

# Plasma Kynurenine-to-Tryptophan Ratio, a Highly Sensitive Blood-Based Diagnostic Tool for Tuberculosis in Pregnant Women Living With Human Immunodeficiency Virus (HIV)

Clement Adu-Gyamfi,<sup>1,2</sup> Dana Savulescu,<sup>1</sup> Lillian Mikhathani,<sup>1</sup> Kennedy Otwombe,<sup>3,5</sup> Nicole Salazar-Austin,<sup>4,6</sup> Richard Chaisson,<sup>4</sup> Neil Martinson,<sup>3,4</sup> Jaya George,<sup>2</sup> and Melinda Suchard<sup>1,2</sup>

<sup>1</sup>Center for Vaccines and Immunology, National Institute for Communicable Diseases, a division of the National Health Laboratory Service, Johannesburg, South Africa; <sup>2</sup>Department of Chemical Pathology, School of Pathology, Faculty of Health Sciences, University of The Witwatersrand and National Health Laboratory Service, Johannesburg, South Africa; <sup>3</sup>Perinatal Health Research Unit (PHRU), Soweto Matlosana Collaborating Centre for HIV/AIDS and TB, Faculty of Health Sciences, University of The Witwatersrand, Johannesburg, South Africa; <sup>4</sup>Johns Hopkins University Centre for TB Research, Baltimore, Maryland USA; <sup>5</sup>School of Public Health, Faculty of Health Sciences, University of The Witwatersrand, Johannesburg, South Africa; and <sup>6</sup>Johns Hopkins School of Medicine, Baltimore, Maryland USA

**Background.** For pregnant women living with human immunodeficiency virus (HIV), concurrent active tuberculosis (TB) disease increases the risk of maternal mortality and poor pregnancy outcomes. Plasma indoleamine 2,3-dioxygenase (IDO) activity measured by kynurenine-to-tryptophan (K/T) ratio has been proposed as a blood-based TB biomarker. We investigated whether plasma K/T ratio could be used to diagnose active TB among pregnant women with HIV.

**Methods.** Using an enzyme-linked immunosorbent assay (ELISA), we measured K/T ratio in 72 pregnant women with and active TB and compared them to 117 pregnant women with HIV but without TB, matched by age and gestational age.

**Results.** Plasma K/T ratio was significantly elevated during pregnancy compared to sampling done after pregnancy ( $P < .0001$ ). Pregnant women who had received isoniazid preventive therapy (IPT) before enrollment had decreased plasma K/T ratio compared to those who had not received IPT ( $P = .0174$ ). Plasma K/T ratio was elevated in women with active TB at time of diagnosis compared to those without TB ( $P < .0001$ ). Using a cutoff of 0.100, plasma K/T ratio gave a diagnostic sensitivity of 94% (95% confidence interval [CI]: 82–95), specificity of 90% (95% CI: 80–91), positive predictive value (PPV) 85% and negative predictive value (NPV) 98%. A receiver operating characteristic curve (ROC) gave an area under the curve of 0.95 (95% CI: .92–.97,  $P < .0001$ ).

In conclusion, plasma K/T ratio is a sensitive blood-based diagnostic test for active TB disease in pregnant women living with HIV. Plasma K/T ratio should be further evaluated as an initial TB diagnostic test to determine its impact on patient care.

**Keywords.** IDO; kynurenine/tryptophan ratio; sensitivity; specificity; Target product profile.

Despite global efforts to end tuberculosis (TB), TB remains a global health threat, associated with more than a million deaths annually [1]. Active TB in pregnancy is risky for both mothers and infants. TB diagnosis has challenges, particularly using methods which rely on sputum samples. Sputum is difficult to obtain in severely ill patients and is not useful in patients with extrapulmonary TB [2]. As a result, there is a gap between the number with active TB and the number diagnosed.

Undiagnosed cases fuel TB transmission [3] and cause maternal and fetal morbidity and mortality. The World Health Organization (WHO) has highlighted the urgent need for a rapid, quantitative, non-sputum-based marker that does not depend on mycobacterial direct detection and can indicate pulmonary or extra-pulmonary TB, as a key intervention to attain TB control targets [4].

A significant burden of TB occurs in women of reproductive age (15–45 years) [1]. Pregnant women are at an increased risk of developing active TB disease [5–7]. Concurrent TB-human immunodeficiency virus (TB-HIV) infection in pregnancy is a significant cause of mortality in settings of high HIV prevalence [6, 8]. In Johannesburg, Black et al reported that about 70% of deaths in pregnant women with HIV were due to TB infection [9].

Indoleamine 2,3-dioxygenase (IDO) breaks down tryptophan to kynurenines [10]. IDO is the first and rate-limiting enzyme in the de novo biosynthesis of nicotinamide adenine dinucleotide (NAD) [11, 12]. IDO activity suppresses T-cell function, resulting

Received 19 December 2020; editorial decision 9 March 2021; published online 15 March 2021.

Correspondence: C. Adu-Gyamfi, Centre for Vaccines and Immunology, National Institute for Communicable Diseases, 1 Modderfontein Rd, Sandrigtham., Johannesburg, South Africa (clementgascua@yahoo.com).

Clinical Infectious Diseases® 2021;73(6):1027–36

© The Author(s) 2021. Published by Oxford University Press for the Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com  
DOI: 10.1093/cid/ciab232

in generation of regulatory T cells and apoptosis of other T cells [13, 14]. IDO is highly expressed at the placenta and declines to nonpregnancy levels after delivery [15, 16].

Recently, we reported that IDO activity measured by product-to-substrate ratio (kynurenine/tryptophan [K/T]) is a suitable blood-based TB biomarker in HIV-infected and uninfected patients [17, 18]. In this study, we investigated whether pregnancy acts as a potential confounder of IDO activity as a screening tool for active TB disease.

## METHODS

### Study Subjects

This was a nested case-control study. Study samples were from the Tshepiso cohort recruited from 2011 to 2014 [19]. Tshepiso was a prospective cohort study of HIV-infected pregnant women with active TB disease (cases) and without TB (controls) in Soweto, South Africa [19]. Pregnant women were recruited at the time of presentation for antenatal care. Eligible women were 18 years or older and had gestational age  $\geq 13$  weeks with a confirmed HIV diagnosis. Participants (cases and controls) were evaluated for active TB disease at enrolment with the WHO symptom screen [20] and a sputum specimen for mycobacterial liquid culture (BACTEC™ MGIT™ 960 System, Becton Dickinson, California, USA). For each case enrolled, 2 HIV-infected pregnant women without TB with similar maternal age, gestational age, and whose delivery was planned to be at the same institution as the woman with TB, were enrolled as controls. Cases were defined as those with a positive *Mycobacterium tuberculosis* culture result, whereas a probable TB case was a patient who was smear positive for acid-fast bacilli (AFB) with a negative or unknown culture result, and a possible TB case was a patient with clinical symptoms of active TB but negative or unconfirmed sputum smear and culture result. Participants were followed up to 1 year postpartum.

