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MAJOR ARTICLE

Macrophage Activation Marker Soluble CD163 Is a Dynamic Marker of Liver Fibrogenesis in Human Immunodeficiency Virus/Hepatitis C Virus Coinfection

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Background. Coinfection with human immunodeficiency virus (HIV) accelerates hepatitis C virus (HCV)–related liver fibrosis. Macrophages are triggered during both viral infections and are critical in liver inflammation/fibrogenesis. Liver fibrosis strongly associates with serum soluble CD163 (sCD163, a macrophage activation marker); comprehensive evaluation in HIV/HCV coinfection is lacking.

Methods. We retrospectively analyzed sCD163 (enzyme-linked immunosorbent assay) and hepatic CD163 (immunofluorescent CD163/CD68 costaining) in patients infected with HIV/HCV, HCV, or HIV, pre– and post–antiviral therapy.

Results. sCD163 was significantly higher in HIV/HCV compared to either monoinfection, and decreased following successful antiviral therapy, although did not fully normalize. In HIV/HCV, sCD163 was associated with necroinflammation, Ishak fibrosis scores, and noninvasive fibrosis scores. We observed a novel trend whereby sCD163 levels progressively increase with increasing Ishak fibrosis score, peaking at stage 4, above which levels plateaued. Periportal $CD163^+$ macrophage frequency was also higher with increasing fibrosis score. When stratified by fibrosis stage, sCD163 levels were higher in HIV/HCV than HCV but only in individuals with mild to moderate fibrosis.

Conclusions. In HIV/HCV, increasing sCD163 levels accompanied periportal $CD163^+$ macrophage enrichment in mild to moderate fibrosis, but not in established cirrhosis, suggesting that sCD163 is a dynamic biomarker of fibrogenesis rather than accumulated fibrosis. Our findings implicate HIV-related macrophage activation in accelerated fibrosis progression in HIV/HCV coinfection.

Keywords. hepatic fibrogenesis; antiretroviral therapy; interferon-based therapy.

Human immunodeficiency virus (HIV) coinfection occurs in approximately 10% of individuals infected with hepatitis C virus (HCV) and is associated with accelerated liver fibrosis progression [1, 2]. However, the underlying mechanism(s) remain unknown. Despite significant advances in anti-HCV therapy, HIV/HCV-coinfected persons with preexisting hepatic fibrosis remain at risk for disease progression and hepatocellular carcinoma. Furthermore, liver disease has now emerged as the second most common cause of non-AIDS mortality in HIV-infected individuals in the post–antiretroviral therapy (ART) era [1]. In addition, the lack of biomarkers predicting individuals at highest risk for accelerated fibrosis progression or that can be used to follow liver disease progression highlight key areas of unmet clinical need.

The immunopathogenesis of liver disease in HIV/HCV coinfection involves a complex interplay of host and viral interactions [2, 3] in which macrophages play an important role. Both HCV and HIV infection activate macrophages (tissue resident and infiltrating macrophages), and increasing evidence supports their critical role in all stages of liver inflammation and fibrosis that characterizes chronic hepatitis [4–7]. Viral infection and tissue injury both trigger macrophage polarization across a spectrum of M1/proinflammatory (associated markers/cytokines/chemokines include CD80, inducible nitric oxide synthase, Toll-like receptor [TLR] 2, TLR4, tumor necrosis factor alpha, monocyte chemotactic protein-1), and M2/anti-inflammatory/profibrogenic and fibrolytic phenotypes (markers/cytokines/chemokines include arginase 1, CD206, TLR1, TLR8, interleukin 10, transforming growth factor β, metalloproteinases) [6, 8–10]. These M1/M2 markers have mostly been identified from mouse and in vitro cell culture studies, but remain

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not clearly defined in human liver disease. Macrophage activation can be assessed by measuring serum soluble CD163 (sCD163), a membrane lineage protein. CD163 is enriched in M2 macrophages [11] and is cleaved as sCD163 in response to inflammation [12]. Therefore, sCD163 is commonly elevated in inflammatory diseases, including HIV infection [13–19]. Furthermore, sCD163 strongly associates with liver inflammation and fibrosis in HCV, hepatitis B virus (HBV), and nonalcoholic steatohepatitis [15–17, 20], underscoring the crucial role of macrophages in liver disease pathogenesis. HIV itself has also been implicated in liver disease pathogenesis [2, 4, 10, 21], thought to be mediated through macrophage activation with immune dysregulation and gut $CD4^+$ T-cell depletion leading to increased microbial translocation and oxidative stress [2], and HIV-mediated induction of profibrotic cytokines from hepatocytes and hepatic stellate cells [4, 21].

In HIV/HCV coinfection, sCD163 levels are higher compared with HIV or HCV monoinfection [18, 22, 23]. However, little is known regarding the role of sCD163 in assessing liver fibrosis progression and response to antiviral therapy, and no studies have comprehensively explored the relationship between hepatic CD163 and fibrosis stage. In the present study, we evaluated sCD163 in several well-characterized cohorts with chronic HIV/HCV, HCV, or HIV infection. Importantly, we compared intrahepatic and serum CD163 levels in HCV- and HIV/HCV-infected individuals, and correlated these profiles with liver histology and treatment response. Our findings suggest involvement of macrophage activation as a key event in early stage disease, which may contribute to accelerated fibrogenesis in HIV/HCV coinfection, and implicates sCD163 as a useful biomarker for active fibrogenesis.

