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# Long-Lasting Imprint in the Soluble Inflammatory Milieu Despite Early Treatment of Acute Symptomatic Hepatitis C

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***Background.*** Treatment with direct-acting antivirals (DAAs) in patients with chronic hepatitis C infection leads to partial restoration of soluble inflammatory mediators (SIMs). In contrast, we hypothesized that early DAA treatment of acute hepatitis C virus (HCV) with DAAs may normalize most SIMs.

***Methods.*** In this study, we made use of a unique cohort of acute symptomatic hepatitis C patients who cleared HCV with a 6-week course of ledipasvir/sofosbuvir. Plasma samples were used for proximity extension assay measuring 92 proteins.

***Results.*** Profound SIM alterations were observed in acute HCV patients, with marked upregulation of interleukin (IL)-6 and CXCL-10, whereas certain mediators were downregulated (eg, monocyte chemoattractant protein-4, IL-7). During treatment and follow-up, the majority of SIMs decreased but not all normalized (eg, CDCP1, IL-18). Of note, SIMs that were downregulated before DAA treatment remained suppressed, whereas others that were initially unchanged declined to lower values during treatment and follow-up (eg, CD244).

***Conclusions.*** Acute hepatitis C was associated with marked changes in the soluble inflammatory milieu compared with both chronic hepatitis patients and healthy controls. Whereas early DAA treatment partly normalized this altered signature, long-lasting imprints of HCV remained.

**Keywords.** acute infection; direct-acting antivirals; hepatitis C virus; proximity extension assay; soluble inflammatory milieu.

Symptomatic acute hepatitis C virus (HCV) occurs in only approximately 15%–30% of patients who are infected with HCV. The incidence of acute hepatitis C has declined in recent years; however, new infections do still occur in high-risk populations [1]. In the absence of an HCV vaccine, it is imperative to focus on the available treatment options and their durations to prevent the spread of HCV. Historically, acute and chronic HCV infections were treated with interferon (IFN)-alpha [2] or pegylated-IFN-alfa [3]. Over the years, the treatment for chronic hepatitis C with the administration of IFN in combination with ribavirin raised sustained virological response (SVR) rates from 30% to 90% depending on the HCV genotype and the stage of liver disease [4]. On the other hand, for acute HCV infection, higher cure rates (89%–95%) were observed with IFN without coadministration of ribavirin [5]. Albeit more than 90% of patients could be cured with IFN, treatment caused side effects, and many patients could not be treated due to contraindications. Since 2013, antiviral treatment options improved dramatically with the introduction of potent direct-acting antiviral agents (DAAs) [6]. Direct-acting antiviral agents have been used in patients with acute and chronic HCV infections with high efficacy and safety [7].

Viral infections are tightly regulated by host immune responses [8]. In a recent study, our group showed that distinct soluble inflammatory mediators (SIMs) as well as natural killer (NK) cell-T cell interactions may contribute to spontaneous early control of acute HCV infection [9]. When HCV is not cleared, persistent infection may lead to profound imprints on immune cells populations with altered frequencies, phenotypes, and function of many innate and adaptive immune cell [10, 11]. Clearance of chronic hepatitis C with novel DAAs does reverse impaired immune cell function to some extent [12], but, interestingly, and importantly, there is no complete restoration of immune responses even in the long-term after chronic HCV clearance [13–17]. It is currently unknown whether the effects of HCV on SIMs are reversible if antiviral treatment is initiated early during infection, compared with treatment of chronic hepatitis in which the infection has been established for several decades. Soluble inflammatory mediators are produced upon

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activation of the immune system. Although there are studies that have investigated SIMs during both chronic [18] and acute phase of infection [19], only limited studies have been performed to investigate the broad spectrum of the inflammatory milieu upon treatment with antivirals [13, 20–22]. Some data suggest that several SIMs that were upregulated before DAA treatment of chronic hepatitis C, decreased but did not normalize, with the exception of IL-12B [13].