In total, Tshepiso enrolled 83 pregnant women with active TB and 155 women without TB [21]. Tshepiso was a noninterventional study, and women received care in public sector clinics according to South African guidelines. Standard of care from 2011 to 2013 depended on CD4 count. For CD4 counts above 350 cells/mL women were given zidovudine (AZT) monotherapy with single dose nevirapine during labor, intrapartum AZT, and 1 postpartum dose of tenofovir and emtricitabine (option A). Women with CD4 counts below 350 cells/mL received WHO option B (zidovudine, lamivudine, and efavirenz triple therapy). In 2013, guidelines changed to lamivudine or emtricitabine with tenofovir and efavirenz for those with CD4 counts below 350 cells/mL. At enrollment, the study clinician offered isoniazid preventive therapy (IPT) to participants who had not previously received IPT. Plasma samples were stored at  $-70^{\circ}\text{C}$  at the Clinical Laboratory Services in Johannesburg until testing.

After study completion, we accessed plasma samples and clinical data of participants from enrollment to 1 year postpartum. For this secondary analysis, we excluded 6 women because nontuberculous mycobacteria were identified on culture and 5 women diagnosed either prior to or after pregnancy. All active TB cases were diagnosed at the time of recruitment but at varying weeks of gestation. We combined probable and possible TB cases from the Tshepiso study as “probable/possible” TB in this analysis. In total, we analyzed 72 women with active TB that occurred during pregnancy and compared to 117 control specimens available in the repository.

### ELISA for Plasma K/T Ratio

Plasma samples were tested at the National Institute for Communicable Diseases in Johannesburg. EDTA-anticoagulated plasma was used to measure plasma kynurenine and tryptophan by competitive ELISA using a commercial kit (ImmuSmol, Inc. France). Briefly, acylated or derivatized plasma kynurenine or tryptophan competed with solid phase bound kynurenine or tryptophan for antibody binding over a 15-hour incubation period (overnight). Free antigen and free antigen-antibody complexes were removed by washing. The antibody bound to the solid phase was detected by an anti-rabbit immunoglobulin G (IgG)-peroxidase conjugate using 3,3', 5,5'-tetramethylbenzidine as a substrate. The reaction was monitored at 450 nm using a microplate reader. Quantification of unknown samples was achieved by comparing their absorbance with a reference curve prepared with known standards provided with the kit. IDO activity was calculated as the ratio of kynurenine ( $\mu\text{mol/L}$ )/tryptophan ( $\mu\text{mol/L}$ ). Investigators were blind to TB status of samples during analysis.

### Statistical Analysis

We used GraphPad Prism 6 software (GraphPad Software, USA) and SAS Enterprise Guide 7.15 (SAS Institute., USA) for analysis. Normally distributed data were shown as mean  $\pm$  standard deviation (SD) whereas non-normally distributed data were expressed as median with interquartile range [IQR]. Wilcoxon and Man-Whitney tests were used to determine significant differences between 2 nonparametric paired and unpaired groups, respectively. We used the Kruskal-Wallis test to compare multiple nonparametric groups.

Sensitivity, specificity, and positive and negative predictive values were calculated. We derived receiver operating characteristic (ROC) curves to evaluate the most suitable diagnostic cutoff value for K/T ratio to discriminate women with active TB from those without TB. For controls in the ROC curve analysis, we included multiple time points, excluding labor, from each subject; that is, for controls, we included all other time points. For cases, we included all other time points except for enrollment as a control time point during which the women did not have active TB. We used this approach because each person

could visit the clinic multiple times and require a TB test; thus each time point is a valid time point for inclusion as a control. We excluded the labor time point from the analysis. Labor has many physiological alterations that may affect the IDO activity; labor, however, is not a typical TB screening time point for a patient. We repeated the ROC curve analysis using only the enrollment time point for both cases and controls in our supplementary analysis.

We assessed association between K/T ratio and clinical parameters such as body mass index (BMI), CD4 count, and viral load using Spearman correlation. Using baseline data, we modeled factors associated with K/T ratio by running univariate and multivariate linear regression to determine the parameter estimate and standard error (SE). The covariates included study arm (TB cases vs controls), age, CD4 count, BMI, gestational age (in weeks), and viral load (in log<sub>10</sub>).

The Tshepiso cohort study was approved by the John Hopkins University Institutional Review Board. The University of the Witwatersrand Human Research Ethics Committee approved the Tshepiso cohort study (M10336), and this nested substudy (M170476).

## RESULTS

### Baseline Clinical Parameters

Bacteriologically confirmed, probable and possible TB were combined as active TB cases. Of the 72 women with active TB, 35 (49%) were bacteriologically confirmed by culture (Table 1). Furthermore, 66 (92%) had pulmonary TB, whereas 6 patients had extrapulmonary TB (pleural, peritoneal, pericardial, and disseminated TB). One woman was diagnosed with

drug-resistant TB. At baseline, 70 (97%) women with active TB and 110 (94%) of the controls were receiving antiretroviral therapy (ARTs). There was no difference in the proportion of women with active TB and controls on ART; however, controls had been on ART longer than cases (Table 1).

Regarding isoniazid preventive therapy (IPT), 58% of controls had received IPT for an average of 63 days before baseline sampling. No patient with active TB disease had received IPT prior to enrolment into the study. Lower BMI and CD4 cell count were apparent in TB patients at baseline compared with controls. There was a trend toward higher viral loads in TB patients compared to controls (Table 1).

### Plasma K/T Ratio Is Elevated During Pregnancy

First, we investigated whether plasma K/T ratio was increased during pregnancy using only specimens from the control group. The median K/T ratio at enrollment was 0.075 (IQR 0.050–0.12), significantly higher than at 6 weeks after delivery (0.061 [IQR 0.045–0.082], *P* < .0001, Figure 1).

