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Systemic inflammation and liver damage in HIV/HCV co-infection

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Abstract

Objective—Chronic viral infections, HCV and HIV, are characterized by systemic inflammation. Yet the relative levels, drivers and correlates of inflammation in these settings are not well defined.

Methods—We studied seventy-nine HIV-infected patients who had been receiving antiretroviral therapy (ART) for more than two years and had suppressed plasma HIV levels (<50 copies/ml). Two patient groups: HCV^+/HIV^+, HCV^-/HIV^+, and a control group comprised of healthy volunteers (n=20) were examined. Markers of systemic inflammation (IL-6, IP-10, sTNF-RI, and sTNF-RII), monocyte/macrophage activation (sCD163, sCD14, and neopterin), intestinal epithelial barrier loss (I-FABP and LPS), and coagulation (D-dimers) were analyzed. $CD4^+$ naïve T cells and $CD4^+$ recent thymic emigrants (RTE) were enumerated.

Results—Plasma levels of IP-10, neopterin, and sCD163 were higher in HCV/HIV co-infection than in HIV monoinfection and were positively correlated with indices of hepatic damage (AST, ALT, and APRI). Levels of I-FABP were comparably increased in both HIV monoinfection and HIV/HCV co-infection but LPS concentrations were highest in HCV/HIV co-infection suggesting impaired hepatic clearance of LPS. Plasma HCV levels were related to no inflammatory indices but for sCD163. In co-infected subjects, a previously recognized relationship of $CD4^+$ naïve T cell and RTE counts to hepatocellular injury was defined more mechanistically by an inverse relationship to sCD163.

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Conflicts of interest

The authors have no conflict of interests.

Conclusion—Hepatocellular injury in HCV/HIV co-infection is linked to elevated levels of certain inflammatory cytokines and an apparent failure to clear systemically translocated microbial products. A related decrease in $CD4^+$ naïve T cells and recent thymic emigrants also merits further exploration.

Keywords

Antigens; CD31; Antiretroviral Therapy; Highly Active; Hepatitis C; HIV Infections; Inflammation Mediators

Introduction

An estimated 10–15% of the 35 million people living with HIV-infection worldwide are also infected with hepatitis C virus (HCV) (1). These two viral diseases can adversely influence each other. HIV speeds the course of hepatitis C infection, accelerating liver fibrosis and cirrhosis, and promoting liver cancer (2, 3). In turn, HCV co-infection has been linked to $CD4^+$ and $CD8^+$ T cell activation (4, 5), increased $CD4^+$ T cell apoptosis (6, 7), and in some studies, has been associated with diminished $CD4^+$ T lymphocyte restoration with antiretroviral therapy (ART) (8).

Indices of systemic inflammation and coagulation are now recognized as important predictors of morbidity and mortality in treated HIV infection (9–11). Here we ask if HIV infected patients with suppressed viremia on combination antiretroviral therapy have different systemic levels of inflammation or coagulation than HCV co-infected and if so, are these levels related to indices of hepatic damage.

Patients and methods

This work was approved by the Institutional Review Board of Perm Regional Center for Protection against AIDS and Infectious Diseases (IRB00008964). All patients provided their written informed consent.

Seventy-nine HIV-infected patients receiving ART for more than two years and twenty healthy controls participated. All patients had a confirmed diagnosis of HIV-infection, were adherent to their ART regimen, and had plasma HIV RNA levels <50 copies/ml. ART regimens included 2 nucleoside reverse transcriptase inhibitors (NRTIs) together with a ritonavir-boosted protease inhibitor or a non-nucleoside reverse transcriptase inhibitor. Hepatitis C virus co-infection was confirmed by the demonstration of HCV RNA in plasma by a PCR-based assay (“Quantitative RT-Gepatogen C” kit; DNA-Technology, Russia); HCV uninfected subjects each had a negative test for serum antibodies to HCV. Patients who had been exposed to interferon/ribavirin treatment were excluded from the study. HIV-infection duration was timed from the date of the first positive western blot analysis. HCV-infection duration was calculated from when the first positive ELISA was received. A report describing lymphocyte phenotype in these subjects has been published previously (12).

