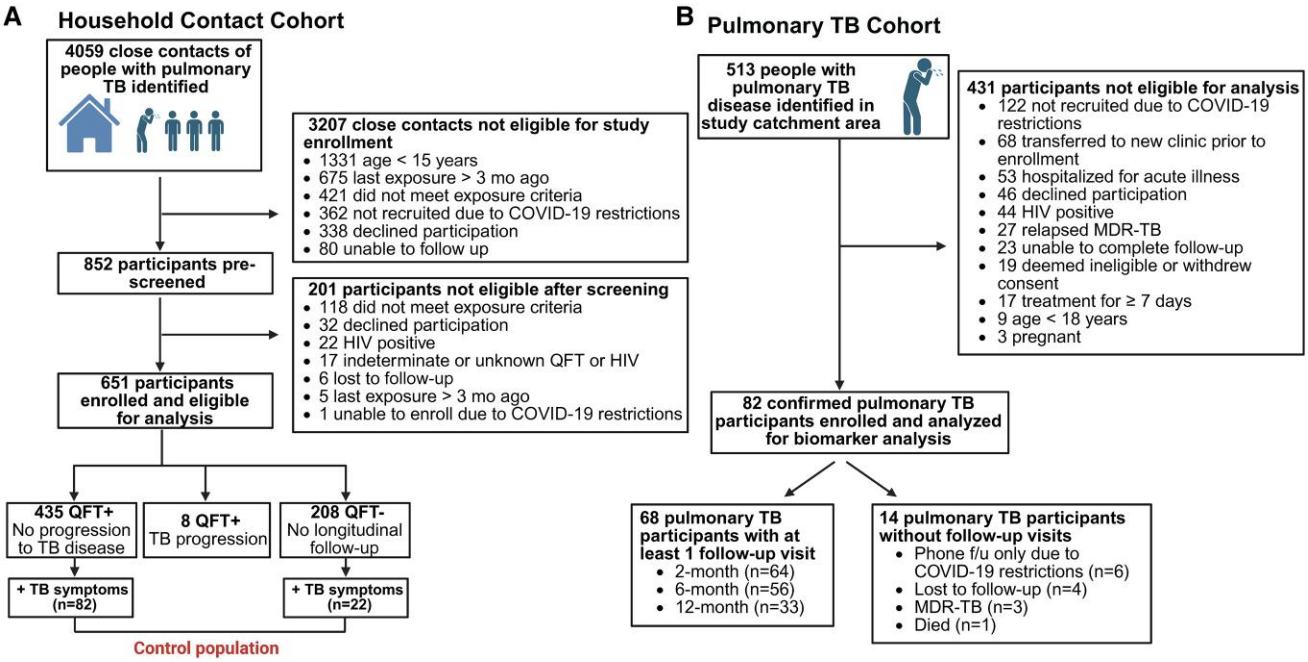

Figure 1. *A*, Flow diagram of Ethiopian study participants enrolled in the household contact cohort. This included participants who progressed to tuberculosis (TB) disease (n = 8) as well as controls who reported TB symptoms but did not progress to TB disease (n = 104). *B*, Flow diagram of study participants enrolled in the pulmonary TB cohort (n = 82). Created in BioRender. Collins, J.M. (2025) https://BioRender.com/7iqrl7g. Abbreviations: COVID-19, coronavirus disease 2019; HIV, human immunodeficiency virus; MDR-TB, multidrug-resistant tuberculosis; QFT, QuantiFERON Gold In-Tube Test; TB, tuberculosis.