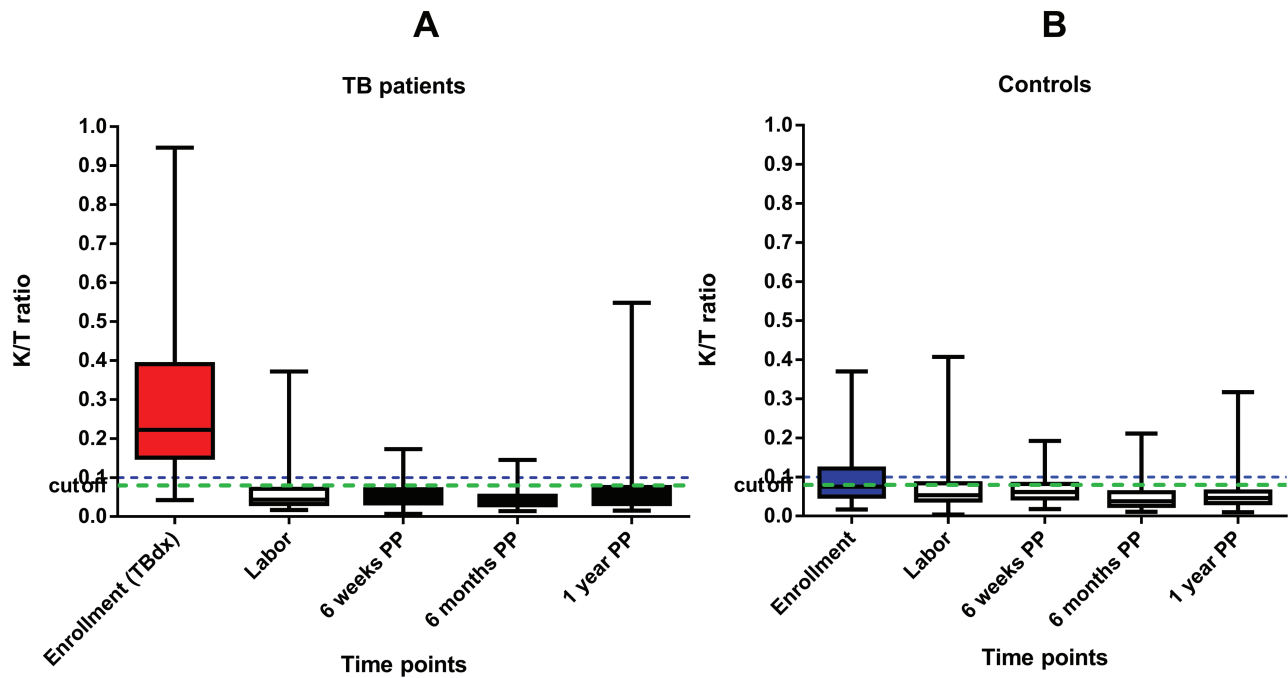
Next, we analyzed intra-individual variability over time using only nonpregnant time points (6 weeks, 6 months, and 1 year postpartum) in controls. The mean plasma K/T ratio was 0.050 with SD of 0.022 and a coefficient of variation (CV) of 31% (Supplementary Figure 1)

We further evaluated whether plasma K/T ratio was elevated in women with active TB. In women with active TB, median K/T ratio was 0.222 (IQR 0.151–0.391) compared to 0.075 (IQR 0.050–0.121) in controls at enrollment, *P* < .0001 (Figure 1).

**Table 1. Subjects' Demographics and Baseline Clinical Data**

Maternal characteristics at baseline			
	TB cases (n = 72)	Controls (n = 117)	<i>P</i> value
Age (years)	36 ± 4	36 ± 4	.9838
Gestational age (weeks)	29 (25–34)	30 (26–35)	.3031
BMI	24 (22–28)	27 (24–30)	<.0001
CD4 cell (count/ml)	243 (128–314)	374 (248–483)	<.0001
HIV viral load (copies/mL)	19 (19–77)	19 (17–29)	.0836
Classification of TB disease			
Confirmed TB	35 (49%)	-	
Possible/probable TB	37 (51%)	-	
Site of TB location			
Pulmonary TB	66 (92%)	-	
Extrapulmonary TB	6 (8%)	-	
ART regimen at enrollment			
Proportion of individuals on/off ART	70/2	110/7	.4865
Median number of days on ART (IQR)	48 (20–106)	90 (41–430)	.0023
Isoniazid preventive treatment			
Number of individuals on/off IPT	0/72	68/49	<.0001
Number of days on IPT (days) <sup>A</sup>	0	63 ± 100	-

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IPT, Isoniazid preventive therapy; IQR, interquartile range; TB, tuberculosis.



**Figure 1.** Plasma K/T ratio. Abbreviations: K/T, kynurenine-to-tryptophan; TB, tuberculosis.

#### Effect of TB Treatment on Plasma K/T Ratio

We determined the effect of TB treatment on plasma K/T ratio using 44 women who had samples available from enrollment, 6 weeks postpartum and 6 months postpartum. At 6 weeks postpartum, TB patients had received anti-TB therapy for a median of 16 (12–19) weeks. We observed that plasma K/T ratio declined significantly in all patients 16 (12–19) weeks after starting TB therapy and remained at low level even 6 months postpartum. The median K/T ratio at enrollment (time of TB)

was 0.300 (IQR 0.164–0.440) compared to 0.042 (0.031–0.057) and 0.033 (0.027–0.050) at 6 weeks postpartum and 6 months postpartum, respectively, with overall  $P < .0001$  (Figure 2).

