Associations Between Plasma Immunomodulatory and Inflammatory Mediators With VACS Index Scores Among Older HIV-Infected Adults on Antiretroviral Therapy

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The prevalence of age-related comorbidities is increased in people living with HIV, even in those well-controlled on combination antiretroviral therapy (ART). Persistent immune activation and inflammation may play pivotal roles in the pathogenesis; however, the burden of morbidities in the older HIV infected population may be exacerbated and driven by distinct mechanisms. In a cross sectional study of 45 HIV-infected participants 60 years or older, we examined the relationships between 14 immunomodulatory and inflammatory factors and the Veterans Aging Cohort Study (VACS) Index, a metric of multimorbidity and mortality comprised of age, CD4 count, hemoglobin, Fibrosis-4 [FIB-4], and estimated glomerular filtration rate [eGFR], by linear regression analysis. All participants were virally suppressed (<50 HIV RNA copies/mL), on ART, and primarily Caucasian (86.7%), and male (91.1%). Plasma levels of monocyte/macrophage-associated (neopterin, IP-10, sCD163, sCD14, and MCP-1) and glycan-binding immunomodulatory factors (galectin (Gal)-1, Gal-3, and Gal-9) were assessed, as well as inflammatory biomarkers previously linked to the VACS Index (i.e., CRP, cystatin C, TNF-α, TNFRI, IL-6, and D-dimer) for comparison. In regression analysis, higher VACS index scores were associated with higher levels of neopterin, cystatin C, TNFRI, and Gal-9 (all $p < 0.05$), potentially driven by correlations found with individual VACS components, including age, CD4 count, FIB-4, and eGFR. Gal-9, cystatin C, and TNFRI directly correlated with the extent of multimorbidity. Multiple correlations among markers were observed, suggesting an interplay of overlapping, but distinct, pathways. Collectively, in addition to cystatin C and TNFRI, both galectin-9 and neopterin, independently emerged as novel fluid markers of the VACS Index and

burden of comorbidity and may further guide in understanding pathogenic mechanisms of age-related disorders in older HIV-infected individuals on suppressive ART.

Keywords: HIV, aging, inflammation, morbidity, anti-retroviral therapy

INTRODUCTION

In the current era of effective suppressive combination anti-retroviral therapy (ART) regimens people aging with HIV (PAWH) have an increased risk for and earlier onset of age-related comorbidities including cardiovascular, kidney, liver, bone, and neurologic disease (1, 2). Nearly half of the population living with HIV in the United States is older than 50 years of age and non-AIDS-defined events and age-related comorbidities are now the leading cause of mortality in the ART era (3–5). This demographic shift in the HIV-infected population further complicates the clinical care and management of PAWH, particularly as the increased burden of multimorbidity, frailty, geriatric syndromes, and polypharmacy become the norm (6, 7). Although multifactorial, evidence suggests chronic inflammation, particularly monocyte activation, is a key driver of early development of these comorbidities (8–11). This chronic inflammation is thought to stem from occult viral replication and the senescence, exhaustion, and premature aging of the immune system (12–14).

Soluble biomarkers of immune activation, inflammation, and coagulation, such as TNF-α and D-dimer, have been previously associated with clinical indices of morbidity and mortality in people with HIV on suppressive ART (15, 16). As organ system injury is strongly related to immune perturbations, linking clinical predictors and outcomes of morbidity and mortality with mediators of chronic inflammation and immune dysfunction can inform potential molecular pathways involved in the early onset of these comorbidities and, thus, guide therapeutic interventions. The Veterans Aging Cohort Study (VACS) Index is a validated predictor of morbidity and mortality for people with HIV by incorporating markers of organ system injury with traditional HIV disease estimates (17–19). The VACS Index is shown to correlate with age-related complications (i.e., frailty and cognitive impairment) and can inform mechanistic studies on the pathophysiologic effects of aging with HIV (15, 20–25). Evaluating soluble mediators with these clinical index scores and discovering novel correlations can provide a deeper understanding of the interplay between biological function and outcomes of morbidity and mortality in PAWH (26).