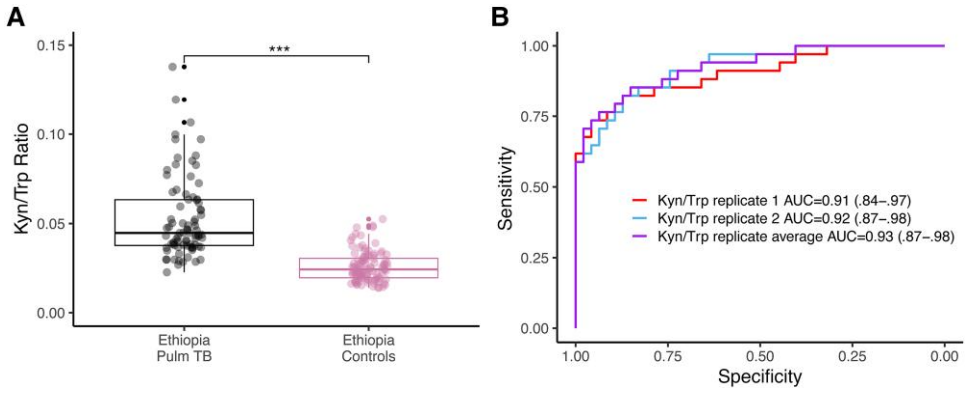

Figure 2. *A*, In Ethiopia, the kynurenine-to-tryptophan (Kyn/Trp) ratio was significantly higher in participants with tuberculosis (TB) disease (n = 82) vs household contacts with TB symptoms (n = 104). Participants with TB disease were compared with controls using a linear regression model controlling for age and sex (***$P < .001$). The box plots depict the minimum and maximum values (whiskers), the upper and lower quartiles, and the median. The length of the box represents the interquartile range. *B*, Receiver operating characteristic curves showing the classification accuracy for TB disease of each Kyn/Trp ratio replicate and their average. The area under the curve (AUC) was calculated using logistic regression, and the 95% confidence intervals for each estimate are displayed in parentheses.

[13–15, 17, 18, 20]. We found that plasma tryptophan concentrations were significantly decreased in persons with TB disease versus controls (Supplementary Figure 1*A*; $P < .001$) while plasma kynurenine was significantly increased (Supplementary Figure 1*B*; $P < .001$), with the plasma Kyn/Trp ratio providing greater separation between groups (Figure 2*A*; $P < .001$). The plasma Kyn/Trp ratio had good classification accuracy for TB disease with an AUC of 0.91 (Figure 2*B*). To determine whether the classification accuracy was affected by technical variability, we repeated the Kyn/Trp ratio measurement in a second plasma sample from the same participants. We found the measure had excellent reproducibility across the 2 technical replicates ($r = 0.87$, $P < .001$; Supplementary Figure 1*C*) and classification accuracy only improved modestly by averaging the 2 replicates (AUC = 0.93). These findings indicate that the plasma Kyn/Trp ratio is an accurate and reproducible marker of TB disease in Ethiopian adults.