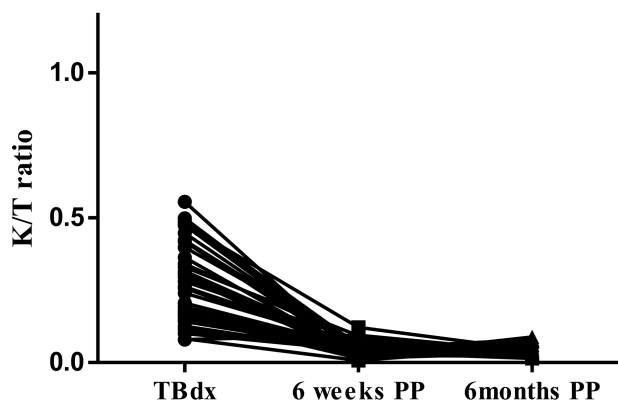
#### Diagnostic Value of Plasma K/T Ratio

We evaluated the diagnostic significance of plasma K/T ratio to detect TB disease. We included all time points from enrollment to 1 year postpartum, except the labor time point. We calculated sensitivity, specificity, positive and negative predictive values (PPV and NPV) to determine the presence of active TB (Table 2). We used a receiver operating characteristics (ROC) curve to determine the optimal cutoff. At a cutoff of 0.100, plasma K/T ratio gave an optimal sensitivity of 94% (95% confidence interval [CI]: 82–95), specificity of 90% (95% CI: 80–91), PPV 85% and NPV 98% to diagnose active TB (Table 2). A ROC gave AUC of 0.95 (95% CI: .92–.97,  $P < .0001$ ) (Figure 3). Even when using a lower cutoff of 0.080, previously optimized in non-pregnant HIV-infected patients, K/T ratio gave a sensitivity of 96% (95% CI: 88–98), a specificity of 86% (95% CI: 73–85), and PPV 77% and NPV of 99% for diagnosis of TB (Table 2). We repeated the analysis using K/T ratio measurements from enrollment only (Supplementary Figure 2).

#### Pulmonary TB Compared to Extrapulmonary TB

We evaluated whether plasma K/T ratio differed by site of TB. We found no significant difference in K/T ratio between patients with pulmonary TB (median 0.230 [IQR 0.145–0.382]) and those with extrapulmonary TB (0.205 [IQR 0.155–0.445],  $P = .956$ , Supplementary Figure 3A).

#### Longitudinal K/T ratio data for the individuals treated for active TB



**Figure 2.** Effect of TB treatment on plasma K/T ration. Abbreviations: K/T, kynurenine-to-tryptophan; TB, tuberculosis.

**Table 2. Diagnostic Significance of K/T Ratio in Active TB Disease**

IDO Activity for Diagnosing Active TB Disease						
Cutoff	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV (%)	NPV (%)
0.010	100	95–100	30	14–24	22	100
0.020	100	95–100	37	21–31	27	100
0.030	100	95–100	39	24–33	30	100
0.040	100	95–100	42	31–39	32	100
0.050	99	92–99	49	43–51	35	100
0.060	98	92–99	62	57–66	45	99
0.070	97	90–99	77	66–74	58	99
0.080	96	88–98	86	73–85	77	99
0.090	95	85–96	90	78–85	79	98
<b>0.100</b>	<b>94</b>	<b>82–95</b>	<b>90</b>	<b>80–91</b>	<b>85</b>	<b>98</b>
0.110	90	70–90	92	85–90	86	97
0.120	87	67–87	93	88–92	87	97
0.130	82	66–86	94	89–94	90	96
0.140	80	64–85	95	90–95	92	96
0.150	79	63–84	96	92–96	94	95

TB positives: All active TB cases at time of enrollment (n = 72). TB negatives: TB cases at time points other than TB diagnosis or labor, and controls at all times excluding labor (n = 510 specimens from women with HIV but no TB).

Abbreviations: CI, confidence interval; IDO, indoleamine 2,3-dioxygenase; NPV, negative predictive value; PPV, positive predictive value; TB, tuberculosis.

Next, we compared plasma K/T ratio in patients with bacteriologically confirmed TB to possible/probable TB patients. There was no significance difference between bacteriologically confirmed TB cases (median 0.222 [IQR 0.160–0.385]) compared to possible/probable TB cases (median 0.185 [0.098–0.383],  $P = .375$ , [Supplementary Figure 3B](#)).

#### Association of K/T Ratio With Body Mass Index, CD4 Count, and HIV Viral Load

We analyzed association of plasma K/T ratio with clinical parameters at the baseline visit. Plasma K/T ratio was inversely correlated with BMI in patients with active TB,

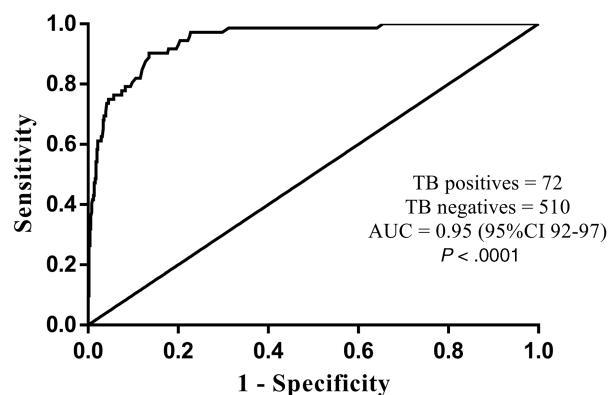
although in controls there was no correlation ([Figure 4](#)). In controls but not in TB patients, plasma K/T ratio showed a weak correlation with viral load. For CD4 count, there was no correlation with plasma K/T ratio in either group ([Figure 4](#)).

In a univariate regression, factors significantly associated with plasma K/T ratio were BMI, CD4, and presence of active TB disease ([Table 3](#)). In the multivariate analysis, women with active TB had a K/T ratio of 0.153 higher than controls ( $P < .0001$ ).

