HHS Public Access

Author manuscript

Alcohol Clin Exp Res. Author manuscript; available in PMC 2021 May 01.

Published in final edited form as:

Alcohol Clin Exp Res. 2020 May ; 44(5): 1075–1087. doi:10.1111/acer.14331.

Persistent Hyperactivation of Endothelial Cells in Patients with Alcoholic Hepatitis

Ying Xia[1,2,3], **Jing Yang**[2,3], **Arun Sanyal, MD**[4], **Vijay Shah, MD**[5], **Naga Chalasani, MD**[6], **Qigui Yu, PhD/MD**[3,*], **Xiaoqun Zheng, PhD**[1,2,*], **Wei Li, PhD**[3,*]

[1]Department of Clinical Laboratory, the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

[2]School of Laboratory Medicine and Life Sciences, Wenzhou Medical University, Wenzhou, Zhejiang, China

[3]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN

[4]Division of Gastroenterology and Hepatology, Department of Medicine, Virginia Commonwealth University, Richmond, VA 23298

[5]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN 55905

[6]Division of Gastroenterology and Hepatology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202-5175

Abstract

Background: Alcoholic hepatitis (AH) is a severe inflammatory liver disease that develops in some heavy drinkers. AH patients have intense hepatic infiltration of leukocytes. Upregulation of cell adhesion molecules (CAMs) upon endothelial cell (EC) activation plays an important role in leukocyte trans-endothelial migration. CAMs can shed from EC cell surface and accumulate in the blood, serving as soluble markers for EC activation. In this study, we examined the impact of heavy drinking on expression of soluble forms of EC activation markers (CD146, ICAM-1, VCAM-1, and VEGF-A) and the effect of alcohol abstinence on the reversal of these abnormalities in patients with and without AH.

Methods: ELISA and multiplex immunoassays were used to measure soluble EC markers in plasma samples from 79 AH patients, 66 heavy drinkers without overt liver disease (HDC), and 44 healthy controls (HC) at baseline, 31 AH patients and 30 HDC at 6-month follow-up, and 18 AH patients and 25 HDC at 12-month follow-up.

Results: At baseline, the 4 soluble markers were significantly upregulated in AH patients compared to HDC and HC; whereas only sVCAM-1 was elevated in HDC relative to HC. At

[*]Address correspondence Dr. Wei Li (wl1@iupui.edu) or Dr. Qigui Yu (andyu@iupui.edu), Department of Microbiology and Immunology, Indiana University School of Medicine, Medical Science Building MS420, 635 Barnhill Drive, Indianapolis, IN 46202, Tel: 317-274-4653, Fax: 317-278-3331, or Dr. Xiaoqun Zheng (jszhengxq@163.com), School of Laboratory Medicine and Life Sciences, Wenzhou Medical University, Wenzhou, Zhejiang, China, Tel: 0577-86699196, Fax: 0577-86689779.

CONFLICT OF INTEREST

The authors declare no conflicts of interest that pertain to this manuscript.

follow-ups, plasma levels of CD146, VCAM-1, and VEGF-A remained higher in AH patients, even for those who stopped drinking. These dysregulated markers correlated with AH disease severity, clinical parameters, and several soluble factors.

Conclusions: The levels of soluble CD146, ICAM-1, VCAM-1, and VEGF-A were highly elevated in AH patients, and alcohol abstinence did not completely reverse these abnormalities.

Keywords

alcoholic hepatitis; endothelial activation; adhesion markers; soluble forms; longitudinal study

INTRODUCTION

Heavy alcohol consumption causes liver damage through impacts of highly toxic chemicals and oxidative stress produced by ethanol and its metabolites (Younossi et al., 2018), triggers pro-inflammatory cascades (Wang et al., 2012, Gao et al., 2019, Dhanda and Collins, 2015), and leads to a range of severe alcoholic liver disease (ALD), including alcoholic hepatitis (AH), fibrosis/cirrhosis, and hepatocellular carcinoma (HCC) (O'Shea et al., 2010). A portion of the heavy drinkers develop AH, a severe inflammatory liver disease with significant morbidity and mortality, for which few effective medical treatments are available (Chayanupatkul and Liangpunsakul, 2014, Saberi et al., 2016). AH is characterized by hyper-inflammation and infiltration of the liver tissue by a range of inflammatory leukocytes, including neutrophils, macrophages, and lymphocytes (Bautista, 2002, Sandahl et al., 2014, Haydon et al., 2002). Although the mechanisms by which those inflammatory cells are recruited to the liver in AH are not fully understood, inflammatory cells must first adhere to endothelial cells (ECs) before they transmigrate from circulation into tissue. The binding of leukocytes to vascular endothelium is critical for the inflammatory process, which is regulated by EC activation and cell adhesion molecules (CAMs) expressed on ECs and leukocytes (Muller, 2003). Inflammatory cytokines/chemokines and growth factors induce upregulation of CAMs on ECs and inflammatory cells, which promotes trans-endothelial migration of the later (Langer and Chavakis, 2009, Shaik-Dasthagirisaheb et al., 2013).

Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are key CAMs that mediate the firm adhesion of leukocytes to endothelium in physiological and pathological inflammatory processes, such as experimental cutaneous inflammation and atherosclerotic plaques (Norris et al., 1991, Poston et al., 1992). CD146, a CAM member, is expressed mainly in the endothelial tight junction and plays an important role in cell-cell cohesion, permeability, and monocyte trans-endothelium migration (Bardin et al., 2001, Bardin et al., 2009). CD146 is also expressed on approximately 2% of peripheral blood T cells from healthy humans (Elshal et al., 2007, Elshal et al., 2005, Pickl et al., 1997). Upregulation of CD146 on T cells has been observed in multiple human diseases, such as connective tissue diseases, musculoskeletal diseases, multiple sclerosis, and graft-versus-host disease (Li et al., 2016, Hadjinicolaou et al., 2013, Dagur et al., 2010, Larochelle et al., 2012).

As inflammation progresses, the CAMs that mediate aberrant interactions between inflammatory cells and the vascular endothelium can be shed from cell surface and

accumulate in the blood as soluble isoforms. Studies have suggested that several soluble CAMs could be explored as potential prognostic biomarkers and monitors of disease activity (Rothlein et al., 1991, Sasseville et al., 1992, Yuan et al., 2013). Elevated levels of soluble CD146 (sCD146) are observed in cirrhotic patients of alcoholic, viral, and other etiology and could serve as a sensitive soluble marker to predict decompensation of cirrhosis (Nomikou et al., 2015). Soluble CAMs can also play functional roles in cell trafficking and angiogenesis. For example, soluble VCAM-1 (sVCAM-1) induces chemotaxis of human ECs in *in vitro* assays (Koch et al., 1995). Soluble CD146 is known to have both chemotactic and angiogenetic properties (Harhouri et al., 2010). Soluble CD146 can also increase monocyte transmigration through endothelial cells, and the levels of sCD146 could influence the magnitude of monocyte transmigration (Bardin et al., 2009). In addition to CAMs, some growth factors can also affect endothelial function. For example, vascular endothelial cell growth factor-A (VEGF-A) is a growth factor that can potently stimulate endothelial proliferation and increase vascular permeability (Kevil et al., 1998).

Upregulation of CAMs on the vascular ECs is one of the critical indicators of endothelial dysfunction (Fries et al., 1993). In addition, soluble CAMs in circulation can also serve as a marker for EC dysfunction. Excessive alcohol consumption is associated with endothelial dysfunction and increased risk for development of cardiovascular diseases (Di Gennaro et al., 2007, Bau et al., 2007). In fact, sICAM-1 and sVCAM-1 levels are elevated in patients of different liver diseases with excess alcohol ingestion, including AH and alcoholic cirrhosis (Adams et al., 1994). However, the relationship between the vital CAM CD146 and alcohol consumption, as well as the expressions of sCD146 and membrane-bound CD146 on T cells in chronic heavy drinkers remain unclear. Although several researches have investigated the relationship between alcohol-related disease and CAMs, clinical studies focusing on expression of sCD146, sICAM-1, sVCAM-1 and VEGF-A in chronic heavy drinkers with or without AH and the influence of alcohol abstinence on CAM dysregulation are still limited.

The present study compared expression of soluble EC activation markers including sCD146, sICAM-1, sVCAM-1, and VEGF-A and membrane-bound CD146 on peripheral blood T cells in a large cohort of heavy drinkers with AH, heavy drinkers without overt clinic evidence of liver disease (HDC), and healthy controls (HC). We correlated plasma levels of these EC activation markers with patients' clinical parameters and plasma levels of inflammatory cytokines and growth factors that are related to EC activation and function. Cross-sectional and longitudinal analysis were performed to determine the impact of long-term alcohol abstinence on reversing abnormalities in plasma levels of these EC activation makers. We found that levels of sCD146, sICAM-1, sVCAM-1, and VEGF-A, which could be considered as surrogates for EC dysfunction, were highly elevated and were associated with AH disease severity, and that alcohol secession did not completely reverse these abnormalities.