We studied three groups:

1. HIV/HCV co-infected patients (n=42);

2. HIV monoinfected patients (n=37);

3. Uninfected volunteers (n=20).

The two infected groups had no differences in nadir $CD4^+$ T cell count (table) or prior AIDS defining conditions. No information on the alcohol consumption and smoking was provided.

HIV and HCV levels in plasma

Plasma levels of HIV RNA were assessed using a Versant 440 amplifier (Siemens) and «Versant HIV 1 RNA 3.0 assay b» kits (Bayer, Germany). HCV RNA levels in plasma were measured using an iCycler IQ5 (Bio-Rad, USA) and real-time PCR «Quantitative RT-Gepatogen C» kits (DNA-Technology; Russia).

Blood samples for T cell phenotyping

Approximately 30 ml of blood was taken from each participant in Vacutainer tubes containing EDTA (Becton Dickinson). $CD4^+$ T cell numbers were counted in real time using the IMK-Lymphocyte Kit (San Jose, CA) and a BD FACSCalibur flow cytometer. Peripheral blood mononuclear cells (PBMC) were isolated using Diacoll-1077 (Dia-M; Russia) density sedimentation. PBMC were cryopreserved in fetal calf serum and dimethyl sulfoxide, and then stored at −196°C.

Monoclonal antibodies

Fluorochrome tagged monoclonal antibodies (anti-CD3-PerCP, anti-CD4-AF700, anti-CD27-APC-Cy7, anti-CD45RA-APC, anti-CCR7-PE-Cy7, and anti-CD31-FITC) and isotype control antibodies were obtained from Becton Dickinson (San Jose, CA). LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit was obtained from Life Technologies (Grand Island, NY).

Flow cytometry

PBMC were thawed and stained and viable cells were enumerated using a Becton Dickinson LSR II Flow Cytometer. Naïve $CD4^+$ T cells were identified as $CD3^+CD4^+CD27^+CD45RA^+CCR7^+$. Naïve $CD31^+$ T lymphocytes were considered to be recent thymic emigrants. At least 100,000 events in the lymphocyte gate were collected for each sample. Relative values were determined from the cytometer data. Absolute lymphocyte subpopulations were calculated based on $CD4^+$ T cell numbers detected in fresh blood.

ELISA

ELISA kits for the detection of interleukin-6 (IL-6), interferon gamma-induced protein 10 (IP-10), soluble CD163 (sCD163), soluble CD14 (sCD14), soluble tumor necrosis factor receptor-I (sTNF-RI), soluble tumor necrosis factor receptor-II (sTNF-RII), and intestinal fatty acid binding protein (I-FABP) were purchased from R&D Systems (Minneapolis, MN). D-dimer kits were purchased from Diagnostica Stago (Asnieres, France). Neopterin competitive ELISA kits were purchased from IBL International (Hamburg, Germany). Lipopolysaccharide (LPS) levels were assessed using a Hycult Biotech Limulus amebocyte

lysate chromogenic endpoint assay kit (Uden, Netherlands). Assays were performed according to kit instructions. Plasma samples were diluted as needed to assure that results were within the linear range of the assay.

Statistical analysis

Data were reported as medians and interquartile ranges. Groups were compared by the Mann-Whitney test. Multiple regression analysis was used to control for the effects of possible confounding factors. Correlation analysis was performed using the Spearman method. All statistical analyses were done using “STATISTICA 6.0” software.

Results

Clinical characteristics

The ages of the HIV infected patient groups and healthy controls were comparable (table). The median age was 33 years in HIV/HCV co-infected, 34 years in HIV monoinfected and 31 years in the uninfected group. Men were overrepresented (61.9%) among HIV/HCV co-infected patients reflective of the epidemic features of HCV infection in Russia (13). In contrast, the HIV monoinfected patients in this study were predominantly (78.4%) women. The difference in the gender ratio was significant ($P<0.001$). In the healthy control group 40% were men. The known duration of infection was longer in “dually” infected subjects than in HIV monoinfected subjects (11 vs. 8 years, respectively). In the two infected groups, there were no differences in $CD4^+$ T cell numbers before or after ART initiation. In HIV/HCV co-infected subjects median HCV RNA levels exceeded 1,000,000 copies/ml and liver enzymes were elevated compared with the levels in HIV monoinfected patients, while albumin levels and platelet counts in co-infected and monoinfected persons were not significantly different. Aspartate transaminase (AST) to platelet ratio index (APRI) for predicting fibrosis and cirrhosis (14) was higher in the HIV/HCV co-infected group than in the HIV monoinfected group.