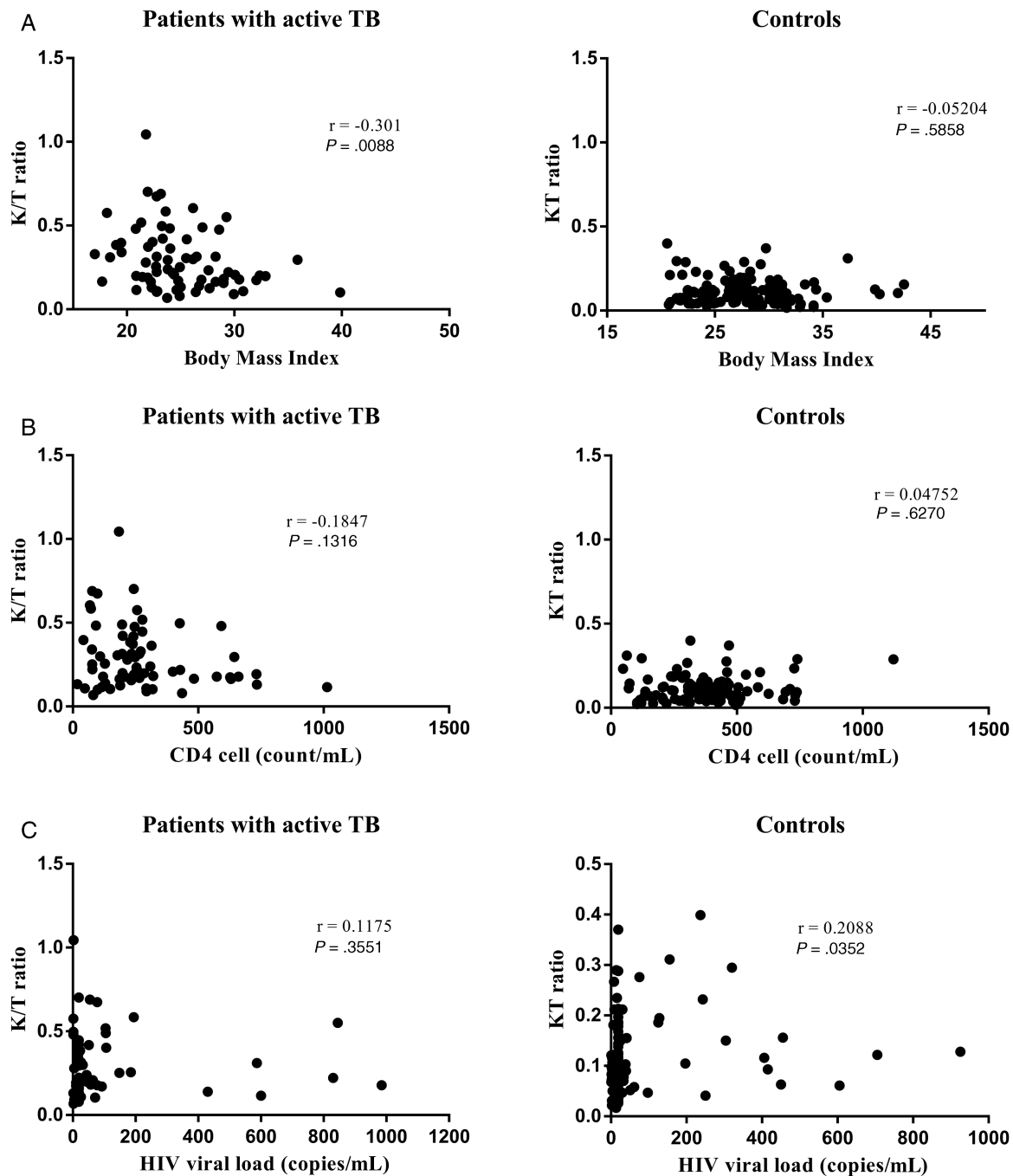
#### The Effect of IPT

We explored the effect of IPT on plasma K/T ratio in the control group. Of the 117 controls, 68 (58%) had used IPT, whereas 49 (52%) had not used IPT before enrollment. The median plasma K/T ratio of women who had received IPT before enrollment was 0.068 (IQR 0.047–0.103), significantly lower than in IPT naive women (median 0.092 [0.050–0.155],  $P = .0174$ , [Figure 5A](#)). Among 68 pregnant women who had taken IPT for an average of 63 ( $\pm 114$ ) days, we found no correlation between number of days on IPT and plasma K/T ratio ( $r = -0.117$ ,  $P = .9451$ , [Figure 5B](#)). Of the 49 women who were IPT naive, 35 were initiated on IPT at enrollment, whereas 14 were not. We compared plasma K/T ratio at enrollment to 8 (IQR 2–11) weeks after IPT use. In a paired analysis, the median plasma K/T ratio declined from 0.091 (IQR 0.042–0.139) to 0.039 (IQR 0.030–0.058) ( $P < .0001$ ). Conversely, in 14 controls who never took IPT, median plasma K/T ratio at enrollment increased from 0.097 (IQR 0.052–0.213) to 0.101 (IQR 0.053–0.263) at the 32-week visit ( $P = .0042$ , [Figure 5C](#)).

**Diagnosing active TB disease using plasma K/T ratio in HIV-infected pregnant women**



**Figure 3.** Diagnostic value of plasma K/T ratio. Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; K/T, kynurenine-to-tryptophan; TB, tuberculosis.



**Figure 4.** Association of K/T ratio with BMI, CD4, and HIV viral load. Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; K/T, kynurenine-to-tryptophan; TB, tuberculosis.

#### The Effect of ART

We investigated whether ART affected K/T ratio. In the control subjects, median plasma K/T ratio of those who were on ART was 0.070 (IQR 0.050–0.120), significantly lower than those not on ART (0.130 [IQR 0.065–0.307],  $P = .0331$ , [Supplementary Figure 4A](#)). We found no significant difference between individuals on AZT-based options compared to late regimen B ( $P = .2252$ , [Supplementary Figure 4B](#)). Furthermore, there was

no significant correlation between length of time on ART and K/T ratio in patients or controls ([Figure 6](#)).

In TB patients at enrollment, we found no significant difference in K/T ratio between patients who had been on ART compared to those who had not been on ART ( $P = .6445$ , data not shown). Additionally, there was no significant difference in K/T ratio among TB patients who were given AZT-based options compared to late option B ( $P = .2252$ ).

**Table 3. Regression Analysis for Factors Associated With KT Ratio**

Variables Arm	Univariate		Multivariate	
	Estimate (SE)	P-value	Estimate (SE)	P-value
Active TB vs control	0.188 (0.019)	<.0001	0.153 (0.023)	<b>&lt;.0001</b>
Age	-0.001 (0.003)	.689	...	...
CD4 Count	-0.018 (0.006)	.003	-0.01(0.005)	.057
BMI	-0.011 (0.002)	<.0001	-0.006 (0.002)	<b>.006</b>
Gestational age (weeks)	0.001 (0.002)	.595	...	...
Log <sub>10</sub> viral load	0.011 (0.010)	.266	...	...