METHODS

Patients and Samples

We performed retrospective analyses of serum sCD163, liver CD163 expression, and liver histology using samples obtained from several multicenter studies (below). Inclusion/exclusion criteria are described in the parent publications [24, 25]. All participants provided written informed consent for future testing of stored samples under protocols approved by corresponding institutional review boards.

1. AIDS Clinical Trials Group (ACTG): Serum and liver tissue from 80 patients with HIV/HCV coinfection were obtained from the ACTG-A5071 study, a randomized controlled trial comparing pegylated interferon alpha (IFN-α) plus ribavirin with standard IFN-α plus ribavirin for 48 weeks in HIV/HCV coinfection [24]. Patients were receiving ART for HIV for a minimum of 12 weeks prior to study entry, but were treatment naive for HCV. ART continued during HCV therapy. Baseline liver biopsies and pretreatment/24 weeks posttreatment sera were obtained.
2. Liver Disease Tissue Repository (LDTR): Serum and liver tissue from 47 untreated HCV-monoinfected patients without HIV were obtained through the Massachusetts General Hospital (MGH) LDTR, which follows patients with suspected or established liver disease from any etiology.
3. SCOPE: Serum from 45 HIV and 33 HIV/HCV-infected patients (HIV and HCV treatment-naive), pre-/24 weeks post-ART were obtained from SCOPE, an observational Study of the Consequences of the HIV Protease Inhibitors Era [25].
4. Controls: 22 serum from uninfected volunteers without HCV, HBV, or HIV infection were obtained from a serum repository at MGH.

Liver Fibrosis Assessment

De-identified liver biopsies were scored for fibrosis and necroinflammation (Ishak scoring system) [26]. Aspartate aminotransferase (AST)–to-platelet ratio index (APRI) and Fibrosis 4 (FIB-4) index, a serum index based on age, AST, alanine aminotransferase (ALT), and platelet count, were used as noninvasive measures of fibrosis [27].

sCD163 Measurement

Soluble CD163 levels were measured by enzyme-linked immunosorbent assay (R&D Systems) at baseline for all groups, after anti-HCV therapy for ACTG patients, and following ART for SCOPE patients.

Liver Immunofluorescence Staining for CD163 and CD68

Formalin-fixed, paraffin-embedded liver sections were obtained from a subset of ACTG HIV/HCV (n = 16) and LDTR HCV patients (n = 13) at baseline. In brief, liver sections were deparaffinized, subjected to heat-/citrate-based antigen retrieval, blocked, and permeabilized. Slides were incubated with antihuman mouse-CD163 monoclonal antibody (LifeSpan Biosciences) and rabbit-CD68 polyclonal antibody (Abcam), followed by incubation with antimouse (AlexaFluor 488) and antirabbit (AlexaFluor 568) donkey secondary antibodies (Life Technologies). Negative controls were the primary isotype control and secondary antibody without primary antibodies. Slides were mounted with ProLong Gold antifade containing 4′,6-diamidino-2-phenylindole (Life Technologies) and imaged using the EvosFL fluorescence microscope (ThermoFisher). $CD163^+$ and $CD68^+$ cells were counted in 3–5 periportal and 5 lobular areas per biopsy using ImageJ (version 1.49, National Institutes of Health). To study the proportion of total macrophages that express CD163, results are reported as the median ratio of $CD163^+$ cells normalized to the total number of macrophages ($CD68^+$ cells).

Statistical Analysis

Statistical analyses were performed using SAS software (version 9.4). Continuous data are presented as median (interquartile range) and categorical data as frequency. Associations

were evaluated using the Spearman correlation coefficient. Group comparisons were analyzed by Mann–Whitney *U* test (unpaired) and Wilcoxon signed-rank test (paired). *P* values < .05 were considered significant.

RESULTS

Patient Characteristics

Multiple independent cohorts were used for this study (Table 1); therefore, groups were unmatched. In brief, HCV- and/or HIV-infected participants were predominantly male, while controls were predominantly female. LDTR, SCOPE, and control patients were predominantly white. LDTR patients were older ($P = .0001$). HIV RNA loads prior to ART were similar among SCOPE HIV-infected and HIV/HCV-infected patients. In contrast, lower HIV RNA loads and higher CD4 counts (both $P < .0001$) were observed in ACTG HIV/HCV-infected patients, as these individuals had been receiving ART for at least 12 weeks prior to enrollment. All groups had similar noninvasive fibrosis scores (APRI, FIB-4), and Ishak scores, where available (ACTG and LDTR). Histologic Ishak inflammation scores (only available for the ACTG cohort) were significantly associated with Ishak fibrosis scores ($r = 0.54$, $P < .0001$) (not shown).