In order to inform our understanding of the biology of morbidity risk in older PAWH, we assessed correlations between the VACS Index and soluble monocyte/macrophage activation (neopterin, IP-10) and glycan-binding (galectins) immunomodulatory factors. Several of these factors are elevated in the plasma of untreated people living with HIV and, to a lesser degree, those on ART (27–29). Furthermore, many of these factors are shown to be involved in modulating metabolism

and inflammation, directly involved in HIV replication, and linked to comorbidities in the general population (30–52). We investigated these immunomodulatory factors in addition to biomarkers traditionally associated with the VACS Index in a cohort of older adults living with HIV (over the age of 60) with long-term viral suppression to better understand the relationship between aging, HIV, and inflammation.

MATERIALS AND METHODS

Participants

We conducted a cross sectional study of HIV-infected participants enrolled in the UCSF HIV Elders Study or HIV Over 60 Cohort. These studies were approved by the UCSF Institutional Review Board at the University of California, San Francisco. All participants were aged 60 or older, virally suppressed (defined as plasma HIV RNA <50 copies/mL), and reported adherence to ART for at least 12 months. All participants underwent neuropsychological testing and had endorsed cognitive symptoms on the Patient Assessment of Own Functioning questionnaire as previously described (53). Individuals with confounding conditions such as major neurological or psychiatric conditions were excluded. VACS index was calculated using age, current CD4 t-lymphocyte count, plasma HIV RNA level, hemoglobin, Fibrosis-4 (FIB-4) index, estimated glomerular filtration rate (eGFR), and active hepatitis C infection in a weighted manner, per the scoring system developed by the VACS Project Team (19). Self-reported current CD4 T-lymphocyte count was employed for two individuals as laboratory measures were not available. FIB-4 was calculated using routine liver function tests (AST and ALT), platelet count, and age. eGFR was calculated using serum creatinine levels, age, gender, and race.

Quantification of Plasma Markers

Plasma aliquots were thawed and prepared following kit manufacturer guidelines. All samples were analyzed in duplicate. MCP-1, soluble (s)CD163, IP-10, galectin-1, galectin-3, galectin-9, IL-6, TNF-α, TNFR1, D-dimer, and cystatin C were measured using custom Luminex kits from (R&D Systems) and CRP was measured using the Procartaplex kit (Thermofisher). Data was acquired on a Luminex 200™ analyzer (Luminex) and analyzed using MILLIPLEX® Analyst software (Millipore). Neopterin and sCD14 were measured via ELISA (Neopterin competitive enzyme immunoassay, ALPCO, NH, USA; Human CD14 Quantikine ELISA kit, R&D Systems). Optical density was read with a microplate spectrophotometer (Bio-Rad) and data analysis, including four parameter logistic standard

interpolation, was carried out using the online MyAssays Ltd. data analysis tool.

Statistical Analysis

Demographic and HIV-related characteristics were described using the median, first quartile (Q1), and third quartile (Q3) for continuous variables, and frequency for categorical variables. Relationships among soluble markers and clinical parameters were examined by Pearson correlation for continuous variables or Spearman correlation for count variables. A multiple linear regression model was fit to examine predictors of the VACS index. Soluble marker values were log transformed to conform to normality prior to analysis. All statistical tests were performed with GraphPad Prism version 8.0 (Graphpad Software Inc., CA, USA) or SPSS version 26 (IBM SPSS Statistics, NY, USA). Correlation matrix was constructed using GraphPad Prism. Statistical significance is indicated as $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. P-values ≤ 0.100, but not significant, are noted as statistical trends.

RESULTS

Participants were 60 years of age or older, predominantly male (91.1%), and Caucasian (86.7%) (**Table 1**). All individuals were on ART and had undetectable plasma HIV RNA (viral load; VL). Most participants self-reported being infected with HIV for 20+ years (89%). More than half of participants (65%) had CD4 T-lymphocyte counts over 500 cells/µL; only one individual had CD4 T-lymphocyte count <200 cells/µL. Only one individual had a hemoglobin level <12 g/dL, three individuals had a FIB-4 score consistent with liver fibrosis (>3.25), 23% had some compromise in renal function (eGFR <60), and six had past co-infection with hepatitis C virus (HCV) and at least one active infection. We observed a mean VACS index score of 30 for the cohort. Based on prior reports, this suggests a 11.9% all-cause 5-years mortality risk (19, 54). Recent comorbid conditions of cohort participants were also assessed (**Table 2**), showing 14% were obese defined by a BMI >30, three had coronary disease, almost half (49%) had hyperlipidemia/hypercholesterolemia, more than half (58%) had hypertension, 22% had diabetes mellitus, one individual had liver disease, 22% had chronic obstructive pulmonary disease (COPD), three previously experienced kidney failure (two had stage III chronic kidney disease, and one had chronic renal insufficiency), and one individual had cancer.