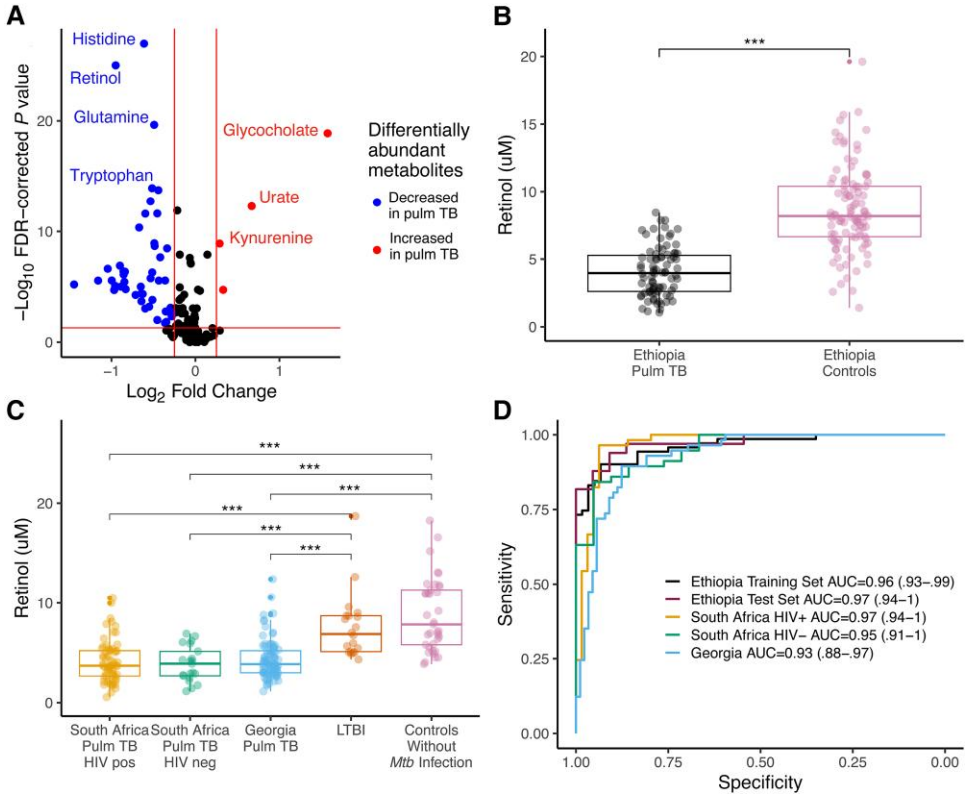

Figure 3. *A*, Volcano plot showing the $\log_2$ fold change and -log *q* value for a targeted panel of 153 plasma metabolites compared between Ethiopian participants with tuberculosis (TB) disease (n = 82) and household contacts with TB symptoms and no evidence of TB disease (n = 104). *B*, Plasma retinol concentrations were significantly decreased in Ethiopian TB disease participants. *C*, Retinol was also significantly decreased in participants with pulmonary TB from South Africa with (n = 64) and without (n = 21) human immunodeficiency virus (HIV) coinfection, as well as from the country of Georgia (n = 89). Participants with TB disease were compared with controls using a linear regression model controlling for age and sex (***$P < .001$). The box plots depict the minimum and maximum values (whiskers), the upper and lower quartiles, and the median. The length of the box represents the interquartile range. *D*, Receiver operating characteristic curves showing the classification accuracy for TB disease of the metabolic signature including the kynurenine-to-tryptophan ratio and retinol in the Ethiopian training set, Ethiopian test set, South Africans living with HIV, South Africans without HIV, and Georgians. The 95% confidence intervals for each area under the curve estimate are displayed in parentheses. Abbreviations: AUC, area under the curve; FDR, false discovery rate; HIV, human immunodeficiency virus; LTBI, latent tuberculosis infection; *Mtb*, *Mycobacterium tuberculosis*; Pulm TB, pulmonary tuberculosis.

Development of a Metabolic Signature for TB Disease

We then examined a targeted set of 153 validated and quantified metabolites in plasma to determine whether adding other metabolites to the plasma Kyn/Trp ratio could improve classification accuracy. Metabolites were selected and quantified from a reference standard panel as described previously [28, 29]. In addition to kynurenine and tryptophan, we found that the most differentially abundant metabolites between cases and controls included histidine, retinol, glutamine, urate, and glycocholate (Figure 3*A*). Retinol (Figure 3*B*), histidine, and glutamine (Supplementary Figures 1*D* and 1*E*) were all significantly decreased in Ethiopian participants with TB while urate (Supplementary Figure 1*F*) was significantly increased ($q < .0001$ for all).

To further assess generalizability of these metabolic changes, we examined the 5 metabolites with the lowest *q* values in a previously collected metabolomics dataset from 2 independent cohorts of persons with TB disease from Georgia and South Africa, which were compared to asymptomatic persons with and without LTBI (Supplementary Table 2). Similar to the Ethiopia cohort, we found that both validation cohorts had significantly decreased plasma retinol concentrations compared to controls (Figure 3*C*). While histidine was also significantly decreased in the Georgia cohort versus controls, it was significantly elevated in South African participants with TB disease (Supplementary Figure 2*A*). Glutamine was significantly decreased in the South African cohort but not the Georgian cohort (Supplementary Figure 2*B*), and urate was only modestly elevated in both validation cohorts (Supplementary Figure 2*C*).