Virologic Response to Therapy

Following IFN-based therapy for HCV, 23% (18/80) of ACTG HIV/HCV-infected ART-treated patients achieved a sustained virologic response (SVR), defined as an HCV RNA level <60 IU/mL 24 weeks posttherapy [24]. ART initiation in SCOPE patients (HIV and HIV/HCV) led to complete HIV suppression in 89% (HIV RNA median [range], 54 222 [470–500 001] copies/mL vs <50 [<50–15 750] copies/mL before and 24 weeks after ART commencement; $P < .05$), and an increase in CD4 cell count median (range) (266 [10–975] cells/mL vs 424 [89–1476] cells/mL before and 24 weeks after ART commencement, $P < .05$).

HIV/HCV Coinfection Cooperatively Increases Serum sCD163

Prior to antiviral therapy initiation, LDTR (HCV) and SCOPE (HIV and HIV/HCV) subjects had significantly higher sCD163 levels compared to uninfected controls ($P < .0001$; Figure 1). sCD163 levels were similar among HIV- and HCV-monoinfected patients; however, sCD163 levels were significantly higher in HIV/HCV coinfection compared with either HIV or HCV monoinfection ($P < .003$). In ART-treated HIV/HCV coinfection (ACTG), sCD163 levels continued to be higher than with either monoinfection (Figure 1).

sCD163 Decreases With Effective Antiviral Therapy

In HIV treatment-naive patients (SCOPE), sCD163 levels decreased significantly following 24 weeks of ART, both in HIV-infected ($P = .002$) and HIV/HCV-infected ($P = .03$) patients, but levels did not return to those observed in uninfected controls (Figure 2A). In HCV treatment-naive HIV/HCV-coinfected

Table 1. Baseline Patient Characteristics

	ACTG	LDTR	SCOPE		Controls
	HIV/HCV	HCV	HIV	HIV/HCV	Uninfected
Characteristic	(n = 80)	(n = 47)	(n = 45)	(n = 33)	(n = 22)
Age, y	45 (30–60)	59 (22–73)[a]	41 (23–53)	46 (28–58)	27 (22–62)
White race, No. (%)	38 (48)[b]	37 (79)	29 (64)	19 (58)	22 (100)
Male sex, No. (%)	65 (81)	27 (57)	38 (84)	18 (55)	8 (36)[c]
Treatment status	ART >3 mo, HCV Tx-naive	HCV Tx-naive	HIV Tx-naive	HIV/HCV Tx-naive	Tx-naive
HIV viral load, copies/mL	39 (6–22 500)[d]	NA	62 816 (4815–500 000)	48 762 (470–408 264)	NA
HCV viral load, IU/mL	1 915 000 (68 000–7 470 000)	1 280 000 (3280–25 000 000)	NA	823 164 (65 943–7 692 310)	NA
HCV genotype 1, No. (%)	61 (76)	39 (83)	NA	NA	NA
CD4 cell count/mL	470 (131–1376)[e]	NA	275 (10–661)	266 (38–975)	NA
Platelet count, $\times 10^3/\mu L$	206 (100–396)	220 (83–354)	265 (110–426)	233 (40–606)	NA
AST level, IU/mL	60 (22–271)	47 (16–285)	36 (19–162)	43 (17–281)	NA
ALT level, IU/mL	70 (24–347)	50 (13–404)	35 (11–199)	43 (11–287)	NA
APRI	0.77 (0.24–4.63)	0.61 (0.20–8.58)	NA	0.65 (0.10–12.94)	NA
FIB-4	1.48 (0.68–6.28)	1.89 (0.63–11.50)	NA	1.58 (0.35–22.68)	NA
Fibrosis Ishak stage	2 (0–6)	3 (0–6)	NA	NA	NA

Data are presented as median (range) unless otherwise indicated. Significant differences ($P < .05$): compared to each of the other groups.

Abbreviations: ACTG, AIDS Clinical Trials Group; ALT, alanine aminotransferase; APRI, aspartate aminotransferase–to-platelet ratio index; ART, antiretroviral therapy; AST, aspartate aminotransferase; FIB-4, fibrosis index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LDTR, Massachusetts General Hospital Liver Disease Tissue Repository; NA, not available/not applicable; SCOPE, Study of the Consequences of the HIV Protease Inhibitors Era; Tx, treatment.

[a]Older LDTR patients.

[b]Less frequently white ACTG patients.

[c]Predominantly female uninfected controls.

[d]Compared to SCOPE, ACTG patients had lower HIV viral load, due to ART.

[e]Compared to SCOPE, ACTG patients had higher CD4 cell count, due to ART.

