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Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios as Prognostic Inflammatory Biomarkers in Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), and HIV/HCV Coinfection

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Background. Inflammation in human immunodeficiency virus (HIV)-infected patients is associated with poorer health outcomes. Whether inflammation as measured by the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) adds information to existing prognostic indices is not known.

Methods. We analyzed data from 2000 to 2012 in the Veterans Aging Cohort Study (VACS), overall and stratified by HIV/hepatitis C virus status (n = 89 786). We randomly selected a visit date at which all laboratory values of interest were available within 180 days; participants with HIV received at least 1 year of antiretroviral therapy. We followed patients for (1) mortality and (2) hepatic decompensation (HD) and analyzed associations using Cox regression, adjusted for a validated mortality risk index (VACS Index 2.0). In VACS Biomarker Cohort, we considered correlation with biomarkers of inflammation: interleukin-6, D-dimer, and soluble CD-14.

Results. Neutrophil-to-lymphocyte ratio and PLR demonstrated strong unadjusted associations with mortality ($P < .0001$) and HD ($P < .0001$) and were weakly correlated with other inflammatory biomarkers. Although NLR remained statistically independent for mortality, as did PLR for HD, the addition of NLR and PLR to the VACS Index 2.0 did not result in significant improvement in discrimination compared with VACS Index 2.0 alone for mortality (C-statistic 0.767 vs 0.758) or for HD (C-statistic 0.805 vs 0.801).

Conclusions. Neutrophil-to-lymphocyte ratio and PLR were strongly associated with mortality and HD and weakly correlated with inflammatory biomarkers. However, most of their association was explained by VACS Index 2.0. Addition of NLR and PLR to VACS 2.0 did not substantially improve discrimination for either outcome.

Keywords. HCV; hepatic decompensation; inflammation; NLR; PLR.

Human immunodeficiency virus (HIV) infection is associated with chronic proinflammatory states, even in patients receiving antiretroviral therapy (ART) [1, 2]. Inflammation in HIV, even when viral loads are low or undetectable, is associated with worsened outcomes including mortality, development of acquired immunodeficiency syndrome, and cirrhosis (in hepatitis C virus [HCV] coinfection) [3–8]. Most inflammatory biomarkers are not measured in standard practice and may be expensive or impractical to monitor routinely. Two established markers of subclinical inflammation, the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), are easily determined from a complete blood count.

Neutrophil-to-lymphocyte ratio and PLR are associated with mortality in populations with and without HIV [9–13]. Platelet-to-lymphocyte ratio is also associated with hepatic fibrosis in HCV [14, 15]. However, the clinical impact of these biomarkers is not clear. If they are useful for prognosis, they may eventually be incorporated into a weighted risk index, although it is unknown whether they add information to existing indices of this type (such as the VACS Index and its successor, VACS 2.0) [16]. Furthermore, if NLR and PLR correlate with known inflammatory pathways, they could be applied in large-scale research as a surrogate for less widely available biomarkers [16]. There has been substantial interest in inflammatory biomarkers in HIV, including interleukin (IL)-6, a marker of systemic inflammation and neutrophil activation, D-dimer, a marker of altered coagulation activity, and soluble CD-14 (sCD-14), a marker of monocyte activation [3, 17, 18].

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Table 1. Characteristics of Veterans Randomly Selected From VACS 2.0 Measurements Between 2000 and 2012, With NLR and PLR Measured Within 180 Days of VACS 2.0, and Receiving ART for ≥1 Year (If HIV$^+$)[a]

Measurement or Characteristic	Overall	HIV$^-$/HCV$^-$	HIV$^+$/HCV$^-$	HIV$^-$/HCV$^+$	HIV$^+$/HCV$^+$
N	89 786	47 570	11 881	8955	3713
Demographics					
Age (years)	52.9 ± 10.4	52.4 ± 10.4	51.5 ± 11.1	53.5 ± 6.2	54.0 ± 6.9
Male sex (%)	97.3	97.0	97.1	98.9	98.6
Race (%)					
White	39.7	39.6	43.3	29.2	25.9
Black	48.5	48.0	45.5	61.1	62.6
Hispanic	8.8	9.8	7.2	8.6	9.5
Other	3.0	2.7	4.0	1.2	2.1
BMI (kg/m^2)	28.1 (24.5–32.2)	29.2 (25.6–33.4)	25.7 (22.9–29.1)	27.1 (23.9–30.8)	24.5 (21.8–27.6)
Prevalent Comorbidities					
CAD	14.3	15.3	9.8	12.4	9.5
CHF	4.9	5.0	3.5	4.7	4.9
BMI >30	33.5	39.2	19.8	27.0	12.9
Alcohol abuse or dependence	26.0	24.4	20.4	52.7	47.8
Alcohol-related complications	2.2	1.8	0.8	7.1	3.0
Cirrhosis	2.1	1.0	0.9	9.3	7.6
Diabetes mellitus	21.7	23.9	11.7	23.7	15.2
COPD	15.2	15.0	13.4	17.7	20.2
Cancer	3.2	3.0	4.1	2.6	2.8
CD4 (cells/mm^3)					
≥500	40.6	–	43.5	–	30.8
350–499	21.5	–	21.3	–	22.8
300–349	21.1	–	19.9	–	25.3
100–299	9.5	–	8.5	–	12.3
50–99	3.4	–	3.0	–	4.6
<50	4.0	–	3.8	–	4.3
HIV-1 RNA (copies/mL)					
≤500	90.0	–	82.8	–	76.2
500–10 000	14.6	–	13.3	–	18.4
≥10 000	4.4	–	3.9	–	5.4
Hemoglobin (g/dL)					
≥14	60.4	63.0	56.5	55.9	42.6
12–13.9	29.2	28.6	31.3	29.3	35.1
10–11.9	8.1	6.6	9.3	11.5	17.1
<10	2.3	1.8	2.9	3.3	5.3
FIB-4					
<1.45	68.4	76.1	65.8	41.1	27.6
1.45–3.25	25.9	21.1	29.8	39.9	45.6
>3.25	5.8	2.8	4.5	19.1	26.8
eGFR (mL/min per 1.73 m^2)					
≥60	90.3	91.0	90.0	90.5	86.9
45–59.9	5.5	5.2	6.2	3.9	6.0
30–44.9	2.0	1.9	1.8	1.9	2.3
<30	2.2	1.9	2.1	3.7	4.7
VACS Index 2.0	34 (26–45)	30 (24–37)	46 (36–58)	42 (36–51)	65 (54–79)
Died (%)					
Within 180 days	3.7	2.4	4.1	5.0	8.4
No	83.6	88.1	83.7	76.7	66.4
After 180 days	12.7	9.6	12.3	18.3	25.2
HD (%)					
Pre-existing (within 180 days)	2.9	1.9	2.2	8.7	9.0
No	95.9	97.5	97.0	87.1	86.4
Yes	1.2	0.7	0.8	4.2	4.6
White blood cells (×10^3/μL)	5.2 (4.6–8.3)	6.8 (5.5–8.6)	5.5 (4.4–7.0)	6.4 (4.8–8.3)	5.0 (3.8–6.6)
Neutrophils (×10^3/μL)	3.8 (2.7–5.2)	4.0 (2.9–5.5)	2.9 (2.1–4.1)	3.4 (2.3–5.0)	2.5 (1.7–3.8)