Given that TB disease had a highly significant association with the plasma Kyn/Trp ratio and retinol concentrations in all groups, we tested the classification accuracy of this 3-metabolite signature. To minimize the likelihood of biased estimates, we first randomly divided the control and TB disease participants from Ethiopia into training (70% of participants) and test sets (30% of participants). We found classification accuracy was excellent for pulmonary TB in both training (AUC = 0.96) and test sets (AUC = 0.97)

Table 2. Test Performance by Country

Cohort	Specificity at 90% Sensitivity (95% CI)	Sensitivity at 70% Specificity (95% CI)	Sensitivity at 95% Specificity (95% CI)	Sensitivity at 98% Specificity (95% CI)
Target Product Profile	70%	90%	NA	65%
Ethiopia (training set)	83% (.70–.98)	96% (.90–1)	85% (.69–.96)	75% (.65–.92)
Ethiopia (test set)	91% (.73–1)	97% (.91–1)	88% (.73–1)	82% (.70–1)
Georgia	81% (.64–.93)	96% (.88–1)	54% (.23–.86)	35% (.07–.68)
South Africa, HIV positive	94% (.88–.98)	100% (1–1)	82% (.44–1)	54% (.16–.98)
South Africa, HIV negative	76% (.52–1)	95% (.84–1)	84% (.54–.95)	63% (.51–.93)

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; NA, not applicable.

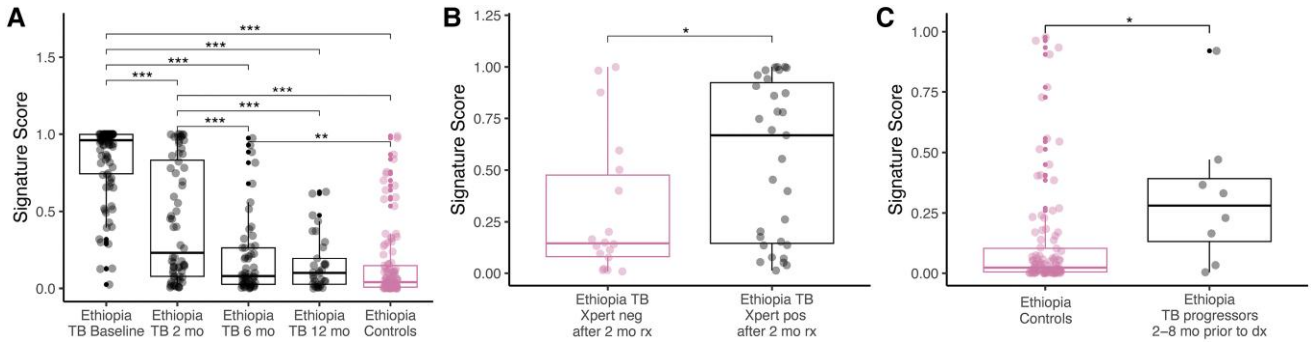

Figure 4. *A*, Trajectory of the 3-metabolite signature scores in Ethiopian adults with drug-susceptible pulmonary tuberculosis (TB) during and after treatment. *B*, Signature score in participants with a negative gene Xpert result after 2 months of treatment vs those with a persistently positive Xpert. *C*, Signature scores in TB progressors 76–225 days prior to TB diagnosis vs controls who did not progress to TB disease. Changes between time points were compared using a Wilcoxon signed-rank test. The cross-sectional comparisons between groups were performed using a linear regression model controlling for age and sex (*$P \leq .05$, **$P < .01$, ***$P < .001$). The box plots depict the minimum and maximum values (whiskers), the upper and lower quartiles, and the median. The length of the box represents the interquartile range. Abbreviations: dx, diagnosis; rx, treatment; TB, tuberculosis.