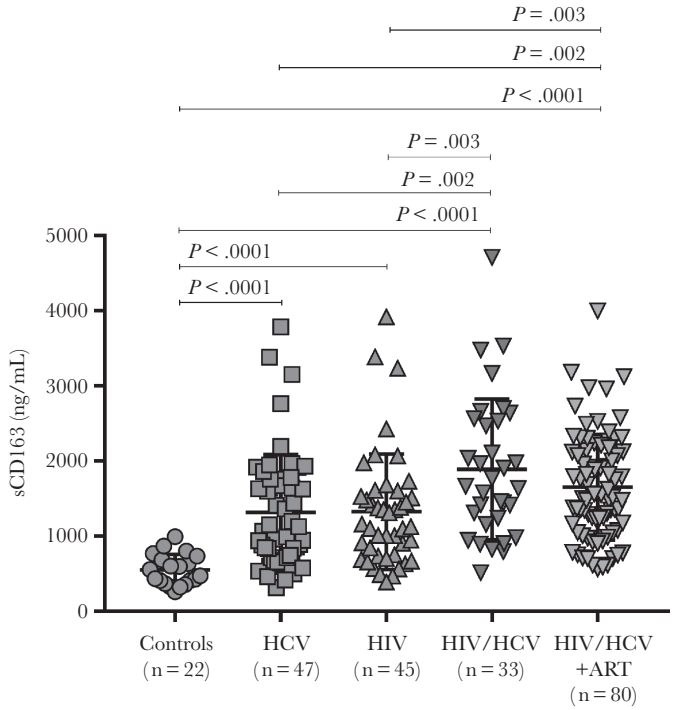

Figure 1. Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) coinfection cooperatively elevate soluble CD163 (sCD163) levels. sCD163 levels in treatment-naive subjects with chronic HCV, HIV, or HIV/HCV coinfection; antiretroviral therapy (ART)–experienced subjects with HIV/HCV coinfection; and uninfected controls. Mann–Whitney *U* test.

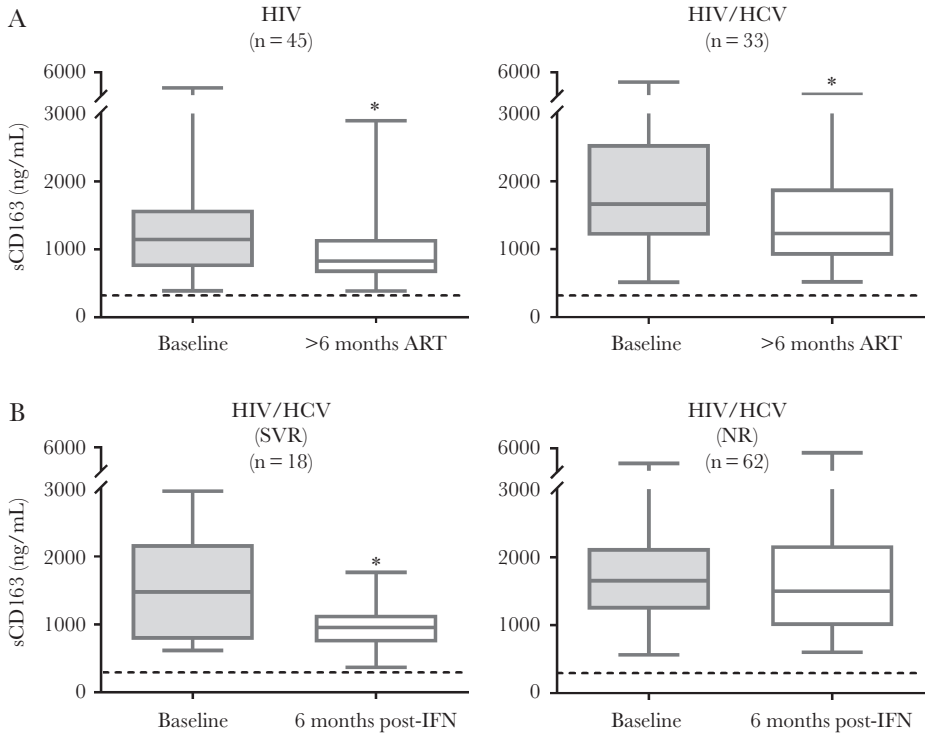

Figure 2. Soluble CD163 (sCD163) decreases upon successful antiretroviral therapy (ART) (*A*) and only in sustained virologic responders (SVR) to anti-HCV therapy (*B*). *A*, sCD163 levels in human immunodeficiency virus (HIV) and HIV/hepatitis C virus (HCV)–infected subjects, prior to ART and 6 months post-ART. Patients were HCV and HIV treatment-naive at baseline. *B*, sCD163 levels before and following completion of interferon (IFN)–based therapy in SVR and nonresponders (NR). Patients were ART-treated for >3 months, and HCV treatment-naive at baseline. Dashed lines are median sCD163 levels in uninfected controls. The box plots represent the median and interquartile ranges, and the whiskers depict the minimum and maximum of the data set. Wilcoxon signed-rank test (paired) and Mann–Whitney *U* test (unpaired). **P* < .05.

patients established on ART (ACTG), sCD163 levels decreased significantly only in SVR patients at 24 weeks after anti-HCV therapy (P = .005; Figure 2B). However, as observed post-ART, levels did not completely normalize.

sCD163 Correlates With Liver Disease

At baseline, sCD163 correlated significantly with noninvasive markers of fibrosis (APRI and FIB-4; P = .0001) in HIV/HCV-infected (ACTG/HCV treatment-naive on ART), and HCV-infected (LDTR/HCV treatment-naive) patients (Figure 3A). These findings were validated with histology: sCD163 levels correlated with Ishak fibrosis stage in both HIV/HCV-infected (ACTG: r = 0.34, P = .002) and HCV-infected (LDTR: r = 0.61, P = .0001) patients (Figure 3B). sCD163 also correlated significantly with the surrogate marker for liver inflammation ALT, in both HIV/HCV (ACTG: r = 0.36,