Table 1. Continued

Measurement or Characteristic	Overall	HIV⁻/HCV⁻	HIV⁺/HCV⁻	HIV⁻/HCV⁺	HIV⁺/HCV⁺
N	89 786	47 570	11 881	8955	3713
Platelets (×10³/μL)	230 (189–276)	237 (199–281)	219 (180–263)	206 (157–258)	188 (140–240)
Lymphocytes (×10³/μL)	1.9 (1.4–2.4)	1.9 (1.5–2.4)	1.8 (1.3–2.3)	1.9 (1.4–2.5)	1.6 (1.1–2.2)
NLR	2.0 (1.4–3.0)	2.1 (1.5–3.1)	1.7 (1.1–2.5)	1.7 (1.1–2.7)	1.6 (1.0–2.6)
PLR	122 (92–165)	125 (95–166)	122 (92–169)	104 (75–147)	113 (79–166)
Albumin (g/dL)	4.1 (3.8–4.4)	4.1 (3.9–4.4)	4.1 (3.8–4.4)	3.9 (3.6–4.2)	3.8 (3.4–4.1)

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; CAD, coronary artery disease; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HD, hepatic decompensation; HIV, human immunodeficiency virus; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RNA, ribonucleic acid; VACS, Veterans Aging Cohort Study.

[a]Values presented are median (Q1–Q3), mean ± standard deviation, or column percentage. Patients were followed for death and HD for up to 5 years.

We sought to elucidate the importance of NLR and PLR in patients with HIV on ART, with or without HCV coinfection. Our primary aim was to assess the prognostic significance of NLR and PLR, with respect to both mortality and hepatic decompensation (HD). Of particular interest was whether these markers improve discrimination of the VACS Index 2.0, a validated risk index. Our second aim was to further investigate the relationship between NLR, PLR, and 3 alternative inflammatory biomarkers (IL-6, D-dimer, and sCD-14).

METHODS

We analyzed data from the Veterans Aging Cohort Study (VACS), a prospective longitudinal observational study of HIV-infected veterans that includes age, race, and site-matched HIV-negative veterans in care as controls [19]. The institutional review boards associated with participating VA sites and the coordinating center approved the VACS. We randomly selected a visit date between 2002 to 2012, at which all results of interest

