Plasma CD163 and FABP4 are associated with residual liver disease and fibrosis recovery during treatment-induced clearance of chronic HCV infection

Jean-Baptiste Gorin[1], David F. G. Malone[1], Benedikt Strunz[1], Tony Carlsson[2], Soo Aleman[2,3], Niklas K. Björkström[1], Karolin Falconer[2], and Johan K. Sandberg[1*]

[1]Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden.

[2]Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden.

[3]Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden.

* Corresponding author

Correspondence

Dr. Johan K. Sandberg, CIM, Department of Medicine, Karolinska Institutet, 14152 Stockholm, Sweden. E-mail: johan.sandberg@ki.se

Abstract

Direct-acting antivirals (DAAs) have dramatically improved the management of chronic hepatitis C (CHC). In this study, we investigated the effects of hepatitis C virus clearance on markers of systemic inflammation measured in plasma samples from CHC patients before, during and after DAA therapy. We identified an inflammatory profile specifically associated with CHC, and not shared with alcohol-induced non-viral liver disease. Successful DAA therapy rapidly normalised the plasma inflammatory milieu, with the notable exception of soluble (s) CD163, a marker of macrophage activation, which remained elevated after viral clearance and segregated patients with high and low levels of cirrhosis. Patients who received Ribavirin in combination with DAA maintained high levels of CXCL10, consistent with an immune-stimulatory role of Ribavirin. DAA-treated patients experienced durable improvement in liver fibrosis measurements and, interestingly, pre-treatment levels of fatty acid-binding protein 4 (FABP4) were inversely associated with fibrosis reduction during treatment. Together, these results support the notion of a rapid restoration of many aspects of the inflammatory state in CHC patients in response to DAA therapy. Furthermore, the associations with sCD163 and FABP4 suggest roles of persistent macrophage activation and altered fatty acid metabolism in residual liver disease and fibrosis improvement after viral clearance.

Introduction

Over 70 million individuals are infected with Hepatitis C virus (HCV) worldwide.[1] During the last decade, direct acting antivirals (DAA) have become available for treatment of chronic hepatitis C (CHC) and have dramatically improved clinical outcomes. DAA combinations have proven to be highly efficient, allowing for sustained virologic response (SVR) rates approaching 100%,[2] with a standard treatment course of 12 weeks that can even be shortened to 8 weeks in some instances.[3] These treatments have sparked hopes of eradicating HCV and the WHO has set the goal of a 90% reduction in new cases of chronic infection by 2030.[4] Nevertheless, the current burden of HCV remains high and chronic infection occurs in approximately 70% of cases. The chronic inflammation resulting from HCV-infection eventually leads to cirrhosis[5] and promotes tumorigenesis.[6,7] According to WHO approximately 400 000 people die each year from CHC, mostly because of cirrhosis and hepatocellular carcinoma (HCC). Despite the progress in treatment of CHC, it is therefore important to understand the dynamics of systemic and hepatic inflammation during and after successful DAA treatment.

Although HCV infection localizes to the liver, chronic hepatic inflammation causes systemic changes in blood cytokine and chemokine levels.[8,9] Numerous studies have investigated whether plasma levels of such soluble markers could predict clinical outcome of interferon (IFN)α-based therapy.[10,11] In the present study, we investigated how viral clearance in response to DAA therapy affects plasmatic levels of cytokines and inflammatory markers, and whether the levels of these soluble components are associated with clinically important measures, in a cohort of CHC patients initiating DAA. We observed a rapid restoration of the plasma cytokine milieu upon treatment with IFN-free DAA combination, with the exception of soluble (s)CD163, a marker of macrophage activation, which remained elevated. Interestingly, sCD163 differentiated Child Pugh A and B patients (i.e. patients with varying

degrees of liver cirrhosis) throughout treatment and may therefore be an indicator of residual liver disease. As expected, clearance of HCV was associated with significantly decreased liver stiffness measurement (LSM), Aspartate transaminase-to-platelet ratio Index (APRI) and Fibrosis index based on 4 factors (FIB-4) scores. We also investigated whether plasmatic protein levels could predict improvement in liver status and found that pre-treatment levels of fatty acid-binding protein 4 (FABP4) were significantly associated with the decrease in APRI and FIB-4 scores by the end of treatment. Our results thus support the notion that DAA therapy reduces inflammation and fibrosis upon successful elimination of HCV in chronically infected patients, and prompt further investigation of the associations between FABP4 and sCD163 with fibrosis improvement and residual liver disease after HCV clearance.

Results

Chronic hepatitis C-associated alterations in the plasma cytokine milieu

To investigate the impact of HCV clearance on the immune system, 28 CHC patients were sampled before, during and after successful DAA treatment (Table 1). In addition, blood samples from 20 healthy donors (HD), and 12 patients suffering from alcohol-induced cirrhosis (AC) were included as non-HCV infected comparison groups. Plasma concentrations of 25 soluble factors, all known to be related to the immune response or inflammation, were measured in each plasma sample (Supplementary Table S1 online). Mann-Whitney U comparisons were then performed between HD and CHC patients who were going to initiate IFN-free DAA therapy. Out of the 25 soluble factors measured, five were significantly different ($p < 0.05$) between the HD and CHC groups at baseline: CCL5, IL-4, CXCL19, sCD14 and sCD163. Both CCL5 and IL-4 were detected at lower levels ($p = 0.0018$ and 0.045, respectively), whereas CXCL10, sCD14 and sCD163 were considerably higher in the CHC patients compared to HD ($p < 0.0001$, $p = 0.0039$ and $p < 0.0001$, respectively) (Fig. 1a). Interestingly, there were no significant differences in CCL5 and IL-4 levels between CHC patients and AC patients ($p = 0.135$ and 0.0512), indicating that the lower levels of CCL5 and IL-4 in CHC relative to HD subjects may be driven by non-viral effects of liver disease rather than by HCV infection *per se* (Fig. 1b). In contrast, CXCL10, sCD14 and sCD163 were all significantly higher in CHC patients compared to AC ($p = 0.0203$, 0.0186 and 0.0215, respectively), indicating that levels of these soluble factors are primarily driven by HCV infection.

Longitudinal patterns of the plasma cytokine profile during treatment

We next analysed changes in the plasma cytokine profile of CHC patients during the course of therapy. In the DAA group, most baseline differences with HD rapidly normalized by four

weeks of therapy and only sCD163 was still significantly elevated at end of treatment (EOT) as compared to HD (Fig. 2a). In contrast, in patients undergoing IFN therapy, all of the observed plasma protein alterations at baseline were maintained and six additional markers were altered during treatment such that eight markers were significantly different from HD at EOT (Fig. 2a). This effect was further illustrated by the evolution over the course of treatment of the fold-change differences in median concentrations for the different cytokines when compared to HD (Fig. 2b). Notably, sCD14 and IL-18 were both increased during IFN therapy as previously observed.[12,13] With DAA therapy, the concentrations quickly normalized to levels observed in HD (dotted line), whereas in patients receiving IFN the median concentration of many of the analysed soluble proteins diverged from normal values during treatment.