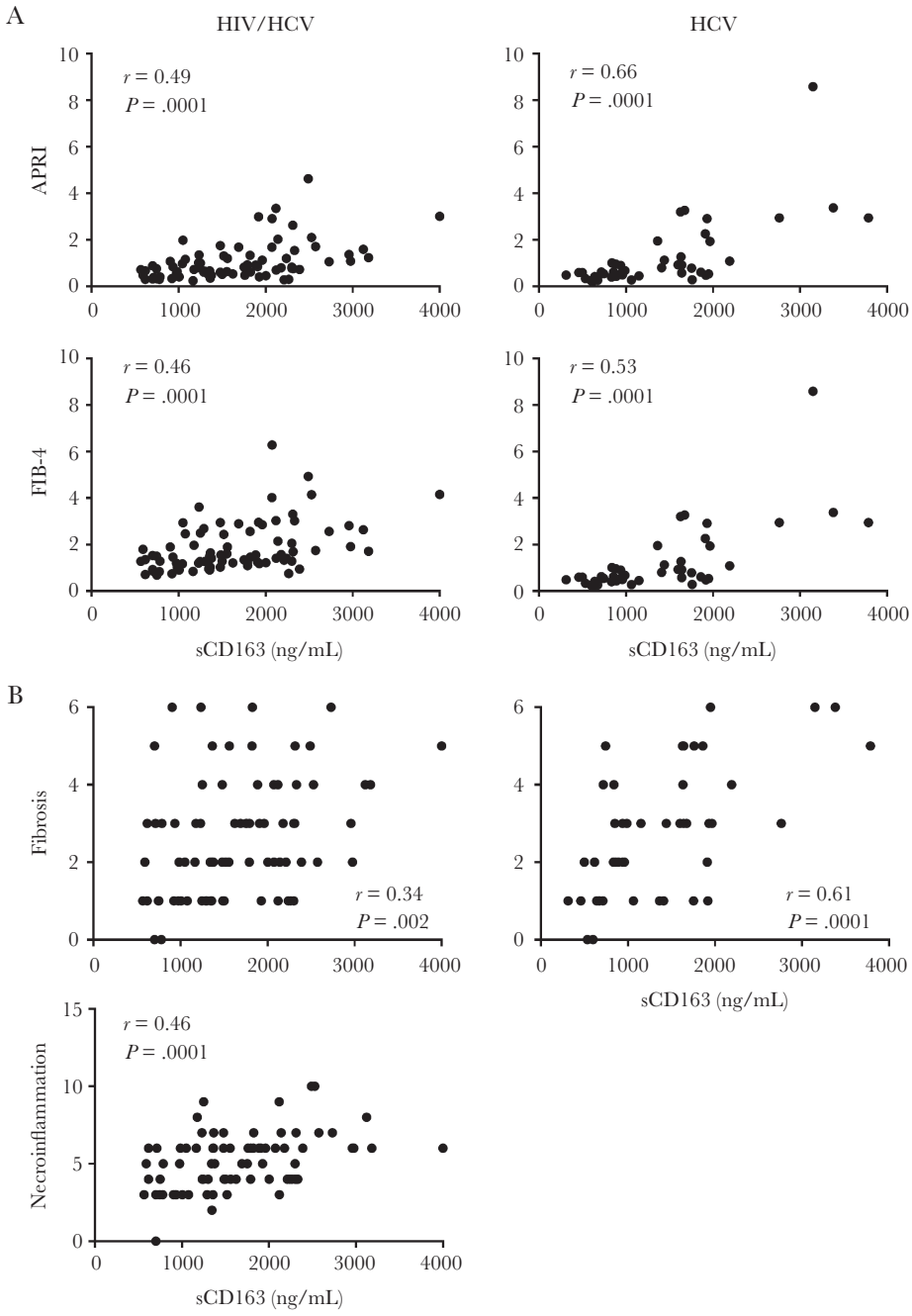

Figure 3. Soluble CD163 (sCD163) levels correlate with noninvasive assessment (*A*) and histological assessment (*B*) of liver fibrosis. Baseline sCD163 in 80 human immunodeficiency virus/hepatitis C virus (HCV) (antiretroviral therapy–treated, HCV treatment-naive) and 47 HCV (HCV treatment-naive). Histological liver fibrosis and necroinflammation assessed using Ishak scoring system [26]. Spearman correlation test. Abbreviations: APRI, aspartate aminotransferase–to-platelet ratio index; FIB-4, fibrosis index; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

P = .001) and HCV (LDTR: r = 0.64, P < .0001) infections (not shown). Furthermore, in HIV/HCV coinfection, sCD163 levels strongly correlated with liver necroinflammatory score (r = 0.42, P = .0001), similar to HCV monoinfection [15]. Ishak inflammation grading for HCV/LDTR subjects was unavailable.

sCD163 Increases With Incremental Fibrosis Stage, Peaking Prior to Cirrhosis Establishment

Intriguingly, in ART-treated HIV/HCV-infected patients (ACTG), we observed incremental rises in sCD163 levels with increasing Ishak fibrosis stage from 0 to 4 (absent fibrosis to bridging fibrosis, P < .03), whereas levels plateaued in established cirrhosis (Ishak stage 5–6) (Figure 4). In HCV-monoinfected patients (not shown), sCD163 also increased with incremental fibrosis stage in early fibrosis; however, a plateau was not observed in advanced fibrosis, although the number of patients with Ishak fibrosis scores 4–6 in this group was limited (13 in HCV vs 24 in HIV/HCV; Figure 3B).