MATERIALS AND METHODS

Study Subjects

The alcoholic study subjects (79 AH patients and 66 HDC at baseline, 31 AH patients and 30 HDC at 6-month follow-up, and 18 AH patients and 25 HDC at 12-month follow-up)

were part of the participants recruited to the multicenter prospective Translational Research and Evolving Alcoholic Hepatitis Treatment 001 study (TREAT 001, NCT02172898), which also serves as the study cohort for our previous studies (Li et al., 2017, Li et al., 2019). The inclusion and exclusion criteria as well as detailed definitions of AH and HDC were previously published (Liangpunsakul et al., 2016). HDC were individuals with a comparable history of heavy drinking but had no overt clinical liver disease. Both AH and HDC subjects were advised to abstain from alcohol and were followed-up at 6 months and 12 months or until death. Table 1 summarizes the demographic and clinical characteristics and drinking patterns of the alcoholic cohort. This study was performed with the approval of the Institutional Review Boards at Indiana University School of Medicine, Mayo Clinic, and Virginia Commonwealth University. Blood samples were drawn after participants provided a written informed consent form.

Blood Samples

Peripheral blood was collected in heparin-coated tubes (BD Biosciences, Franklin Lakes, NJ) and separated into plasma and peripheral blood mononuclear cells (PBMCs), which were stored at −80°C until analysis. Plasma and PBMCs from 46 age- and sex- matched HC were also used in the study.

Enzyme-linked Immunosorbent Assay (ELISA), lipopolysaccharide (LPS) Detection, and Multiplex Immunoassay

Levels of sCD146, sICAM-1, and sVCAM-1 in plasma samples were quantified using the Human CD146 DuoSet ELISA Kit, the Human ICAM-1 DuoSet ELISA Kit, and the Human VCAM-1 DuoSet ELISA Kit (all from R&D Systems, Minneapolis, MN), respectively. LPS amounts were determined using the LAL assay as previously descried (Li et al., 2019). Plasma levels of soluble CD163, CD14, and LBP were quantified using the Human CD163 Quantikine Kit, the Human CD14 Quantikine Kit, and the Human LBP DuoSet ELISA Kit (all from R&D Systems), respectively. Plasma concentration of VEGF-A and 44 other cytokines/chemokines/growth factors were measured by multiplex immunoassays as previously descried (Li et al., 2019).

Flow Cytometry

For cell surface staining of CD146 on peripheral blood T cells, PBMCs from AH patients, HDC, and HC were stained with a fixable viability dye (eBioScience, ThermoFisher Scientific) to exclude dead cells for analysis. Cells were then stained with fluorochrome-conjugated antibodies against CD3, CD4, CD19, CD14, and CD146. All antibodies were purchased from BioLegend (San Diego, CA). For intracellular cytokine staining, PBMCs from AH patients and HC were stimulated with 100 ng/ml Phorbol 12-myristate 13-acetate (PMA) and 1 μm ionomycin (both from Sigma, MA) in the presence of GolgiStop (BD Biosciences, CA) for 6 hours. Cells were collected and stained with a fixable viability dye, followed by fluorochrome-conjugated antibodies against CD3, CD4, CD8, and CD146. Cells were then fixed in 2% paraformaldehyde, permeabilized with the Cytofix/Cytoperm reagents (BD Biosciences, CA), and stained with antibodies against fluorochrome-conjugated IL-17 (BioLegend, CA). Stained cells were subsequently acquired using a BD LSRFortessa flow

cytometer (BD Biosciences, CA). FlowJo v10 software (Tree Star, San Carlos, CA) was used to analyze flow data.

Statistical Analysis

Chi-square test was performed to compare groups of categorical variables. Mann-Whitney test and Kruskal-Wallis test with Dunn's corrections were used to calculate differences in continuous variables between 2 groups and among 3 groups in cross-sectional analysis, respectively. The linear association of the EC activation markers with clinical parameters and other soluble factors was analyzed using the Spearman correlation test. Multiple linear regression analyses were performed to identify variables to predict plasma levels of EC activation markers. Friedman rank sum test with Dunn's corrections was used to calculate the differences in longitudinal analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the Study Cohort

To study the role of EC activation markers in the inflammatory process of AH, we analyzed peripheral blood samples obtained from 79 AH patients, 66 HDC individuals at the time of recruitment, and 46 HC, as well as 31 AH patients and 30 HDC at 6-month follow-up, and 18 AH patients and 25 HDC at 12-month follow-up. Among the 79 AH patients, 42 (53%) were treated with the corticosteroid prednisone alone (n=29) or together with pentoxifylline (n=13). Table 1 summarizes the demographic and clinical characteristics of the participants at enrollment and follow-ups. AH patients had higher MELD score, liver biochemistries (AST, ALT, and total bilirubin) and longer prothrombin time than HDC at enrollment and follow-ups. Levels of baseline creatinine were not different between HDC and HC, whereas total bilirubin, AST, and ALT levels were slightly higher in HDC than HC. At follow-ups, both AH patients and HDC had much fewer drinks and their liver biochemistries greatly improved but not normalized in AH patients and remained unchanged for HDC. For patients who reported complete alcohol abstinence at follow-ups, the MELD score, total bilirubin, AST, and prothrombin time were still higher in AH patients than HDC (Supporting Table S1).

Plasma Levels of Soluble EC Activation Markers Were Highly Upregulated in AH Patients

To assess the EC activation marker profiles in the peripheral blood from AH patients, HDC, and HC, we used ELISA and multiplex immunoassays to measure plasma levels of CD146, VCAM-1, ICAM-1, and VEGF-A. Compared to HC and HDC, AH patients had significantly higher baseline levels of sCD146, sICAM-1, sVCAM-1, and VEGF-A (Fig. 1A–D). HDC also had higher levels of soluble VCAM-1 than HC (Fig. 1C). These data are additionally summarized as median and 1st and 3rd quartile in Supporting Table S2 and as fold change in median concentrations in Supporting Table S3. Soluble CD146 was the most highly upregulated marker in AH patients, followed by sVCAM-1, sICAM-1 and VEGF-A.

Next, we analyzed follow-up samples from AH patients and HDC, which included both abstinent and non-abstinent subjects. Levels of EC activation markers tended to normalize in AH patients, and remained similar in HDC. However, at 6-month follow-up, all 4

upregulated markers (sCD146, sVCAM-1, sICAM-1, and VEGF-A) were still elevated in AH patients relative to HDC. At 12-month follow-up, sCD146 and sVCAM-1 remained higher, while sICAM-1 and VEGF-A became similarly expressed between AH patients and HDC (Fig. 1A–D, Supporting Table S2 and Supporting Table S3). Together, our data indicated that plasma levels of soluble EC activation markers were highly upregulated in AH patients and that there was an incomplete reversal of those abnormalities at follow-ups.

Association of Plasma Levels of EC Activation Markers with Clinical Profiles and Inflammatory Factors in AH Patients

To explore whether soluble EC activation markers might be related to pathogenesis of AH, we determined their correlation with AH disease severity as represented by MELD score, creatinine, liver biochemical measurements (total bilirubin, AST, ALT), prothrombin time, and mDF score in AH patients. Baseline and follow-up data were pooled for these analyses, and the significant correlations with a correlation coefficient > 0.25 are listed in Table 2. All 4 upregulated EC activation markers positively correlated with the MELD score. Additional correlations were found between the EC markers and the mDF score (sCD146 and sVCAM-1), creatinine (sICAM-1 and VEGF-A), total bilirubin (sCD146 and sICAM-1), AST (sCD146 and VEGF-A), and prothrombin time (sCD146, sVCAM-1, and VEGF-A). Among the 79 AH patients, 13 died within 90 days after recruitment. We did not find significant difference in levels of the 4 soluble markers between the survivors and the deceased (data not shown). Together, our data suggested that hyper-activation of endothelium might be involved in liver damage.

Bacterial translocation (BT) is one of the critical mechanisms in the development of ALD (Ponziani et al., 2018). Therefore, we also determined whether plasma levels of EC activation markers correlated with 4 surrogate markers of BT: LPS, soluble CD163 (sCD163), soluble CD14 (sCD14), and LBP. The levels of the BT markers were shown in Supporting Table S4 and the correlations were summarized in Table 2. Plasma levels of LPS, sCD163, sCD14, and LBP positively correlated with sICAM-1. Some of them also showed positive correlations with sCD146 (sCD163) and sVCAM-1 (LPS and sCD163).

Cytokines and growth factors are known to regulate EC activation (Mantovani et al., 1992, Sulpice et al., 2009). Next, we analyzed the correlations between plasma levels of EC activation markers and inflammatory/growth factors in AH patients. The results were summarized in Table 2, and the levels of correlated inflammatory/growth factors were shown in Supporting Table S5. Circulatory levels of all 4 soluble EC activation markers correlated with the pro-inflammatory cytokine IL-8. In addition, they had correlations with multiple other cytokines/chemokines, including IL-6, MCP-1, or IFN-γ. Interestingly, the plasma levels of sICAM-1 and VEGF-A positively correlated with the anti-inflammatory factors (IL-9, IL-10 and IL-1RA). Furthermore, there were also correlations between EC activation markers and growth factors (GRO-α, HGF and SDF-1α). In addition, levels of sCD146 and sVCAM-1 highly correlated with each other ($r = 0.50$; $p < 0.001$). These results suggested that stimulation by inflammatory factors and growth factors could contribute to dysregulation of EC activation.