When the plasma protein profile at follow-up (FU) approximately six months after EOT was analysed, an interesting pattern emerged with elevated levels of CXCL10 and IL-6 in DAA patients (Fig. 2b). To investigate this, we further stratified the DAA group according to use of Ribavirin (RBV) in their treatment regimen. RBV was previously found to have immunomodulatory effects,[14,15] and to induce ISG expression in vitro.[16,17] Half of the patients (14 patients) received RBV as part of their DAA regimen while the other half did not (Supplementary Fig. S1 online). There were no other significant differences at baseline between these two subgroups regarding age, BMI, liver health indicators (ALT, AST, albumin, bilirubin, PT-INR, thrombocytes, LSM) or any of the measured soluble markers. The patients that received RBV-free therapy quickly normalized all soluble factors in their plasma except for sCD163, which remained elevated throughout treatment. In contrast, patients that received RBV in combination with DAA maintained elevated CXCL10 throughout treatment as well as slightly elevated sCD14 at follow-up (Supplementary Fig. S2

online). Thus, among the measured soluble plasma proteins, sCD163 appears to remain uniquely elevated in a treatment-independent manner after viral clearance.

Levels of sCD163 distinguish patients with different degrees of liver cirrhosis throughout DAA therapy

To assess whether the stage of liver disease was associated with the plasmatic protein profile, we stratified cirrhotic patients in the DAA group according to Child Pugh class. Most of the patients initiating DAA treatment were categorized as Child Pugh A at baseline (18 out of 28) and, the results for these patients did not differ from the overall combined DAA group (Fig. 3a). However, the seven patients classified as Child Pugh B presented significant differences in plasma concentrations for 11 out of the 25 analysed soluble components at baseline in comparison to HD (Fig. 3a). This suggested that the degree of cirrhosis might be linked to a distinct cytokine profile at baseline. Furthermore, four weeks after the start of therapy, LIF and sCD163 were still different from HD, and by EOT sCD14 and sCD163 remained significantly elevated (Fig. 3a). Despite these differences, analysis of the median fold change over the course of therapy, revealed similar response kinetics for patients classified as Child Pugh A or B at the start of treatment (Fig. 3b).

At the EOT there were two significant differences between the Child Pugh A or B subgroups, in the plasma concentrations of PDGF-BB and sCD163 (Fig. 3b). Interestingly, a study by Hengst et al. that found PDGF-BB to be negatively correlated to liver stiffness[18], and Kazankov et al. observed an independent association between sCD163 and liver fibrosis in CHC-patients before treatment.[19] Thus, sustained low levels of PDGF-BB and high levels of sCD163 in treated Child Pugh B patients may therefore suggest limited improvement in fibrosis even six months after the end of therapy. To investigate this hypothesis, we next analysed changes in indicators of liver fibrosis and inflammation throughout therapy.

IFN-free DAA combinations improve fibrosis indicators

Liver stiffness measurements were available from time points before and after therapy in 18 of the 28 DAA-treated patients (two non-cirrhotic, 14 Child Pugh A and two Child Pugh B). Wilcoxon signed-rank test of paired LSMs demonstrated a significant decrease in stiffness values ($p = 0.0002$), between baseline and follow-up measurements in the DAA group. This pattern was also evident in the subgroup of Child Pugh A patients ($p = 0.0039$), with a similar trend in Child Pugh B patients (Fig. 4a). This pattern indicated decreased liver fibrosis and inflammation. Since DAA therapy efficiently eliminates the virus and quickly normalizes most aspects of the cytokine milieu in plasma, it is likely that this treatment reduces liver inflammation and a decrease in LSM may thus be insufficient to conclude that there is a decrease in fibrosis. We therefore calculated APRI[20] and FIB-4[21] scores for all treated patients. Both APRI and FIB-4 decreased significantly by EOT ($p < 0.0001$), and this was maintained at follow-up ($p < 0.0001$) (Fig. 4b and 4c). This pattern was also evident when stratifying patients by Child Pugh score at baseline. Indeed, both APRI and FIB-4 were significantly lower at EOT in Child Pugh A ($p < 0.0001$ and $p = 0.0003$) and Child Pugh B ($p = 0.0156$ and 0.0313) patients. This is consistent with the notion that DAA therapy reduces liver fibrosis, including in patients with advanced cirrhosis. In support of these results, six out of seven patients classified as Child Pugh B before the start of therapy were re-classified as Child Pugh A at FU, further indicating an improvement of liver status.

Baseline levels of soluble FABP4 are associated with improvement of fibrosis indicators in response to DAA therapy

To investigate whether soluble plasma markers might be associated with fibrosis improvement, we performed Spearman correlation analyses between baseline levels of the 25

cytokines and the reduction in LSM, APRI and FIB-4 scores observed at FU (Fig. 5). IL-18 levels at baseline correlated positively with LSM reduction ($\rho = 0.54$, $p = 0.028$), and FABP4 levels at baseline correlated negatively with reductions in both APRI ($\rho = -0.53$, $p = 0.0035$) and FIB-4 ($\rho = -0.65$, $p < 0.001$). FABP4 appeared to be the marker most strongly associated with reduction in fibrosis indicators, since it showed the strongest correlation and was associated with two of the three indicators.

Discussion

The introduction of IFN-free DAA combinations has drastically improved the management of CHC with high SVR rates, shorter treatment course and fewer side effects compared to previous IFN containing regimens. Nevertheless, continuing risks of residual liver disease and development of HCC is a concern in patients that have cleared their HCV infection, and it is therefore important to understand the dynamics of systemic and hepatic inflammation during and after successful DAA treatment. Here, we identify an inflammatory cytokine profile associated with CHC, but not with AC, including elevated levels of sCD163, sCD14 and CXCL10. We show that DAA therapy rapidly normalizes the plasmatic cytokine milieu in peripheral blood of both Child Pugh A and B patients, with the exception of sCD163, which distinguishes patients with different levels of cirrhosis. Furthermore, our data set identifies a strong association between baseline levels of FABP4 and fibrosis improvement during DAA treatment. The associations with sCD163 and FABP4 support roles of macrophage activation and fatty acid metabolism in residual liver disease and fibrosis improvement after clearance of HCV.