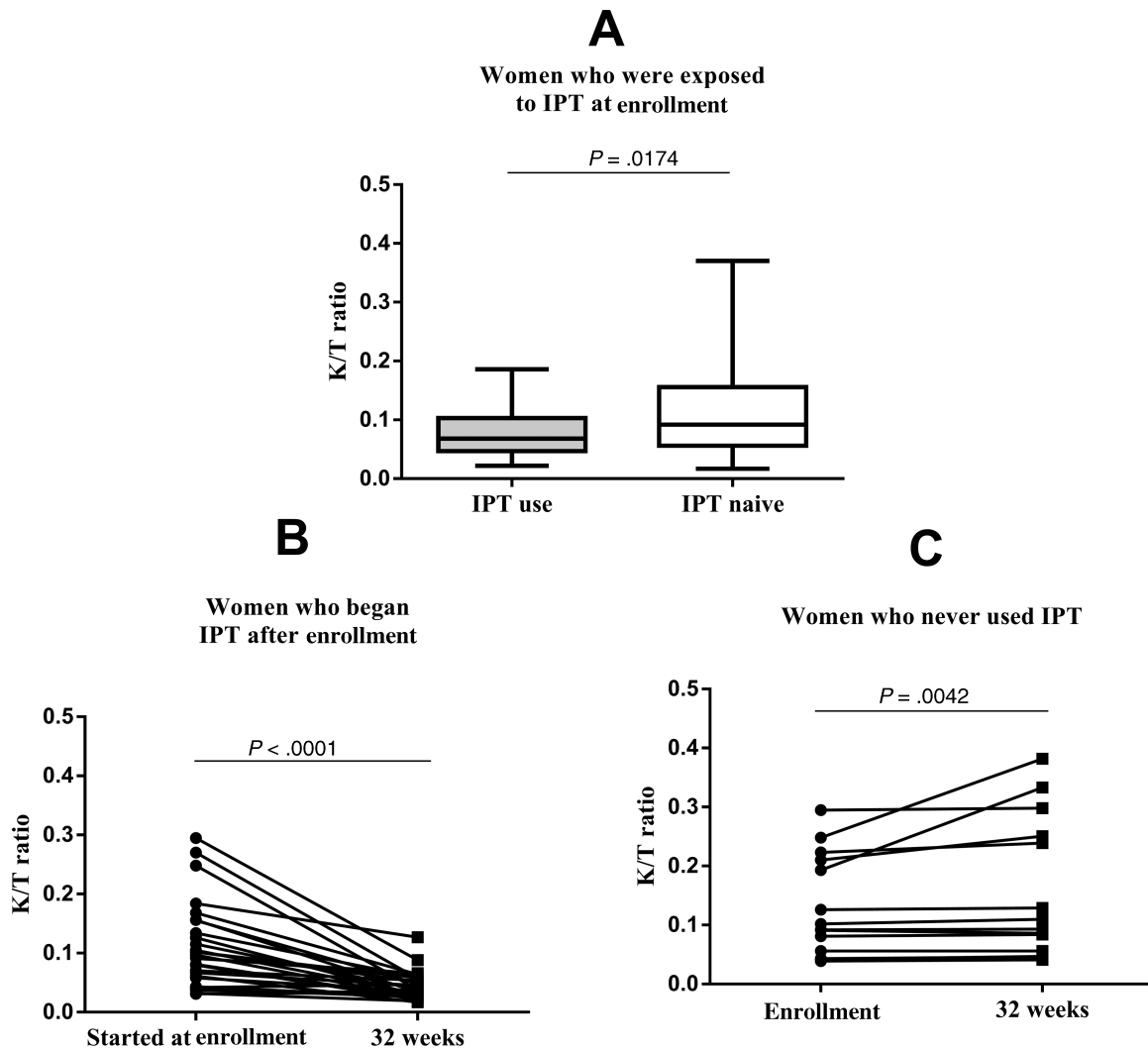
Abbreviations: BMI, body mass index; SE, standard error; TB, tuberculosis.

**DISCUSSION**

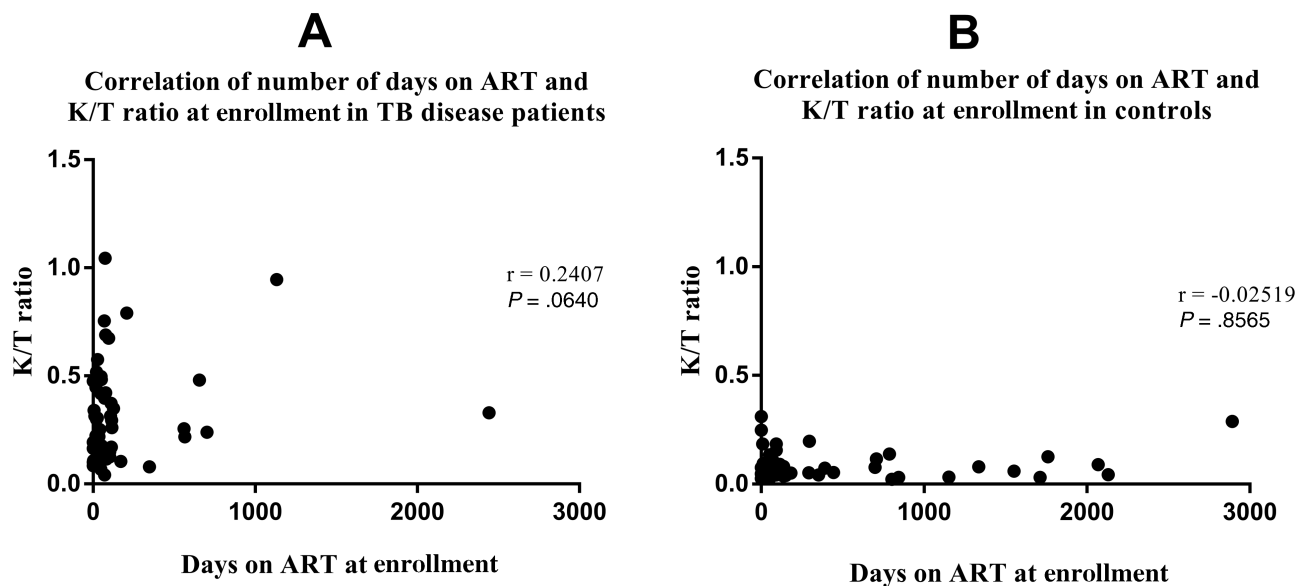
The lack of a reliable TB biomarker is an obstacle to controlling TB. Recently, we and others reported that increased IDO activity detected by plasma K/T ratio was a promising TB biomarker in HIV-infected and uninfected patients [18, 22, 23]. In this study,

we evaluated the potential of plasma K/T ratio to serve as a host-derived TB biomarker in pregnant women with HIV.

