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Red blood cell distribution width as an easily measurable biomarker of persistent inflammation and T cell dysregulation in antiretrovirally treated HIV-infected adults

Zao Zhang[1,2], **Glen M. Chew**[3], **Cecilia M. Shikuma**[1], **Louie M. Gangcuangco**[3], **Scott A. Souza**[1], **Bruce Shiramizu**[1,3], **Beau K. Nakamoto**[1,4], **Ting Gong**[3], **Santhosh R Mannem**[2], **Brooks I. Mitchell**[3], **Kalpana J. Kallianpur**[1], **Lishomwa C. Ndhlovu**[1,3], and **Dominic C. Chow**[1]

[1]Hawaii Center for AIDS, University of Hawaii John A. Burns School of Medicine, Honolulu, HI, USA

[2]The Queen's Medical Center, Honolulu, HI, USA

[3]Department of Tropical Medicine, Medical Microbiology & Pharmacology, University of Hawaii John A. Burns School of Medicine, Honolulu, HI, USA

[4]Straub Medical Center, Hawaii Pacific Health, Honolulu, HI, USA

Abstract

Background: Chronic inflammation and immune dysfunction occur in human immunodeficiency virus (HIV)-infection despite stable antiretroviral therapy (ART). Red blood cell distribution width (RDW) has been shown to correlate with markers of inflammation in non-HIV conditions. The study objective was to determine associations between RDW with cellular markers of immune activation and immune dysfunction including soluble inflammatory mediators in ART treated HIV infection.

Methods: We performed a cross-sectional analysis of the Hawaii Aging with HIV-Cardiovascular study. RDW was defined as one standard deviation of RBC size divided by mean corpuscular volume multiplied by 100%. Correlations were analyzed between RDW, soluble inflammatory biomarkers and T cell activation (CD38 + HLA-DR+), senescence (CD28-CD57+), and immune exhaustion (PD-1, TIGIT, TIM-3 expression).

Results: Of 158 participants analyzed, median age was 50 years, duration of ART 12.6 years, virally suppressed 84.4%, and CD4 count 503 cells/mm3. Significant positive correlations were identified between RDW and soluble biomarkers including sICAM, IL-8, IL-6, SAA, TNF-α, sE-selection, fibrinogen, D-dimer, CRP, CD4/CD8 ratio, and frequency of multiple CD8 T-cell populations such as CD38 + HLA-DR + T-cells, single TIGIT+, and dual expressing of TIGIT + PD1+, TIGIT + TIM3+, and TIM3 + PD1+ CD8+ T-cell subsets ($p < .05$). Frequencies of CD38 + HLA-DR + CD8+ T-cells and TIGIT + CD8+ T-cells remained significant adjusting for baseline variables ($p < .01$).

Correspondence to: Dominic C. Chow, Hawaii Center for AIDS, University of Hawaii John A. Burns School of Medicine, 651 llalo Street, BSB 231, Honolulu, HI 96813, USA. dominicc@hawaii.edu.

Disclosure statement

No authors have any conflict of interest to report.

Conclusion: Our study revealed correlations between RDW with systemic inflammatory biomarkers and CD8+ T-cell populations related to immune activation and exhaustion in HIV-infected individuals on ART. Further studies are warranted to determine the utility of RDW as a marker of immune dysregulation in HIV.

Keywords

human immunodeficiency virus; red blood cell distribution width; immune checkpoint; inflammatory biomarker; T cell dysregulation; immune activation and exhaustion

Introduction

Although effective antiretroviral therapy (ART) can achieve durable viral suppression in most persons with human immunodeficiency virus (HIV) infection, it does not completely restore immunologic function. Higher risk of cardiovascular (CVD), renal and liver diseases, and non-acquired immune deficiency syndrome (AIDS)-related cancers are observed in virally suppressed patients.[1] Chronic HIV infection is associated with persistent T cell immune activation, which drives disease progression. Furthermore, senescent HIV-specific CD8 T cells expressing high levels of negative immune checkpoint receptors such as PD-1, CTLA-4, and TIGIT, exhibit impaired proliferative capacity, cytokine production, and killing activity.[2]

Red cell distribution width (RDW) measures size variability of red blood cells (RBC), and is an easily measurable parameter in complete blood count (CBC). Besides anemia, elevated RDW is seen in conditions with persistent inflammation including inflammatory bowel disease, coronary artery disease, congestive heart failure (CHF), stroke, and peripheral artery disease in the general population.[3,4] We hypothesize that an association exists between subclinical inflammation, immune activation and exhaustion with anisocytosis, and aim to identify whether RDW correlates with biomarkers of inflammation and T cell immune activation in HIV-infected adults on stable ART.