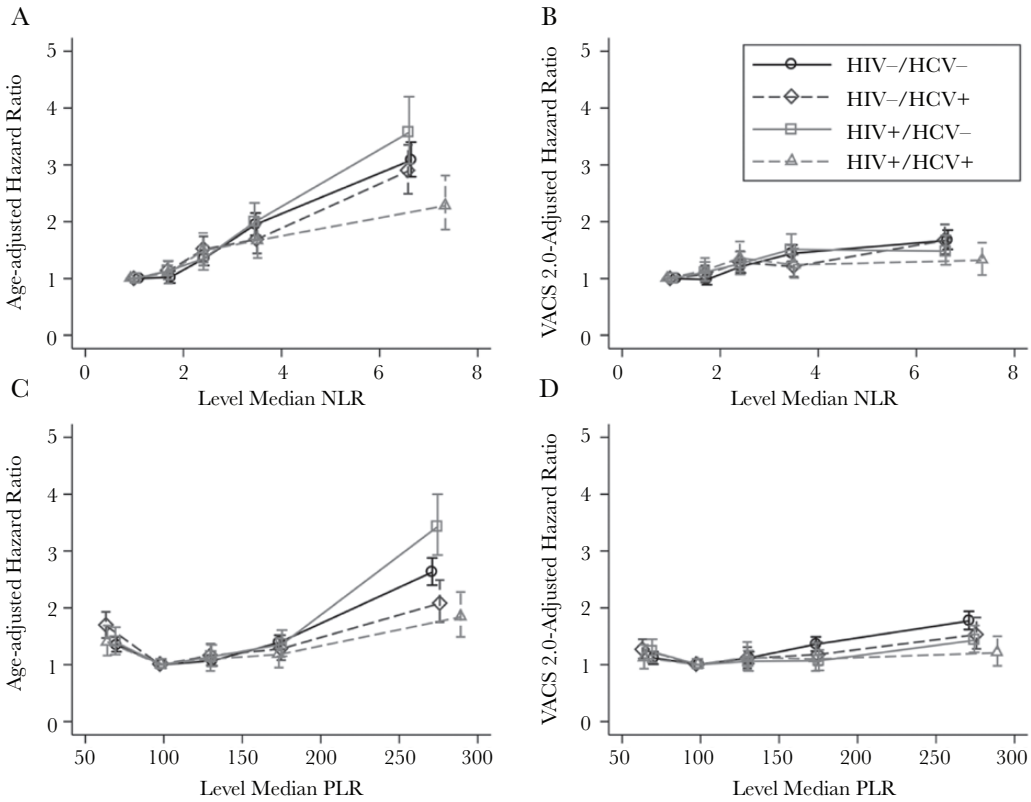

Figure 1. Hazard ratios for all-cause mortality associated with neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR). Levels defined by equal numbers of deaths. Error bars represent 95% confidence intervals. A and C are age-adjusted; B and D are adjusted for Veterans Aging Cohort Study (VACS) 2.0. A and B analyze NLR as an independent variable; C and D analyze PLR as an independent variable. HCV, hepatitis C virus; HIV, human immunodeficiency virus.

Table 2. Model Fit and Discrimination for Endpoint of Death

	Endpoint: Death									
	Overall (N = 86 444)		HIV⁻/HCV⁻ (N = 46 447)		HIV⁺/HCV⁻ (N = 11 395)		HIV⁻/HCV⁺ (N = 8505)		HIV⁺/HCV⁺ (N = 3400)	
Covariates in Model	C-statistic	AIC	C-statistic	AIC	C-statistic	AIC	C-statistic	AIC	C-statistic	AIC
VACS Index 2.0	0.758	247 201	0.773	92 309	0.784	25 254	0.725	28 035	0.735	14 189
VACS 2.0, NLR	0.766	246 270	0.778	92 150	0.786	25 227	0.732	27 997	0.738	14 183
VACS 2.0, PLR	0.764	246 825	0.779	92 143	0.787	25 239	0.728	28 018	0.736	14 193
VACS 2.0, NLR, PLR	0.767	246 157	0.780	92 094	0.788	25 217	0.735	27 976	0.738	14 188

Abbreviations: AIC, Akaike's information criterion; HCV, hepatitis C virus; HD, hepatic decompensation; HIV, human immunodeficiency virus; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; VACS, Veterans Aging Cohort Study.

were available (allowing values to carry forward 180 days); participants with HIV had received at least 1 year of ART therapy. We assessed whether NLR and PLR added prognostic information to the VACS Index 2.0 using multivariate adjustment. VACS 2.0 is a validated risk score incorporating age, CD4 count, HIV-1 ribonucleic acid (RNA), hemoglobin, FIB-4 (a composite marker of liver fibrosis), estimated glomerular filtration rate, HCV coinfection, body mass index, albumin, and white blood cell count [20–22].

Active HCV infection was defined as (1) a positive HCV RNA, (2) a positive HCV genotype, or (3) 1 inpatient or 2 outpatient HCV *International Classification of Diseases, Ninth Revision* (ICD-9) codes. Hepatitis C virus-negative status was defined as a negative HCV antibody test. All other patients were considered to have unknown HCV status. Those with positive HCV status at any timepoint during follow-up were classified as HCV positive.

Primary endpoints were mortality and HD. Death dates were determined from the VA vital status file, which combines information from inpatient mortality, social security data, and national death benefits data; this method has been shown to provide excellent mortality ascertainment [23]. Hepatic decompensation was defined by ascites, spontaneous bacterial peritonitis, or esophageal varices; 1 inpatient