Our data set identified clear differences in plasma inflammatory markers between CHC patients and HD. Interestingly, CCL5 and IL-4 that were low in CHC patients were even more so in AC patients, suggesting that those changes were not specific to HCV infection *per se* but instead reflective of liver damage or liver inflammation. On the other hand, CXCL10, sCD14 and sCD163 were markedly higher in CHC than in AC patients, indicating that those markers may be more tightly associated with immune activation in response to HCV infection. CXCL10 has been previously associated with viral load and it is known that HCV triggers its production through NF-κB activation.[20] Soluble CD14 is a marker of monocyte activation,[21] sCD163 is a marker of macrophage activation,[22] and all these three markers have been associated with liver fibrosis in untreated CHC.[19,23,24] Thus, the higher plasma levels of

CXCL10, sCD14 and sCD163 in CHC compared to AC might collectively reflect a combination of anti-viral immune activation as well as more severe liver disease.

DAA therapy quickly normalized the concentrations of most of the analysed soluble proteins, with the notable exception of sCD163, whereas IFN-containing regimens induced broad changes in the cytokine milieu. This pattern likely reflects the very different mechanisms of action of the two treatments. DAA drugs specifically interfere with viral replication, whereas IFN is immune activating and modulates the expression of hundreds of IFN-regulated genes, which themselves can influence cytokine secretion via a complex network of interactions.[25] These findings are in accordance with recent work by Burchill et al. that showed a rapid decrease in the expression of genes associated with inflammation in PBMCs of HCV infected patients undergoing DAA therapy.[26] Certain features of T-cell and NK cell phenotype and function are also restored following DAA therapy,[27-30] supporting the idea of a general immune system normalization after HCV clearance. However, recent studies also indicate that other immune cell traits, such as the appearance of regulatory T cells,[31] NK cell receptor repertoire diversity,[32] and the MAIT cell compartment,[33] are still altered after DAA therapy. Thus, the impact on the cellular immune system might in part persist after successful viral clearance. Hengst et al. observed a partial but incomplete restoration of the cytokine milieu during DAA therapy and, notably, CXCL10 remained elevated throughout treatment.[18] Interestingly, all patients in that cohort received RBV in combination with DAA, and our observations indicate that patients who receive RBV maintain higher levels of CXCL10. Studies have also shown that RBV can induce ISGs in vitro.[16,17] In our study, only sCD163 remained significantly elevated in patients that received DAA regimen without RBV. This is in accordance with work by Mascia et al. showing sustained high levels of sCD163 in DAA-treated CHC patients,[34] and taken together these findings by us and others suggest that

residual macrophage activation plays an important role in residual liver disease despite a general dampening of inflammation.

It is important to understand the response of patients with more severe cirrhosis (Child Pugh B) to DAA combinations in comparison to patients with milder liver damage (Child Pugh A). Our data indicate that IFN-free DAA therapy normalizes the cytokine profile, with the exception of sCD163, in both groups, and thus suggest that advanced liver damage is not a barrier to a normalized plasma cytokine milieu. Interestingly, six out of the seven Child Pugh B patients from our cohort were re-classified as Child Pugh A at the EOT, in line with improved liver function. We also observed a clear improvement in LSMs after treatment, as has been observed by others.[35-37] However, this measurement does not allow clear discrimination between reduction in liver inflammation, improvement of fibrosis, or a combination of the two. It has also been argued that the predictive power of liver elasticity measurements for fibrosis is reduced following therapeutic eradication of HCV.[38] To complement our observation, we therefore investigated the evolution of APRI and FIB-4 scores during therapy and showed a clear reduction in both of these indicators of liver fibrosis. Nevertheless, further studies comprising liver biopsies will be required to determine the extent of liver regeneration after successful DAA therapy.

Lastly, we investigated associations between plasmatic proteins and fibrosis improvement and found that baseline levels of FABP4 inversely correlated with the improvement in both APRI and FIB-4 observed after treatment. Although this association may be partly explained by the correlation between FABP4 levels and platelet counts, this alone does not account for the relatively strong correlation with both fibrosis indicators. FABP4 is expressed in adipocytes and macrophages, and high serum concentrations of FABP4 are associated with inflammation and risk for metabolic and vascular diseases.[39] Recently, increased FABP4 levels in the blood has also been shown to correlate with poor

prognosis in cirrhosis.[40] In the same study, it was shown that FABP4 gene expression was increased in cirrhotic livers and that liver macrophages seemed to be responsible for that increase. Interestingly, sCD163 is also released by macrophages and hepatic macrophages are known to play a critical role in liver inflammation, fibrosis and resolution of inflammation.[41] Investigating the mechanisms leading to sCD163 and FABP4 release during CHC may thus help better understand the mechanisms of fibrosis resolution during DAA therapy.

In conclusion, these results support the notion of a rapid restoration of the inflammatory state in CHC in response to DAA therapy. Furthermore, our findings indicate important roles of macrophage activation and fatty acid metabolism in residual liver disease and fibrosis improvement after clearance of HCV infection.

Methods

Patients

To investigate the impact of HCV clearance on the immune system, CHC patients above 18 years of age scheduled to receive DAA therapy were sampled before, during and after treatment at the Department of Infectious Diseases and the Department of Gastroenterology and Hepatology at Karolinska University Hospital (Table 1). Blood samples were collected from 28 CHC patients who received DAA therapy and successfully achieved SVR, and 13 CHC patients who received pegylated-IFN therapy and successfully achieved SVR (Supplementary Fig. S1 and S3 online). Exclusion criteria included concurrent Hepatitis B virus or HIV co-infections. In addition, blood samples from 20 HD, and 12 patients suffering from AC were included as non-HCV infected comparison groups. Of note, a proportion (64%) of patients in the DAA treated group previously underwent IFNα-based therapy without achieving SVR. Consequently, the DAA group was older than the IFN group and comprised a larger proportion of cirrhotic patients (89% vs 38% in the IFN cohort). The HD group was matched in gender and age to the DAA group. The study was conducted in accordance with the declaration of Helsinki, approved by the Stockholm Regional Ethics Review Board (approval number 2012/63-31/1) and all participants gave informed consent.

Liver Stiffness Measurement

Liver stiffness measurement was performed using transient elastography (FibroScan®). Paired LSMs before and after therapy were obtained for 18 of the 28 DAA-treated patients (2 non-cirrhotic, 14 Child Pugh A and 2 Child Pugh B liver cirrhosis patients). Measurements with an IQR > 30 or a success rate < 50% were excluded from the analysis.

Sample collection

Venous blood was collected in heparin-coated tubes and spun 10 min at 680 g at room temperature to separate plasma, which was stored at -80°C immediately after separation. CHC patients receiving DAA therapy had blood collected at four time-points: before initiation of therapy, four weeks after initiation of therapy (W4), at the end of therapy (EOT, either 12 or 24 weeks after initiation of therapy) and at a follow-up time point collected approximatively six months after the EOT. For the IFN-treated group, 13 baseline samples, 11 W12 and 6 EOT samples (either 24 or 48 weeks after initiation of therapy) were included in the analysis.