Interestingly, although male and female AH patients had similar heavy alcohol consumption at enrollment, female had significantly higher plasma levels of ICAM-1, whereas sICAM-1 levels were not different between male and female HDC (Fig. 2). The ratio of median sICAM-1 levels between AH patients and HDC in the male and female groups was 3.6 and 5.1, respectively. On the other hand, levels of sCD146, sVCAM-1, and VEGF-A were not different between the genders (data not shown). To clarify which factors were able to predict the plasma levels of ICAM-1, multiple linear regression analysis was performed. All nine continuous variables (age, ALT, AST, MELD score, INR, mDF score, total bilirubin, creatinine, and CRP) and one categorical variable (gender) were included in the analysis. Multiple linear regression analysis with stepwise method resulted in an optimized model consisting of gender, creatinine, CRP, and the constant. In addition to gender, creatinine and CRP were the statistically significant variables to predict plasma ICAM-1 concentration among the 10 variables (Supporting Table S6).

We also determined the effect of the therapeutical treatment on EC activation in AH patients by comparing plasma levels of EC activation markers in patients treated with prednisone/pentoxifylline and untreated AH patients (Supporting Table S7). Treated patients had higher mDF score, MELD score, total bilirubin level, and longer prothrombin time than untreated patients at enrollment. Among the 4 upregulated EC activation markers, only sICAM-1 levels were significantly higher in treated patients, and the levels of the other 3 markers were not different. At 6- and 12-month follow-up, there were no differences in soluble levels of EC activation markers between the treated and untreated abstinent AH patients. Our data suggested that steroid/pentoxifylline treatment did not appear to have long-term EC functional benefits in AH patients.

Dysregulation in Soluble EC Activation Markers Was Not Completely Reversed in AH Patients by Alcohol Abstinence

To determine the impact of alcohol abstinence on recovery of EC activation marker abnormalities in AH patients, we did cross-sectional analysis on follow-up samples between abstinent AH patients and HDC, and the results are shown in Figure 3 and Supporting Table S8. All 4 EC activation markers remained higher in AH patients than HDC at 6-moth follow-up. Furthermore, compared to HDC, AH patients at 12-month follow-up still had elevated plasma levels of sCD146, sVCAM-1, and VEGF-A. Those data provided additional evidence that alcohol abstinence did not lead to complete recovery from hyper-activation of EC in AH patients.

Next, we performed longitudinal analysis of soluble levels of EC activation markers in AH patients and HDC who were completely abstinent at follow-ups. Except sVCAM-1, the other 3 soluble markers in AH patients were significantly reduced at the 12-month follow-up (Fig. 4, and Supporting Fig. S1). For HDC, elevated sVCAM-1 was downregulated at 12-month follow-up, whereas sCD146, sICAM-1, and VEGF-A remained unchanged throughout the study (Fig. 4, and Supporting Fig. S1). Taken together, our results indicated that the upregulated EC activation markers recovered greatly, but not completely, in abstinent AH patients.

Dysregulated Membrane-bound CD146 on CD4 T cells from AH Patients

It has been shown that CD146 is also expressed on approximately 2% of $CD4^+$ T cells in healthy humans and that CD146 expression on $CD4^+$ T cells is upregulated in several inflammatory diseases (Li et al., 2016, Hadjinicolaou et al., 2013, Dagur et al., 2010, Larochelle et al., 2012, Elshal et al., 2005, Elshal et al., 2007). We then measured expression levels of the membrane-bound CD146 on T cells in PBMC samples from AH patients, HDC and HC. The results are shown in Fig. 5C. Compared to HDC and HC, AH patients had significant higher expression of membrane CD146 on $CD4^+$ T cells (median % of $CD146^+CD4^+$ T cells in HC, AH and HDC were 1.28, 4.41 and 0.96, respectively), but not on $CD8^+$ T cells. Many studies have linked CD146 expression with the production of the pro-inflammatory Th-17 cytokine IL-17 (Brucklacher-Waldert et al., 2009, Dagur et al., 2011, Larochelle et al., 2012). Since CD146+ T cells have shown increased IL-17 production, we next explored whether IL-17 increased in AH patients. Consistent with previous studies, we found that AH patients had higher expression of IL-17 on $CD4^+$ T cells than HC (Fig. 5D).

DISCUSSION

In the present study, cross-sectional and longitudinal analyses were performed to compare 4 circulatory soluble EC activation markers from a large cohort of AH patients, matched HDC, and healthy controls (Table 1). We observed that AH patients had elevated plasma levels of sCD146, sICAM-1, sVCAM-1, and VEGF-A, and an increased frequency of CD146-expressing $CD4^+$ T cells (Fig. 1 and Fig 5). On the other hand, only sVCAM-1 was upregulated in HDC compared to HC. We showed that plasma levels of EC activation markers positively correlated with disease severity, multiple upregulated BT markers (LPS, sCD163, sCD14, and LBP), and pro-inflammatory cytokines/chemokines, growth factors (IFN-γ, IL-6, IL-8, IL-18, IL-23, IP-10, MCP-1, GRO-α, HGF, and SDF-1α) in AH patients (Table 2). We also found that alcohol abstinence significantly improved but did not fully reverse EC activation abnormalities in AH patients (Fig. 3 and Fig. 4).

Excessive recruitment of leukocytes to the endothelium and subsequent transmigration from circulation to tissues are the most common and important characteristics of inflammation (Osborn, 1990). CAMs, such as ICAM-1 and VCAM-1, play a pivotal role in facilitating leukocyte transmigration and are associated with the development of ALD through recruitment of neutrophils and mononuclear cells to the liver (Sacanella and Estruch, 2003). In addition to expression on activated EC and leukocytes, CAMs are also released into the circulation. Circulating levels of VCAM-1 likely reflect endothelial function *in vivo* (Hwang et al., 1997). Although ICAM-1 is a vascular adhesion molecule, it is also widely expressed on activated lymphocytes, which might be another source of sICAM-1 (Rothlein et al., 1991). The levels of sICAM-1 and sVCAM-1 are increased in alcoholic cirrhosis and AH (Adams et al., 1994). Here, we confirmed that plasma levels of ICAM-1 and VCAM-1 were upregulated in AH patients. We also found that plasma levels of ICAM-1 were higher in female AH patients than in male patients. In addition, we found that gender could serve as an independent predictor of sICAM-1 level in our cohort. Women tend to have higher blood alcohol concentrations per unit of alcohol consumed (Frezza et al., 1990, Nolen-Hoeksema,

2004), and female are at a greater risk of developing AH (Liangpunsakul et al., 2016). Currently, it is unknown whether female AH patients suffer more impairment in alcohol-induced EC function. Here, we found higher levels of sICAM-1 in female patients, suggesting that female might have a more severe EC dysregulation. Future study is warranted to address the relevance of this gender difference in sICAM-1 levels in AH pathogenesis. Interestingly, baseline levels of sVCAM-1 were elevated in HDC, who did not have overt liver diseases. Soluble VCAM-1 can be considered as a prognostic marker for cirrhosis, as it has been previously shown that sVCAM-1 levels are higher in patients with alcoholic cirrhosis than AH patients (Adams et al., 1994). Soluble VCAM-1 could also act as a sensitive marker for EC dysfunction in those heavy drinking individuals without overt liver diseases.

VEGF-A can enhance expression of ICAM-1 and VCAM-1 (Kim et al., 2001) and promotes vascular permeability (Shibuya, 2011). Consistent with our results, elevated VEGF-A levels have also been observed in patients with a wide range of inflammatory diseases, such as erythroderma (Miyamoto et al., 2017) or virus infections (Alkharsah, 2018). Significant correlations between VEGF-A and clinical parameters and inflammatory factors suggested the potential biomarker of VEGF-A in liver inflammatory disease.

Compared to other CAMs, CD146 is less well studied. Membrane-bound CD146 is mainly expressed at the endothelial junction and participates in the control of cell-cell cohesion. Soluble CD146 is generated by shedding of membrane CD146, which is considered as a marker of endothelial dysfunction (Saito et al., 2008, Figarella-Branger et al., 2006). Soluble CD146 has been reported as a biomarker in various diseases, such as heart failure, active multiple sclerosis, and chronic liver disease (Arrigo et al., 2017, Duan et al., 2013, Nomikou et al., 2015). However, it has not been evaluated in the immune-pathogenesis of AH. In this study, sCD146 levels were highly upregulated in AH patients. We also showed that sCD146 correlated with inflammatory factors and the other EC markers (ICAM-1 and VCAM-1) that can promote leukocytes recruitment. Soluble CD146 is known to promote lymphocyte transmigration through endothelium (Guezguez et al., 2007). Therefore, it is likely that the high levels of sCD146 could facilitate inflammatory cell recruitment to tissues including the liver, which is at least partially involved in the pathogenesis of AH.

CD146 is also expressed on a subset of human effector memory CD4 T cells (Elshal et al., 2005). $CD146^{+}CD4$ T cells express higher levels of genes associated with the pro-inflammatory Th17 cells, such as IL-17, compared to $CD146^{-}CD4$ T cells (Dagur et al., 2011, Brucklacher-Waldert et al., 2009, Larochelle et al., 2012). Also, it has been shown that CD146 expression on T cells can mediate or strengthen adhesion of $CD146^{+}$ T cells to ECs (Elshal et al., 2007, Guezguez et al., 2007). Consistent with published studies showing an increased frequency of $CD146^{+}CD4^{+}$ T cells in several inflammatory diseases (Dagur et al., 2011, Wu et al., 2015, Larochelle et al., 2012, Li et al., 2016), we found that the percentage of these cells was also significantly increased in the peripheral blood of AH patients, suggesting that CD146 expression on those T cells may play an important role in their trafficking into the liver and contribute to liver inflammation and injury. Interestingly, mining of microarray data (Gene Expression Omnibus DataSet GDS4389, Series GSE28619) (Affo et al., 2013) revealed that intrahepatic CD146 was hyper-expressed in AH

liver tissue compared with controls (p=0.003, Supporting fig. S2). It would be interesting to analyze expression of CD146 on hepatic T cell infiltrates as well as hepatic ECs in the future.

