White Blood Cell Counts, Lymphocyte Subsets, and Incident Diabetes Mellitus in Women Living With and Without HIV

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Abstract

Diabetes mellitus (DM) is associated with expansion of proinflammatory lymphocyte subsets. We investigated the relationship of total white blood cell (WBC) count and lymphocyte subsets with incident DM in the Women's Interagency HIV Study (WIHS). Higher CD4 and CD8 T cell counts, lymphocyte count, and total WBC count were associated with incident DM among both women with and without HIV, although the association of CD8 was not statistically significant among women without HIV.

Keywords: HIV-1, diabetes mellitus, white blood cell, lymphocyte, CD4, CD8, women

Type 2 diabetes mellitus (DM) is a chronic low-grade inflammatory state with alterations in adipose tissue, liver, pancreas, vasculature, and circulating leukocytes, and has been associated with expansion of proinflammatory T cells, and inflammatory cytokines.[1] Emerging data suggest that higher lymphocyte counts are associated with decreased insulin sensitivity and greater risk of DM.[2] Furthermore, individuals with DM have increased memory CD4 T cell populations, increased production of interferon-γ, and decreased regulatory T cells, which create a proinflammatory state.[1,3] Taken together, these data suggest that individuals with DM may have more lymphocytes characterized by a functional profile skewed toward a proinflammatory state.

People living with HIV (PLWH) may have an increased risk of DM,[4,5] but few data exist on the relationship of total white blood cell (WBC) count and its components, including lymphocyte subsets, with diabetes risk. Lower nadir CD4 has been associated with incident DM in a Canadian HIV cohort,[6] whereas higher geometric mean CD4 count has been positively associated with prevalent DM in PLWH in the Medical Monitoring Project.[7] In the latter study, the lack of T cell data in the population-based controls limited the ability to determine if the relationship between CD4 count and DM is specific to HIV. To clarify these relationships, we assessed associations of WBC and lymphocyte subsets with incident DM in women living with and without HIV.

The Women's Interagency HIV Study (WIHS) prospectively follows a cohort of women with HIV and demographically similar women without HIV initially enrolled at six urban sites in the United States in 1994–1995, 2001–2002, 2011–2012, and at four new sites in the southern United States, 2013–2015.[8] Women are assessed semiannually and

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demographic, clinical, and behavioral data are collected. This analysis included women who had not been diagnosed with DM either before visit 13 (October 2000–March 2001, the first visit at which fasting metabolic laboratories were drawn) or at/before entry for new recruits. Informed consent was obtained from all participants and human experimentation guidelines of the U.S. Department of Health and Human Services, and those of the authors' institutions, were followed.

DM was defined based on laboratory parameters, report of anti-DM medications, and self-report of DM as previously described.[4] Cox proportional hazards models stratified by HIV serostatus analyzed time to incident DM, based on the following time-updated hematologic measures examined separately: CD4 T cell count, CD8 T cell count, lymphocyte count, and total WBC count. Each model controlled for baseline age, WIHS site, race, date of initial study visit, and time-updated body mass index (BMI). Statistical analysis was performed using SAS 9.4 software (SAS Institute, Cary, NC).

A total of 2,606 nondiabetic women living with HIV and 972 nondiabetic women living without HIV were assessed with potential follow-up until visit 47 (October 2017–March 2018). At baseline, women living with HIV had a median age of 39 years (interquartile range [IQR]: 33–45), and 65%, 20%, and 15% self-identified as black, white, and ''other,'' respectively. Women living without HIV had a median age of 36 years (IQR: 28–44) at baseline with 67%, 20%, and 13% identifying as black, white, and ''other,'' respectively. Median BMI was in the overweight range 27 (IQR: 24–33) and 29 (IQR: 24–35) kg/m^2 in women with and without HIV, respectively.

Median duration of follow-up for incident diabetes was 6.6 years (range 0.2–17.5) for women with HIV and 9.3 years (range 0.2–17.5) for women without HIV. There were 338 incident cases of DM during 21,664 person-years of follow-up in women living with HIV (incidence 1.56 per 100 person-years [95% CI: 1.40–1.74]) compared with 137 cases with 8,766 person-years of follow-up in women without HIV (incidence 1.56 per 100 person-years [95% CI: 1.32–1.85]).