We found several significant correlations between VACS Index scores and the plasma levels of inflammatory mediators, including the monocyte/macrophage-associated markers, glycan-binding immunomodulatory proteins, as well as inflammatory biomarkers previously linked to the VACS Index (**Table 3**). VACS index scores were significantly associated with neopterin ($r = 0.40$, $p = 0.007$), Gal-9 ($r = 0.38$, $p = 0.012$), cystatin C ($r = 0.54$, $p = 0.0002$), and TNFRI ($r = 0.50$, $p = 0.0007$). Furthermore, these correlations found with neopterin, Gal-9, cystatin C, and

TABLE 1 | Demographics, HIV disease characteristics, and VACS components ($n = 45$).

Variable	
Age, yrs	65.0 (62.0, 66.0)
Gender (male)	41, 91.1%
Ethnicity (caucasian)	39, 86.7%
EDI, yrs	25.3 (23.0, 30)
HIV RNA <50 copies/mL	45, 100%
BMI[a]	26.5 (24.4, 28.4)
CD4 Nadir (cells/µL)[a]	173 (50, 243)
Current CD4 (cells/µL)[b]	623 (403, 804)
≥500	28, 65.1%
350–499	9, 20.9%
200–349	6, 13.0%
<200	1, 2.0%
Hemoglobin (g/dL)[b]	14.8 (14.2, 15.8)
FIB-4[a]	1.8 (1.2, 2.0)
<1.45	16, 36.4%
1.45–3.25	25, 56.8%
>3.25	3, 6.8%
eGFR[a]	73.7 (60.3, 87.2)
≥60	34, 77.3%
30–59.9	10, 22.7%
HCV infection	7, 15.6%
VACS index score[c]	30 (18, 39)

Continuous variables are listed as mean (Q1, Q3) and categorical variables as n, %. EDI, estimated duration of infection; BMI, body mass index; FIB-4, fibrosis index 4; eGFR, estimated glomerular filtration rate; HCV, Hepatitis C virus.
$^a n = 36$, $^b n = 44$, $^c n = 43$.

TNFRI remained statistically significant when adjusting for estimated duration of infection (EDI). Additionally, we observed trends between VACS index scores with IP-10 ($r = 0.30$, $p = 0.051$), Gal-1 ($r = 0.30$, $p = 0.053$), and TNF-α ($r = 0.27$, $p = 0.082$). However, markers previously being associated with the VACS index, including sCD163, sCD14, CRP, D-dimer, and IL-6, were not observed here.

Upon correlation analysis of individual VACS components (**Figure 1**), we observed that levels of neopterin, cystatin C, TNF-α, and TNFRI were positively linearly associated with age (all $p < 0.05$). Neopterin, IP-10, and TNF-α inversely related with CD4 count (all $p < 0.01$). Hemoglobin levels were inversely associated with Gal-1 and cystatin C levels, but directly correlated with levels of IP-10 (all $p < 0.05$). Direct correlations between FIB-4 and IP-10, sCD163, Gal-9, cystatin C, TNF-α, and TNFRI were present (all $p < 0.05$). And finally, levels of neopterin, MCP-1, Gal-1, Gal-9, cystatin C, and TNFRI inversely related with eGFR (all $p < 0.05$). No soluble markers differed significantly among HCV status, except for higher Gal-3 levels observed in individuals with past HCV co-infection ($p = 0.048$; data not shown). Soluble markers levels were compared among individuals differing in CD4 nadir (>100, 100–200, >200)

TABLE 2 | Prevalence of comorbid conditions.