Our results demonstrate minimal intra-individual fluctuation of plasma K/T ratio over a one-year period with CV of 31%. Pregnant women had higher plasma K/T ratio than nonpregnant



**Figure 5.** Effect of IPT. Abbreviation: IPT, isoniazid preventive therapy; K/T, kynurenine-to-tryptophan.



**Figure 6.** Effect of ART. Abbreviations: ART, antiretroviral therapy; K/T, kynurenine-to-tryptophan; TB, tuberculosis.

women. An increase IDO activity during pregnancy ties in with similar findings of others [24–27]. IDO expression and activity plays a critical role in maintaining pregnancy to term [24].

Most importantly, pregnant women with TB could be distinguished from pregnant women without active TB using a K/T ratio cutoff of 0.100. We found that pregnant patients with HIV and active TB had higher plasma K/T ratio than those without active TB disease. At a cutoff value of 0.100, K/T ratio showed a sensitivity of 94%, a specificity of 90%, and NPV of 98%. This suggests that plasma K/T ratio could be used as a rule-out first diagnostic test for active TB disease in women with HIV. We suggest this higher cutoff value of 0.100 is more suitable for pregnant patients.

Additionally, in patients with active TB, we noticed that K/T ratio rapidly declined concurrent with starting anti-tuberculous treatment. The finding of K/T ratio decreasing after initiation of TB treatment is consistent with our previous work and that of others [18, 22, 23, 28]. A similar difference was also observed in controls taking IPT compared to those never initiated on IPT. We conclude that administration of isoniazid alone decreases K/T ratio. The finding of K/T ratio decreasing after isoniazid preventive therapy is novel. Isoniazid was originally developed as a nicotinamide analog [29]. Thus, isoniazid administration may bypass the need for endogenous synthesis of nicotinamide through the IDO-catalyzed pathway. Notably, no patients in the TB group had been taking IPT prior to enrolment, which leads us to speculate that one of the ways isoniazid prevents development of active TB is by bypassing the need for endogenous nicotinamide synthesis [30].

Plasma K/T ratio showed a significant inverse correlation with BMI in TB patients but not in controls. The relation

between K/T ratio and BMI in TB patients is consistent with our previous findings and those of others in nonpregnant populations [31, 32]. As weight loss is a cardinal sign of TB, an appropriate TB biomarker should be expected to show inverse correlation with BMI in TB patients. There was a weak association between plasma K/T ratio and viral load in controls; in keeping with literature [33], however, the relation was not observed in TB patients. Controls on ART had lower K/T ratios than those who were ART-naive, but in TB patients there was no difference in K/T ratio between those on ART and those who were ART-naive. There was no association of plasma K/T ratio with CD4 cell count in either group. Thus, high K/T ratio in TB patients is not merely due to correlation of kynurenine or tryptophan with immune activation or HIV progression.

Plasma K/T ratio showed no difference between bacteriologically confirmed and possible/probable TB, nor between pulmonary and extrapulmonary TB. This finding is in keeping with our previous research [17]. Although our numbers with extrapulmonary disease were small, our results suggest that plasma K/T ratio may be useful for diagnosing extrapulmonary TB.

This study has several strengths. We detected K/T ratio from plasma, which is an easily collected clinical sample. Our patients were from a TB endemic area, implying that many of our controls were likely latently infected with TB, although latency was not tested. Most blood-based diagnostics fail to discriminate active disease from previous infection or latent TB and perform poorly in HIV infection. We further used an ELISA method that does not require complicated equipment and is simple to perform.



Study limitations include the lack of inclusion of patients with pneumonia or other pulmonary pathology. Our reported positive predictive values may be affected due to the case-control study design in which prevalence of the disease is predetermined. Our assay should be assessed in nonpregnant women with symptoms suggestive of TB.

These findings, together with our previous work [17, 32], suggest that plasma K/T ratio can be used to diagnose active TB disease in individuals with HIV, including pregnant women. We report here that elevated K/T ratio could detect almost all active TB cases among pregnant women. In those who tested negative for K/T ratio (below a cutoff of 0.100), 99% did not have TB. Our results suggest that K/T ratio has the potential to be used in pregnancy with an adjusted cutoff value of 0.100. The PPV of 85% would be of great clinical value and ideally could be confirmed by a second testing method. A negative result would, however, confidently exclude TB. An advantage of this assay over other existing diagnostics is its applicability to plasma as a sample type, avoiding the need for sputum collection.

In conclusion, our findings suggest that plasma K/T ratio, measured by ELISA with a cutoff of 0.100, may serve as a sensitive TB diagnostic tool in pregnant women with HIV. TB treatment decreased plasma K/T ratio, suggesting that plasma K/T ratio may prove a potential tool for monitoring TB therapy. Additionally, IPT decreased K/T ratio, suggesting that prospective monitoring of K/T ratio in latently infected individuals may be predictive of risk of progression to active TB disease.

### Supplementary Data

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

### Notes

**Acknowledgements.** We thank all the participants in the Tshepiso cohort, the principal investigator and the Perinatal HIV Research Unit, University of the Witwatersrand, Johannesburg.

**Author contributions.** C. A. G. performed the literature search, conducted experiments, analyzed data, produced the figures, wrote and revised the manuscript.

L. M. conducted laboratory experiments and revised the manuscript.

K. O. assisted with statistical analysis and reviewed manuscript

J. A. G. assisted with data interpretation and edited the manuscript.

D. S. performed data mining, assisted with data interpretation, and edited the manuscript.

N. S. A., N. M., and R. C. assisted with trial design and patient recruitment and edited the manuscript.

M. S. S., senior author, designed the hypothesis, supervised data analysis and interpretation, and edited the manuscript.

**Funding support.** This study was supported with Strategic Health Innovation Partnerships (SHIP) Unit of the South African Medical Research Council with funds received from the South African Department of Science and Technology. We were also assisted with funds from the Soweto Matlosana Collaborating Centre for HIV/AIDS and TB (SoMCHAT), the International Society for Infectious Diseases (ISID), and the Discovery Health Academic Fellowship Award. The Tshepiso study was supported by the National Institute of Child Health and Human Development (grant number R01HD064354).