sCD163 Is Higher in HIV/HCV Coinfection Than HCV Monoinfection in Mild to Moderate Fibrosis

Dichotomizing fibrosis measurement into mild to moderate (Ishak stage 0–3) and advanced fibrosis (bridging fibrosis/cirrhosis, Ishak stage 4–6) reflects a more clinically meaningful fibrosis assessment. According to these criteria, as expected, sCD163 levels were significantly higher in advanced fibrosis compared with mild to moderate fibrosis, in both HCV/HIV-infected (ACTG, P = .009) and HCV-infected (LDTR, P = .005) individuals (Figure 5).

We then compared the contribution of infection status, stratified according to fibrosis stage. Importantly, for patients with mild to moderate fibrosis, sCD163 levels were significantly higher in HIV/HCV coinfection compared to HCV monoinfection (P = .002) (Figure 5), while liver enzymes, APRI, FIB-4, platelet count, and HCV viral load remained comparable (not shown). In contrast, no significant difference in sCD163 levels were observed between HIV/HCV-infected and HCV-infected individuals with advanced fibrosis. These findings suggest that HIV contributes to additional sCD163 elevation specifically at early stages of fibrosis, which may contribute to accelerated liver fibrogenesis.

Periportal CD163 Liver Expression Increases With Higher Liver Fibrosis

CD68 and CD163 liver tissue co-staining was performed in a subset of 16 ACTG HIV/HCV-infected and 13 LDTR HCV-infected patients. The proportion of CD163$^+$ macrophages was enriched in periportal regions with increasing levels of fibrosis in both HIV/HCV (r = 0.55, P = .04) and HCV infection (r = 0.60, P = .03). Given that we observed increasing sCD163 levels from Ishak stage 0–4 with a plateau beyond Ishak stage 4, we restricted the analysis of liver CD163 to precirrhotic stages (Ishak stage 0–4) and found an even stronger association between the periportal proportion of CD163$^+$ macrophages and Ishak fibrosis score in HIV/HCV coinfection (r = 0.68, P = .01) but not in HCV monoinfection, although fewer patients with HCV monoinfection were included in this subanalysis (HCV, n = 11 vs HIV/HCV, n = 14; Figure 6A). As there were only 2 subjects with cirrhosis in each group, we were unable to evaluate CD163 expression in established cirrhosis. Interestingly, in patients without established cirrhosis, the proportion of liver CD163$^+$ macrophages was significantly higher in HIV/HCV coinfection (n = 14) compared with HCV monoinfection (n = 11)

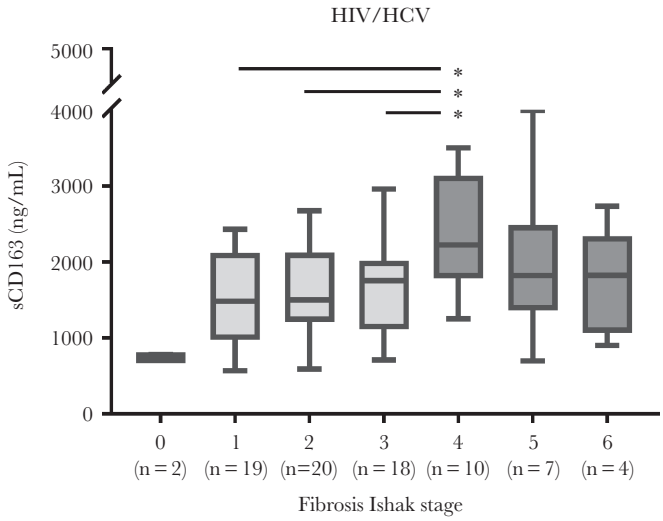

Figure 4. Soluble CD163 (sCD163) levels rise with increasing fibrosis stage, peaking prior to cirrhosis establishment. Baseline sCD163 per Ishak fibrosis stage in human immunodeficiency virus (HIV)/hepatitis C virus (HCV)–coinfected patients (antiretroviral therapy–treated, HCV treatment-naive). Light bars: stages 0–3 (mild to moderate fibrosis), dark bars: stages 4 (bridging fibrosis) and 5–6 (cirrhosis). The box plots represent the median and interquartile ranges, and the whiskers depict the minimum and maximum of the data set. Mann–Whitney *U* test. *P < .05.