Comorbid condition	Recent	%
Obesity (BMI > 30)[a]	6	13.6
Myocardial infarction / cardiac arrest	0	0
Heart failure	0	0
Coronary artery disease	3	6.7
Hyperlipidemia/hypercholesterolemia	22	48.9
Hypertension	24	53.3
Diabetes mellitus	9	20.0
Kidney failure	3	6.7
Liver disease[b]	1	2.3
Chronic obstructive pulmonary disease	10	22.2
Current smoker	6	13.3
Osteopenia or osteosclerosis	6	13.3
Cancer	1	2.2

BMI, body mass index; [a]n = 44, [b]n = 43.

and no differences were observed (**Supplementary Figure 1**). Associations among sCD14, CRP, and IL-6 with individual VACS components were not found. We next evaluated these markers with the burden of multimorbidity (**Figure 2**). We observed that levels of Gal-9 (rho = 0.34, $p = 0.021$), TNFRI (rho = 0.36, $p = 0.015$), and cystatin C (rho = 0.44, $p = 0.003$) were directly correlated with the total number of comorbid conditions. Differences in soluble marker levels for specific comorbidities were also evaluated (**Supplementary Figure 2**). Only higher levels of CRP in individuals with obesity ($p = 0.022$) and higher Gal-3 with COPD ($p = 0.023$) were observed.

We also conducted an exploratory analysis to determine if there exists an interplay of multiple immune pathways by evaluating intercorrelations among the 14 soluble mediators quantified (**Figure 3**). A positive interaction between neopterin and Gal-9 was noted ($p < 0.001$) and multiple correlations among several plasma markers were observed, including neopterin levels positively associating with IP-10, cystatin C, TNF-α, and TNFRI ($p < 0.05$). Gal-9 levels positively associated with IP-10, CRP, cystatin C, IL-6, TNF-α, TNFRI, and Gal-1 ($p < 0.05$). Among the other monocyte/macrophage activation markers, IP-10 also correlated with sCD163, cystatin C, and TNF-α; sCD14 only correlated with cystatin C ($p < 0.05$). And Gal-1 correlated with Gal-3, CRP, cystatin C, IL-6, TNF-α, and TNFRI ($p < 0.05$).

DISCUSSION

In an assessment of immunomodulatory factors in older PAWH on suppressive ART, we illuminated potential plasma mediators while highlighting the importance of previously evaluated markers that could lead to a better understanding of the biological complexity of the aging process within the context of HIV and assess those most vulnerable of experiencing morbidity and mortality. In addition to cystatin C and TNFRI, we identified a relationship between myeloid-associated and

glycan-binding immunomodulators neopterin and Gal-9, and to a lesser extent IP-10 and Gal-1, with the VACS Index and several key VACS components. Furthermore, several of these factors correlated with the extent of comorbidities and with one another.

Chronic inflammation, particularly through monocyte/macrophage activation, is hypothesized as a key predictor of increased morbidity and all-cause mortality in HIV infection even in the context of viral suppression (55–57). Neopterin and IP-10 are markers of monocyte/macrophage activation that are associated with IFN-γ-induced pathways and demonstrate immune regulatory functions (58–61). Prior literature has demonstrated that both neopterin and IP-10 are implicated in the progression of HIV disease, principally associating with viremia, decreased CD4 counts, and all-cause mortality (62–64). We extend these observations by demonstrating that neopterin and, to a lesser extent, IP-10 associated with increased risk of mortality in older PAWH on suppressive ART. Furthermore, our correlative findings between eGFR, an indicator of kidney function, with these markers are consistent with previous findings demonstrating that neopterin associates with chronic kidney disease severity and IP-10 blockade promotes renal dysfunction (65, 66). Interventions that target monocyte activation, specifically IFN-γ-induced pathways, may potentially be useful in lowering chronic immune activation, and reduce morbidity incidence in PAWH during suppressive ART.

Glycan-glycan-binding protein mediated immune responses, particularly through the galectin family of proteins, is an emerging field in HIV research and implicated to play a role in HIV pathogenesis (67). Gal-9 and Gal-1, are demonstrated as drivers of HIV transcription and cell entry, respectively, as well as pro-inflammatory mediators (33–35, 37, 38). Gal-9 specifically has been shown to rapidly increase in the plasma as part of the cytokine storm in early HIV infection and remains elevated during chronic infection despite viral suppression (28). Furthermore, in the cerebrospinal fluid of people living with HIV higher Gal-9 levels were closely linked to increased viremia and immune activation as well as lower cognitive performance (68). Mechanistically, Gal-9 is hypothesized to potentially contribute to NK cell dysfunction and the non-specific activation of T cells in HIV infection (69, 70). Whether elevated endogenous Gal-9 levels contribute or sustain the state of chronic inflammation and immune activation and contribute to the progression of comorbid conditions during suppressive ART warrants further investigation. Here, we observed Gal-9 directly associated with morbidity risk and the extent of multimorbidity and our findings with VACS components are consistent with correlations previously demonstrated with FIB-4, a predictor of liver fibrosis, and eGFR in people without HIV (42, 44). As Gal-9 plays significant roles in both immune function and HIV transcription, elevated levels in PAWH may provide additional insights beyond biomarkers previously assessed with the VACS Index.