Methods

Study design and participants

We conducted a cross-sectional analysis of participants from the Hawaii Aging with HIV-Cardiovascular (HAHC-CVD) study, which is a 5-year longitudinal cohort study that enrolled patients greater than 40 years old with documented HIV infection on ART for more than 3 months.[5] The study was approved by Committee on Human Subjects of University of Hawaii with informed consent.

CBC and RDW measurement

RDW was defined as one standard deviation of RBC size divided by mean corpuscular volume (MCV) then multiplied by 100%. RDW and other cell counts were obtained from the CBC. Samples were measured on a Sysmex SE-2100 analyzer at Diagnostic Laboratory Service Inc. (Honolulu, Hawaii), a CLIA-certified laboratory.

Inflammatory biomarkers

Plasma was assayed for biomarkers of systemic inflammation including C-reactive protein (CRP), interleukin (IL)-6, IL-8, IL-10,) tumor necrosis factor (TNF)-α, monocyte chemoattractant protein-1 (MCP-1), serum amyloid A (SAA), myeloperoxidase (MPO), coagulation factors (D-dimer and fibrinogen), endothelial function makers (soluble vascular cell adhesion molecule-1 [sVCAM-1], soluble intercellular adhesion molecule-1 [sICAM-1], and soluble E-selectin [sE-selectin]. Antibody-coated beads were used in a high sensitivity Milliplex Human CVD biomarker panel (Millipore, Billerica, Massachusetts) as previously described.[6] The minimum detectable concentration of CRP of this assay is 0.001 ng ml^{-1}. Samples were acquired on a Labscan 200 analyzer (Luminex, Austin, Texas) using Bio-Plex manager software (Bio-Rad, Hercules, California). The average coefficient of variation of all biomarker measurements was less than 10%.

T cell assessment

Cryopreserved PBMC were rapidly thawed in warm 10% supplemented RPMI (RPMI 1640 medium; Hyclone, Logan, Utah) for viability with an aqua amine reactive dye (AARD; Invitrogen, Carlsbad, California) and incubated with a panel of conjugated anti-human monoclonal antibodies (mAbs) against TIGIT, PD-1, TIM-3, CD38, HLA-DR, CD28, CD57, CD4, CD8, and CD3 as previously described.[2] Between 100,000 and 500,000 lymphocyte events were collected for each sample. Isotype controls or fluorescence minus one (FMO) samples were prepared to facilitate gating. Anti-mouse or anti-rat IgG-coated beads (BD Biosciences) were individually stained with each fluorochrome-conjugated mAbs and used for software-based compensation. DataVwere analyzed using Flowjo Software version 9.5 (Treestar, Ashland, Oregon).

Statistical analysis

Baseline demographics, clinical and immunological characteristics were described using the median, first quartile (Q1), and third quartile (Q3) for continuous variables, and frequency in percentage for categorical variables. Shapiro-Wilk test, Q-Q plot, and box plot were used to test the data normality. Non-normally distributed variables were log base-10 transformed for normalization. Pearson correlation and multivariable regression analysis were performed to assess cell counts, inflammatory and immune biomarkers for their J association with RDW. The statistical analyses were conducted using SPSS Statistics version 23 (IBM, Armonk, New York). Tests were all two-sided, and a p-value <.05 was considered statistically significant.

Results

Study population

Of all participants enrolled in the HAHC-CVD study, 158 participants had RDW results available on record. Inflammatory biomarkers were available in 138 participants, and immune checkpoint molecules in 43 participants. The cohort's median age was 50 years, 88% were males, 58% were Caucasian and 84% were virally suppressed (defined by an HIV RNA viral load ≤48 copies mL^{-1}). The median duration of ART was 12.6 years, CD4 count

was 503 cells mm^{-3} and RDW was 13.4%. The median body mass index was 25.7kg m^{-2}, systolic blood pressure was 121mmHg, serum creatinine was 1.0 mg dL^{-1}, and hemoglobin was 14.5 g dL^{-1}. Among all participants, 16% had diabetes mellitus and 25% were current cigarette smokers. Other demographic and clinical characteristics are shown in Table 1.

Association between inflammatory biomarkers and immune markers with RDW

Pearson correlation analysis (Table 2) revealed significant associations between RDW and inflammatory biomarkers including log CRP, TNF-α, IL-6, IL-8, MCPA 1, log SAA, log MPO, D-dimer, fibrinogen, sE-selectin, sVCAM, and sICAM. RDW was significantly associated with log CRP, IL-6, IL-8, TNF-α, log SAA, D-dimer, fibrinogen, sVCAM, and sICAM after adjustment for age, sex, race (Caucasian vs. non-Caucasian), status of current cigarette smoking, serum creatinine, and hemoglobin (Table 2). Platelet count was not found to have a correlation with RDW.