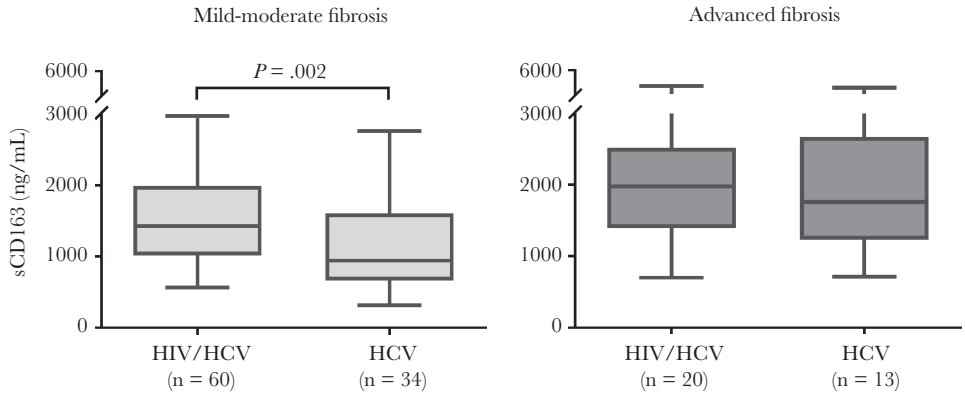

Figure 5. Soluble CD163 (sCD163) levels are higher in human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfection than in HCV monoinfection in mild to moderate fibrosis but not in advanced fibrosis. Baseline sCD163 levels in HIV/HCV-coinfected (antiretroviral therapy–treated, HCV treatment-naive) and HCV-monoinfected (HCV treatment-naive) patients stratified by liver fibrosis stage: mild to moderate fibrosis (Ishak stage 0–3) and advanced fibrosis (bridging fibrosis/cirrhosis, Ishak stage 4–6). The box plots represent the median and interquartile ranges, and the whiskers depict the minimum and maximum of the data set. Mann–Whitney *U* test.

(P = .049; Figure 6B), and a similar trend was also observed when restricted to mild to moderate fibrosis only (Ishak stage 0–3; P = .08) (not shown), mirroring sCD163 findings. No correlation between liver CD163 and sCD163 levels was observed.

DISCUSSION

Availability of liver tissue in early-stage liver disease without clinical indication has been largely lacking owing to potential complications of liver tissue sampling. Therefore, previous studies of macrophages in HIV/HCV coinfection have relied on noninvasive fibrosis assessment, which are suboptimal in discriminating intermediate stages of fibrosis. We accessed valuable serum and paired liver tissue repositories in HCV- and HIV/HCV-infected patients, allowing interrogation of relationships between fibrosis stage and serum/liver CD163, a surrogate marker for macrophage activation and M2 polarization.

We observed higher sCD163 levels in HIV/HCV coinfection compared to HIV or HCV monoinfection. Viral control significantly decreased sCD163 levels; however, levels did not fully normalize post-ART or post–HCV therapy (consistent with previous reports [14, 18, 19, 23, 28]). These findings suggest that both HIV and HCV cooperatively elevate sCD163, likely due to both interactive and additive effects in HIV/HCV coinfection. They further implicate residual macrophage activation despite viral suppression, possibly from persistent HIV-related immune activation despite adequate HIV suppression [29]. The failure of sCD163 levels to return to normal following successful anti-HCV therapy (IFN-based/IFN-free) in HCV monoinfection has also been described [15, 18], perhaps attributable to macrophage involvement in reparative/resolution and fibrolytic phases of chronic liver disease, perhaps reflecting as-yet unresolved fibrosis.

We also observed that sCD163 levels strongly correlated with both liver necroinflammation and fibrosis in ART-treated HCV/

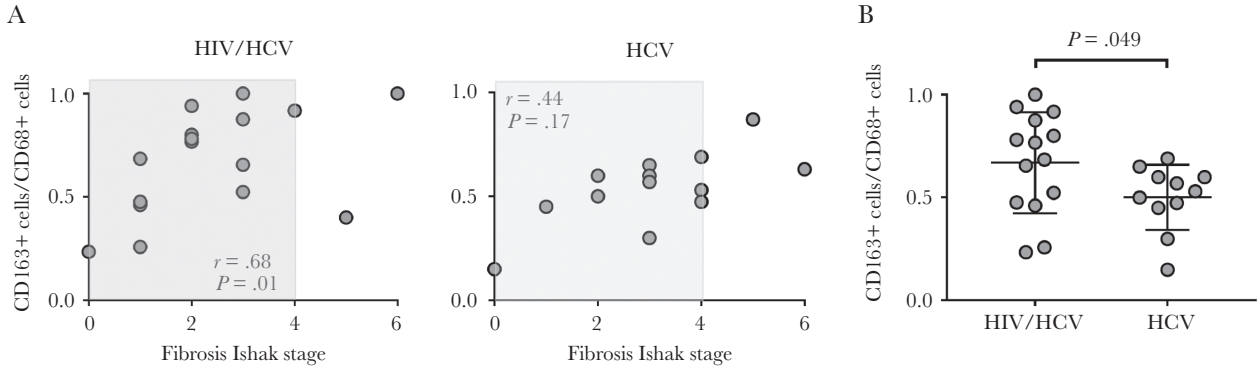

Figure 6. Periportal proportion of CD163⁺ macrophages is enriched with incremental liver fibrosis and higher in human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfection compared with HCV monoinfection. *A*, Liver tissues from 16 HIV/HCV-infected (antiretroviral therapy–treated, HCV treatment-naive) and 13 HCV-infected (treatment-naive) patients were co-stained for CD163/CD68. While the proportion of CD163⁺ macrophages enriched in periportal regions with increasing liver fibrosis in both HIV/HCV (r = 0.55, P = .04) and HCV liver tissue (r = 0.60, P = .03) (not shown), this enrichment was stronger in HIV/HCV liver tissue prior to cirrhosis (Ishak stage 0–4). Spearman correlation test. *B*, Proportion of periportal CD163⁺ macrophages in 14 HIV/HCV-infected and 11 HCV-infected patients without established cirrhosis (Ishak stage 0–4). Mann–Whitney *U* test.