(Figure 3*D*). Signature scores did not differ between those with and without a cavity on chest X-ray (Supplementary Figure 3*A*). There was a trend toward higher signature scores in persons with a higher-grade AFB sputum smear, but this did not meet statistical significance (Supplementary Figure 3*B*). The 3-metabolite signature performed at near the same level in all the validation cohorts including South Africans with MDR-TB with HIV (AUC = 0.97) and without HIV (AUC = 0.95), as well as HIV-negative Georgians with DS-TB (AUC = 0.93). For the Georgia cohort, signature scores did not differ in participants who received high-dose vitamin D, which was given as adjunctive TB treatment to a subset of participants (Supplementary Figure 3*C*) [31]. The diagnostic accuracy of the signature greatly exceeded the target product profile for a TB triage test in all populations and met thresholds for a POC TB diagnostic test in the Ethiopian cohort (Table 2).

The bile acid glycocholate was also found to be significantly increased in Ethiopian participants with TB disease (Supplementary Figure 4*A*) as well as in participants with TB disease from the countries of Georgia and South Africa (Supplementary Figure 4*B*). However, we found a positive association between plasma glycocholate concentrations and the number of days of TB treatment (Supplementary Figure 4*C*). This suggests that while glycocholate was significantly increased in all TB disease participants, the effect was augmented by antibiotic therapy. Adding glycocholate to create a 4-metabolite signature improved classification accuracy versus the 3-metabolite signature (Supplementary Figure 4*D*). This suggests that the addition of glycocholate may further improve signature performance but will need to be examined in participants prior to antibiotic initiation.

A Metabolic Signature for TB Progression and Treatment Response

We then sought to determine whether the 3-metabolite signature could be used to predict TB progression and monitor responses to anti-TB therapy. Similar to our prior study [17], we again found that the plasma Kyn/Trp ratio declined in a stepwise fashion after 2 months and 6 months of treatment ($P < .001$ for both; Supplementary Figure 5*A*). Plasma retinol concentrations significantly increased 2 months after treatment start and again after 6 months (Supplementary Figure 5*B*). The 3-metabolite signature score showed the same trajectory and was not significantly different from controls at 12 months (Figure 4*A*). While only