RDW and T cell markers

In Pearson analysis (Table 2), RDW was significantly correlated with CD4 count, CD8 count, and CD4/CD8 ratio. RDW also had significant positive correlations with the frequencies of single TIGIT + and PD1 + CD8 T cells, dual TIGIT + PD1+, TIGIT + TIM3+, and TIM3 + PD1+ CD8 T cells and with the frequencies of HLA-DR + CD38 + (activated) CD8+ T cells. We found that single PD1+, TIGIT + and dual TIGIT + PD1+ CD4 T cells were correlated with RDW. However, in multivariable regression analyses (Table 2), only CD8+ T cells count, CD4/CD8 ratio, and the frequencies of single TIGIT+, dual TIGIT + PD1+, TIGIT + TIM3+, TIM3 + PD1+, and CD38 + HLA-DR + CD8 T cell subsets remained significantly associated with RDW after adjustment. These findings were unchanged even when the analyses were restricted to participants who were virologically suppressed.

Discussion

The introduction of ART has dramatically reduced AIDS-related morbidity and mortally by restoring immune deficiency, but subclinical inflammation remains existing and relates to development of non-AIDS related HIV morbidities. The fact that subclinical inflammation occurs in the absence of HIV treatment or viremia is supported by the finding of increased atherosclerotic changes that were observed in HIV elite controllers, who remain virally suppressed without any HIV treatment.[7] Here we identified correlations between multiple systemic inflammatory biomarkers, CD4/CD8 ratio and immune checkpoint expressing CD8 T cells and CD8 T cell activation with RDW, in HIV-infected individuals receiving ART.

A prior study reported associations between CRP, IL-6, D-dimer, fibrinogen, CD38 + HLA-DR + CD4 T cells, and PD-1+ CD4 T cells with RDW in a cohort with somewhat similar clinical features.[8] Our study expanded on these findings with the discovery of new correlations between additional systemic inflammatory biomarkers, the CD4/CD8 ratio, the recently described immune checkpoint TIGIT receptor on CD8 T cells. Immune checkpoint molecules are receptors expressing on activated T cells that down-regulate immune activity in order to prevent excessive immune response and minimize cell damage.[9] Overexpression

of immune checkpoint molecules reflects a status of immune exhaustion, which leads to T cell dysfunction in chronic HIV infection.[10] Further, in our analysis, after adjustment for confounders, the correlation was significant between RDW and several dual expressing combinations of TIGIT, PD-1, and TIM3 expressing CD8+ T cells. These significant correlations noted between inflammatory biomarkers, immune checkpoint molecules with RDW suggest that RDW may be related to multiple systemic inflammatory pathways in HIV infection.

The mechanism of RDW elevation in the context of inflammation and immune activation is not well understood. Prior study showed that bone marrow abnormalities, especially myelodysplasia occurs more commonly in AIDS patients.[11] Traditionally, RDW when used together with MCV, aids in differentiate between different causes of anemia. Elevated RDW is known to demonstrate ineffective erythropoiesis, hemolysis, or after blood transfusion.[12,13] In general population, elevated RDW is associated with impaired iron mobilization despite an adequate total iron storage.[14] Hepcidine, a peptide hormone is associated with decreased iron intake from intestine and iron trapping in liver cells and macrophage. Hepcidine is known to be inappropriately up-regulated in chronic diseases including CHF.[15] Additionally, this effect of hepcidine interfering with release of iron from reticuloendothelial storage is known to be induced by the inflammatory cytokine IL-6,[16] which may account for the correlation between anisocytosis and inflammation.

Previous data suggest monitoring RDW change can be useful in assessing treatment efficacy in acute myocardial infarction.[17] Similarly, given the significant correlation between inflammatory and immune biomarkers with RDW, it may potentially serve as a surrogate biomarker demonstrating the level of subclinical inflammation in stable HIV-infected individuals. Further investigation is needed to validate its clinical utility and predictive capacity.

Our study has several limitations. First, the crosssectional design yielded susceptibility to bias. Second, the study participants were mostly male, which in general have a higher prevalence of CVD and underlying chronic inflammation; this may have caused a selection bias. Third, there were no HIV-negative participants. Despite these limitations, the strength of this study was the careful clinical characterization of the cohort along with markers of inflammatory and T cell immune activation.