Table 1 summarizes the results of Cox proportional hazards models. Notably, higher CD4 T cell count, lymphocyte count and total WBC count were each associated with time to incident DM in women with and without HIV. Although the direction and magnitude of the association between CD8 T cell count and DM was similar in both groups, it was only statistically significant in women with HIV. The CD4:CD8 ratio was not associated with time to diabetes in women with or without HIV.

As described hereunder, we performed additional sensitivity analyses to rule out other potential confounders. In analyses stratified on current versus prior/never smoking, the associations of WBC parameters with incident DM in women with and without HIV were qualitatively similar. Neither baseline receipt of antiretroviral therapy, nor specifically stavudine or protease inhibitor use, was associated with incident DM. In analyses excluding women diagnosed with DM based on self-reported use of anti-DM medications ($n = 57$ and 15 women with and without HIV), the associations of WBC parameters with incident DM remained unchanged.

To our knowledge this is the first study examining hematologic parameters with respect to incident DM comparing associations in women with and without HIV. We found positive associations of total WBC, lymphocyte count, and CD4 T cell count with incident DM in both women with and without HIV.

Inflammatory changes mediated by both innate and adaptive immunity have been related to DM. Investigation of adipose tissue suggests that immune cells, specifically T cells, including regulatory T cells and memory T cells resident in adipose, mediate inflammatory changes and affect metabolic pathways.[9] It is possible that increased circulating lymphocytes, and in particular CD4 T cells, are indicative of higher peripheral tissue concentrations of these T cell populations, which mediate inflammation that promotes insulin resistance and development of DM.

Further investigation into the relationship between inflammatory changes in blood versus peripheral tissues is warranted. In a preliminary study of persons living with and without HIV, subcutaneous adipose tissue was found to be enriched with effector memory CD4 and CD8 T cells compared with blood, and participants living with HIV had higher CD4 central memory T cells and lower CD4 effector memory T cells in adipose compared with participants living without HIV.[10] The underlying CD4 T cell depletion in HIV and subsequent

Table 1. White Blood Cell Count with Differential Cell Count Results and Multivariable Cox Proportional Hazard Models of Time to Incident Diabetes Mellitus White Blood Cell Parameters Stratified by HIV Serostatus

	Women living with HIV (n = *2,592**)				*Women living without HIV* (n = *962**)			
WBC parameter	*Baseline cell count (IQR)*	*Hazard ratio (per 100 cells/μL)*	*95% CI*	p	*Baseline cell count (IQR)*	*Hazard ratio (per 100 cells/μL)*	*95% CI*	p
CD4 cells/μL	459 (289–668)	1.045	1.010–1.081	.012	978 (780–1,216)	1.061	1.010–1.115	.019
CD8 cells/μL	779 (555–1,066)	1.038	1.014–1.063	.002	489 (383–632)	1.022	0.947–1.102	.578
CD4:CD8	0.58 (0.34–0.91)	0.972	0.777–1.215	.801	2.04 (1.55–2.62)	1.1 81	0.980–1.423	.081
Lymphocyte count × 10^3 cells/μL	1.7 (1.3–2.2)	1.029	1.015–1.043	.001	2.0 (1.7–2.5)	1.035	1.011–1.060	.004
WBC × 10^3 cells/μL	4.8 (3.8–6.1)	1.019	1.013–1.024	.001	6.3 (5.1–7.7)	1.011	1.003–1.020	.007

Individual models contained only one WBC covariate. Each model controlled for baseline age, Women's Interagency HIV Study (WIHS) site, race, body mass index, and initial study visit.

*For women living with HIV, there were 2,606 total cohort study participants, of whom 2,592 had blood samples collected for analysis. For women living without HIV, there were 972 total cohort study participants, of whom 962 had blood samples collected for analysis.

WBC, white blood cell.

immune reconstitution after initiation of antiretroviral therapy, combined with a shift of the CD4:CD8 ratio, could provoke such inflammatory and insulin-resistant states and increase the risk of DM. The similar magnitude and directions of associations of WBC, lymphocyte, and CD4 cell counts with DM risk in both women with and without HIV, however, suggest that there may be common immunopathogenetic mechanisms of DM regardless of HIV serostatus.

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Authors' Contributions

M.J.G., D.B.H, D.R.H., A.S., and K.A. conceived of and designed the study; K.A., A.S., P.C.T., M.F., D.G., A.S., R.K., A.F., M.S., A.A.A., C.M., and D.K.-P. contributed to data collection and funding; D.R.H. and Q.S. analyzed the data; C.D.J. drafted the article; all authors contributed to data interpretation, revision of the article, and approved the final version of the article.

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