In this study we reveal additional inflammatory-induced pathways that could be contributing to the persistent inflammatory milieu and morbidity risk in treated HIV in

TABLE 3 | Soluble mediator correlations with VACS index scores.

Parameter	VACS	
	Unadjusted	**EDI adjusted**
	Correlation coefficient (*p*-value)	
Neopterin (nMol/L)	**0.40 (0.007)**	**0.43 (0.004)**
IP-10 (pg/mL)	0.30 (0.051)	0.29 (0.055)
sCD163 (pg/mL)	0.03 (0.840)	0.08 (0.637)
sCD14 (pg/mL)	−0.04 (0.781)	−0.07 (0.674)
MCP-1 (pg/mL)	0.18 (0.242)	0.15 (0.327)
Gal-1 (pg/mL)	0.30 (0.053)	0.28 (0.064)
Gal-3 (pg/mL)	0.18 (0.249)	0.20 (0.208)
Gal-9 (pg/mL)	**0.38 (0.012)**	**0.41 (0.006)**
D-dimer (pg/mL)	0.25 (0.101)	0.27 (0.075)
CRP (pg/mL)	−0.09 (0.587)	−0.09 (0.561)
Cystatin C (pg/mL)	**0.54 (0.0002)**	**0.53 (0.0003)**
IL-6 (pg/mL)	0.17 (0.291)	0.16 (0.312)
TNF-α (pg/mL)	0.27 (0.082)	0.27 (0.080)
TNFRI (pg/mL)	**0.50 (0.0007)**	**0.48 (0.002)**

Soluble markers were log-transformed prior to Pearson correlation and multivariate analysis. EDI, estimated duration of infection. Significant values are bold.

the older population. We also conducted an evaluation of markers previously shown to associate with the VACS Index based on a diverse population in terms of age and ART treatment status. In our virally suppressed cohort, we found correlations among the VACS Index with cystatin C and TNFRI levels, as well as a trends with D-dimer and TNF-α, complementing the idea that perturbations in inflammation and coagulation mechanisms are linked to morbidity and mortality progression in PAWH (5). However, we did not observe VACS index correlations with CRP, IL-6, sCD14, and sCD163 as previously described (71). The absence of these correlations could stem from a low incidence of comorbid complications in our cohort that these biomarkers are found to be associated with, such as cardiovascular disease, bone disease, and cancer (72–78). Additionally, previous links between these biomarkers and the VACS Index were observed in relatively younger participants either with incomplete viral suppression (VL detectable but <500 c/mL) or an immunologic non-responder profile (20, 21).

As people living with HIV advance in age measures to determine increased risk for morbidity and mortality are warranted and soluble mediators may be particularly useful as screening tools to identify high-risk groups. However, the synergistic effects of HIV, aging, and multimorbidity complicates the ability to identify "universal" biomarkers. Previous studies demonstrate that PAWH display accelerated or early aging and accentuated inflammatory responses in advancing age, seroconversion at an earlier age associates with higher rates of multiple comorbidities, and specific mediators are associated with distinct comorbidities observed in HIV infection (79–82). Many comorbidities were common in our cohort, similar to other studies of older PAWH, which makes many of these soluble markers informative, as some are disease specific and

others are common among many conditions. Furthermore, we discovered intercorrelations among many of the soluble mediators analyzed, suggesting an interplay of overlapping, but distinct, pathways, leading to the conclusion that a composite panel of clinical and immunomodulatory markers might be a more comprehensive approach for monitoring comorbid progression and the discovery of an effective means of intervention.