Systemic inflammation indices are elevated in HCV/HIV co-infection

Plasma levels of the inflammatory cytokines IL-6, IP-10, and monocyte/macrophage markers (neopterin and sCD163), and sTNF-RII were higher in HIV/HCV co-infected patients than in patients who were singly HIV infected and but for sTNF-RII, were also higher than among healthy controls (fig. 1). As the two infected groups differed in duration of HIV infection and gender composition, we asked if these factors might have confounded our results. After adjustment for these factors the difference between the two HIV positive groups in the levels of IL-6 and TNF-RII lost statistical significance. With correction for gender and duration of HIV infection, levels of IP-10, sCD163, and neopterin remained significantly higher in HCV/HIV co-infected subjects than in HIV monoinfection. Median levels of IP-10, sCD163, and sTNF-RII in singly HIV infected subjects were not different from those in healthy controls, but IL-6, neopterin and sCD14 levels were higher in singly HIV infected patients than in healthy subjects. These differences in inflammatory markers may be associated with intestinal epithelium damage, as plasma I-FABP levels in both groups of HIV infected patients were also significantly higher ($P<0.01$) than those in the control group, and plasma LPS concentrations were higher in HCV/HIV co-infected subjects

than among HIV monoinfected patients and uninfected controls (fig 2). In contrast, plasma D-dimer levels, reflecting coagulation and fibrinolysis, were similar among the three groups.

To explore the possibility that HCV co-infection increases monocyte/macrophage activation (sCD163 and neopterin levels) and stimulates IFN-dependent production of the chemokine IP-10, we assessed the relationship of these markers to indices of HCV replication and hepatic injury.

In the group of HCV/HIV co-infected patients we found highly significant and consistent correlations between indices of hepatic damage (AST, alanine transaminase (ALT), and APRI) and plasma IP-10, sCD163 and neopterin (fig. 3). Correlations with levels of sCD14, I-FABP, sTNF-RI, and sTNF-RII were not significant. AST levels correlated with D-dimers, albeit weakly ($R = 0.326$; $P<0.05$). HCV levels in plasma were also associated with liver enzyme elevations: $R_{AST\text{-}HCV} = 0.527$ ($P<0.001$), $R_{ALT\text{-}HCV} = 0.483$ ($P<0.01$), $R_{APRI\text{-}HCV} = 0.361$ ($P<0.05$), but among all the markers of systemic inflammation, correlated only with sCD163 concentration ($R_{sCD163\text{-}HCV} = 0.316$; $P<0.05$).

In an earlier report in this cohort, we found inverse significant relationships between the magnitude of hepatic damage (ALT, AST, and APRI) and absolute numbers of circulating $CD4^+$ recent thymic emigrants (12). Having found that indices of hepatocellular injury are linked to inflammatory markers in HIV/HCV co-infection, we examined here the relationship between these inflammatory markers and $CD4^+$ RTE and found that higher levels of sCD163 were associated with fewer circulating $CD4^+$ RTE and with fewer $CD4^+$ naïve T cells (fig. 4).