In the present study, we made use of a unique cohort of patients with acute symptomatic hepatitis C who were treated for only 6 weeks with ledipasvir/sofosbuvir [23]. We investigated how the inflammatory milieu, such as cytokines, chemokines, and growth factors, was affected by acute HCV infection and were altered during and after short duration treatment with DAAs. We applied a high-throughput multiplex immunoassay technology called the proximity extension assay (PEA) [24, 25], and we comprehensively analyzed a large panel of inflammatory biomarkers to understand whether different kinetics of restoration of the soluble inflammatory milieu occurs depending on the duration of HCV infection and treatment.

## MATERIALS AND METHODS

### Patient and Patient Material

In this study, a total of 63 subjects were evaluated: 20 patients with acute symptomatic, 23 with chronic HCV infection both treated with ledipasvir plus sofosbuvir for 6 and 8/12 weeks, respectively; and 20 healthy donors. In brief, acute infection was defined as either documented seroconversion anti-HCV or known or suspected exposure to HCV within the last 4 months and HCV ribonucleic acid (RNA) viral load of more than 10 000 IU/mL at screening and with raised alanine aminotransferase (ALT) concentration more than 10 times the upper limit of normal. Patient characteristics are presented in Table 1, and Figure 1A provides a scheme of the administration of therapy. For further details including the study protocol, refer to the original publication [23] and Supplementary Figure 1. Blood plasma was collected from ethylene diamine tetraacetic acid-treated peripheral blood samples at different time points and stored at −20°C for analysis.

### Protein Quantification

Plasma samples were thawed, and 20 µL of each sample was sent to Olink AB where PEA was performed. A predefined panel (Inflammation) simultaneously measuring 92 inflammation-related proteins in plasma was used (Supplementary Table 1). The PEA for IL-12b measures IL-12 beta subunit (p40), which means it detects IL-12p40 monomers or heterodimers containing IL-12 beta: ie, IL-12 and IL-23. All samples were tested in 1 run; different batches were not used. Samples from the same donor were on the same plate, but the clinical groups (acute, chronic, and healthy) were equally distributed over all of the plates. Internal controls from Olink were used for normalization of intraexperimental variation. For detailed description of the method, please refer to Supplementary Method and Materials.

### Statistical Analysis

Data were analyzed using GraphPad Prism version 8 (GraphPad Software, La Jolla, CA). In general, quantitative comparisons were performed using parametric Student *t* test for normally distributed values. Comparisons of normally distributed multiple groups were calculated with analysis of variance or Kruskal-Wallis test for not-normally distributed groups. Principal component analysis (PCA) was performed with both Qlucore Omics Explorer v3.2 (Qlucore, Lund, Sweden) and an R-based script (R version 3.6.1.) For Qlucore-based PCA, values for analysis were set to *P* values of .05 and a q value of <0.2. Spearman correlation coefficients were calculated using GraphPad Prism version 8. Adjusted *P* values were corrected for multiple comparisons using the Benjamini Hochberg method. The Heatmap was created

**Table 1. Baseline Characteristics (Mean ± Range) of Patients With Acute and Chronic Hepatitis C**

| Characteristics | Healthy | Chronic Hepatitis C | Acute Hepatitis C |
|---|---|---|---|
| Number | 18 | 23 | 20 |
| Gender | | | |
| Male | 5 | 10 | 8 |
| Female | 13 | 13 | 12 |
| Age, years | 43 (32–63) | 54 (24–82) | 49 (23–63) |
| HCV Genotype | | | |
| 1a | … | 9 (39%) | 11 (55%) |
| 1b | … | 14 (61%) | 9 (45%) |
| HCV RNA ($log_{10}$ IU/mL) | … | 6 (4.56–6.53) | 4.71 (3.27–6.75) |
| Alanine aminotransferase (U/L) | … | 50 (24–273) | 506 (42–1982) |
| Aspartate aminotransferase (U/L) | … | 40 (18–146) | 174 (36–1128) |
| Bilirubin (µmol/L) | … | 10 (3–18) | 27.2 (6.0–215.5) |
| Fibroscan score (kPa) | … | 5.4 (4–7.1) | … |
| Lymphocytes (Tsd/µL) | … | 1.8 (0.7–3.3) | 2.05 (1.72–3.98) |

Abbreviations: HCV, hepatitis C virus; RNA, ribonucleic acid.