Conclusion

Our study revealed novel correlations between RDW with systemic inflammatory biomarkers and CD8+ T cell populations related to immune activation and exhaustion in HIV-infected individuals who are on stable ART. Further studies are warranted to determine the utility of RDW as a marker of perturbed immune dysregulation in ART treated HIV infection.

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Table 1

Clinical and laboratory characteristics

N	**158**
Age, years	50 (45, 57)
Male	139 (88)
Caucasian	92 (58.2)
History of hypertension	64 (40.5)
History of diabetes mellitus	8 (5.1)
Current cigarette smoker	39 (24.7)
Former cigarette smoker	65 (41.4)
Body mass index (BMI), kg m^{-2}	25.7 (23.65, 27.88)
Systolic BP, mmHg	121 (114,132)
Diastolic BP, mmHg	75 (69, 81)
RDW, %	13.4 (12.9, 14.1)
Hemoglobin, g L^{-1}	14.5 (13.7, 15.3)
MCV, fL	96.2 (92.68, 99.2)
Platelet, × 10^9 L^{-1}	198 (172, 233)
Creatinine, mg dL^{-1}	1.0 (0.9, 1.1)
HDL cholesterol, mg dL^{-1}	42 (33, 53)
LDL cholesterol, mg dL^{-1}	108 (89, 132)
Total Cholesterol, mg dL^{-1}	178 (155, 207)
Total/HDL ratio	4.14 (3.42, 5.17)
Triglycerides, mg dL^{-1}	117 (87, 169)
Therapeutic duration on ART, years	12.6 (7.0, 16.2)
HIV RNA <48 copies mL^{-1} (undetectable viral load)	135 (84.4)
CD4+ T cell count, cells mm^{-3}	503 (341, 661)
CD8+ T cell count, cells mm^{-3}	737 (560, 1033)
CD4/CD8 cell count ratio	0.68 (0.42, 0.98)

All categorical variables are summarized with frequencies and percentages, while continuous variables are displayed as medians = (Q1, Q3).

Table 2

Association between RDW with markers of inflammatory and T cell immune activation by Pearson correlation and multivariable linear regression

	Pearson correlation		Multivariable linear regression[a]	
	Pearson correlation coefficient (*r*)	***p*-value**	**Beta efficient (*β*)**	***p*-value**
Laboratory parameters and markers of inflammatory[b]				
CRP*	0.245	**.004**	0.205	**.026**
IL-1b	0.130	.127	0.092	.328
IL-6	0.350	**<.001**	0.253	**.002**
IL-8	0.418	**<.001**	0.296	**<.001**
IL-10*	0.167	.050	0.146	.115
TNF-a	0.315	**<.001**	0.258	**.002**
MCP-1	0.220	**.009**	0.095	.282
SAA*	0.317	**<.001**	0.271	**.003**
MPO*	0.185	**.030**	0.139	.133
D-dimer	0.252	**.001**	0.161	**.035**
Fibrinogen	0.246	**.002**	0.166	**.038**
sVCAM	0.221	**.009**	0.199	**.028**
sICAM	0.468	**<.001**	0.485	**<.001**
sE-selectin	0.280	**.001**	0.244	**.005**
CD4+ T cell	−0.161	**.043**	−0.157	.065
CD8+ T cell	0.249	**.002**	0.276	**.001**
CD4/CD8 ratio	−0.174	**.029**	−0.187	**.030**
Plateletb	−0.110	.172	−0.083	.332
T cell immune activation[c]				
CD8 + HLA-DR + CD38+	0.206	**0.015**	0.279	**0.002**
CD4 + TIGIT+	0.315	**0.040**	0.238	0.138
CD4 + PD1 +	0.337	**0.027**	0.278	0.083
CD4 + TIM3+	0.06	0.701	0.117	0.504
CD4 + TIGIT + PD1 +	0.311	**0.042**	0.254	0.113
CD4 + TIGIT + TIM3+	0.297	0.053	0.265	0.107
CD4 + TIM3 + PD1 +	0.226	0.146	0.257	0.141
CD8 + TIGIT+	0.424	**0.005**	0.437	**0.008**
CD8 + PD1 +	0.343	**0.024**	0.308	0.070
CD8 + TIM3+	0.175	0.262	0.361	**0.037**
CD8 + TIGIT + PD1 +	0.372	**0.014**	0.361	**0.026**
CD8 + TIGIT + TIM3+	0.383	**0.011**	0.411	**0.010**
CD8 + TIM3 + PD1 +	0.364	**0.016**	0.349	**0.039**

*In log.

[a]Adjusted for age, sex, race, cigarette smoking, serum creatinine and hemoglobin level.

[b] $n = 138$.

[c] $n = 43$.