HIV coinfection, validating previous associations of sCD163 noninvasive fibrosis markers in HIV/HCV coinfection [19, 22]. Our new finding is that periportal macrophage CD163 expression was enriched with increasing liver fibrosis degree, both in HIV/HCV and HCV infection. Given that extracellular matrix deposition begins in portal regions and expands toward the hepatic lobule center as fibrosis progresses, our finding implicates that $CD163^+$ macrophages specifically contribute to liver fibrosis. It is likely that persistent liver inflammation results in continual macrophage sCD163 shedding, further exacerbated by increased CD163 expression upon macrophage maturation and M2 polarization (anti-inflammatory but profibrotic phenotype). The lack of a direct correlation between sCD163 and liver CD163 may be due to the limited number of paired samples, and that sCD163 reflects total CD163 cleaved from liver and nonliver sources. Nonetheless, both serum and portal CD163 significantly correlated with liver fibrosis.

Another new finding is the pattern of sCD163 levels observed in HIV/HCV coinfection, rising in mild to moderate fibrosis accompanied by enrichment of periportal $CD163^+$ macrophages, peaking prior to establishment of cirrhosis without further increase once cirrhosis is present. This finding implicates sCD163 as a dynamic marker of early fibrogenesis rather than total accumulated fibrosis. The lack of increasing sCD163 levels in established cirrhosis may reflect an equilibrium between ongoing inflammation, sCD163 cleavage, and subsequent M2 polarization. Furthermore, we observed that sCD163 levels were higher in HIV/HCV than HCV infection in patients with mild to moderate fibrosis; however, levels were comparable in advanced fibrosis irrespective of infection states. A similar trend was observed in the liver in early fibrosis, with greater frequency of periportal $CD163^+$ macrophages in HIV/HCV compared to HCV infection. It is likely that HIV-mediated monocyte/macrophage activation patterns enhance inflammation, inducing greater infiltration of inflammatory cells, and predispose them to greater responsiveness during chronic inflammatory-mediated liver injury such as HCV, leading to intensified anti-inflammatory feedback responses, and subsequently greater profibrotic responses and M2 polarization, all contributing to higher sCD163 levels. While paired longitudinal observations are required, our data strongly suggest that activation of $CD163^+$ macrophages play a critical role in driving hepatic fibrogenesis, and that sCD163 may be useful as a dynamic longitudinal marker of fibrosis progression, which may identify individuals at highest risk of accelerated disease progression in the context of HIV/HCV coinfection.

Importantly, macrophage recruitment and activation is pivotal in inflammation and fibrosis in cardiovascular disease and gut-associated tissue fibrosis in HIV [30–33]. While HIV does have direct effect on many cell types in the liver, alone this is insufficient to develop cirrhosis in the majority, and a second hit with additional liver-specific injury is likely required, such as HCV infection, allowing cooperative immune activation of macrophages leading to greater liver fibrogenesis.

Our study strengths include the 3 independent, well-characterized cohorts in both HIV/HCV and HCV infection, allowing the first comprehensive evaluation of serum sCD163 and paired CD163 liver expression in relation to liver histology, the gold standard of fibrosis assessment. Importantly, we further validated histology with noninvasive fibrosis markers, confirming they can be applied to assess fibrogenesis when interpreting sCD163 in HIV/HCV coinfection. Limitations include unmatched demographic and patient characteristics between the retrospective cohorts, the small number of high-quality liver biopsies for CD163 staining, and the relatively small number of patients within each fibrosis stage. Therefore, our findings need to be validated in larger and longitudinal cohorts. Additionally, our study focused on the activation and polarization state of macrophages, as determined by CD163. Although sCD163 is an overly simplistic macrophage activation marker, it is biologically plausible and a readily available biomarker that could be employed in the clinical arena. However, macrophages are extremely heterogeneous and plastic, and complex changing phenotypes exist in any given disease state. More in-depth macrophage characterization at the transcriptome and protein levels may provide more detailed insights into the complex function and phenotypes of macrophages that drive fibrogenesis.

In conclusion, our new findings reveal increasing sCD163 levels accompanied by periportal $CD163^+$ macrophage enrichment in mild to moderate fibrosis, but not established cirrhosis, implicating sCD163 as a dynamic biomarker of fibrogenesis rather than total accumulated fibrosis. Therefore, sCD163 kinetics have strong potential as a clinical tool to determine fibrosis progression rate, which may in turn identify individuals likely to have accelerated fibrosis progression and those who may benefit from more intensive follow-up and earlier antiviral therapeutic intervention to prevent further fibrosis accumulation. Additionally, our results support the hypothesis that HIV-related macrophage activation contributes to accelerated liver fibrogenesis, implicating macrophages as potential therapeutic targets in HIV/HCV coinfection. A better understanding of the role of macrophages and macrophage-associated molecules may reveal novel targets for future therapeutic development to ameliorate and prevent fibrosis progression not only in HIV-infected individuals, but also in other fibrotic inflammatory diseases.

Notes

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