Discussion

HIV and HCV infections are each characterized by increases of various inflammatory marker levels in blood (15–17). With suppressive antiretroviral therapy, plasma concentrations of inflammatory markers tend to decrease but do not always normalize (18). Here, we compared plasma levels of inflammatory and coagulation markers in HIV infected and HIV/HCV co-infected patients who are receiving suppressive antiretroviral therapy. In both groups, HIV levels in plasma were suppressed while HCV replication was uncontrolled – providing a window by which to explore the effects of HCV replication in the setting of chronic HIV infection while attenuating the direct effects of HIV replication. Plasma concentrations of IL-6, IP-10, sCD163, neopterin, and sTNF-RII were significantly higher in co-infected subjects than in HIV infected patients not infected with HCV. As the patient groups were not comparable in gender composition and recognized duration of HIV infection, adjustment for these two factors left only differences in IP-10, sCD163, and neopterin remaining significantly and independently higher in HCV/HIV co-infected patients than among HIV infected subjects not infected with HCV. Although a contribution of ART-induced hepatotoxicity in the setting of HCV^+HIV^+ co-infection cannot be excluded, a simpler and more plausible explanation is that the observed effects are related to HCV-HCV mediated liver damage (19, 20).

Whereas there are numerous sources of IP-10, in HCV infection, IP-10 is synthesized by liver sinusoidal lining cells (21) and is induced by interferons and co-stimulated by TNF-α and IL-1 (22, 23). The primary role of the chemokine IP-10 is to recruit $CD4^+$ Th1 cells, $CD8^+$ cytotoxic T cells, and NK cells through interaction with CXCR3 to provide a proinflammatory antiviral immune response (24–27).

During chronic HCV infection an increase in the number of macrophage-like Kupffer cells is observed (28, 29). These cells acquire an activation phenotype (30), and express greater levels of CD33 and CD163 (31, 32). Blood levels of sCD163 may reflect systemic macrophage activation. Higher levels of sCD163 are seen in subjects with HCV/HIV co-infection than in HIV monoinfected patients (33) and in HCV infection are linked to the development of cirrhosis (34).

While effective antiretroviral therapy does not reliably result in complete suppression of immune activation and inflammatory responses in HIV infected patients (35, 36), we find here that plasma levels of IL-6, neopterin and sCD14 in subjects not co-infected with HCV remain higher than among controls while levels of IP-10, sCD163 and TNF-RII are similar to control levels. The drivers of persistent inflammation in treated HIV infection and HIV/HCV co-infection are not entirely clear but in these settings, damage to the gut epithelial barrier has been implicated in promoting translocation of microbial products from the gut lumen into the systemic circulation (37). Elevated plasma levels of I-FABP are regarded as a marker of this intestinal damage (38, 19). Interestingly, we found increased plasma levels of LPS in HIV infection and even higher levels in HIV/HCV co-infection, while in both HIV monoinfection and HIV/HCV co-infection the elevated levels of I-FABP and the LPS co-receptor sCD14 were comparable. These data suggest that in both HIV infected/HCV uninfected and HIV/HCV co-infected individuals the gut barrier defect may be comparable, allowing bacterial products access to the portal vein. However, impaired hepatic clearance of LPS in HCV co-infection may result in even higher levels of LPS in peripheral blood of HIV/HCV co-infected compared with HIV^+ patients not infected with HCV. It is also possible that the impaired clearance of LPS and other microbial products not measured here may contribute to the profound increases in other inflammatory mediators that we found in HCV/HIV co-infection when compared to levels in HIV infected patients negative for HCV.

While sCD14 levels were elevated in both HIV infection and HCV/HIV co-infection sCD14 levels did not distinguish between HIV monoinfection and HIV/HCV co-infection. In contrast, Sandler *et al.* found that sCD14 level was correlated with markers of hepatic destruction (AST) and abnormal liver function (γ-glutamyl transpeptidase, alkaline phosphatase, and α-fetoprotein) (19), and French *et al.* showed that levels of sCD14 were higher in HIV/HCV co-infected women during periods of liver disease progression than during intervals when minimal or no progression occurred (39). We did not find a relationship between sCD14 and any indices of hepatocellular damage in this cohort where APRI scores are consistent with a low probability of advanced liver damage. On the other hand, we found here that plasma levels of two other markers of macrophage activation, sCD163 and neopterin and levels of the interferon-inducible protein IP-10 were significantly higher in HCV/HIV co-infection than in HIV^+ patients not infected with HCV and while

only sCD163 levels were correlated with plasma levels of HCV, each was correlated with three indices of hepatic damage (AST, ALT, and APRI).