Chronic inflammation and immune activation are proposed as significant drivers of comorbidity development in PAWH, but this could be propelled by several factors, including biological aging, ARV regimens, HIV infection, or rather a synergistic effect. Chronic, low-grade inflammation occurs as people age, termed inflammaging, and soluble markers shown to be elevated with age, such as Gal-9 and neopterin, could be a reflection of this normal biological aging process (83). Adverse effects of several ARVs are linked to inflammation and the development of specific comorbidities; however, initiation of ART at diagnosis irrespective of CD4 count is now mandated given the overriding benefit of viral suppression in reducing transmission and lowering immune damage and higher states of inflammation (84–89). Current efforts to use ARV drugs that are safer and in combinations with anti-inflammatory agents are being pursued. To date, no therapy has been approved to decrease inflammation in PAWH despite numerous clinical trials targeting multiple proposed mechanisms of chronic inflammation in HIV (90–97). Any successes in interventions to lower immune activation and inflammation would be of significant value for PAWH and our panel of identified markers could potentially assist in monitoring interventions and identifying novel pathways to target.

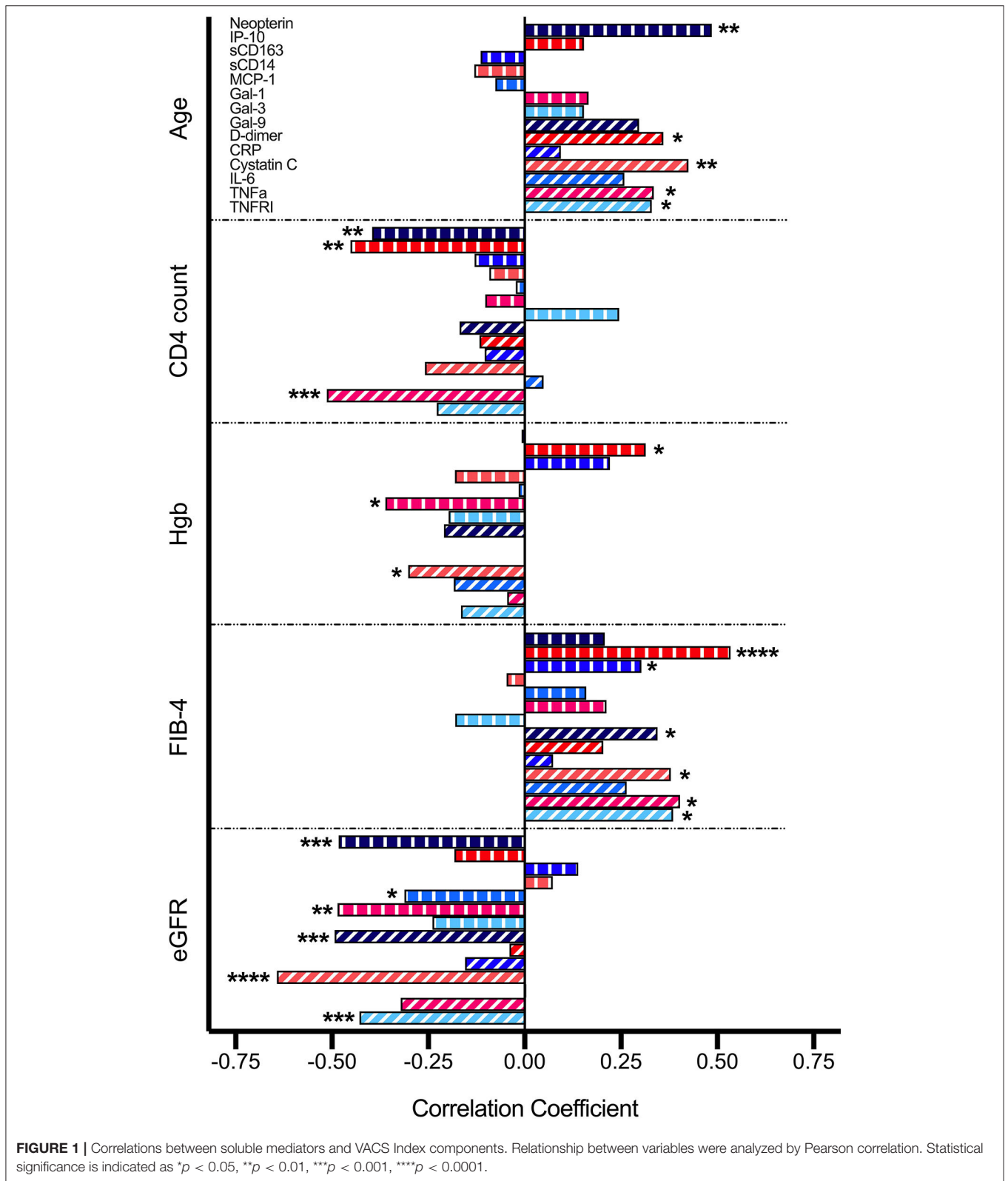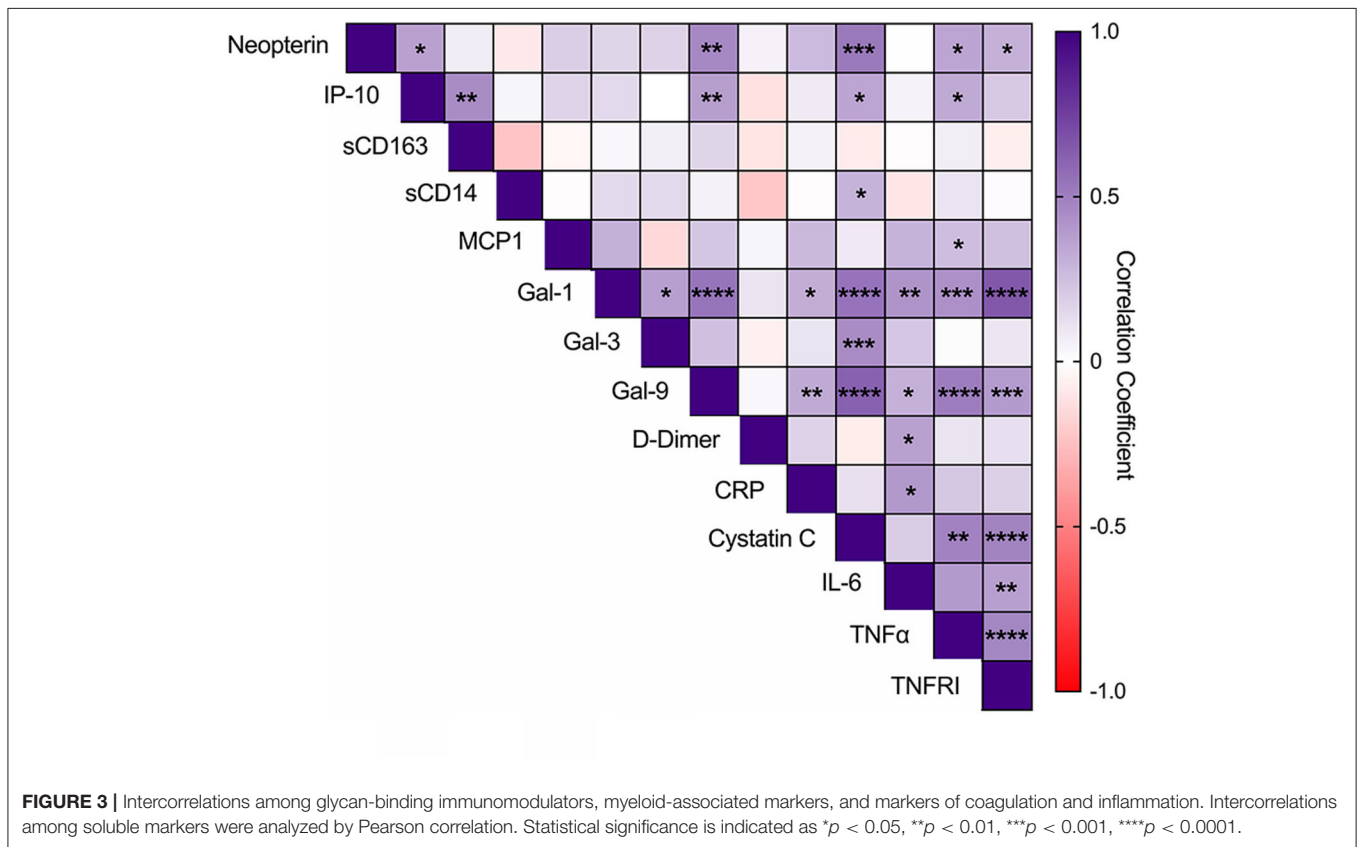Our study has several limitations that should be acknowledged. Our sample was relatively small and, thus,
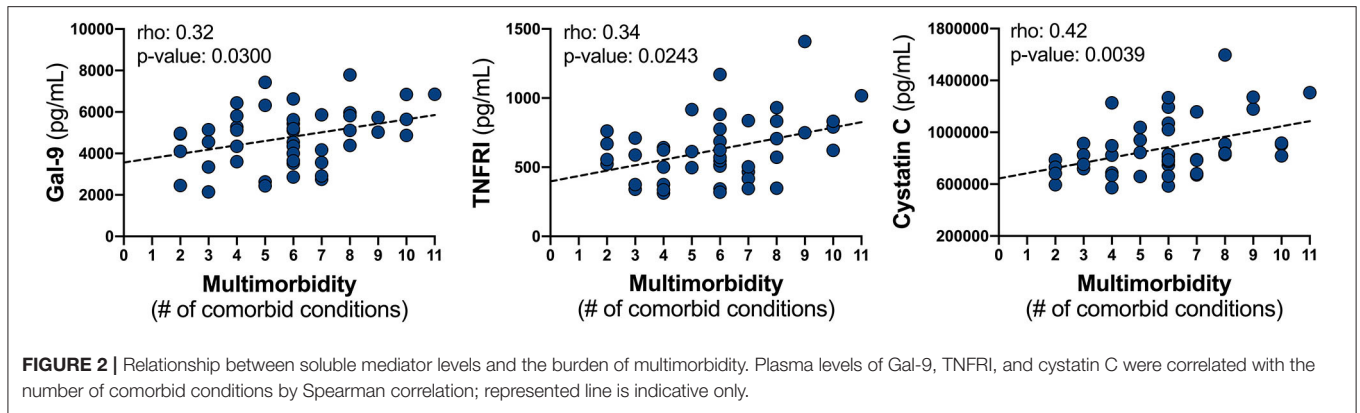
FIGURE 1 | Correlations between soluble mediators and VACS Index components. Relationship between variables were analyzed by Pearson correlation. Statistical significance is indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