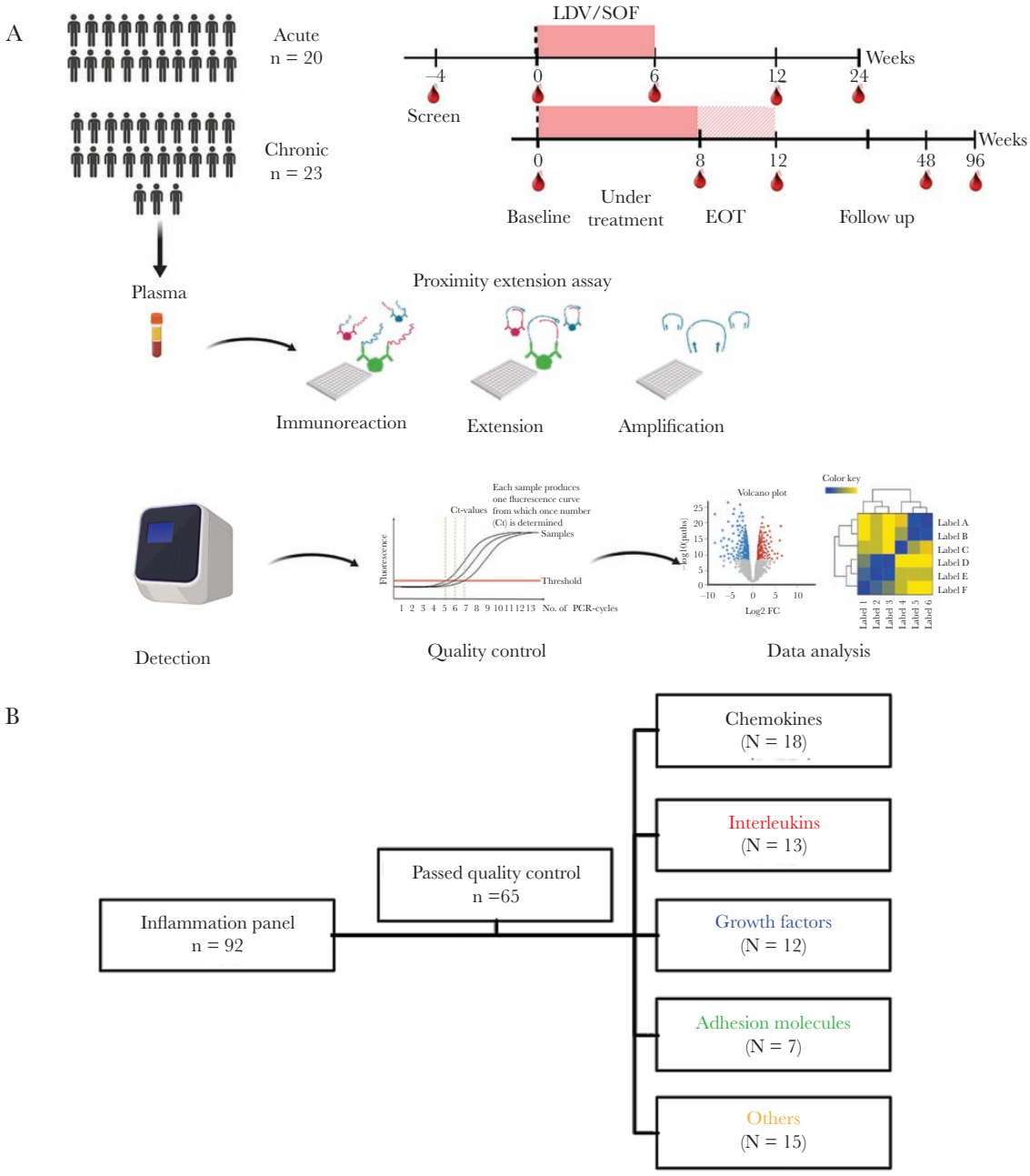


**Figure 1.** Study population and the overview of the proximity extension assay. (A) Patients with acute symptomatic hepatitis C virus (HCV) infection (n = 20) from the HepNET Acute HCV IV study, treated early with ledipasvir (LDV) plus sofosbuvir (SOF) for 6 weeks were analyzed. As control, chronic patients (n = 23) treated with the same regimen for 8–12 weeks were included. In addition, healthy donors (n = 20) were used. Plasma was collected from all of these cohorts. Proximity extension assay was performed profiling a total of 92 cellular proteins across 96 samples simultaneously. Ninety-two antibody pairs, labeled with deoxyribonucleic acid (DNA) oligonucleotides, bind their target antigen in solution. Oligonucleotides that are in proximity hybridize and are extended by a DNA polymerase. This newly created piece of DNA barcode is amplified by polymerase chain reaction (PCR). The amount of each DNA barcode is quantified by microfluidic quantitative PCR. All samples were analyzed in 1 run. (B) Inflammation panel: in total, 92 soluble inflammatory molecules were measured, 65 of which were selected and categorized into chemokines, cytokines, growth factor, ligands, and others. The remaining 27 soluble inflammatory mediators were below the limit of detection and were excluded from analysis.

using Heatmapper (http://www2.heatmapper.ca) using the complete linkage clustering method (dendrogram on columns) and Spearman's rank correlation distance measurement method.

### Ethics Statement

Patients gave informed written consent for the study. The protocol for sample collection and investigations were reviewed and approved by the local ethics committee of Hannover Medical School.

## RESULTS

### Distinct Expression of Soluble Inflammatory Mediators in Acute Compared With Chronic Hepatitis C Virus Infection

To investigate the expression of various SIMs, we made use of the PEA described in Figure 1A, and we curated the data by eliminating measured proteins and patient samples that either did not pass quality control or had values that were too low. We identified 65 markers from a panel of 92, which were above the limit of detection, and categorized the 65 different biomarkers according to their function, as listed in Figure 1B, namely, chemokines (n = 18), cytokines (n = 13), growth factors (n = 12), adhesion molecules (n = 7), and others (n = 15). These 65 proteins were further screened for their expression comparing acute and chronic HCV as well as healthy controls. This is depicted by a heat map that shows the unique profile of various SIM to be either upregulated (purple) or downregulated (green) based on the normalized protein expression values before the start of treatment (Figure 2A). Patients with acute (screen) and chronic (baseline) HCV