Luminex assay and ELISA

Plasma samples were analysed using a custom 24-plex magnetic Luminex assay (R&D systems). All samples were diluted 1:2 and assays were performed according to manufacturer's protocol. Samples were acquired using the Bio-Plex Magpix multiplex reader (Bio-Rad) and analysed using the Bio-Plex Manager software. Soluble CD14 (sCD14) concentration in plasma samples was measured using the human CD14 Quantikine ELISA kit from R&D systems (DC140) according to manufacturer's protocol.

Statistical analysis

Statistical analyses were performed using the Scipy python library version 1.0.0 and Prism 6 software. The statistical tests used are specified in the legend for each figure and p-values < 0.05 were considered statistically significant throughout the study.

Acknowledgements

We thank Britt-Mare Löfberg and Pia Loqvist for their help with collecting blood samples. We also thank the Swedish Research Council (2016-03052) and the Swedish Cancer Society (CAN 2017/777) for their financial support.

Author Contributions

JBG collected samples, performed experiments, analysed data and co-wrote the manuscript. DFGM participated in study design, collected samples and performed experiments. BS collected samples. TC, SA and KF participated in patient recruitment. NKB, SA and KF participated in study design and data analysis. JKS coordinated the study, participated in study design, data analysis, and co-wrote the manuscript. All authors read and approved the final manuscript.

Competing Interests

JBG, DFGM, BS, TC, NKB, KF and JKS report no competing interests. SA has served as a speaker and a consultant for AbbVie, Gilead, BMS and MSD, and has received research funding from AbbVie and Gilead for an investigator-initiated study, not related to this study.

Data Availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

References

1. Blach, S. *et al.* Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *The Lancet Gastroenterology & Hepatology* **2,** 161–176 (2017).
2. Burstow, N. J. *et al.* Hepatitis C treatment: where are we now? *Int J Gen Med* **10,** 39–52 (2017).
3. European Association for the Study of the Liver. Electronic address: easloffice@easloffice.euEuropean Association for the Study of the Liver. EASL Recommendations on Treatment of Hepatitis C 2018. *J. Hepatol.* (2018). doi:10.1016/j.jhep.2018.03.026
4. World Health Organization. Global Hepatitis Programme. *Global Health Sector Strategy on Viral Hepatitis, 2016-2021*. (2016).
5. Tanaka, M. & Miyajima, A. Liver regeneration and fibrosis after inflammation. *Inflamm Regen* **36,** 19 (2016).
6. Brownell, J. & Polyak, S. J. Molecular Pathways: Hepatitis C Virus, CXCL10, and the Inflammatory Road to Liver Cancer. *Clinical Cancer Research* **19,** 1347–1352 (2013).
7. Yu, L.-X., Ling, Y. & Wang, H.-Y. Role of nonresolving inflammation in hepatocellular carcinoma development and progression. *npj Precision Onc* **2,** 87 (2018).
8. Mascia, C. *et al.* Active HCV infection is associated with increased circulating levels of interferon-gamma (IFN-γ)-inducible protein-10 (IP-10), soluble CD163 and inflammatory monocytes regardless of liver fibrosis and HIV coinfection. *Clin Res Hepatol Gastroenterol* (2017). doi:10.1016/j.clinre.2017.04.007
9. Baskic, D. *et al.* Cytokine profile in chronic hepatitis C: An observation. *Cytokine* **96,** 185–188 (2017).

10. Neesgaard, B., Ruhwald, M. & Weis, N. Inducible protein-10 as a predictive marker of antiviral hepatitis C treatment: A systematic review. *World J Hepatol* **9,** 677–688 (2017).

11. Yoneda, S. *et al.* Association of serum cytokine levels with treatment response to pegylated interferon and ribavirin therapy in genotype 1 chronic hepatitis C patients. *J. Infect. Dis.* **203,** 1087–1095 (2011).

12. Anthony, D. D. *et al.* Baseline levels of soluble CD14 and CD16+56- natural killer cells are negatively associated with response to interferon/ribavirin therapy during HCV-HIV-1 coinfection. *J. Infect. Dis.* **206,** 969–973 (2012).

13. Malone, D. F. G., Falconer, K., Weiland, O. & Sandberg, J. K. The dynamic relationship between innate immune biomarkers and interferon-based treatment effects and outcome in hepatitis C virus infection is altered by telaprevir. *PLoS ONE* **9,** e105665 (2014).

14. Graci, J. D. & Cameron, C. E. Mechanisms of action of ribavirin against distinct viruses. *Rev. Med. Virol.* **16,** 37–48 (2006).

15. Chung, R. T. *et al.* Mechanisms of action of interferon and ribavirin in chronic hepatitis C: Summary of a workshop. in **47,** 306–320 (2008).

16. Thomas, E. *et al.* Ribavirin potentiates interferon action by augmenting interferon-stimulated gene induction in hepatitis C virus cell culture models. *Hepatology* **53,** 32–41 (2011).

17. Wang, Y. *et al.* Ribavirin Contributes to Hepatitis C Virus Suppression by Augmenting pDC Activation and Type 1 IFN Production. *PLoS ONE* **10,** e0135232 (2015).

18. Hengst, J. *et al.* Direct-Acting Antiviral-Induced Hepatitis C Virus Clearance Does Not Completely Restore the Altered Cytokine and Chemokine Milieu in Patients With Chronic Hepatitis C. *J. Infect. Dis.* **214,** 1965–1974 (2016).

19. Kazankov, K. *et al.* Soluble CD163, a macrophage activation marker, is independently associated with fibrosis in patients with chronic viral hepatitis B and C. *Hepatology* **60,** 521–530 (2014).

20. Brownell, J. *et al.* Direct, interferon-independent activation of the CXCL10 promoter by NF-κB and interferon regulatory factor 3 during hepatitis C virus infection. *Journal of Virology* **88,** 1582–1590 (2014).

21. Shive, C. L., Jiang, W., Anthony, D. D. & Lederman, M. M. Soluble CD14 is a nonspecific marker of monocyte activation. *AIDS* **29,** 1263–1265 (2015).

22. Møller, H. J. Soluble CD163. *Scand. J. Clin. Lab. Invest.* **72,** 1–13 (2012).

23. Sandler, N. G. *et al.* Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. *Gastroenterology* **141,** 1220–30–1230.e1–3 (2011).

24. Zeremski, M., Dimova, R., Astemborski, J., Thomas, D. L. & Talal, A. H. CXCL9 and CXCL10 Chemokines as Predictors of Liver Fibrosis in a Cohort of Primarily African-American Injection Drug Users With Chronic Hepatitis C. *J. Infect. Dis.* **204,** 832–836 (2011).

