The Association Between the Ratio of Monocytes: Lymphocytes and Risk of Tuberculosis Among HIV-Infected Postpartum Women

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Abstract: Recent human studies support historical animal studies that suggested an association between peripheral blood monocyte: lymphocyte (ML) ratio and tuberculosis (TB) disease. To evaluate generalizability of this finding, we modeled the association between peripartum ML ratio and incident TB disease within 18 months postpartum among 1202 HIV-infected women in South Africa, Tanzania, Uganda, and Zimbabwe. The ML ratio was associated with increased risk of TB disease independently to combination antiretroviral therapy, World Health Organization stage, or CD4 count (adjusted hazard ratio = 1.22, 95% confidence interval: 1.07 to 1.4, $P = 0.003$ per 0.1 unit increase in ML ratio).

Key Words: tuberculosis, HIV, pregnancy, monocytes, lymphocytes, ML ratio

(*J Acquir Immune Defic Syndr* 2014;67:573–575)

Received for publication March 15, 2014; accepted August 11, 2014.

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The HIV Prevention Trials Network (HPTN) 046 study was funded by the US National Institutes of Health, initially through the HPTN and later through the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) research network. The HPTN (U01AI46749) has been funded by the National Institute of Allergy and Infectious Diseases (NIAID), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institute of Drug Abuse, and National Institute of Mental Health (NIMH). IMPAACT (U01AI068632) has been funded by NIAID, NICHD, and NIMH. The study products were provided for free by Boehringer-Ingelheim.

The authors have no conflicts of interest to disclose.

V.N., H.F., H.M., A.V.S.H., and D.M. designed the secondary analysis. D.M., T.C., L.S.-C., C.N., M.K., P.M., K.M., K.G., L.M.E., P.R., P.A., and M.F. conducted the HIV Prevention Trials Network 046 clinical trial, for which H.M.C. was the protocol chair; all contributed to the study design and manuscript review. The analysis and data interpretation were performed by V.N. and supervised by D.M. and A.V.S.H. The decision for submission was made jointly by the authors.

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Current methods to predict the development of tuberculosis (TB) are poor. Neither tuberculin skin tests nor interferon gamma release assays are good predictors of risk of TB in high-risk populations.[1] Pregnant women with HIV are at particularly high risk of TB disease because of suppression of Th1 responses during pregnancy and HIV-induced immunosuppression.[2] New methods to predict TB may allow targeting of preventive interventions. Moreover, predictive correlates of TB disease may help to reveal pathophysiology. Although gratifying advances in prevention of mother-to-child transmission of HIV have been achieved in sub-Saharan Africa,[3] TB in mothers has a profoundly negative direct effect on infants' risk of TB and indirect effect through maternal illness.[4]

Fletcher[5] observed that infants in the Western Cape, South Africa, who developed TB disease before 2 years of age could be differentiated from those who did not by the ratio of lymphoid and myeloid transcripts in blood collected at 10 weeks of age. Because these transcripts largely originate from monocytes and lymphocytes, respectively, we hypothesized that the ratio of these cells may be similarly predictive. We recently confirmed this hypothesis in infants born to mothers with HIV[6] and separately found that among combination antiretroviral therapy (cART)–naive adults with AIDS, the ratio of monocytes:lymphocytes (ML) was predictive of incident TB disease during the subsequent 5 years on cART. These findings are consistent with animal studies from 80 years ago[8–10] and historical human studies[11] in which the ML ratio was reported to be a marker of TB disease course and prognosis. If these findings could be extended to pregnant

women, they would add to the generalizability of the association and be of potential use in stratifying risk of TB in this high-risk group.

We hypothesized that increased ML ratio of peripheral blood may be associated with incident TB disease in pregnant women. To test this, we performed a secondary analysis of a large multinational clinical trial. The HIV Prevention Trials Network (HPTN) 046 study (clinicaltrials.gov: NCT00074412) was a phase 3 randomized controlled trial conducted in South Africa, Uganda, Zimbabwe, and Tanzania to determine the efficacy and safety of an extended regimen of nevirapine (NVP) to prevent breast-feeding transmission of HIV in infants born to HIV-infected women.[12,13] HIV-infected pregnant women (>18 years) who provided informed consent and were free of serious medical conditions that could interfere with study compliance were screened and enrolled in the third trimester of their pregnancy or before 7 days postpartum. The local ethics committee at each site approved the study. At screening, women provided a medical and obstetric history and underwent a physical examination. Contemporary HIV treatment guidelines informed maternal HIV treatment, and women who were receiving cART or had previous NVP exposure were not excluded. At the time this study was conducted, routine isoniazid preventive therapy was not implemented at the study sites but TB symptom screening was done. At a minimum, to prevent mother-to-child transmission, participants received the HIV Network for Prevention Trials 012 2-dose intrapartum/neonatal regimen of NVP.[14] A physical examination was performed, and $CD4^+$ T-cell enumeration and complete blood count, including white blood cell differential, were conducted in accredited laboratories using standardized automated counters. These clinical procedures were repeated in the women and their infants within 7 days of delivery, at 2 and 6 weeks, and at 3, 6, 12, and 18 months postpartum. For this analysis, participants with active TB or TB diagnosed in the puerperium were excluded to avoid reverse causality. A priori, we specified that a fractional polynomial model would be used to model maternal ML ratios against TB outcomes, adjusting for baseline $CD4^+$ T-cell count, World Health Organization stage, and cART receipt. Analyses were conducted in R using the *mfp*, *epiR*, and *rms* packages. Because of insufficient power, we elected not to evaluate infant ML ratio and TB disease.