differed significantly from the healthy donors but also from each other. As expected, approximately 60% of the SIMs were upregulated in both acute and chronic HCV patients, but higher upregulation was observed in acute compared with chronic infection. In addition, we performed hierarchical clustering on the expression of SIM as shown by the dendrograms for both acute and chronic infection. We observed that not all markers clustered based on their function. By comparing correlation matrices for all parameters studied between patients with acute and chronic hepatitis C, we discovered slightly different patterns of coexpression between the 2 groups, in particular for growth factors and ILs (Supplementary Figure 2).

In total, expression of 44 proteins in acute and chronic differed significantly compared with healthy controls and were either up- or downregulated (Supplementary Table 2). Representative examples of upregulated proteins were C-X-C motif chemokine 10 (CXCL-10), IL-12 subunit B (IL-12B), and programmed death ligand 1 (PD-L1), whereas IL-7, chemokine ligand 6 (CXCL-6), and Sirtuin 2 (SIRT-2) were downregulated in comparison to healthy controls (Figure 2B). Taken together, both acute and chronic HCV infections were associated with altered inflammatory proteins signatures with more profound changes in acute patients.

### Soluble Inflammatory Mediators Expression Correlates With Viral Load and Biochemical Activity in Acute but Not Chronic Hepatitis C

Next, we accessed the correlation of various SIMs to clinical parameters such as liver enzymes (ALT, aspartate aminotransferase), HCV RNA level, and bilirubin at the start of treatment. A heat map was generated based on the calculated Spearman's correlation values (Figure 3A). This analysis revealed a high positive correlation between SIMs and clinical parameters in patients with acute HCV in contrast to chronic infection. Within the acute group, the IL-18R1, the growth factor FGF-19, the chemokine CXCL-10, and adhesion molecule CDCP1 are examples of proteins that all showed high positive correlations to the measured clinical parameters (Figure 3A). Moreover, within the acute group, CXCL-10, FGF-19, and IL-18R1 were positively correlated to ALT, whereas stem cell factor (SCF) was the only protein that showed a negative correlation with ALT (Figure 3B). Compared with ALT, only positive correlations were observed between plasma proteins and HCV RNA levels, such as for IL-8, SLAMF-1, IL-10, and CXCL-10 (Figure 3C). In addition, some of the proteins in the acute HCV patients that showed a positive correlation with HCV RNA levels were also significantly elevated in their expression compared with healthy controls (eg, CXCL-10, CCL4, and CD40). They are highlighted in bold, and markers that showed no significance are in italics (Figure 3A).