Complete alcohol abstinence plays a key role in improving the disease outcome and survival of AH, but it does not bring about complete recovery in all patients (O'Shea et al., 2010). Consistently, although clinical outcomes and liver function of the AH patients significantly improved, abnormalities in EC activation were not fully reversed with alcohol secession (Supporting Table 1). We previously showed that plasma levels of several pro-inflammatory cytokines, such as IL-8 and TNF-α, were still higher in abstinent AH patients than HDC at 12-month follow-up (Li et al., 2017). Here, we found that levels of sCD146, sVCAM-1 and VEGF-A remained highly upregulated in abstinent AH patients at the end of the study. These data suggested that endothelial dysfunctions persistently remained even after long-term alcohol abstinence, and that a longer abstinence might be required for a complete recovery of EC dysregulation in AH patients.

In conclusion, we found that AH patients had a broad dysregulation of soluble EC activation markers (sCD146, sICAM-1, sVCAM-1, and VEGF-A) in the peripheral circulation, and that alcohol abstinence greatly, but not fully, reversed these abnormalities. In addition, several significant correlations were found between the levels of soluble EC activation markers and measures of disease activity, bacterial translocation, and inflammatory/growth factors in AH patients. Further mechanistic studies are required to address the role of soluble CAMs in the pathogenesis of AH. As levels of the soluble EC activation markers in the peripheral blood can be easily monitored, they can be further explored as potential prognostic and/or predictive markers for AH. Furthermore, therapies targeting those dysregulated markers, especially CD146, might represent a potential strategy to restore immune homeostasis in AH patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We thank the members of the TREAT consortium (Translational Research and Evolving Alcoholic Hepatitis Treatment) at Indiana University (NIAAA AA021883), Mayo Clinic (NIAAA AA021788), and Virginia Commonwealth University (NIAAA AA021891); and at NIAAA (Scientific/Program Collaborator, Dr. Svetlana Raedeva), and the support of the National Institute on Alcohol Abuse and Alcoholism (NIAAA U01 AA021840). This work was also funded by NIAAA grant (UH2AA026218 to Q.Y.), the grant (OPP1035237 to Q.Y.) from the Bill & Melinda Gates Foundation, the Zhejiang Provincial Natural Science Foundation of China (No. LY18H200006 to X.Z.) and Wenzhou Science and Technology Project (No. Y20180108 to X.Z.).

Abbreviations

AH	alcoholic hepatitis
AST	aspartate aminotransferase
ALT	alanine aminotransferase

BT	bacterial translocation
CAMs	cell adhesion molecules
CRP	C-reactive protein
EC	endothelial cells
GRO-α	growth-regulated oncogene-α
HC	healthy control
HDC	heavy drinking control
HGF	hepatocyte growth factor
ICAM-1	intercellular adhesion molecule 1
INR	international normalized ratio
IP10	interferon-inducible protein 10
LPS	lipopolysaccharides
MCP-1	monocyte chemoattractant protein 1
MELD	model for end stage liver disease
mDF	Maddrey's discriminant function
NIAAA	National Institute on Alcohol Abuse and Alcoholism
PBMC	peripheral blood mononuclear cell
SDF-1α	stromal cell-derived factor 1α
TREAT	Translational Research and Evolving Alcoholic Hepatitis Treatment
VCAM-1	vascular cell-adhesion molecule 1
VEGF-A	vascular endothelial growth factor A

REFERENCES

ADAMS DH, BURRA P, HUBSCHER SG, ELIAS E & NEWMAN W 1994 Endothelial activation and circulating vascular adhesion molecules in alcoholic liver disease. Hepatology, 19, 588–94. [PubMed: 7509770]

AFFO S, DOMINGUEZ M, LOZANO JJ, SANCHO-BRU P, RODRIGO-TORRES D, MORALES-IBANEZ O, MORENO M, MILLAN C, LOAEZA-DEL-CASTILLO A, ALTAMIRANO J, GARCIA-PAGAN JC, ARROYO V, GINES P, CABALLERIA J, SCHWABE RF & BATALLER R 2013 Transcriptome analysis identifies TNF superfamily receptors as potential therapeutic targets in alcoholic hepatitis. Gut, 62, 452–60. [PubMed: 22637703]

ALKHARSAH KR 2018 VEGF Upregulation in Viral Infections and Its Possible Therapeutic Implications. Int J Mol Sci, 19.

ARRIGO M, TRUONG QA, ONAT D, SZYMONIFKA J, GAYAT E, TOLPPANEN H, SADOUNE M, DEMMER RT, WONG KY, LAUNAY JM, SAMUEL JL, COHEN-SOLAL A, JANUZZI JL

JR., SINGH JP, COLOMBO PC & MEBAZAA A 2017 Soluble CD146 Is a Novel Marker of Systemic Congestion in Heart Failure Patients: An Experimental Mechanistic and Transcardiac Clinical Study. Clin Chem, 63, 386–393. [PubMed: 28062630]

BARDIN N, ANFOSSO F, MASSE JM, CRAMER E, SABATIER F, LE BIVIC A, SAMPOL J & DIGNAT-GEORGE F 2001 Identification of CD146 as a component of the endothelial junction involved in the control of cell-cell cohesion. Blood, 98, 3677–84. [PubMed: 11739172]

BARDIN N, BLOT-CHABAUD M, DESPOIX N, KEBIR A, HARHOURI K, ARSANTO JP, ESPINOSA L, PERRIN P, ROBERT S, VELY F, SABATIER F, LE BIVIC A, KAPLANSKI G, SAMPOL J & DIGNAT-GEORGE F 2009 CD146 and its soluble form regulate monocyte transendothelial migration. Arterioscler Thromb Vasc Biol, 29, 746–53. [PubMed: 19229070]

BAU PF, BAU CH, ROSITO GA, MANFROI WC & FUCHS FD 2007 Alcohol consumption, cardiovascular health, and endothelial function markers. Alcohol, 41, 479–88. [PubMed: 17980786]

BAUTISTA AP 2002 Neutrophilic infiltration in alcoholic hepatitis. Alcohol, 27, 17–21. [PubMed: 12062632]

BRUCKLACHER-WALDERT V, STUERNER K, KOLSTER M, WOLTHAUSEN J & TOLOSA E 2009 Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis. Brain, 132, 3329–41. [PubMed: 19933767]

CHAYANUPATKUL M & LIANGPUNSAKUL S 2014 Alcoholic hepatitis: a comprehensive review of pathogenesis and treatment. World J Gastroenterol, 20, 6279–86. [PubMed: 24876748]

DAGUR PK, BIANCOTTO A, WEI L, SEN HN, YAO M, STROBER W, NUSSENBLATT RB & MCCOY JP JR. 2011 MCAM-expressing CD4(+) T cells in peripheral blood secrete IL-17A and are significantly elevated in inflammatory autoimmune diseases. J Autoimmun, 37, 319–27. [PubMed: 21959269]

DAGUR PK, TATLICI G, GOURLEY M, SAMSEL L, RAGHAVACHARI N, LIU P, LIU D & MCCOY JP JR. 2010 CD146+ T lymphocytes are increased in both the peripheral circulation and in the synovial effusions of patients with various musculoskeletal diseases and display pro-inflammatory gene profiles. Cytometry B Clin Cytom, 78, 88–95. [PubMed: 19834966]

DHANDA AD & COLLINS PL 2015 Immune dysfunction in acute alcoholic hepatitis. World J Gastroenterol, 21, 11904–13. [PubMed: 26576079]

DI GENNARO C, BIGGI A, BARILLI AL, FASOLI E, CARRA N, NOVARINI A, DELSIGNORE R & MONTANARI A 2007 Endothelial dysfunction and cardiovascular risk profile in long-term withdrawing alcoholics. J Hypertens, 25, 367–73. [PubMed: 17211243]

DUAN H, LUO Y, HAO H, FENG L, ZHANG Y, LU D, XING S, FENG J, YANG D, SONG L & YAN X 2013 Soluble CD146 in cerebrospinal fluid of active multiple sclerosis. Neuroscience, 235, 16–26. [PubMed: 23333866]

ELSHAL MF, KHAN SS, RAGHAVACHARI N, TAKAHASHI Y, BARB J, BAILEY JJ, MUNSON PJ, SOLOMON MA, DANNER RL & MCCOY JP JR. 2007 A unique population of effector memory lymphocytes identified by CD146 having a distinct immunophenotypic and genomic profile. BMC Immunol, 8, 29. [PubMed: 17999761]

ELSHAL MF, KHAN SS, TAKAHASHI Y, SOLOMON MA & MCCOY JP JR. 2005 CD146 (Mel-CAM), an adhesion marker of endothelial cells, is a novel marker of lymphocyte subset activation in normal peripheral blood. Blood, 106, 2923–4. [PubMed: 16204154]