**Potential conflicts of interest.** All authors: No reported conflicts of interest. 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. Global tuberculosis report 2019. Geneva: WHO, 2019:243. Available at: [https://www.who.int/tb/publications/global\\_report/en/](https://www.who.int/tb/publications/global_report/en/).
2. Reid MJ, Shah NS. Approaches to tuberculosis screening and diagnosis in people with HIV in resource-limited settings. *Lancet Infect Dis* 2009; 9:173–84.
3. Stop TB. Partnership: the paradigm shift. 2016–2020. Global Plan to End TB. Geneva: United Nations Office for Project Services, UNOPS, 2015.
4. World Health Organization. High-priority target product profiles for new tuberculosis diagnostics. Report of a consensus meeting. Geneva: WHO, 2014.
5. Bates M, Ahmed Y, Kapata N, Maeurer M, Mwaba P, Zumla A. Perspectives on tuberculosis in pregnancy. *Int J Infect Dis* 2015; 32:124–7.
6. Hoffmann CJ, Variava E, Rakgokong M, et al. High prevalence of pulmonary tuberculosis but low sensitivity of symptom screening among HIV-infected pregnant women in South Africa. *PLoS One* 2013; 8:e62211.
7. Zenner D, Kruijshaar ME, Andrews N, Abubakar I. Risk of tuberculosis in pregnancy: a national, primary care-based cohort and self-controlled case series study. *Am J Respir Crit Care Med* 2012; 185:779–84.
8. Pillay T, Sturm AW, Khan M, et al. Vertical transmission of *Mycobacterium tuberculosis* in KwaZulu Natal: impact of HIV-1 co-infection. *Int J Tuberc and Lung Dis* 2004; 8:59–69.
9. Black V, Brooke S, Chersich MF. Effect of human immunodeficiency virus treatment on maternal mortality at a tertiary center in South Africa: a 5-year audit. *Obstet Gynecol* 2009; 114:292–9.
10. Katz JB, Muller AJ, Prendergast GC. Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral immune escape. *Immunol Rev* 2008; 222:206–21.
11. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 2008; 9:46–56.
12. Jaronen M, Quintana FJ. Immunological relevance of the coevolution of IDO1 and AHR. *Front Immunol* 2014; 5:521.
13. Mellor AL, Lemos H, Huang L. Indoleamine 2,3-dioxygenase and tolerance: where are we now? *Front Immunol* 2017; 8:1360.
14. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol* 2010; 185:3190–8.
15. Zong S, Li C, Luo C, et al. Dysregulated expression of IDO may cause unexplained recurrent spontaneous abortion through suppression of trophoblast cell proliferation and migration. *Sci Rep* 2016; 6:19916.
16. Yamazaki F, Kuroiwa T, Takikawa O, Kido R. Human indolylamine 2,3-dioxygenase: its tissue distribution, and characterization of the placental enzyme. *Biochem J* 1985; 230:635–8.
17. Adu-Gyamfi CG, Snyman T, Hoffmann CJ, et al. Plasma indoleamine 2,3-dioxygenase, a biomarker for tuberculosis in human immunodeficiency virus-infected patients. *Clin Infect Dis* 2017; 65:1356–8.
18. Suzuki Y, Suda T, Asada K, et al. Serum indoleamine 2,3-dioxygenase activity predicts prognosis of pulmonary tuberculosis. *Clin Vaccine Immunol* 2012; 19:05402–11.
19. Salazar-Austin N, Cohn S, Lala S, et al. Isoniazid preventive therapy and pregnancy outcomes in HIV-infected women in the Tshepiso cohort. *Clin Infect Dis* 2019; 71:1419–26.
20. World Health Organization. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. Geneva: WHO, 2011. Available at: [http://whqlibdoc.who.int/publications/2011/9789241500708\\_eng.pdf](http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf).
21. Salazar-Austin N, Hoffmann J, Cohn S, et al.; TSHEPISO Study Team. Poor obstetric and infant outcomes in human immunodeficiency virus-infected pregnant women with tuberculosis in South Africa: the Tshepiso study. *Clin Infect Dis* 2018; 66:921–9.
22. Adu-Gyamfi CG, Snyman T, Hoffmann CJ, et al. Plasma indoleamine 2,3-dioxygenase, a biomarker for tuberculosis in human immunodeficiency virus-infected patients. *Clin Infect Dis* 2017; 65:1356–8.
23. Shi W, Wu J, Tan Q, et al. Plasma indoleamine 2,3-dioxygenase activity as a potential biomarker for early diagnosis of multidrug-resistant tuberculosis in tuberculosis patients. *Infect Drug Resist* 2019; 12:1265–76.
24. Chang RQ, Li DJ, Li MQ. The role of indoleamine-2,3-dioxygenase in normal and pathological pregnancies. *Am J Reproduct Immunol* 2018; 79:e12786.

25. Munn DH, Zhou M, Attwood JT, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* **1998**; 281:1191–3.
26. Schröcksnadel H, Baier-Bitterlich G, Dapunt O, Wachter H, Fuchs D. Decreased plasma tryptophan in pregnancy. *Obstet Gynecol* **1996**; 88:47–50.
27. Kudo Y, Boyd CA, Sargent IL, Redman CW. Decreased tryptophan catabolism by placental indoleamine 2,3-dioxygenase in preeclampsia. *Am J Obstet Gynecol* **2003**; 188:719–26.
28. Almeida AS, Lago PM, Boechat N, et al. Tuberculosis is associated with a down-modulatory lung immune response that impairs Th1-type immunity. *J Immunol* **2009**; 183:718–31.
29. Murray MF. Nicotinamide: an oral antimicrobial agent with activity against both *Mycobacterium tuberculosis* and human immunodeficiency virus. *Clin Infect Dis* **2003**; 36:453–60.
30. Suchard MS, Adu-Gyamfi C, Cumming B, Savulescu DM. Indoleamine 2,3-dioxygenase and the tryptophan-kynurenine-nicotinamide pathway in tuberculosis: an evolutionary perspective. *BioEssay* **2020**; 42:bies201900220.
31. Chen J, Xun J, Yang J, et al. Plasma indoleamine 2, 3-dioxygenase activity is associated with the size of the human immunodeficiency virus reservoir in patients receiving antiretroviral therapy. *Clin Infect Dis* **2018**; 68:1274–81.
32. Adu-Gyamfi CG, Snyman T, Makhathini L, et al. Diagnostic accuracy of plasma kynurenine/tryptophan ratio, measured by enzyme-linked immunosorbent assay, for pulmonary tuberculosis. *Int J Infect Dis* **2020**; 99:441–8.
33. Adu-Gyamfi CG, Savulescu D, George JA, Suchard MS. Indoleamine 2, 3-dioxygenase-mediated tryptophan catabolism: a leading star or supporting act in the tuberculosis and HIV pas-de-deux? *Front Cell Infect Microbiol* **2019**; 9:372.