In earlier works, we and others had found an inverse relationship between indices of hepatocellular damage and the frequency of circulating $CD4^+$ T cell recent thymic emigrants as determined by expression of CD31 (12, 40). In the current work we find that circulating levels of sCD163 that are linked to indices of hepatocellular inflammation are also correlated inversely with the numbers of circulating naïve CD4 T cells and $CD4^+$ recent thymic emigrants. The relationship among these indices remains incompletely understood but it is possible that processes taking place in the liver may play a role in alteration of $CD4^+$ T cell recovery during ART in the setting of HCV/HIV co-infection.

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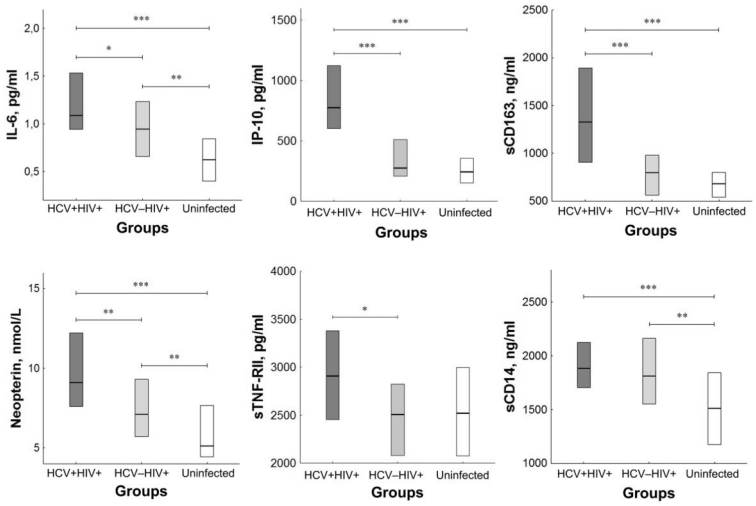

Fig. 1. Plasma indicators of systemic inflammation in HCV/HIV co-infected patients

Plasma concentrations of IL-6, IP-10, sCD163, neopterin, sTNF-RII and sCD14 are shown in three patient groups: co-infected with HCV/HIV, HIV monoinfected, and healthy volunteers without HIV or HCV infection. Columns with horizontal lines show medians with interquartile ranges. * – P<0.05; ** – P<0.01; *** – P<0.001 (Mann-Whitney test).

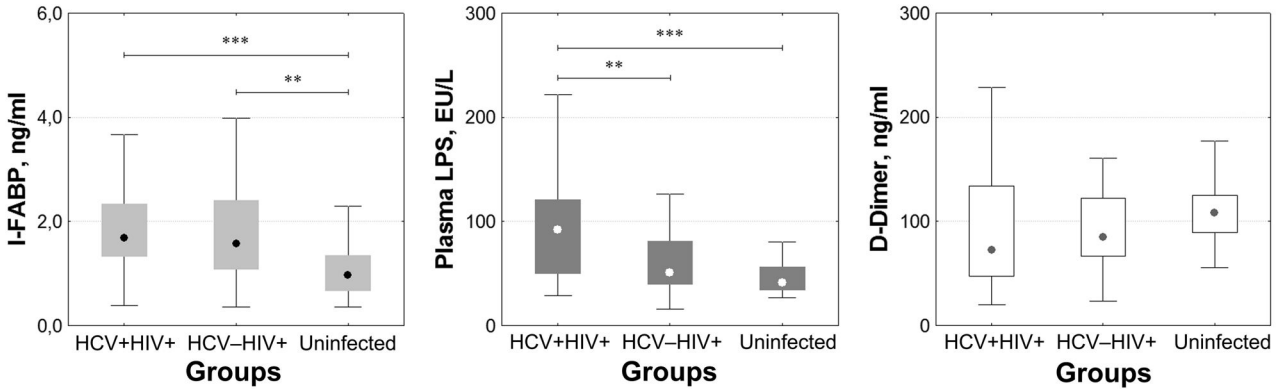

Fig. 2. Plasma concentrations of I-FABP, LPS and D-dimers in HCV/HIV coinfected and HIV monoinfected patients

Three patient groups are shown: HCV/HIV co-infected, HIV monoinfected, and healthy uninfected volunteers. Medians, interquartile ranges, upper and lower ranges are presented. ** – P <0.01; *** – P <0.001 (Mann-Whitney test).