FIGARELLA-BRANGER D, SCHLEINITZ N, BOUTIERE-ALBANESE B, CAMOIN L, BARDIN N, GUIS S, POUGET J, COGNET C, PELLISSIER JF & DIGNAT-GEORGE F 2006 Platelet-endothelial cell adhesion molecule-1 and CD146: soluble levels and in situ expression of cellular adhesion molecules implicated in the cohesion of endothelial cells in idiopathic inflammatory myopathies. J Rheumatol, 33, 1623–30. [PubMed: 16881117]

FREZZA M, DI PADOVA C, POZZATO G, TERPIN M, BARAONA E & LIEBER CS 1990 High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. N Engl J Med, 322, 95–9. [PubMed: 2248624]

FRIES JW, WILLIAMS AJ, ATKINS RC, NEWMAN W, LIPSCOMB MF & COLLINS T 1993 Expression of VCAM-1 and E-selectin in an in vivo model of endothelial activation. Am J Pathol, 143, 725–37. [PubMed: 7689792]

GAO B, AHMAD MF, NAGY LE & TSUKAMOTO H 2019 Inflammatory pathways in alcoholic steatohepatitis. J Hepatol, 70, 249–259. [PubMed: 30658726]

GUEZGUEZ B, VIGNERON P, LAMERANT N, KIEDA C, JAFFREDO T & DUNON D 2007 Dual role of melanoma cell adhesion molecule (MCAM)/CD146 in lymphocyte endothelium interaction: MCAM/CD146 promotes rolling via microvilli induction in lymphocyte and is an endothelial adhesion receptor. J Immunol, 179, 6673–85. [PubMed: 17982057]

HADJINICOLAOU AV, WU L, FANG B, WATSON PA, HALL FC & BUSCH R 2013 Relationship of CD146 expression to activation of circulating T cells: exploratory studies in healthy donors and patients with connective tissue diseases. Clin Exp Immunol, 174, 73–88. [PubMed: 23738744]

HARHOURI K, KEBIR A, GUILLET B, FOUCAULT-BERTAUD A, VOYTENKO S, PIERCECCHI-MARTI MD, BERENGUER C, LAMY E, VELY F, PISANO P, OUAFIK L, SABATIER F, SAMPOL J, BARDIN N, DIGNAT-GEORGE F & BLOT-CHABAUD M 2010 Soluble CD146 displays angiogenic properties and promotes neovascularization in experimental hind-limb ischemia. Blood, 115, 3843–51. [PubMed: 20185588]

HAYDON G, LALOR PF, HUBSCHER SG & ADAMS DH 2002 Lymphocyte recruitment to the liver in alcoholic liver disease. Alcohol, 27, 29–36. [PubMed: 12062634]

HWANG SJ, BALLANTYNE CM, SHARRETT AR, SMITH LC, DAVIS CE, GOTTO AM JR. & BOERWINKLE E 1997 Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation, 96, 4219–25. [PubMed: 9416885]

KEVIL CG, PAYNE DK, MIRE E & ALEXANDER JS 1998 Vascular permeability factor/vascular endothelial cell growth factor-mediated permeability occurs through disorganization of endothelial junctional proteins. J Biol Chem, 273, 15099–103. [PubMed: 9614120]

KIM I, MOON SO, KIM SH, KIM HJ, KOH YS & KOH GY 2001 Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. J Biol Chem, 276, 7614–20. [PubMed: 11108718]

KOCH AE, HALLORAN MM, HASKELL CJ, SHAH MR & POLVERINI PJ 1995 Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. Nature, 376, 517–9. [PubMed: 7543654]

LANGER HF & CHAVAKIS T 2009 Leukocyte-endothelial interactions in inflammation. J Cell Mol Med, 13, 1211–20. [PubMed: 19538472]

LAROCHELLE C, CAYROL R, KEBIR H, ALVAREZ JI, LECUYER MA, IFERGAN I, VIEL E, BOURBONNIERE L, BEAUSEIGLE D, TEROUZ S, HACHEHOUCHE L, GENDRON S, POIRIER J, JOBIN C, DUQUETTE P, FLANAGAN K, YEDNOCK T, ARBOUR N & PRAT A 2012 Melanoma cell adhesion molecule identifies encephalitogenic T lymphocytes and promotes their recruitment to the central nervous system. Brain, 135, 2906–24. [PubMed: 22975388]

LI W, AMET T, XING Y, YANG D, LIANGPUNSAKUL S, PURI P, KAMATH PS, SANYAL AJ, SHAH VH, KATZ BP, RADAEVA S, CRABB DW, CHALASANI N & YU Q 2017 Alcohol abstinence ameliorates the dysregulated immune profiles in patients with alcoholic hepatitis: A prospective observational study. Hepatology, 66, 575–590. [PubMed: 28466561]

LI W, LIN EL, LIANGPUNSAKUL S, LAN J, CHALASANI S, RANE S, PURI P, KAMATH PS, SANYAL AJ, SHAH VH, RADAEVA S, CRABB DW, CHALASANI N & YU Q 2019 Alcohol Abstinence Does Not Fully Reverse Abnormalities of Mucosal-Associated Invariant T Cells in the Blood of Patients With Alcoholic Hepatitis. Clin Transl Gastroenterol.

LI W, LIU L, GOMEZ A, ZHANG J, RAMADAN A, ZHANG Q, CHOI SW, ZHANG P, GREENSON JK, LIU C, JIANG D, VIRTS E, KELICH SL, CHU HW, FLYNN R, BLAZAR BR, HANENBERG H, HANASH S & PACZESNY S 2016 Proteomics analysis reveals a Th17-prone cell population in presymptomatic graft-versus-host disease. JCI Insight, 1.

LIANGPUNSAKUL S, PURI P, SHAH VH, KAMATH P, SANYAL A, URBAN T, REN X, KATZ B, RADAEVA S, CHALASANI N, CRABB DW, TRANSLATIONAL R & EVOLVING ALCOHOLIC HEPATITIS TREATMENT, C. 2016 Effects of Age, Sex, Body Weight, and Quantity of Alcohol Consumption on Occurrence and Severity of Alcoholic Hepatitis. Clin Gastroenterol Hepatol, 14, 1831–1838 e3. [PubMed: 27320325]

MANTOVANI A, BUSSOLINO F & DEJANA E 1992 Cytokine regulation of endothelial cell function. FASEB J, 6, 2591–9. [PubMed: 1592209]

MIYAMOTO D, SOTTO MN, OTANI CS, FUKUMORI LM, PEREIRA NV, SANTI CG, MARUTA CW, BURNIER MN, REBEIS MM & AOKI V 2017 Increased serum levels of vascular endothelial growth factor in pemphigus foliaceus patients with erythroderma. J Eur Acad Dermatol Venereol, 31, 333–336. [PubMed: 27510449]

MULLER WA 2003 Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. Trends Immunol, 24, 327–34. [PubMed: 12810109]

NOLEN-HOEKSEMA S 2004 Gender differences in risk factors and consequences for alcohol use and problems. Clin Psychol Rev, 24, 981–1010. [PubMed: 15533281]

NOMIKOU E, ALEXOPOULOU A, VASILIEVA L, AGIASOTELLI D, PAVLOU E, THEODOSSIADES G & DOURAKIS SP 2015 Soluble CD146, a novel endothelial marker, is related to the severity of liver disease. Scand J Gastroenterol, 50, 577–83. [PubMed: 25636502]

NORRIS P, POSTON RN, THOMAS DS, THORNHILL M, HAWK J & HASKARD DO 1991 The expression of endothelial leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in experimental cutaneous inflammation: a comparison of ultraviolet B erythema and delayed hypersensitivity. J Invest Dermatol, 96, 763–70. [PubMed: 1708800]

O'SHEA RS, DASARATHY S, MCCULLOUGH AJ, PRACTICE GUIDELINE COMMITTEE OF THE AMERICAN ASSOCIATION FOR THE STUDY OF LIVER, D. & PRACTICE PARAMETERS COMMITTEE OF THE AMERICAN COLLEGE OF, G. 2010 Alcoholic liver disease. Hepatology, 51, 307–28. [PubMed: 20034030]

OSBORN L 1990 Leukocyte adhesion to endothelium in inflammation. Cell, 62, 3–6. [PubMed: 2194672]

PICKL WF, MAJDIC O, FISCHER GF, PETZELBAUER P, FAE I, WACLAVICEK M, STOCKL J, SCHEINECKER C, VIDICKI T, ASCHAUER H, JOHNSON JP & KNAPP W 1997 MUC18/MCAM (CD146), an activation antigen of human T lymphocytes. J Immunol, 158, 2107–15. [PubMed: 9036955]

PONZIANI FR, ZOCCO MA, CERRITO L, GASBARRINI A & POMPILI M 2018 Bacterial translocation in patients with liver cirrhosis: physiology, clinical consequences, and practical implications. Expert Rev Gastroenterol Hepatol, 12, 641–656. [PubMed: 29806487]

POSTON RN, HASKARD DO, COUCHER JR, GALL NP & JOHNSON-TIDEY RR 1992 Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. Am J Pathol, 140, 665–73. [PubMed: 1372160]

ROTHLEIN R, MAINOLFI EA, CZAJKOWSKI M & MARLIN SD 1991 A form of circulating ICAM-1 in human serum. J Immunol, 147, 3788–93. [PubMed: 1682385]

SABERI B, DADABHAI AS, JANG YY, GURAKAR A & MEZEY E 2016 Current Management of Alcoholic Hepatitis and Future Therapies. J Clin Transl Hepatol, 4, 113–22. [PubMed: 27350941]