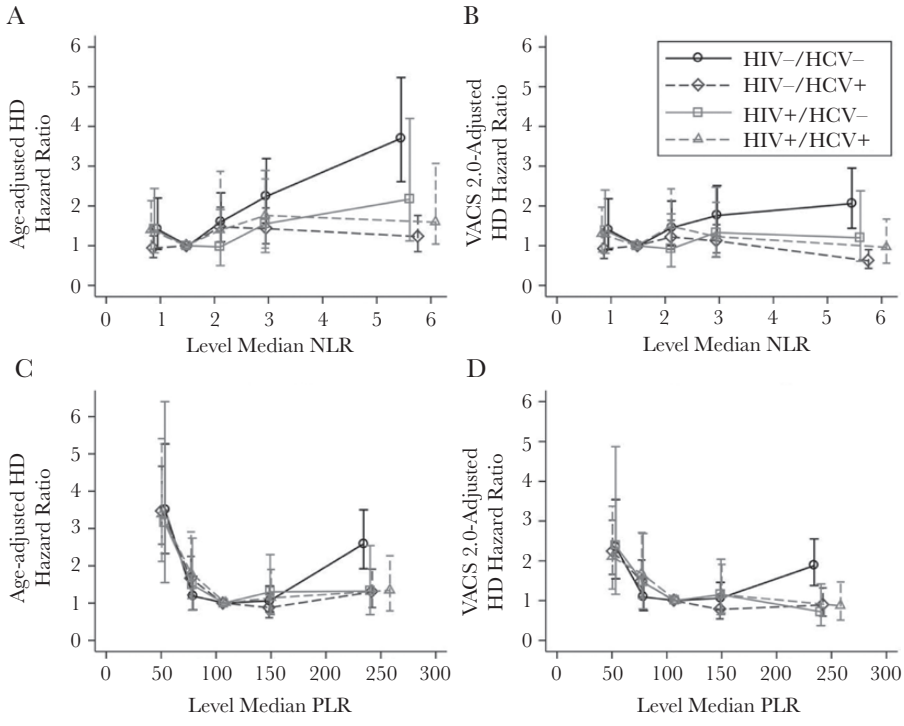

Figure 2. Hazard ratios for hepatic decompensation (HD) associated with neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR). Levels defined by equal numbers of HD events. Error bars represent 95% confidence intervals. A and C are age-adjusted; B and D are adjusted for Veterans Aging Cohort Study (VACS) 2.0. A and B analyze NLR as an independent variable; C and D analyze PLR as an independent variable. HCV, hepatitis C virus; HIV, human immunodeficiency virus.

Table 3. Model Fit and Discrimination for Endpoint of Hepatic Decompensation

Covariates in Model	Endpoint: HD									
	Overall (N = 84 584)		HIV^-/HCV^- (N = 45 750)		HIV^+/HCV^- (N = 11 247)		HIV^-/HCV^+ (N = 7978)		HIV^+/HCV^+ (N = 3195)	
	C-statistic	AIC	C-statistic	AIC	C-statistic	AIC	C-statistic	AIC	C-statistic	AIC
VACS Index 2.0	0.801	23 452	0.778	6808	0.707	1704	0.765	6329	0.766	2541
VACS 2.0, NLR	0.802	23 449	0.786	6798	0.713	1709	0.764	6322	0.767	2545
VACS 2.0, PLR	0.803	23 294	0.791	6785	0.705	1701	0.769	6279	0.778	2531
VACS 2.0, NLR, PLR	0.805	23 247	0.793	6778	0.715	1705	0.776	6256	0.782	2533

Abbreviations: AIC, Akaike's information criterion; HCV, hepatitis C virus; HD, hepatic decompensation; HIV, human immunodeficiency virus; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; VACS, Veterans Aging Cohort Study.

or 2 outpatient ICD-9 codes for any of these conditions were required.

We created a separate dataset using the VACS Biomarker Cohort (VACS BC) substudy to query associations between NLR, PLR, and other inflammatory biomarkers. Interleukin-6, sCD-14, and D-dimer were measured in banked specimens from blood drawn between 2005 and 2007. Assay methods have been previously described [8]. Patients with HIV in our subset of VACS BC had also received at least 1 year of ART. To analyze associations between NLR, PLR, and these biomarkers, we used NLR and PLR values closest to blood draw date (within 90 days).

Statistical Analysis

Mortality was assessed at the first of 5 years of follow-up or September 30, 2016. Hepatic decompensation status was