25. Schneider, W. M., Chevillotte, M. D. & Rice, C. M. Interferon-stimulated genes: a complex web of host defenses. *Annu. Rev. Immunol.* **32,** 513–545 (2014).

26. Burchill, M. A. *et al.* Rapid reversal of innate immune dysregulation in blood of patients and livers of humanized mice with HCV following DAA therapy. *PLoS ONE* **12,** e0186213–17 (2017).

27. Burchill, M. A., Golden-Mason, L., Wind-Rotolo, M. & Rosen, H. R. Memory re-differentiation and reduced lymphocyte activation in chronic HCV-infected patients receiving direct-acting antivirals. *J Viral Hepat* **22,** 983–991 (2015).

28. Martin, B. *et al.* Restoration of HCV-specific CD8+ T cell function by interferon-free therapy. *J. Hepatol.* **61,** 538–543 (2014).

29. Spaan, M. *et al.* Immunological Analysis During Interferon-Free Therapy for Chronic Hepatitis C Virus Infection Reveals Modulation of the Natural Killer Cell Compartment. *J. Infect. Dis.* **213,** 216–223 (2016).

30. Serti, E. *et al.* Successful Interferon-Free Therapy of Chronic Hepatitis C Virus Infection Normalizes Natural Killer Cell Function. *Gastroenterology* **149,** 190–200.e2 (2015).

31. Langhans, B. *et al.* Increased peripheral CD4+ regulatory T cells persist after successful direct-acting antiviral treatment of chronic hepatitis C. *J. Hepatol.* **66,** 888–896 (2017).

32. Strunz, B. *et al.* Chronic hepatitis C virus infection irreversibly impacts human natural killer cell repertoire diversity. *Nat Commun* **9,** 2275 (2018).

33. Hengst, J. *et al.* Nonreversible MAIT cell-dysfunction in chronic hepatitis C virus infection despite successful interferon-free therapy. *Eur. J. Immunol.* **46,** 2204–2210 (2016).

34. Mascia, C. *et al.* Changes in inflammatory biomarkers in HCV-infected patients undergoing direct acting antiviral-containing regimens with or without interferon. *PLoS ONE* **12,** e0179400 (2017).

35. Bernuth, S. *et al.* Early changes in dynamic biomarkers of liver fibrosis in hepatitis C virus-infected patients treated with sofosbuvir. *Digestive and Liver Disease* **48,** 291–297 (2016).

36. Knop, V. *et al.* Regression of fibrosis and portal hypertension in HCV-associated cirrhosis and sustained virologic response after interferon-free antiviral therapy. *J Viral Hepat* **23,** 994–1002 (2016).

37. Bruno, G. *et al.* Rapid improvement in liver fibrosis in HCV-infected patients with or without HIV infection and DAA-induced SVR: A 'turning-off' effect of liver inflammation? *J Viral Hepat* **24,** 174–175 (2017).

38. D'Ambrosio, R. *et al.* The diagnostic accuracy of Fibroscan for cirrhosis is influenced by liver morphometry in HCV patients with a sustained virological response. *J. Hepatol.* **59,** 251–256 (2013).

39. Kralisch, S. & Fasshauer, M. Adipocyte fatty acid binding protein: a novel adipokine involved in the pathogenesis of metabolic and vascular disease? *Diabetologia* **56,** 10–21 (2013).

40. Graupera, I. *et al.* Adipocyte Fatty-Acid Binding Protein is Overexpressed in Cirrhosis and Correlates with Clinical Outcomes. *Sci Rep* **7,** 1829 (2017).

41. Krenkel, O. & Tacke, F. Liver macrophages in tissue homeostasis and disease. *Nature Reviews Immunology* **17,** 306–321 (2017).

Tables

Table 1: Cohort baseline characteristics

Values are count (and percentage) for categorical variables and median [IQR] for continuous variables. ND: Not determined

	HD	***AC***	***DAA***	***IFN***
	n = 20	n = 12	n = 28	n = 13
M / F	50% / 50%	92% / 8%	50% / 50%	92% / 8%
Age (years)	57.00 [7.75]	60.00 [12.25]	60.50 [11.25]	49.00 [17.00]
BMI (kg/m^2)	ND	30.77 [4.38]	25.94 [5.21]	26.80 [3.86]
Diabetes	ND	4 (33%)	4 (14%)	5 (38%)
Alcoholic	ND	12 (100%)	11 (39%)	3 (23%)
Cirrhosis	ND	12 (100%)	25 (89%)	5 (38%)
Child Pugh A / B	-	6 / 5	18 / 7	5 / 0
Viral load (10^6/mL)	-	-	1.06 [3.18]	1.14 [2.55]
Genotype 1/2/3/4	-	-	19 / 2 / 6 / 1	5 / 1 / 6 / 1
Prior treatment	-	-	18 (64%)	0 (0%)
Albumin (g/L)	ND	33.00 [8.75]	34.50 [8.25]	39.00 [5.00]
Bilirubin (µmol/L)	ND	18.50 [24.50]	14.00 [13.25]	13.00 [8.00]
ALT (µkat/L)	ND	0.54 [0.29]	1.27 [0.97]	2.06 [1.48]
AST (µkat/L)	ND	0.82 [0.36]	1.37 [1.04]	1.27 [1.36]
PT (INR)	ND	1.20 [0.23]	1.15 [0.22]	1.00 [0.10]

Thrombocytes (10^9/L)	ND	140.00 [70.00]	127.50 [83.50]	208.00 [104.00]
LSM (kPa)	ND	24.35 [19.25]	21.70 [16.25]	11.50 [10.10]
APRI score	ND	0.90 [0.95]	1.92 [1.99]	0.85 [1.89]
FIB-4 index	ND	4.43 [3.25]	6.22 [5.35]	1.58 [2.72]