SACANELLA E & ESTRUCH R 2003 The effect of alcohol consumption on endothelial adhesion molecule expression. Addict Biol, 8, 371–8. [PubMed: 14690873]

SAITO O, SAITO T, OKUDA K, OKUDA K, KOTODA A, AKIMOTO T, ANDO Y, MUTO S, ISHIKAWA SE & KUSANO E 2008 Serum adiponectin and markers of endothelial injury in hemodialysis patients with arteriosclerosis obliterans. Clin Exp Nephrol, 12, 58–64. [PubMed: 18175063]

SANDAHL TD, GRONBAEK H, MOLLER HJ, STOY S, THOMSEN KL, DIGE AK, AGNHOLT J, HAMILTON-DUTOIT S, THIEL S & VILSTRUP H 2014 Hepatic macrophage activation and the LPS pathway in patients with alcoholic hepatitis: a prospective cohort study. Am J Gastroenterol, 109, 1749–56. [PubMed: 25155228]

SASSEVILLE VG, NEWMAN WA, LACKNER AA, SMITH MO, LAUSEN NC, BEALL D & RINGLER DJ 1992 Elevated vascular cell adhesion molecule-1 in AIDS encephalitis induced by simian immunodeficiency virus. Am J Pathol, 141, 1021–30. [PubMed: 1279978]

SHAIK-DASTHAGIRISAHEB YB, VARVARA G, MURMURA G, SAGGINI A, POTALIVO G, CARAFFA A, ANTINOLFI P, TETE S, TRIPODI D, CONTI F, CIANCHETTI E, TONIATO E, ROSATI M, CONTI P, SPERANZA L, PANTALONE A, SAGGINI R, THEOHARIDES TC &

PANDOLFI F 2013 Vascular endothelial growth factor (VEGF), mast cells and inflammation. Int J Immunopathol Pharmacol, 26, 327–35. [PubMed: 23755748]

SHIBUYA M 2011 Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. Genes Cancer, 2, 1097–105. [PubMed: 22866201]

SULPICE E, DING S, MUSCATELLI-GROUX B, BERGE M, HAN ZC, PLOUET J, TOBELEM G & MERKULOVA-RAINON T 2009 Cross-talk between the VEGF-A and HGF signalling pathways in endothelial cells. Biol Cell, 101, 525–39. [PubMed: 19281453]

WANG HJ, GAO B, ZAKHARI S & NAGY LE 2012 Inflammation in alcoholic liver disease. Annu Rev Nutr, 32, 343–68. [PubMed: 22524187]

WU C, GOODALL JC, BUSCH R & GASTON JS 2015 Relationship of CD146 expression to secretion of interleukin (IL)-17, IL-22 and interferon-gamma by CD4(+) T cells in patients with inflammatory arthritis. Clin Exp Immunol, 179, 378–91. [PubMed: 25113810]

YOUNOSSI ZM, STEPANOVA M, ONG J, YILMAZ Y, DUSEJA A, EGUCHI Y, EL KASSAS M, CASTELLANOS-FERNANDEZ M, GEORGE J, JACOBSON IM, BUGIANESI E, WONG VW, ARRESE M, DE LEDINGHEN V, ROMERO-GOMEZ M, MENDEZ-SANCHEZ N, AHMED A, WONG R, PAPATHEODORIDIS G, SERFATY L, YOUNOSSI I, NADER F, ZIAYEE M, AFENDY A & GLOBAL NC 2018 Effects of Alcohol Consumption and Metabolic Syndrome on Mortality in Patients With Nonalcoholic and Alcohol-Related Fatty Liver Disease. Clin Gastroenterol Hepatol.

YUAN J, GUO Q, QURESHI AR, ANDERSTAM B, ERIKSSON M, HEIMBURGER O, BARANY P, STENVINKEL P & LINDHOLM B 2013 Circulating vascular endothelial growth factor (VEGF) and its soluble receptor 1 (sVEGFR-1) are associated with inflammation and mortality in incident dialysis patients. Nephrol Dial Transplant, 28, 2356–63. [PubMed: 23828162]

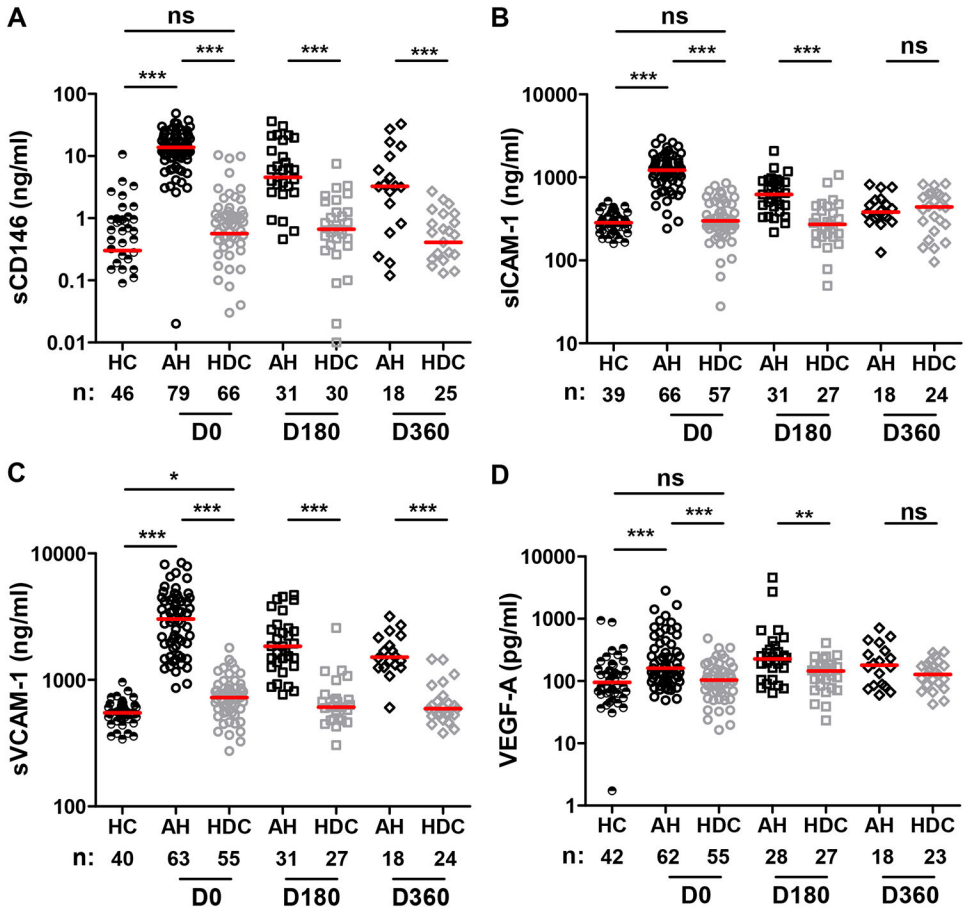

Fig. 1. Plasma levels of soluble EC activation markers in AH patients, HDC and HC.

Scatter plots showing plasma levels of sCD146 (**A**), sICAM-1 (**B**), sVCAM-1 (**C**) and VEGF-A (**D**) in HC, AH patients and HDC controls. Kruskal-Wallis test with Dunn's corrections was performed to compare expression levels among 3 groups at baseline. Mann Whitney test was used for compare AH versus HDC individuals at day 180 and day 360. *p < 0.05, **p < 0.01, ***p < 0.001. ns, not significant. Horizontal lines represent the median. AH, alcoholic hepatitis; HC, healthy control; HDC, heavy drinking control.

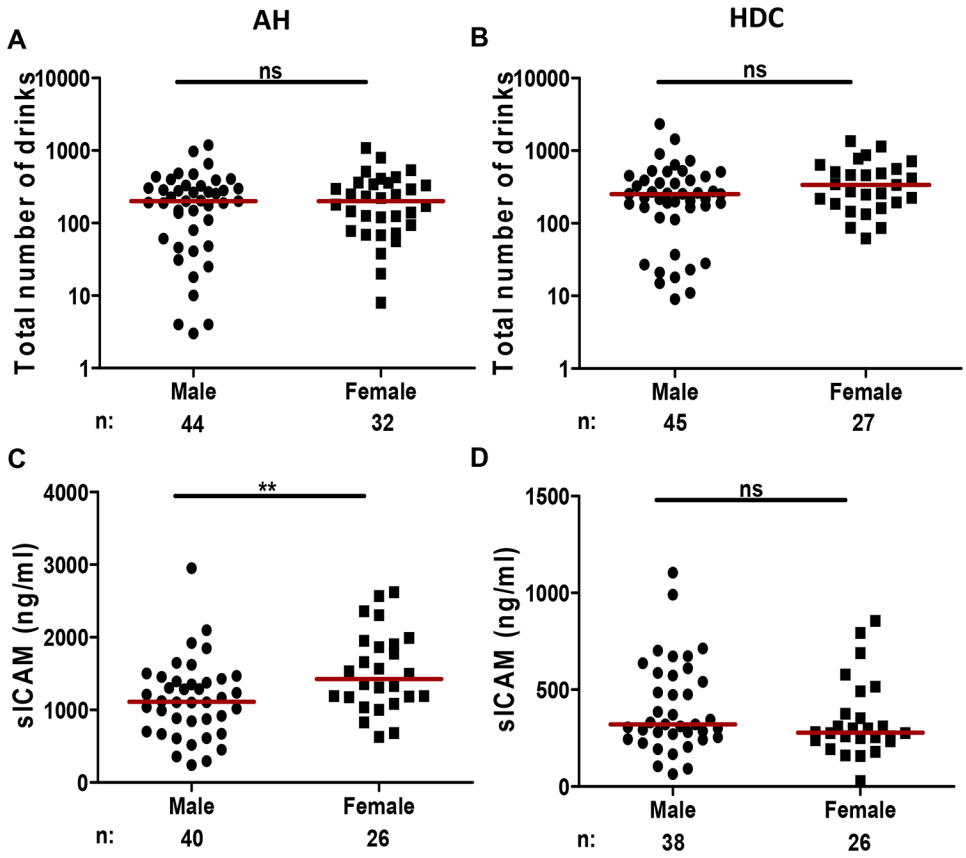

Fig. 2. Higher plasma levels of ICAM-1 in female AH patients.