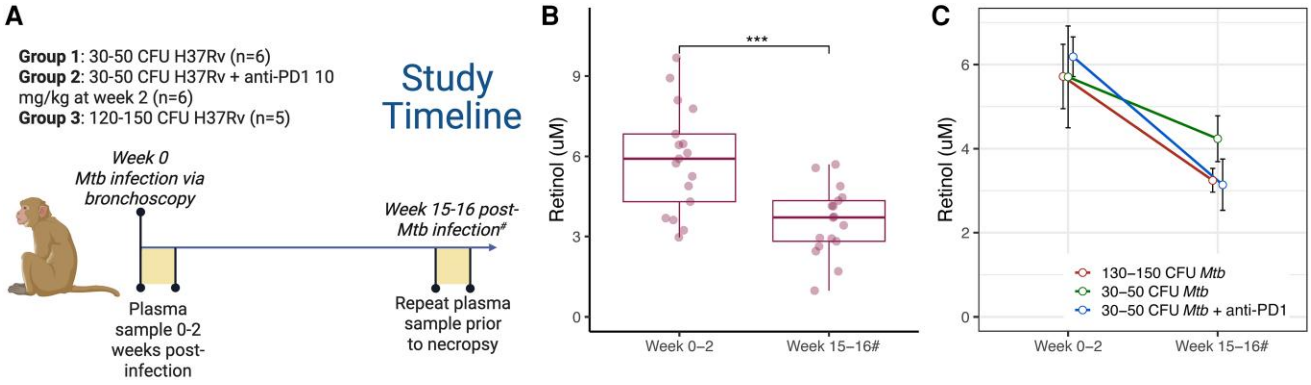

Figure 5. *A*, Design of the rhesus macaque experiments for which plasma samples were analyzed. Created in BioRender. Collins, J.M. (2025) https://BioRender.com/v7p7fy1. *B*, The change in plasma retinol concentrations from 0 to 2 weeks after *Mycobacterium tuberculosis* (*Mtb*) infection vs 15–16 weeks postinfection. *C*, Trajectory of plasma retinol concentrations after *Mtb* infection stratified by infectious dose (colony-forming units [CFU]) and whether the animals received a dose of anti-PD1 antibodies. Changes between time points were compared using a Wilcoxon signed-rank test (***$P < .001$). The box plots depict the minimum and maximum values (whiskers), the upper and lower quartiles, and the median. The length of the box represents the interquartile range. #One animal was sacrificed at 11 weeks due to illness.

3 participants had a positive sputum culture after 2 months of treatment, 2 of these 3 had signature scores ≥0.75. A subset of participants (n = 49) had repeat Xpert testing after 2 months of treatment and those with a positive Xpert had significantly higher signature scores than those who were Xpert negative ($P = .01$; Figure 4*B*). The 3-metabolite signature score significantly decreased after 2 and 4 months of treatment in the cohort from the country of Georgia (Supplementary Figure 5*C*). In a subset of the South Africa cohort with serial plasma samples (n = 17), the signature score also significantly declined after 1 year of treatment (Supplementary Figure 5*D*). Finally, we examined plasma signature scores in Ethiopian TB progressors a median of 156 days prior to diagnosis (range, 76–225 days). We found that signature scores were significantly higher in progressors prior to diagnosis (n = 8) versus controls ($P = .01$; Figure 4*C*).

Mtb Infection in NHPs Alters Retinol Metabolism

Low plasma retinol has been observed at pulmonary TB diagnosis as well as prior to diagnosis in persons progressing to TB disease [36, 37]. This raises the question of whether these metabolic changes are risk factors or early responses to TB disease. We sought to clarify this question by analyzing plasma concentrations of retinol after *Mtb* infection of NHPs in the absence of antibiotic therapy (Figure 5*A*). In 3 cohorts of NHPs (n = 17 total), we found that plasma retinol significantly declined 15–16 weeks after *Mtb* infection ($P < .001$; Figure 5*B*). While the NHP cohorts differed in their infectious dose and some animals also received an anti-PD1 mAb, the trajectory of plasma retinol concentrations was similar for all groups (Figure 5*C*).

DISCUSSION

Discovery of nonsputum TB biomarkers with potential for translation to POC technologies is critical to create new diagnostics that can be performed in primary care settings in resource-limited areas. Herein, we show that a 3-metabolite plasma signature that includes the Kyn/Trp ratio and retinol concentrations had outstanding classification accuracy across clinically and geographically diverse TB cohorts (AUC range, 0.93–0.97). Additionally, we showed that this 3-metabolite plasma signature was elevated in TB progressors prior to diagnosis and normalizes with appropriate TB treatment. This indicates that it has potential for development as a test for TB triage and monitoring treatment response. Finally, we show that plasma retinol concentrations decline in NHPs following *Mtb* infection, providing further evidence that this represents a metabolic response to TB disease.

It is important to note that all 3 metabolites have potential for translation to POC tests. While quantification of these metabolites was performed using mass spectrometry in the present study, there are commercial enzyme-linked immunosorbent assay (ELISA) kits available for detecting all 3 metabolites. This indicates that low-cost, POC, antibody-based detection methods such as lateral flow assays [38] or enzymatically amplified metallization assays [10] could be used for quantification. In the case of the plasma Kyn/Trp ratio, ELISA has had equivalent diagnostic performance to mass spectrometry–based methods [15]. This is an important advantage over other biomarker discovery methods such as transcriptomics, which require PCR amplification [39].