Figure Legends

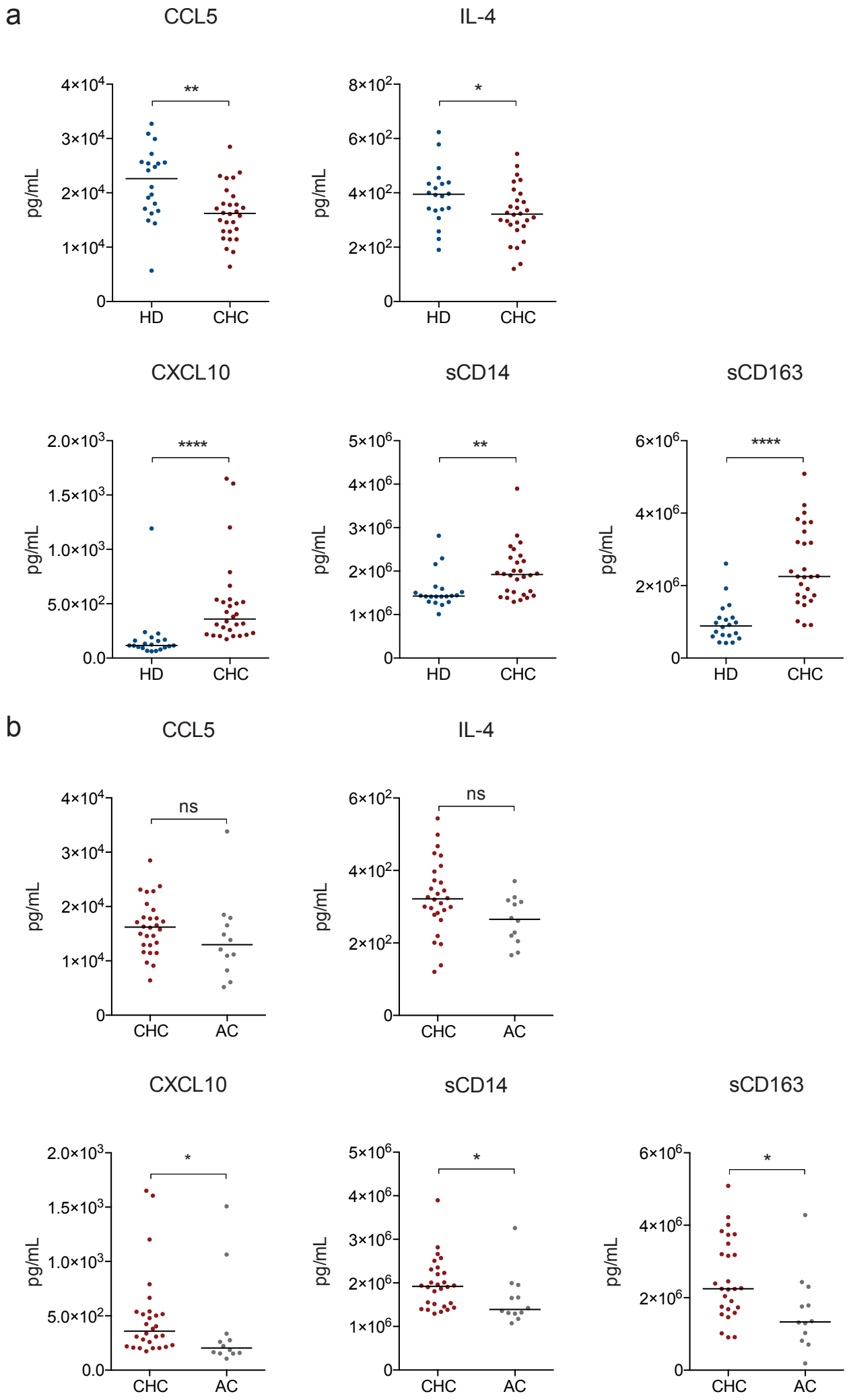
Fig. 1. Baseline differences in plasma levels of soluble markers between healthy controls and patients. (a) Markers significantly different at baseline between chronic hepatitis C patients (CHC) that subsequently received DAA treatment and healthy donors (HD). (b) Comparison between CHC and alcoholic cirrhotic patients (AC) for those same markers. Statistical significance was assessed by Mann-Whitney U comparison. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

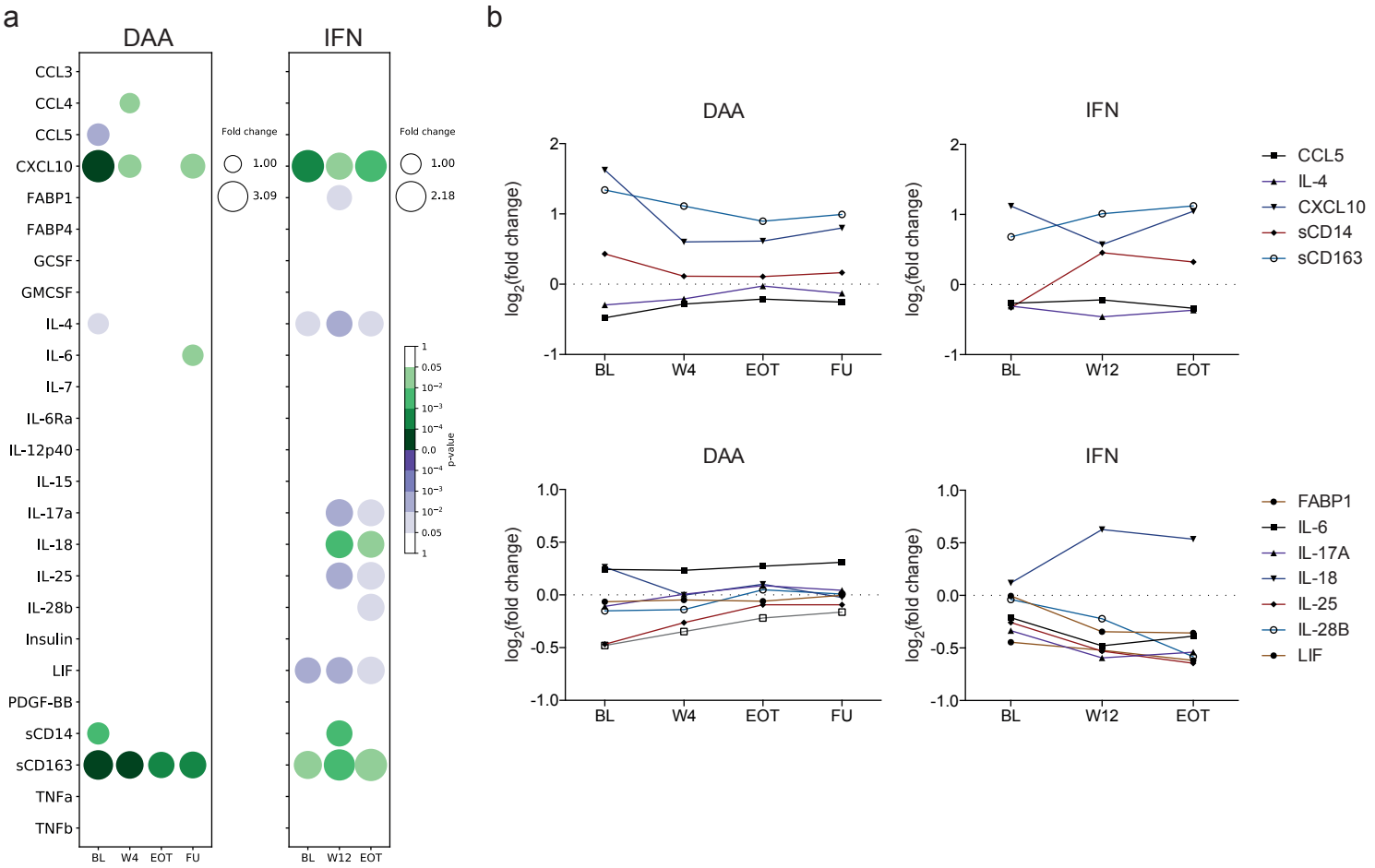
Fig. 2. Changes in plasma levels of soluble markers over the course of therapy. (a) Heatmap of p-values for Mann-Whitney U comparisons for each marker. A purple colour indicates a significant decrease of the marker's level in CHC-patients in comparison to healthy donors while a green colour indicates an increase. (b) Evolution of markers concentration over the course of treatment relative to healthy donors. Fold change was calculated as the median of plasma concentration in patients divided by the median in healthy controls. BL: baseline; W4: week 4; W12: week 12; EOT: end of treatment; FU: follow-up.