Between June 2008 and March 2010, 1678 pregnant women were screened in HPTN 046. For this analysis, 476 women were excluded because of loss to follow-up (n = 146), withdrawal as the infant was not randomized or died (n = 114 and n = 64, respectively), no blood result at baseline (n = 123), or active TB disease during pregnancy or puerperium (n = 29). Therefore, 1202 women were included in this analysis: the baseline blood specimen was obtained at a median of 40 interquartile range (IQR): 20–59] days before delivery. Their median age was 27 (IQR: 23–31) years, and their median $CD4^+$ T-cell count was 435 cells per cubic millimeter (IQR: 299–595). Clinical staging of women performed at the end of the puerperium was as follows: 1006 (83.7%) stage 1, 172 (14.3%) stage 2, 16 (1.3%) stage 3, and 3 (0.3%) stage 4 (4 were missing staging information). Three hundred twenty-eight (27.3%) participants were receiving cART at enrollment and through the course of follow-up. Further clinical characteristics have been reported.[15]

The median ML ratio at enrollment was 0.253 (IQR: 0.192–0.326), and the distribution was similar to that previously reported[7] among nonpregnant HIV-infected women in South Africa of a similar age ($P = 0.76$).

During 1788 years of follow-up, 12 women were diagnosed with TB (11 pulmonary TB and 1 TB adenitis): 4 developed TB by 6 months postpartum, 7 between 6 and 12 months postpartum, and 1 between 12 and 18 months postpartum. In fractional polynomial proportional hazards models, the untransformed ML ratio (ie, exponent = 1) was significantly associated with TB disease (Table 1). After adjusting for cART receipt, $CD4^+$ T-cell count, and World Health Organization stage, the association remained significant (adjusted hazard ratio = 1.22, 95% confidence interval: 1.07 to 1.4, $P = 0.003$). The association between increasing ML ratio and TB disease remained significant when absolute monocyte and absolute lymphocyte counts were included as covariates and when the model was stratified for study site.

We performed a secondary analysis of women who were enrolled in HPTN 046, a large multinational study in postpartum women. The ML ratio in peripheral blood during the last trimester was positively associated with incident TB disease between 6 weeks and 18 months postpartum. A limitation of this study is the relatively low incidence rate suggesting that lack of active case finding may have led to

TABLE 1. Cox Proportional Hazards Models of TB Disease Between 6 Weeks and 18 Months Postpartum in HPTN 046 (n = 1202)*

	Univariate Model		Multivariate Model	
Variable	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
$CD4^+$ T-cell count (per 100 cell increase)	**0.7 (0.5 to 0.98)**	**0.04**	0.61 (0.37 to 1.0)	0.05
World Health Organization clinical stage (per stage increase)	0.94 (0.24 to 3.63)	0.93	0.73 (0.17 to 3.18)	0.68
cART receipt	1.44 (0.41 to 5.1)	0.57	0.75 (0.16 to 3.41)	0.71
ML ratio (per 0.1 unit increase)	**1.26 (1.12 to 1.41)**	**<0.0001**	**1.22 (1.07 to 1.4)**	**0.003**

*A multivariable fractional polynomial model, stratified by site, was used to model the ML ratio as a fractional polynomial so as to allow for nonlinear fits. A linear fit, however, was the most parsimonious, and the variables are therefore presented untransformed.

Bold denotes statistically significant observations.

CI, confidence interval; HR, hazard ratio.

outcome misclassification. The resultant bias toward the null is consistent with the small magnitude of effect. Nevertheless, the result is robust to adjustment for confounding, and the finding is consistent with previous studies in animals[8–10] and humans[7] adding to its generalizability. The ML ratio may be of modest use in stratifying risk of postpartum TB in pregnant women.

ACKNOWLEDGMENTS

The authors thank the mothers and their children who participated in the study, the HPTN 046 study coordinators, counselors, clinicians, pharmacists, data quality and laboratory staff, and those responsible for recruitment and retention for their dedication and hard work on site.

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