### Soluble Inflammatory Mediators Do Not Completely Normalize Despite Early Treatment of Acute Hepatitis C

As we have shown in our previous studies of patients with chronic HCV, SIMs decline over time after treatment, but many of those still remained elevated compared with controls [13]. Thus, we next focused on whether early treatment of acute HCV would lead to normalization of the inflammatory soluble milieu. As shown in Figure 4A, when we assessed the dynamics of plasma proteins over time, 6 different patterns emerged. Furthermore, the patterns were as follows: (1) there was no change compared with healthy controls (eg, ADA, FGF-21); (2) protein levels were upregulated at screen and kept constantly elevated during treatment (CCL11, IL-6); (3) proteins were increased during acute HCV but declined during treatment in a stepwise manner (CDCP1, CXCL-10); (4) proteins that constantly remained suppressed (eg, IL-7, monocyte chemoattractant protein [MCP]-4); (5) proteins that were present at lower levels and were even further suppressed (eg, CD244, CD40); and (4) proteins that were present at lower levels but normalized (only SCF). Of note, most of the proteins present at elevated levels did not decline extensively, neither during therapy nor at follow-up, and the ones that were low remained suppressed. Furthermore, proteins present at elevated levels declined during treatment were still not fully normalized after 24 weeks of follow-up. The 2 most common patterns observed were proteins that were elevated at screening and either declined (n = 21) or remained elevated (n = 19) during and after successful DAA therapy (Figure 4B).

We have previously reported that the soluble inflammatory milieu was not restored upon treatment of chronic hepatitis C [13]. However, in that study, DAAs were administered combined with ribavirin, and thus we could not rule out an effect of ribavirin. Therefore, we analyzed the pattern of serum protein kinetics in the chronic HCV cohort included in this study where only DAA was administered (Supplementary Figure 3). In line with the previous