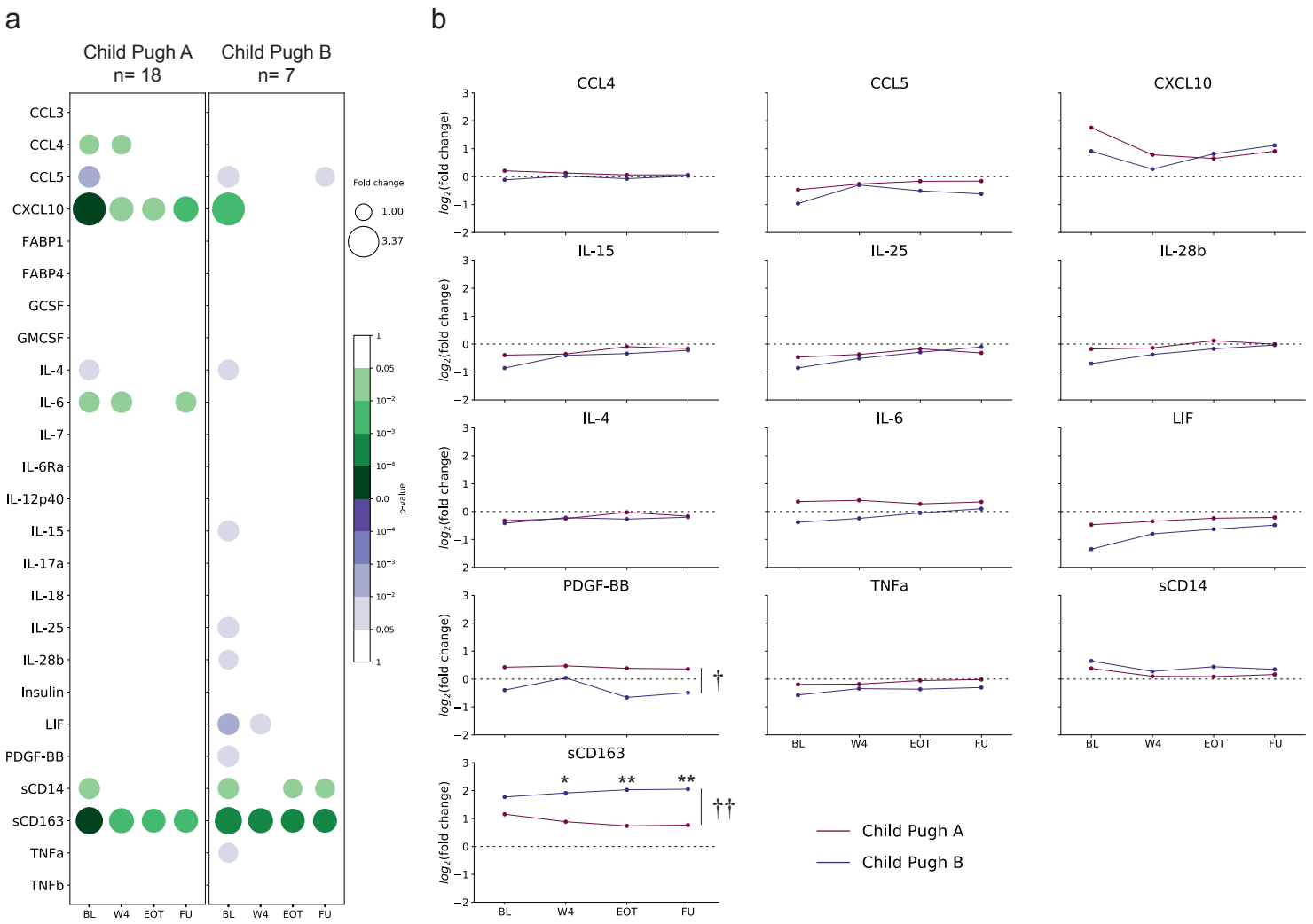
Fig. 3. Changes in plasma levels of soluble markers over the course of therapy. (a) Heatmap of p-values for Mann-Whitney U comparisons for each marker. A purple colour indicates a significant decrease of the marker's level in CHC-patients in comparison to healthy donors while a green colour indicates an increase. (b) Evolution of markers concentration over the course of treatment relative to healthy donors. Fold change was calculated as the median of plasma concentration in patients divided by the median in healthy controls. Crosses and stars indicate statistical difference between Child Pugh A and Child Pugh B patients computed by repeated ANOVA and post hoc Sidak's multiple comparison test, respectively. † $p < 0.05$; †† $p < 0.01$; * $p < 0.05$; ** $p < 0.01$.