Examination of independent research studies provides further evidence that these signatures are present across human populations. Our group and others have previously found that the plasma Kyn/Trp ratio mirrors disease activity in both latent TB and TB disease [13–15, 17]. Similarly, independent studies have shown that this ratio begins to increase 6–12 months prior to TB diagnosis in geographically diverse populations (Ethiopia, South Africa, Uganda, The Gambia) living both

with [13] and without [18] HIV coinfection. Decreased retinol has also been associated with TB disease in multiple geographically diverse populations [40, 41]. Similar to the plasma Kyn/Trp ratio, low plasma retinol concentrations precede clinical symptoms of TB disease in HHCs [36, 37]. Collectively, this shows the metabolic signature observed in the current study has been observed in independent and geographically diverse populations with TB disease. The fact that these metabolic changes are detectable months prior to TB diagnosis suggests they may also have utility in detecting subclinical TB disease.

Because low retinol has been noted to precede development of TB symptoms, it has been put forth as a potential nutritional risk factor for TB progression [36, 37]. The retinol metabolite retinoic acid has a protective effect in in vitro studies of *Mtb* infection by enhancing autophagy in macrophages [42]. Yet the present study provides compelling evidence that TB disease itself causes significant declines in plasma retinol concentrations. Retinol concentrations increased to near the level of controls with TB treatment and without nutritional supplementation, though increased dietary intake cannot be excluded. Further, plasma retinol declined in NHPs following infection with *Mtb*, strongly suggesting this is part of the host response to the pathogen. While our results do not preclude the possibility that retinol supplementation could improve TB outcomes, they do provide rationale for using it as a TB disease biomarker.

The consistent relationship between TB and bile acids warrants further exploration of both their utility as biomarkers of disease and their role in disease pathophysiology. The sulfonated forms of glycocholate and other conjugated bile acids gradually increase in plasma as TB disease progresses [18] and have been shown to differentiate persons with TB disease from those with LTBI [20]. We found concentrations of glycocholate, a conjugated primary bile acid, to be significantly increased in the plasma of persons with TB disease. Yet we also found that glycocholate was further increased by the receipt of antibiotics, potentially due to their impact on the microbiome [43]. In future studies, it will be important to evaluate the utility of bile acids as TB biomarkers prior to antibiotic initiation to determine whether they can further enhance the accuracy of metabolic signatures.

This study is subject to limitations. While the Ethiopian control group did report TB-like symptoms, in nearly all cases these were identified through a symptom screen. Further validation of the signature in groups presenting for medical care related to respiratory illnesses is warranted. In the validation cohorts, the studies were not designed for the evaluation of TB biomarkers and participants received differing treatment regimens. The MDR-TB group had a high prevalence of HIV coinfection and had already received 2–3 months of inadequate treatment prior to enrollment, which could have reduced the accuracy of the signature. Controls used for the validation cohorts were asymptomatic and were from different countries from those with pulmonary TB. It is therefore difficult to exclude the possibility that the differing study protocols could have impacted signatures scores at enrollment and during treatment. All of the participants in this study were adults with pulmonary TB, so the performance of the signature in persons with extrapulmonary TB and children remains unknown.

In summary, we present evidence of a blood-based, 3-metabolic TB biomarker signature. This signature met the target product profile for a TB triage test in 3 independent cohorts, is supported by independent studies, and is reversed with anti-TB therapy. Collectively, these data provide evidence for a metabolic signature of TB disease that could be used to advance TB diagnostics.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. J. M. C. led the data interpretation and manuscript writing. A. N. led data management. J. M. C. and M. S. Y. L. led the data analysis and modeling. J. M. C., N. N., T. R. Z., M. N., and D. P. J. led the metabolomics analysis. L. W., J. R., C. L. D., K. B., H. M. B., and J. D. E. led and designed the parent clinical studies and led project

implementation. N. R. G. and L. W. oversaw participant enrollment and data collection. D. L. B. oversaw data collection for the primate study. T. R. Z., N. T., N. A. I., N. R. G., J. C. M. B., N. S. S., H. M. B., and N. R. G. led enrollment and data acquisition of the validation cohorts. L. W., R. R. K., H. M. B., and J. D. E. assisted with data interpretation. All coauthors read and edited the manuscript and approved publication.

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