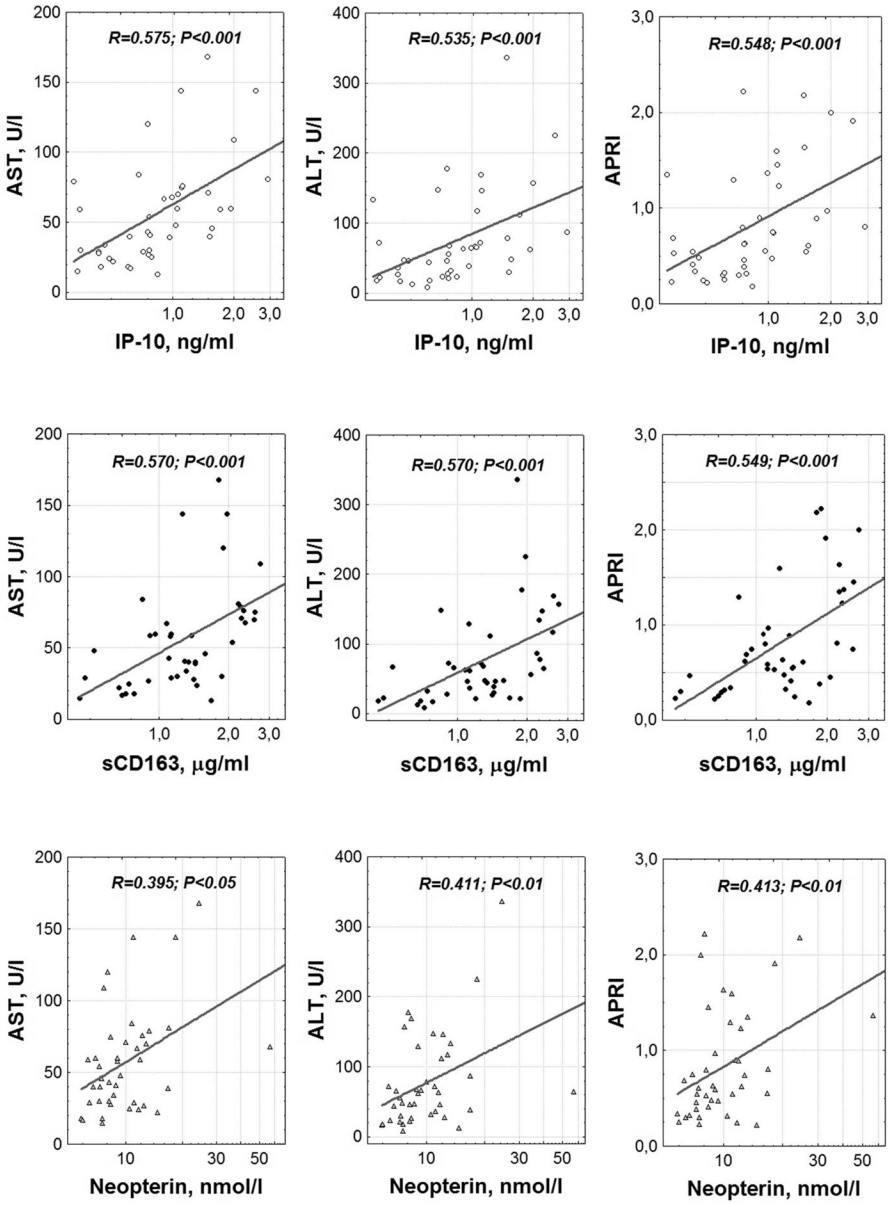

Fig. 3. Relationship between hepatocellular damage and indices of systemic inflammation
Correlation analysis was performed using the Spearman method.