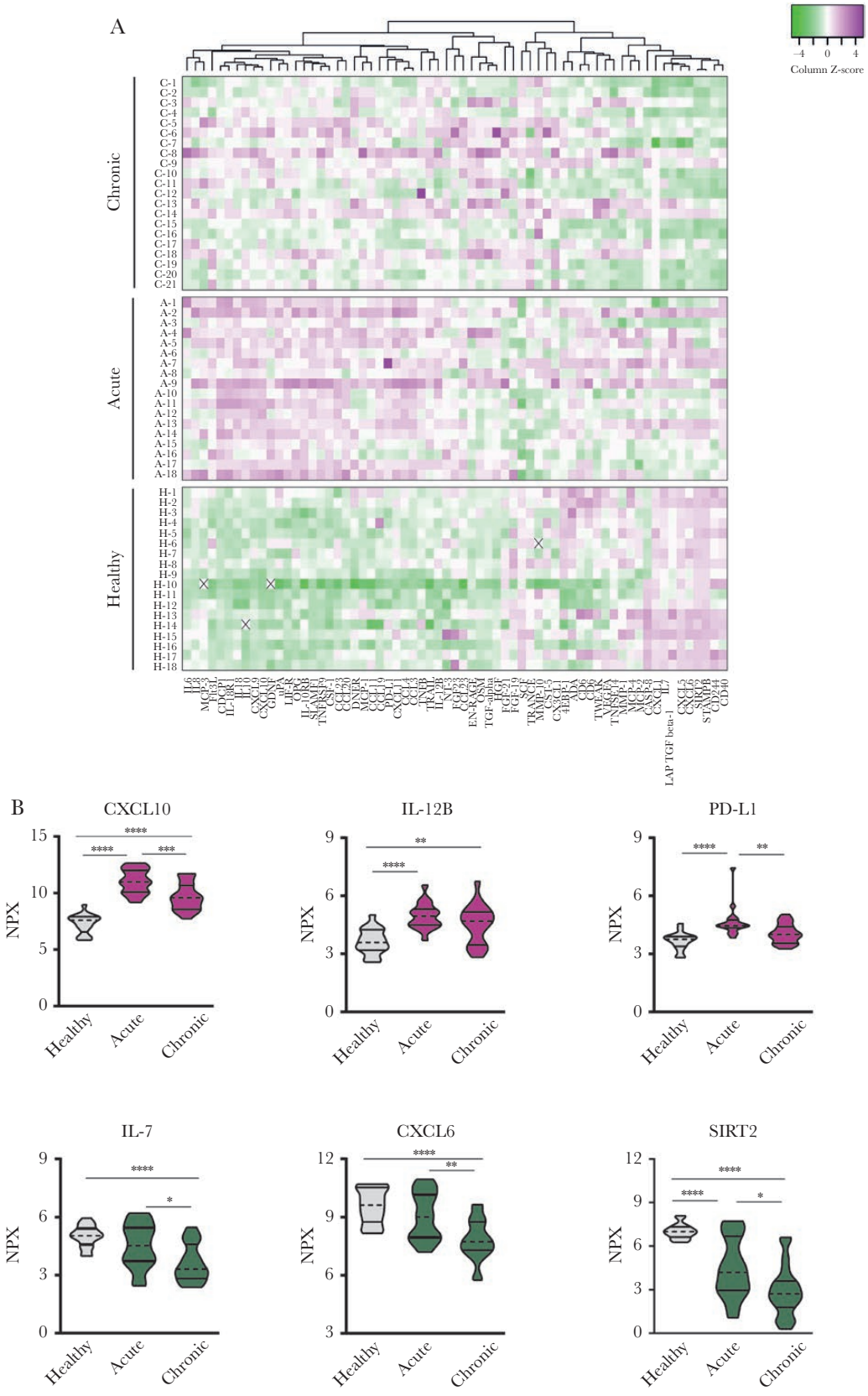


**Figure 2.** Distinct expression of the inflammation markers in acute and chronic hepatitis C virus compared with healthy control. (A) Heat map displaying expression pattern of 65 inflammation markers measured using proximity extension assay. Normalized data are presented with Z-score. Screen of the acute (n = 18), baseline for the chronic (n = 21), and healthy donors (n = 18) are shown. The heat map was created using Heatmapper using complete linkage clustering method (dendrogram on columns)

work, partial restoration was noted, but many proteins remained altered for a prolonged period of time.

To get a broader view of how proteins were altered in acute and chronic infection as well as how they changed in response to treatment, we next performed a PCA analysis (Supplementary Figure 4). Healthy controls clearly separate from both acute and chronic HCV patients before DAA treatment (screening and baseline [acute] and baseline [chronic]). This analysis additionally reveals that the patterns change during and after therapy in acute and chronic HCV patients compared with baseline. However, even after clearing the acute or chronic HCV infection, patients are clearly distinguishable from healthy controls. In a closer analysis that only focused on median SIM values in acute HCV and healthy controls, the screening time point was again separated from the healthy controls (Figure 5). It is interesting to note that a third cluster was formed, which constituted the 3 longitudinal time points (w2, w6, Fu24). This suggests that most changes in SIMs occur early upon treatment of acute HCV. It further shows that patients, at week 24 after follow-up, still clearly separate from controls.

Taken together, early treatment for acute hepatitis C resulted in many SIMs returning close to levels seen in healthy controls, but a long-lasting imprint of the acute infection could still be detected. Most changes were observed during the first weeks of treatment onset.

## DISCUSSION

This is one of the first studies to investigate a broad spectrum of SIMs in patients with acute hepatitis C receiving antiviral therapy with novel DAAs. In this study, we show the following: (1) many SIMs had higher expression levels in patients with acute hepatitis C than in chronically infected patients, but certain markers also displayed a much wider interindividual variability or were even reduced; (2) SIMs correlated with clinical parameters such as HCV viral load or biochemical disease activity in acute but not chronic hepatitis C; (3) 6 different patterns of SIM kinetics were observed over time, during and after antiviral treatment; and (4) despite the decline of many proinflammatory parameters, no complete restoration of the inflammatory milieu was observed after treatment.