(A-D) Scatter plots showing total number of drinks for 30 days before recruitment and sICAM-1 levels in male and female AH patients (**A, C**) and HDC (**B, D**) at baseline. Mann Whitney test was used for compare the difference between the two groups. **p < 0.01. ns, not significant. Horizontal lines represent the median.

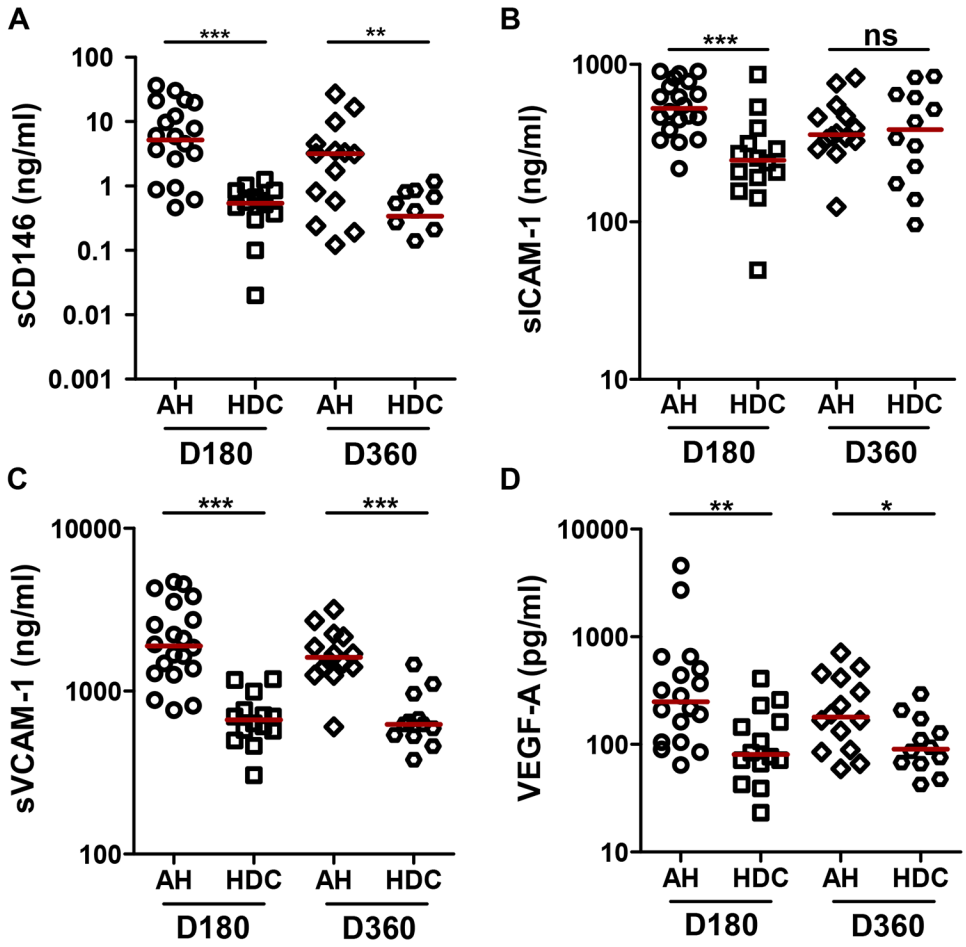

Fig. 3. Comparison of soluble EC activation markers in abstinent subjects at 6-month and 12-month follow-ups.

Scatter plots showing the levels of sCD146 (**A**), sICAM-1 (**B**), sVCAM-1 (**C**), and VEGF-A (**D**) at 6-month (D180) and 12-month (D360) follow-ups in abstinent AH patients and HDC. Mann Whitney test comparing AH vs HDC at D180 and D360. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns: not significant. Horizontal lines represent the median. AH, alcoholic hepatitis; HDC, heavy drinking control.

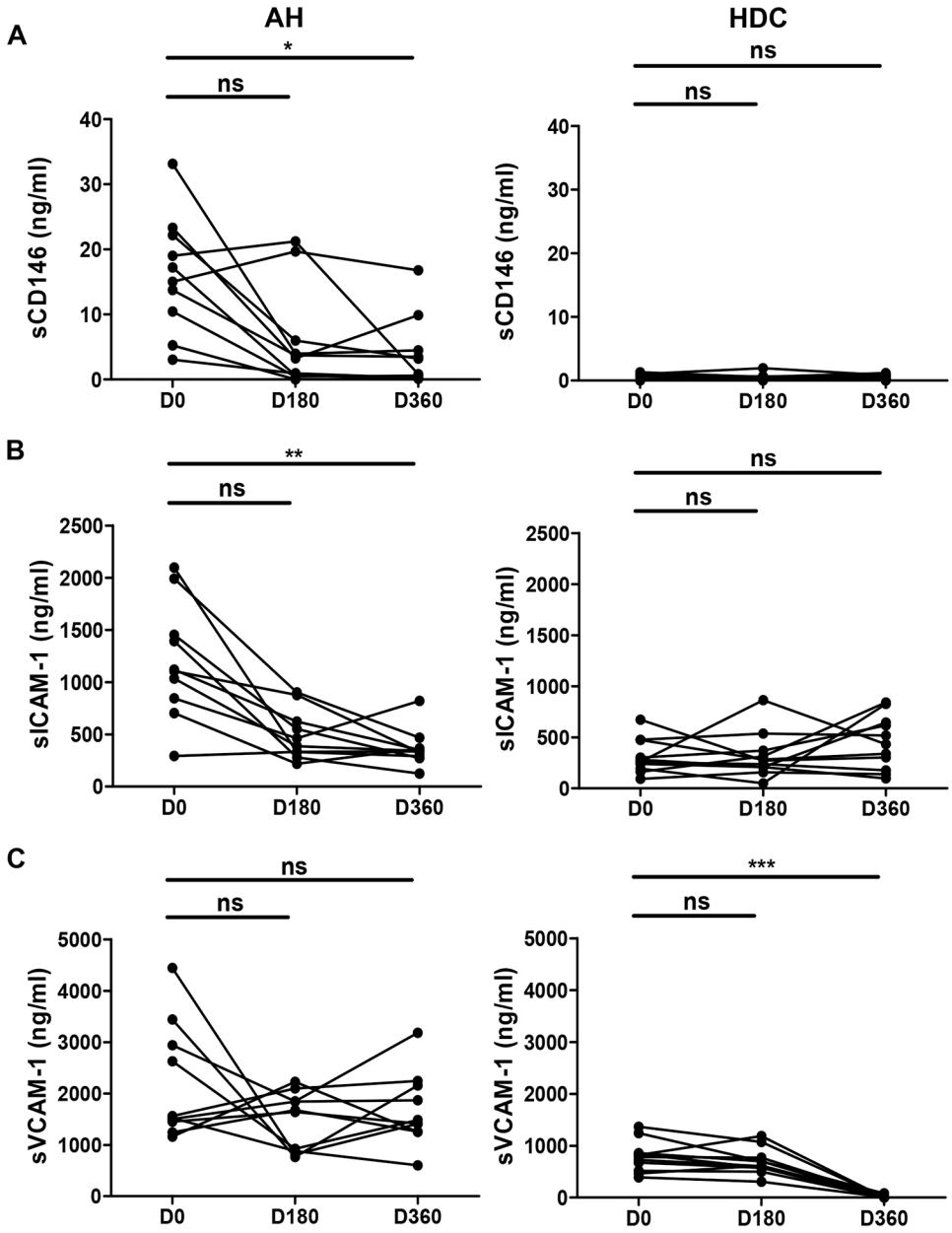

Fig. 4. Longitudinal analysis of plasma levels of soluble CAMs in abstinent subjects.

Before-and-after graphs showing the effect of alcohol abstinence on expression of sCD146 (**A**), sICAM-1 (**B**), and sVCAM-1 (**C**) in AH patients and HDC. Friedman rank sum test with Dunn's corrections for comparing EC activation markers concentrations at day 0 with day 180 or day 360 (n=10 for AH and n=11 for HDC). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ns, not significant. AH, alcoholic hepatitis; HDC, heavy drinking control.