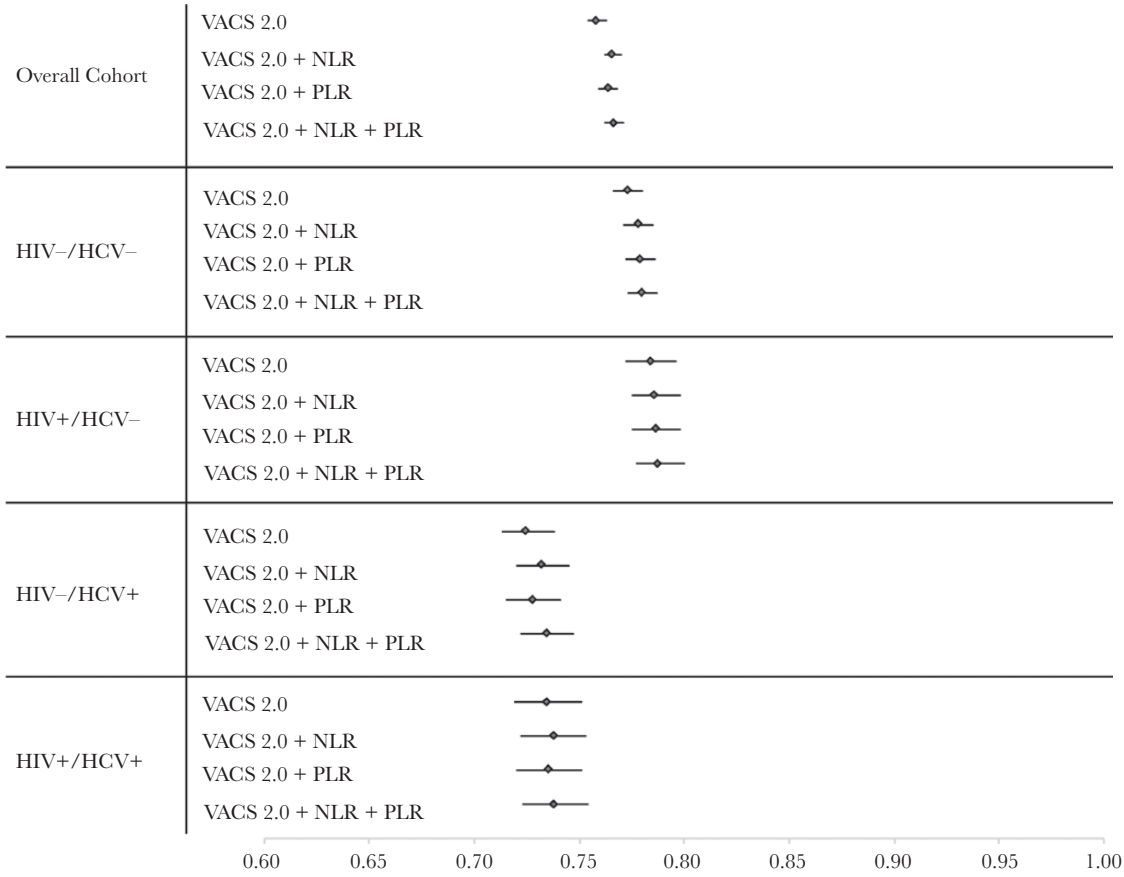

Figure 3. Forest plot of C-statistics (death outcome). Center dots represent Harrell's c; whiskers represent 95% confidence intervals. Left column denotes subgroup; second column indicates covariates in each model. Covariates include categorical neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) and continuous Veterans Aging Cohort Study (VACS) 2.0. Overall cohort includes individuals without documentation of hepatitis C virus (HCV) status. HIV, human immunodeficiency virus.

Table 4. Correlations of NLR and PLR With Inflammatory Biomarkers in VACS Biomarker Cohort[a]

		Overall (N = 1475)		HIV⁻/HCV⁻ (N = 337)		HIV⁻/HCV⁺ (N = 118)		HIV⁺/HCV⁻ (N = 496)		HIV⁺/HCV⁺ (N = 367)	
Biomarker 1	Biomarker 2	r	*P* Value	r	*P* Value	r	*P* Value	r	*P* Value	r	*P* Value
IL-6											
	NLR	0.15	<.0001	0.11	.05	0.22	.02	0.21	<.0001	0.21	<.0001
	PLR	0.049	.06	0.08	.14	0.06	.55	0.08	.07	0.05	.31
sCD-14											
	NLR	0.095	.0002	0.11	.04	0.17	.07	0.15	.001	0.13	.01
	PLR	0.055	.03	0.14	.01	−0.14	.12	0.10	.02	0.03	.51
D-dimer											
	NLR	0.12	<.0001	0.05	.36	0.27	.003	0.08	.09	0.11	.01
	PLR	0.12	<.0001	0.07	.21	0.08	.36	0.14	.001	0.03	.51

Abbreviations: IL-6, interleukin-6; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; sCD-14, soluble CD-14; VACS, Veterans Aging Cohort Study.
[a]Correlations are Spearman's rho.

determined at the first of 5 years of follow-up, the last date of care at a Veteran's Administration, or September 30, 2015, due to changes in ICD coding after this date. We analyzed mortality and HD risk using Cox regression. Initial models assessing NLR and PLR as covariates were age-adjusted. We then adjusted for VACS 2.0 to evaluate whether NLR and PLR added prognostic information. Neutrophil-to-lymphocyte ratio and PLR were analyzed as categorical covariates using 5 levels. For mortality, these levels were defined to have equal number of deaths in each. For HD, levels were defined to have equal number of HD