limited the power for multivariate regression analysis. Participants were primarily white males and, thus, we are limited in our ability to generalize our data to all affected

populations. Our cohort consisted of participants who were virally suppressed and $\geq$ 60 years old, which may limit the predictive capabilities of the VACS Index. The availability

FIGURE 2 | Relationship between soluble mediator levels and the burden of multimorbidity. Plasma levels of Gal-9, TNFRI, and cystatin C were correlated with the number of comorbid conditions by Spearman correlation; represented line is indicative only.

FIGURE 3 | Intercorrelations among glycan-binding immunomodulators, myeloid-associated markers, and markers of coagulation and inflammation. Intercorrelations among soluble markers were analyzed by Pearson correlation. Statistical significance is indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

of an age-matched comparison group comprised of people without HIV could have added more clarity to the relevance of these soluble mediators specifically in the context of aging with HIV. Additionally, many of these soluble mediators are shown to associate with age (98, 99). As calculations for VACS, FIB-4, and eGFR use age as a component adjustments for this variable in regression models were not feasible. Finally, we cannot determine the predictive nor diagnostic ability of these identified soluble mediators for specific non-AIDS clinical events.

In conclusion, we identified several key mediators in our population of PAWH on ART who were over 60, some of which were consistent with previous studies and in particular, novel factors such as neopterin and Gal-9. Further evaluation of these identified immunomodulatory factors could determine if suitable to implement in clinical practice to monitor those at an increased risk of developing comorbidities or mortality in aging HIV-infected individuals on suppressive ART. In light of our studies either independently or collectively, these immune markers could also emerge as novel mediators in understanding

the pathogenic molecular mechanisms of age-related disorders in treated HIV that may ultimately lead to identifying potential targets to prevent, slow, or reverse these complications in this population.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by UCSF Institutional Review Board at the University of California, San Francisco. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TP contributed to biomarker data acquisition, statistical analysis, and wrote the manuscript. SJ, KH, IA, and MG contributed to clinical data acquisition and interpretation. NT contributed to biomarker data acquisition and interpretation. MC, VV, and LN contributed to study design and concept. All authors reviewed the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.01321/full#supplementary-material

Supplementary Figure 1 | Differences in soluble mediator levels in individuals according to CD4 nadir count. Associations among groups were analyzed via Kruskal-Wallis with Dunn's multiple comparison test. Neopterin concentration is in nMol/L.

Supplementary Figure 2 | Differences in soluble mediator levels according to comorbidity. Neopterin concentration is in nMol/L. Associations among groups were analyzed via Mann-Whitney tests. Statistical significance is indicated as *$p < 0.05$.

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