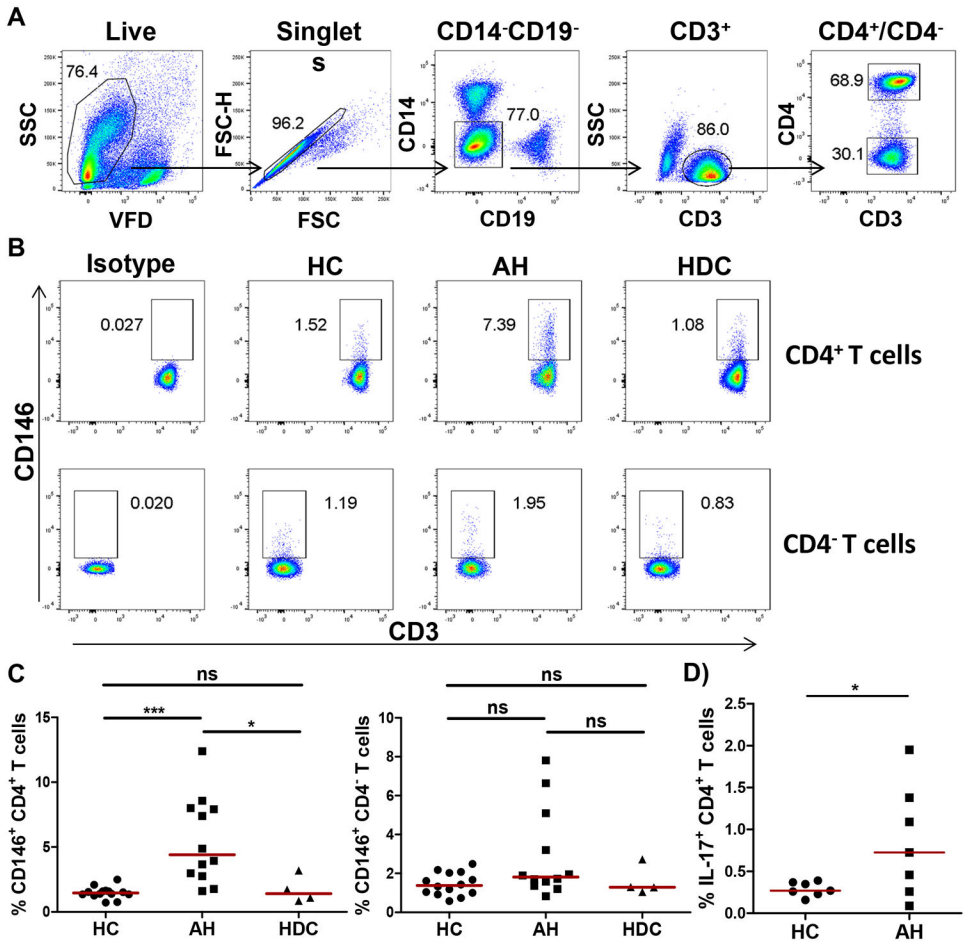

Fig. 5. CD146+ T cells and IL-17+ CD4 T cells in PBMCs from AH patients, HDC, and HC.

(**A**) Gating strategies for analyzing peripheral blood $CD146^+$ T cells. Individual cell population (singlets) among the live PBMCs, which were negative for viability dye (FVD) staining, were identified based on the forward scatter height (FSC-H) versus FSC-A display. Subsequently, CD3 staining was used to identify T cells ($CD3^+$) among the previously gated $CD14^-$ and $CD19^-$ cell population. $CD4^+$ T cells and $CD4^-$ T cells (mainly $CD8^+$ T cells) were identified based on CD4 staining. (**B**) Representative flow plots showing CD146 expression on gated $CD4^+$ and $CD4^-$ T cells in PBMCs from AH patients, HDC, and HC at baseline. Box and number represent the gate and percentage of $CD146^+$ T cells. Isotype control antibody (Isotype) was used to set the $CD146^+$ gate. (**C**) Dot plots summarizing the frequency of CD146-expressing $CD4^+$ and $CD4^-$ T cells. ANOVA test with Bonferroni's corrections was used to compare differences in CD146 expression among the 3 groups of subjects (n=14 for HC, n=12 for AH and n=4 for HDC). (**D**) The frequency of $IL\text{-}17^+$ cells on gated CD4 T cells in PBMCs from AH patients and HC. PBMCs were stimulated with PMA and ionomycin and subsequently subjected to intracellular cytokine staining of IL-17. Unpaired t test was used to calculate the difference in $IL\text{-}17^+$ CD4 T cells (n=7). $*p < 0.05$, $***p < 0.001$. ns, not significant. AH, alcoholic hepatitis; HDC, heavy drinking control; HC, healthy control.

Table 1.

Comparison of characteristics of the study cohort

Variable	Day 0 (baseline)				Day 180			Day 360		
	HC (n=46)	AH (n=79)	HDC (n=66)	*p*	AH (n=31)	HDC (n=30)	*p*	AH (n=18)	HDC (n=25)	*p*
Age (years)	42 (30–52)	44 (52–35)	45 (35–52)	ns	43 (52–35)	47 (35–53)	ns	42 (53–34)	50 (34–53)	ns
Gender (% Male)	59	58	59	ns	61	63	ns	67	64	ns
Total drinks in 30 days		200 (74–330)	270 (191–508)	**	0 (0–8)	6.5 (0–66)	ns	0 (0–1)	2 (0–110)	*
Total drinking days in 30 days		25 (14–30)	28 (23–30)	ns	0 (0–2)	1.5 (0–16)	ns	0 (0–1)	1 (0–24)	ns
MELD score		24 (19–27)	6 (6–8)	***	11 (8–14)	7 (6–8)	***	10 (8–13)	7 (6–8)	***
Creatinine (mg/dL)	0.9 (0.8–1.1)	0.8 (0.6–1.1)	0.8 (0.7–1.0)	ns	0.74 (0.6–1.0)	0.9 (0.8–1.1)	*	1 (0.7–1.2)	1 (0.9–1.1)	ns
Total bilirubin (mg/dL)	0.3 (0.3–0.5)	12.9$^{###}$ (7.1–19.3)	0.6§ (0.4–0.7)	***	1.5 (0.8–3.4)	0.4 (0.3–0.6)	***	0.9 (0.8–1.8)	0.4 (0.3–0.7)	***
AST (IU/L)	17 (13–20)	110$^{###}$ (88–145)	24§§ (18–31)	***	48 (31–70)	20 (16–23)	***	35 (26–57)	21 (17–32)	**
ALT (IU/L)	11 (7–17)	46$^{###}$ (30–60)	22§§§ (16–31)	***	30 (20–44)	16 (11–24)	***	25 (22–35)	18 (13–35)	ns
Prothrombin time (INR)		1.8 (1.5–2.2)	1.0 (0.9–1.0)	***	1.2 (1.1–1.5)	1.0 (1–1.1)	***	1.2 (1.0 – 1.5)	1.0 (1.0–1.1)	***
Treatment with PDN and/or PTX		53%	0%		55%	0%		61%	0%	

Note: Data are represented as median and (interquartile ranges). HC, healthy controls; AH, patients with alcoholic hepatitis; HDC, heavy drinking controls; MELD, model for end-stage liver disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio. Kruskal-Wallis test with Dunn's correction for pairwise comparisons of continuous variables among HC, AH patients and HDC at enrollment (Day 0). Mann Whitney test comparing AH patients versus HDC at day 180 and day 360 follow-up. Chi-square test for analysis of categorical variables.

$^{###}p < 0.001$ for comparison between AH patients and HC at Day 0;

$^{*}p < 0.05$,

$^{**}p < 0.01$,

$^{***}p < 0.001$ for comparison between AH patients and HDC;

$^{§}p < 0.05$,

$^{§§}p < 0.01$,

$^{§§§}p < 0.001$ for comparison between HDC and HC at Day 0; ns, not significant.

Table 2.

Correlations of soluble EC cell activation markers with clinical parameters, BT markers and inflammatory/ growth factors in AH patients

	Variable	sCD146	sICAM-1	sVCAM-1	VEGF-A
Clinical parameters	**MELD**	0.45***	0.54***	0.42***	0.33***
	Creatinine		0.32***		0.64***
	Total Bilirubin	0.32***	0.53***		
	AST	0.30***			0.47***
	ALT				
	INR	0.40***		0.40***	0.26**
	mDF score	0.26**		0.29**	
BT markers	**LPS**		0.30**	0.25**	
	CD163	0.45***	0.69***	0.64***	
	CD14		0.35***		
	LBP		0.43***		
Inflammatory factors	**IFN-γ**	0.44***		0.32***	
	IL-1RA		0.31***		0.33***
	IL-6	0.29**			0.36***
	IL-8	0.33***	0.65***	0.37***	0.40***
	IL-9				0.45***
	IL-10		0.32***		
	IL-18	0.47***		0.28**	
	IL-23	0.37***			0.27**
	IP-10	0.28**			0.34***
	MCP-1	0.41***	0.29**		
	TNF-α				0.32***
GFs	**GRO-α**	0.29**	0.34***		0.34***
	HGF		0.61***	0.30**	0.25**
	SDF-1α				0.67***

Note: AH, alcoholic hepatitis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BT, bacterial translocation; GFs, growth factors; INR, international normalized ratio for prothrombin time; LBP, LPS-binding protein; MELD, model for end-stage liver disease. The numbers represent Spearman's coefficients,

** $p < 0.01$,

*** $p < 0.001$.

Data at enrollment and follow-ups were pooled for these analyses, and the significant correlations with a correlation coefficient > 0.25 are listed.