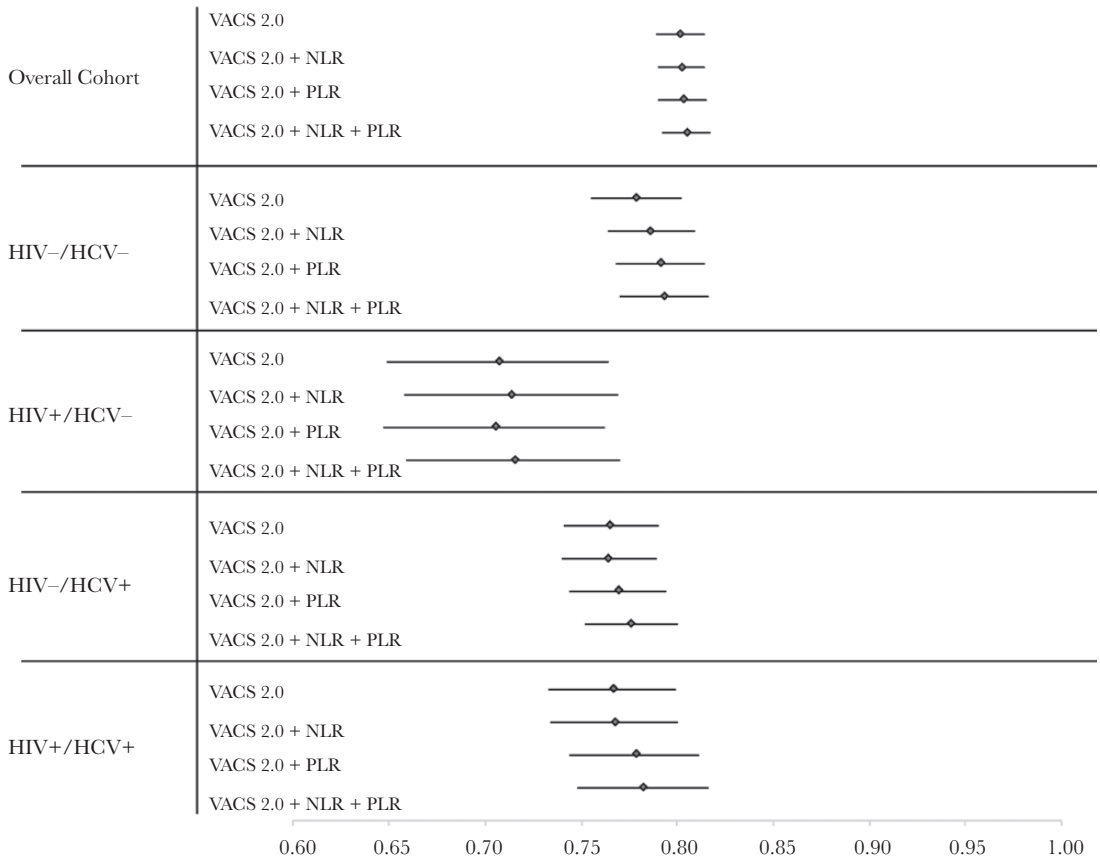

Figure 4. Forest plot of C-statistics (hepatic decompensation outcome). Center dots represent Harrell's c; whiskers represent 95% confidence intervals. Left column denotes subgroup; second column indicates covariates in each model. Covariates include categorical categorical neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) and continuous Veterans Aging Cohort Study (VACS) 2.0. Overall cohort includes individuals without documentation of HCV status.

events. We stratified patients into 4 strata by HIV/HCV infection status for most analyses. The NLR or PLR level with the lowest age-adjusted association in HIV/HCV-coinfected patients was selected as the referent in each analysis. Patients experiencing the endpoint within 180 days of the random visit date were excluded to avoid undue influence of imminent events that might have prompted ordering of a particular test. Given the association between NLR, PLR, and mortality in patients with cancer, we performed a sensitivity analysis excluding patients with malignancy.

We determined discrimination of combinations of covariates using Harrell's c, with 95% confidence intervals (CIs) from the somersd package in Stata. Model fit was assessed using Akaike's information criterion (AIC). In the VACS BC, we assessed the association between NLR, PLR and IL-6, D-dimer and sCD-14, using Spearman's rank correlation.

Statistical significance was defined as a 2-tailed $P < .05$. Statistical analysis was performed in SAS version 9.4 (SAS Institute, Cary, NC) and Stata version 14 (StataCorp, College Station, TX).

RESULTS

Our sample included 89 786 veterans, 72 119 of whom had confirmed HCV status. The most common reason for a missing HCV status was lack of testing. The components of the NLR and PLR were distributed differently among strata defined by HCV and HIV infection, although frank thrombocytopenia, neutropenia, and leukopenia were uncommon (Table 1). As expected, HIV^-/HCV^- patients had the highest median levels of platelets, lymphocytes, and neutrophils. By contrast, HCV and HIV infection were each associated with decreased platelet and neutrophil count, with a synergistic effect in coinfection. Human immunodeficiency virus infection was associated with decreased lymphocyte counts. Consistent with these findings, NLR was overall lower in patients with HIV than those without. Likewise, PLR was lower in patients with HCV compared with those without.

Associations With Mortality

Over 5 years, 14 737 patients died, with 77% of these deaths occurring at least 180 days after the random visit date (Table 1, Supplementary Table 1). Of the deaths occurring after 180 days, 8582 were included in analysis of the relationship between NLR, PLR, and mortality; those that were excluded lacked documentation of HCV status. Adjusting only for age, we found a J-shaped relationship between PLR and mortality and a more linear relationship between NLR and mortality (Figure 1, Supplementary Table 2). After adjustment for VACS 2.0, NLR remained associated with mortality. The association between mortality and NLR or PLR was similar across strata by HIV and HCV status for lower values of NLR or PLR, although hazard ratios (HRs) tended to be higher for uninfected controls than for patients with HIV and HCV among higher levels of NLR or PLR (Figure 1, Supplementary Table 2). Comparing highest to lowest NLR level, the VACS Index 2.0-adjusted HR associated with high NLR was 1.7 for HIV^-/HCV^- (95% CI, 1.5–1.9) and 1.3 for HIV^+/HCV^+ patients (95% CI, 1.1–1.6). A similar trend was observed for PLR, comparing highest to second PLR level (uninfected: HR = 1.8 [95% CI, 1.6–1.9], coinfected: HR = 1.2 [95% CI, 0.98–1.5]). Sensitivity analysis excluding patients with a history of malignancy did not significantly alter these results (data not shown). Addition of NLR, PLR, or both to Cox models using VACS 2.0 did not improve discrimination or model fit; C-statistic improvements did not meet statistical significance in any subgroup, although point estimates increased by 0.003–0.009 (Table 2, Figure 3).

Hepatic Decompensation

Hepatic decompensation occurred in 3727 patients, with 30% of these events occurring at least 180 days after NLR and PLR were measured (Table 1, Supplementary Table 3). Of the HD events occurring at least 180 days after cohort entry, 980 had documented HCV status and were therefore included in this analysis. Hepatic decompensation was more common among patients with HCV than without. Low PLR was a significant predictor of incident HD among all levels, with PLR in the lowest level conferring a VACS 2.0-adjusted HR of 2.1 (95% CI, 1.3–3.4) among coinfected patients and 2.3 (95% CI, 1.6–3.5) among uninfected patients (Figure 2D). Platelet-to-lymphocyte ratio in the highest level also predicted HD in uninfected patients (HR = 1.9; 95% CI, 1.4–2.6), but not in any other stratum. Neutrophil-to-lymphocyte ratio was independently predictive of HD in uninfected patients only (Supplementary Table 4, Figure 2A and B). Addition of NLR, PLR, or both to the VACS 2.0 model resulted in point estimate C-statistic increases of 0.004 in the overall cohort and 0.016 among HIV^+/HCV^+ patients; however, these increases did not meet statistical significance (Table 3, Figure 4).