It is well defined that HCV infection leads to activation of the IFN system by induction of various IFN-stimulated genes (ISGs) that further influences cytokine secretion via a complex network of interactions [26–28]. In the present study, we observed elevated levels of a majority of investigated proteins before the initiation of therapy. In total, 34 proteins were upregulated in acute patients and 27 proteins were upregulated in chronic patients, compared with the healthy controls. An upregulation of proinflammatory molecules has previously been observed in chronic HCV, in which 22 SIMs displayed elevated expression in patients [13]. In general, the expression of the majority of proteins was slightly higher in patients with acute compared with chronic HCV infection. Despite this trend, a cluster of proteins, namely, LAPTGF-Beta 1, CXCL-5, CXCL-6, and SIRT-2, were present at lower levels in chronic compared with acute patients (Figure 2A). Although no clear clustering of markers based on their functionality was observed, some ILs did cluster together, for example, IL-6, IL-8, IL-18R1, IL-10, and IL-18 (Figure 2A), in both acute and chronic HCV patients. A difference between acute and chronic HCV could be observed when we assessed coexpression patterns of proteins (Supplementary Figure 2).

Hepatitis C virus mainly infects hepatocytes, but hepatic inflammation causes systemic changes in blood cytokine and chemokine levels [29]. Most of the studies so far have focused on the chronic phase and only measured very few SIMs. Several studies have independently shown an association between virological response and baseline CXCL-10 concentrations in chronic HCV patients [30–32], and it has been proposed that plasma levels of CXCL-10 can predict the risk of fibrosis progression [33]. CXCL-10 is also increased in patients with acute HCV infection who do not spontaneously clear the infection [34]. Others have observed that not just CXCL-10 but also MIP-1β, IL-12p40, and MIG levels are increased, suggesting that their role in HCV immunopathogenesis starts early in acute HCV [35]. In line with these studies, we also observed high CXCL-10 levels in patients with acute hepatitis C and a positive correlation of this SIM with viral load. This confirms that CXCL-10 is a good prognostic marker. Furthermore, when the expression of all measured 65 markers was correlated to the clinical parameters, more positive correlations were observed in acute as opposed to chronic HCV patients. In most cases, viral load correlated positively with SIMs, suggesting that the infection, per se, triggers immune responses. It further implies that there is general activation of the immune system but no immune exhaustion, which is different from chronic HCV infection where it has been shown to be exhausted [26]. The association of SIMs with ALT in acute HCV was as expected. Of note, high ALT levels in acute HCV are associated with a higher chance to clear HCV, as shown in very early studies [36, 37].

and Spearman's rank correlation distance measurement method. (B) Soluble inflammatory mediators (SIMs) expression differs between acute and chronic hepatitis C before the onset of treatment compared with healthy donors. Expression levels of several SIMs is shown by violin plots either upregulated (purple) or downregulated (green) as compared with healthy controls (gray) before the start of direct-acting antiviral treatment at screen for acute and baseline for chronic. Samples were tested for normal distribution with Shapiro-Wilks test, and, if normally distributed, significant differences were calculated with one-way analysis of variance followed by Tukey's test (CXCL-10, IL-12B, IL-7, CXCL-6, and SIRT-2). Not normally distributed samples (PD-L1) were tested for significance with Kruskal-Wallis test followed by Dunn's multiple comparison test. *, $P < .05$; **, $P < .01$; ***, $P < .001$; ****, $P < .0001$.