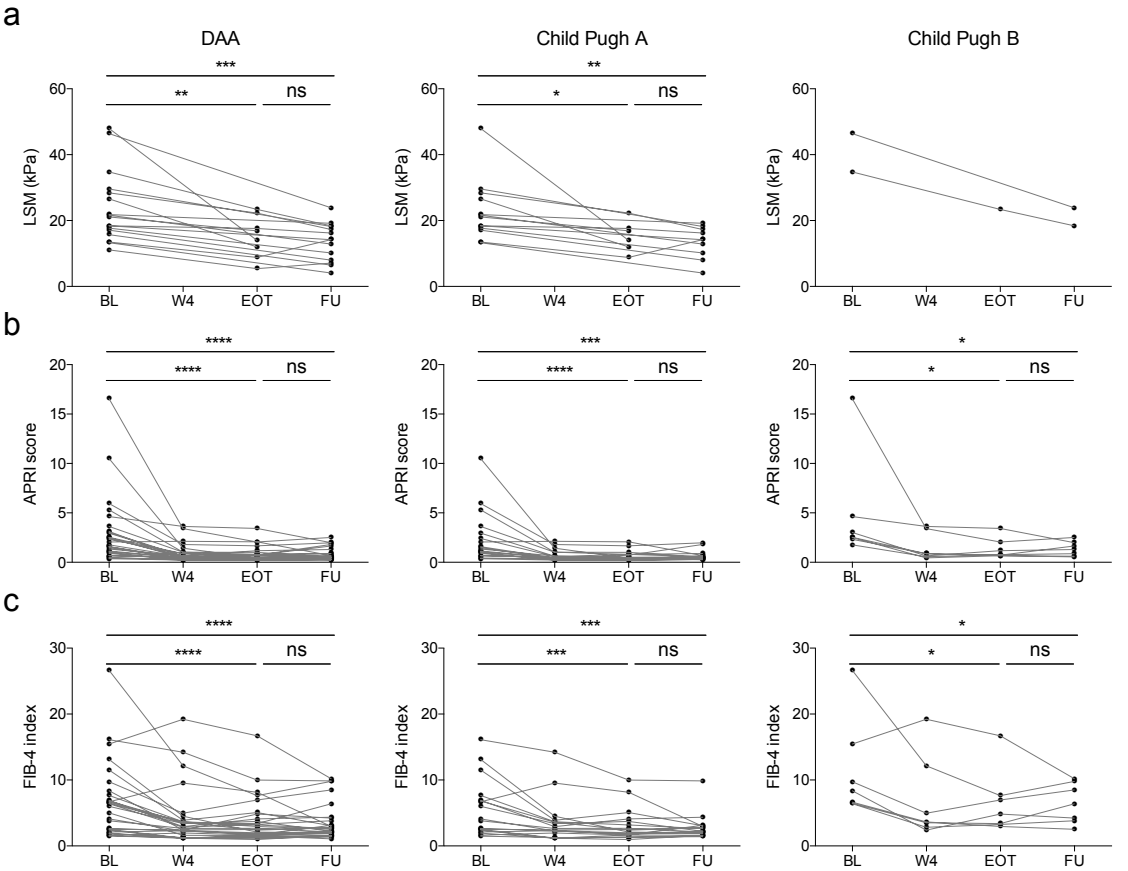
Fig. 4. Improvement in fibrosis indicators values over the course of DAA therapy. Evolution of LSM (a), APRI score (b) and FIB-4 index (c) during the course of DAA therapy. Stars indicate statistical difference between time-points computed with Wilcoxon signed-rank test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

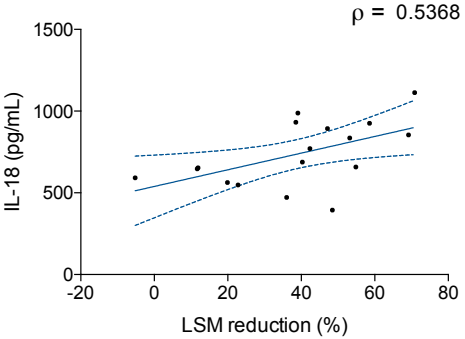
Fig. 5. Correlations between baseline cytokine levels and decrease in fibrosis indicators. Spearman correlations between baseline plasma concentration and the improvement in fibrosis indicator values over the course of DAA treatment were computed for all cytokines. Correlations that were statistically significant ($p < 0.05$) are shown here. Dotted blue lines indicate 95% confidence intervals.

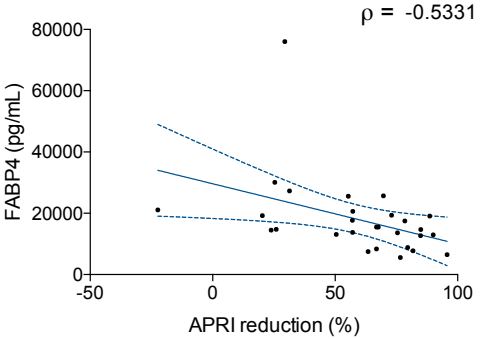

a
CCL5
IL-4
CXCL10
sCD14
sCD163
pg/mL
HD
CHC
**
*
****
b
CHC
AC
ns

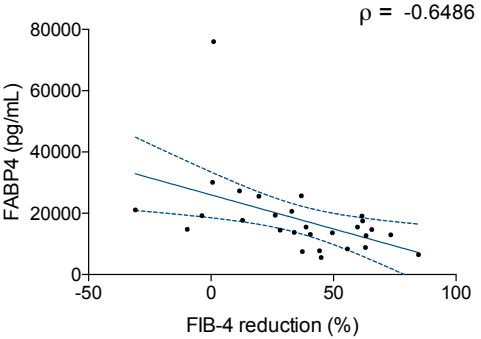

a
DAA
IFN
CCL3
CCL4
CCL5
CXCL10
FABP1
FABP4
GCSF
GMCSF
IL-4
IL-6
IL-7
IL-6Ra
IL-12p40
IL-15
IL-17a
IL-18
IL-25
IL-28b
Insulin
LIF
PDGF-BB
sCD14
sCD163
TNFa
TNFb
Fold change
1.00
3.09
2.18
p-value
BL
W4
EOT
FU
W12
b
DAA
IFN
log2(fold change)
CCL5
IL-4
CXCL10
sCD14
sCD163
FABP1
IL-6
IL-17A
IL-18
IL-25
IL-28B
LIF


a
Child Pugh A
n= 18
Child Pugh B
n= 7
CCL3
CCL4
CCL5
CXCL10
FABP1
FABP4
GCSF
GMCSF
IL-4
IL-6
IL-7
IL-6Ra
IL-12p40
IL-15
IL-17a
IL-18
IL-25
IL-28b
Insulin
LIF
PDGF-BB
sCD14
sCD163
TNFa
TNFb
BL
W4
EOT
FU
Fold change
1.00
3.37
p-value
b
CCL4
CCL5
CXCL10
IL-15
IL-25
IL-28b
IL-4
IL-6
LIF
PDGF-BB
TNFa
sCD14
sCD163
log2(fold change)
Child Pugh A
Child Pugh B


a
DAA
Child Pugh A
Child Pugh B
***
**
ns
**
*
ns
LSM (kPa)
60
40
20
0
BL
W4
EOT
FU
b
****
****
ns
***
****
ns
*
*
ns
APRI score
20
15
10
5
0
c
****
****
ns
***
***
ns
*
*
ns
FIB-4 index
30
20
10
0


ρ = 0.5368
IL-18 (pg/mL)
1500
1000
500
0
-20
0
20
40
60
80
LSM reduction (%)


ρ = -0.5331
FABP4 (pg/mL)
80000
60000
40000
20000
0
-50
0
50
100
APRI reduction (%)


ρ = -0.6486
FABP4 (pg/mL)
80000
60000
40000
20000
0
-50
0
50
100
FIB-4 reduction (%)