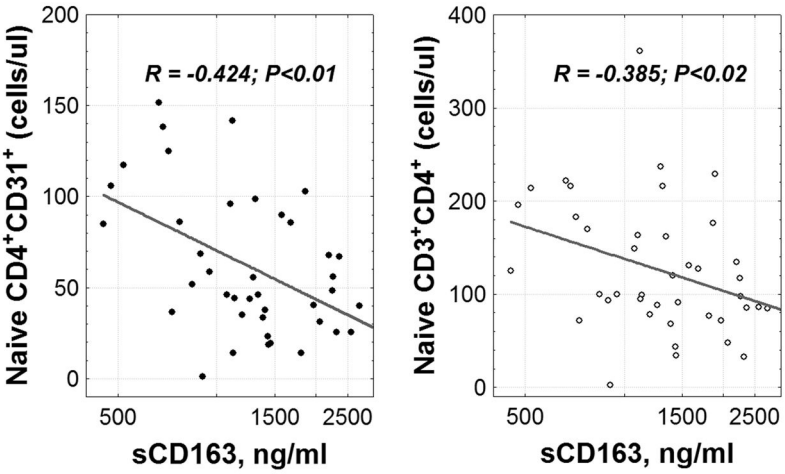

Fig. 4. Relationship between numbers of $CD4^+$ recent thymic emigrants, naïve $CD4^+$ T cell counts and plasma sCD163 levels in HCV/HIV co-infected subjects

Correlation analysis was performed using Spearman method.

Table

Clinical characteristics of HIV/HCV co-infected and HIV mono infected patients

Characteristics	HIV/HCV co-infected	HIV monoinfected	Uninfected
	1	2	3
Examined subjects (n)	42	37	20
Age (years)	33 (32/37)•	34 (31/41)	31 (26/35)
Male	26 (61.9%)	8 (21.6%)	8 (40.0%)
HIV transmission route			
Intravenous	36 (85.7%)	1 (2.7%)	–
Sexual	6 (14.3%)	36 (97.3%)	–
Homosexuals	0	0	0
Sex workers	0	0	0
Active drug users	0	0	0
HIV infection characteristics			
Infection duration (years)	11 (9/12) $P_{1-2}<0.001$	8 (6/10)	–
HAART duration (years)	3.5 (2/5) $P_{1-2}>0.05$	4 (3/5)	–
Nadir $CD4^+$ T cell count (μl^{-1})	140 (100/170) $P_{1-2}>0.05$	150 (106/170)	–
$CD4^+$ T cells at the study (μl^{-1})	350 (260/450) $P_{1-2}>0.05$	410 (290/570) $P_{2-3}<0.001$	1050 (660/1280) $P_{1-3}<0.001$
HIV viral load (copies/ml)	< 50	< 50	–
HCV infection characteristics			
Infection duration (years)	11 (8/12)	–	–
HCV viral load (log_{10} copies/ml)	6.21 (2.88/6.59)	< 2,88	< 2,88
AST (U/l)	47 (29/75) $P_{1-2}<0.001$	19 (17/23) $P_{2-3}>0.05$	19 (15/24) $P_{1-3}<0.001$
ALT (U/l)	59 (28/112) $P_{1-2}<0.001$	18 (14/23) $P_{2-3}>0.05$	19 (15/26) $P_{1-3}<0.001$
γ-GT (U/l)	71 (35/122) $P_{1-2}<0.001$	30 (23/45) $P_{2-3}>0.05$	27 (21/34) $P_{1-3}<0.001$
albumin (g/l)	41.7 (40.9/42.5) $P_{1-2}>0.05$	41.3 (40.4/43.5) $P_{2-3}>0.05$	41.8 (40.8/42.6) $P_{1-3}>0.05$
platelets (10^9/l)	202 (167/244) $P_{1-2}>0.05$	234 (177/276)	–
APRI	0.6 (0.4/1.2) $P_{1-2}<0.001$	0.2 (0.2/0.3)	–

AST – aspartate aminotransferase; ALT – alanine aminotransferase; γ-GT – γ-glutamyl transpeptidase; APRI – AST-to-platelet ratio index.

•Median with interquartile range (25th/75th%); statistics was done by Mann-Whitney method.