Association With Other Biomarkers of Inflammation

In VACS BC (n = 1475) (Table 4, Supplementary Table 5), NLR was correlated with IL-6 (r = 0.15, $P < .0001$) but PLR was not (r = 0.049, $P = .06$). Neutrophil-to-lymphocyte ratio and PLR were correlated with D-dimer (both r = 0.12, $P < .0001$). Soluble CD-14 was correlated with NLR (r = 0.10, $P = .0002$) and PLR (r = 0.06, $P = .03$). Patterns were similar across HIV/HCV strata.

DISCUSSION

The ideal role for NLR and PLR in research and practice has yet to be determined, despite numerous analyses demonstrating their prognostic relevance. In this analysis, we evaluated 2 possible applications: (1) incorporation into a risk index and (2) use as a surrogate for a pathway to end-organ disease (immune dysfunction and systemic inflammation) [16].

We found modest differences in HRs associated with level of PLR and NLR across strata, most of which were attenuated after adjustment for VACS Index 2.0. In particular, individuals with higher NLR or PLR seemed to be at greater relative mortality risk in age-adjusted analyses if they were HIV/HCV-negative than their uninfected counterparts. This trend should be interpreted in light of overall higher death rate in HIV/HCV coinfection, independent of NLR or PLR; although a high NLR or PLR may be a slightly less ominous sign in an HIV/HCV-coinfected patient than an uninfected patient, the absolute 5-year mortality rate for coinfected patients with high NLR was 44% compared with 22% in uninfected patients, with a similar trend for high levels of PLR. This suggests that individuals in the highest levels of NLR and PLR were likely characterized by higher illness severity overall, but that this risk was mostly captured by components of the VACS Index, such as FIB-4 score.

Despite an independent association between NLR and PLR with mortality and HD, NLR and PLR failed to improve discrimination when added to models containing VACS Index 2.0. Because C-statistic changes can be relatively insensitive, it is possible that inflammatory state as measured by NLR and PLR may have an impact on outcome apart from the variables measured in the VACS Index 2.0. However, the lack of improvement in discrimination suggests these markers lack additional prognostic value beyond existing indices, and thus their clinical utility remains unclear.

We observed statistically significant but very weak correlations between most combinations of PLR and NLR with D-dimer, sCD-14, and IL-6. The strongest correlation was between IL-6 and NLR, which is plausible given the documented role of IL-6 in neutrophil differentiation as well as known correlations between IL-6 and NLR in HCV monoinfection [24–26]. However, the use of these ratios as surrogates for systemic inflammation is somewhat confounded in HIV and HCV. Specific considerations include the effects of HIV on neutrophil and lymphocyte counts as well HIV-associated pancytopenia. By adjusting for VACS 2.0, we have controlled for the expected effect of HIV severity (ie, CD4 count and HIV viral load) on mortality; however, VACS Index 2.0 was not designed to predict HD. The mortality-specific weighting of variables incorporated into VACS Index 2.0 to create a single number may oversimplify the relative independent risks of the component variables with respect to a different outcome (eg, FIB-4 score may be more strongly associated with HD than with mortality). In addition, liver fibrosis causes thrombocytopenia, and therefore it may affect the interpretation of PLR as a pure inflammatory marker. Based on weak correlations and possible confounding of interpretation of these ratios, it is unlikely that NLR and PLR can reliably supplant IL-6, sCD-14, or D-dimer as measurements of clinically significant virally induced inflammation.

Limitations of this analysis include its retrospective nature. In particular, although we found an association between NLR, PLR, and outcomes independent of VACS Index, our analysis does not rule out residual confounding, such as the possibility that NLR and PLR may reflect by additional comorbidities or clinical factors not accounted for in the VACS Index. Our dataset included a large number of deaths but fewer HD events, which limits the power of analyses using HD as an outcome. Furthermore, the use of NLR and PLR as biomarkers for HD risk in patients with versus without HCV is limited by the different biological pathways that may lead to HD in HCV-related versus nonrelated liver disease. Our data include participants in care between 2000 and 2012, with outcomes out to 2015 and 2016; it is possible that the increasing number of patients with HCV and sustained viral response may affect the interpretation of NLR and PLR in HCV. Although the exclusion of patients who experienced endpoints within 180 days may introduce bias, sensitivity analyses including events occurring within 180 days of cohort entry found no substantive differences. Finally, we tested correlation between NLR and PLR with IL-6, soluble CD-14, and D-dimer, although many additional inflammatory biomarkers exist. Neutrophil-to-lymphocyte ratio and PLR may be more strongly correlated with other biomarkers.

CONCLUSIONS

Both NLR and PLR were statistically independent of the VACS Index 2.0 for mortality prediction, as was PLR for the prediction of HD. Both ratios demonstrated moderate correlation with inflammatory biomarkers. However, neither NLR nor PLR added meaningfully to the discriminative ability of a validated risk index for mortality. Further research may be indicated to determine the usefulness of NLR and PLR in (1) prognostication for liver fibrosis and hepatocellular carcinoma, especially in HIV/HCV-coinfected patients and (2) surrogacy for alternative inflammatory biomarkers.

Supplementary Data

Supplementary materials are available at *Open Forum 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.

Supplementary Table 1. Mortality rates per level of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) within human immunodeficiency virus (HIV)/hepatitis C virus (HCV) strata.

Supplementary Table 2. Hazard ratios for death associated with levels of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) by human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection status, age-adjusted and with adjustment for Veterans Aging Cohort Study (VACS) Index 2.0.

Supplementary Table 3. Rates of hepatic decompensation per level of NLR and PLR within human immunodeficiency virus (HIV)/hepatitis C virus (HCV) strata.

Supplementary Table 4. Hazard ratios for hepatic decompensation per level of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) within human immunodeficiency virus (HIV)/hepatitis C virus

(HCV) strata, with and without adjustment for Veterans Aging Cohort Study (VACS) Index.

Supplementary Table 5. Characteristics of the Veterans Aging Cohort Study (VACS) Biomarker Cohort.

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References

1. Hunt PW. HIV and inflammation: mechanisms and consequences. Curr HIV/AIDS Rep **2012**; 9:139–47.
2. Armah KA, McGinnis K, Baker J, et al. HIV status, burden of comorbid disease, and biomarkers of inflammation, altered coagulation, and monocyte activation. Clin Infect Dis **2012**; 55:126–36.
3. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med **2008**; 5:e203.
4. Boulware DR, Hullsiek KH, Puronen CE, et al. Higher levels of CRP, D-dimer, IL-6, and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. J Infect Dis **2011**; 203:1637–46.
5. Márquez M, Romero-Cores P, Montes-Oca M, et al. Immune activation response in chronic HIV-infected patients: influence of Hepatitis C virus coinfection. PLoS One **2015**; 10:e0119568.
6. So-Armah KA, Tate JP, Chang CH, et al. Do biomarkers of inflammation, monocyte activation, and altered coagulation explain excess mortality between HIV infected and uninfected people? J Acquir Immune Defic Syndr **2016**; 72:206–13.
7. Mooney S, Tracy R, Osler T, Grace C. Elevated biomarkers of inflammation and coagulation in patients with HIV are associated with higher framingham and VACS risk index scores. PLoS One **2015**; 10:e0144312.
8. Justice AC, Freiberg MS, Tracy R, et al. Does an index composed of clinical data reflect effects of inflammation, coagulation, and monocyte activation on mortality among those aging with HIV? Clin Infect Dis **2012**; 54:984–94.
9. Raffetti E, Donato F, Casari S, et al. Systemic inflammation-based scores and mortality for all causes in HIV-infected patients: a MASTER cohort study. BMC Infect Dis **2017**; 17:193.
10. Guthrie GJ, Charles KA, Roxburgh CS, et al. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. Crit Rev Oncol Hematol **2013**; 88:218–30.
11. Wang X, Zhang G, Jiang X, et al. Neutrophil to lymphocyte ratio in relation to risk of all-cause mortality and cardiovascular events among patients undergoing angiography or cardiac revascularization: a meta-analysis of observational studies. Atherosclerosis **2014**; 234:206–13.
12. Ishizuka M, Nagata H, Takagi K, et al. Combination of platelet count and neutrophil to lymphocyte ratio is a useful predictor of postoperative survival in patients with colorectal cancer. Br J Cancer **2013**; 109:401–7.
13. Pinato DJ, Merli M, Dalla Pria A, et al. Systemic inflammatory response is a prognostic marker in HIV-infected patients with hepatocellular carcinoma. Oncology **2017**; 93:395–400.
14. Meng X, Wei G, Chang Q, et al. The platelet-to-lymphocyte ratio, superior to the neutrophil-to-lymphocyte ratio, correlates with hepatitis C virus infection. Int J Infect Dis **2016**; 45:72–7.
15. Alsebaey A, Elhelbawy M, Waked I. Platelets-to-lymphocyte ratio is a good predictor of liver fibrosis and insulin resistance in hepatitis C virus-related liver disease. Eur J Gastroenterol Hepatol **2018**; 30:207–11.
16. Justice AC, Erlandson KM, Hunt PW, et al. Can biomarkers advance HIV research and care in the antiretroviral therapy era? J Infect Dis **2018**; 217:521–8.
17. Borges AH, O'Connor JL, Phillips AN, et al. Factors associated with D-dimer levels in HIV-infected individuals. PLoS One **2014**; 9:e90978.
18. Borges AH, O'Connor JL, Phillips AN, et al. Factors associated with plasma IL-6 levels during HIV infection. J Infect Dis **2015**; 212:585–95.
19. Fultz SL, Skanderson M, Mole LA, et al. Development and verification of a "virtual" cohort using the National VA health information system. Med Care **2006**; 44:S25–30.
20. Akgün KM, Tate JP, Pisani M, et al. Medical ICU admission diagnoses and outcomes in human immunodeficiency virus-infected and virus-uninfected veterans in the combination antiretroviral era. Crit Care Med **2013**; 41:1458–67.
21. Tate JP, Justice AC, Hughes MD, et al. An internationally generalizable risk index for mortality after one year of antiretroviral therapy. AIDS **2013**; 27:563–72.
22. Tate JP, Brown S, Gibert C, et al. White Blood Count, Albumin, and BMI Enhance VACS Index Prognostic Model, but Nadir CD4 and CD8 Metrics Do Not. Open Forum Infect Dis **2017**; 4:S210.
23. Cowper DC, Kubal JD, Maynard C, Hynes DM. A primer and comparative review of major US mortality databases. Ann Epidemiol **2002**; 12:462–8.
24. Abdel-Razik A, Mousa N, Besheer TA, et al. Neutrophil to lymphocyte ratio as a reliable marker to predict insulin resistance and fibrosis stage in chronic hepatitis C virus infection. Acta Gastroenterol Belg **2015**; 78:386–92.
25. Suda T, Yamaguchi Y, Suda J, et al. Effect of interleukin 6 (IL-6) on the differentiation and proliferation of murine and human hemopoietic progenitors. Exp Hematol **1988**; 16:891–5.
26. Hashizume M, Higuchi Y, Uchiyama Y, Mihara M. IL-6 plays an essential role in neutrophilia under inflammation. Cytokine **2011**; 54:92–9.