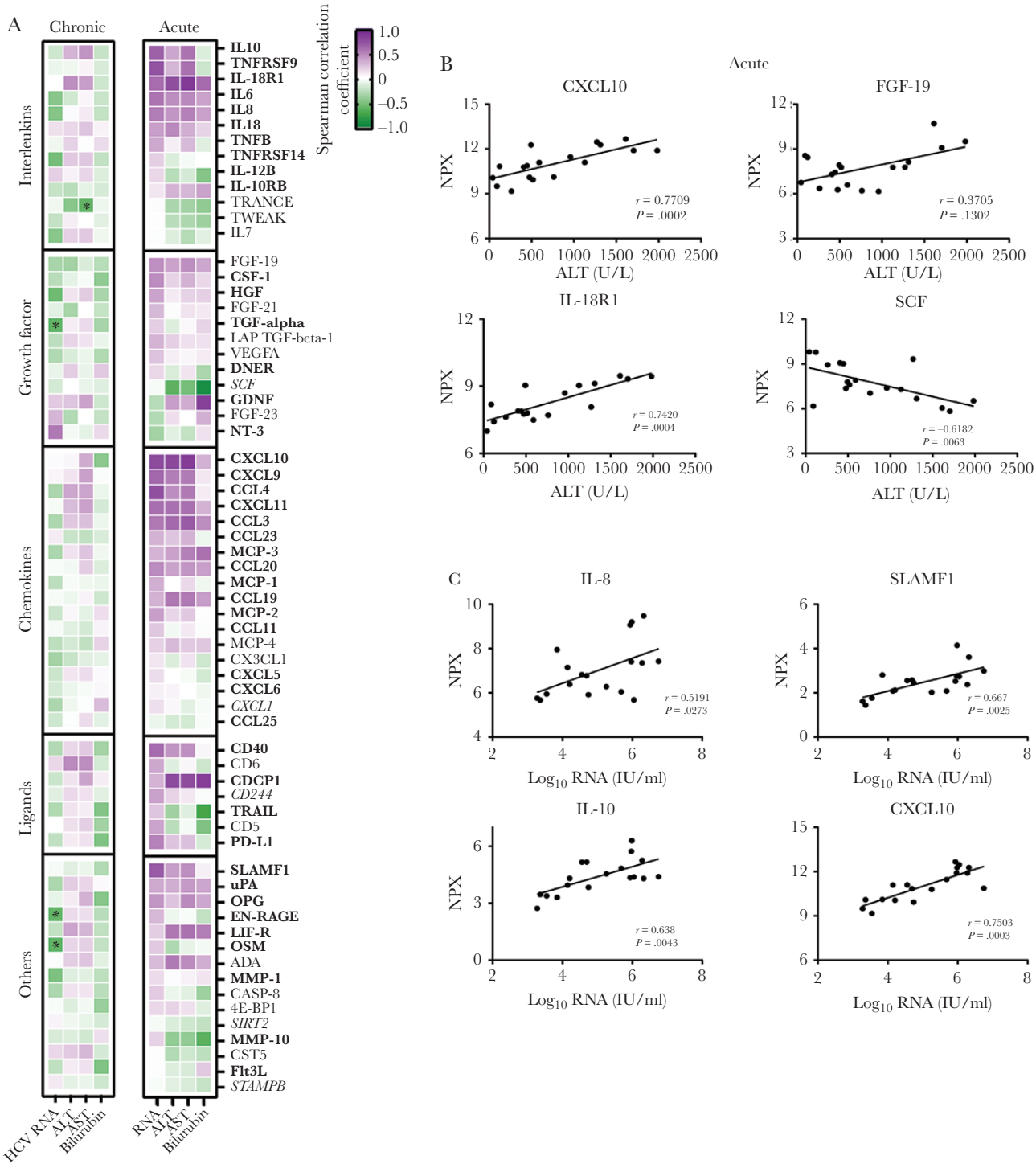


**Figure 3.** Soluble inflammatory mediator (SIM) expression correlates with clinical parameters. (A) Heat map displaying the correlation between selected clinical markers and SIMs for (1) acute and (2) chronic hepatitis C. Correlation strength is indicated by color intensity. Spearman's test was used for calculating correlation. Adjusted *P* values were corrected for multiple comparisons by Benjamini-Hochberg correction method shown by asterisks ($P < .05$). The proteins marked in bold show statistically significant expression compared with healthy donors. (B) Linear regression analysis (alanine aminotransferase [ALT] and (C) hepatitis C virus [HCV] ribonucleic acid [RNA]) for some of the selected markers for acute HCV patients is depicted in the individual plots along with Spearman's correlation coefficient (r). FGF-19, fibroblast growth factor; SCF, stem cell factor; SLAMF1, signaling lymphocytic activation molecule.

Upon initiation of DAA therapy, a majority of measured proteins declined. Analysis of proteins from longitudinal sampling (screening to follow-up week 24) revealed 6 different patterns of SIM alterations. The distribution of various markers is highlighted by the pie chart in Figure 4B. Most of the markers were upregulated due to infection and followed a kinetic where they

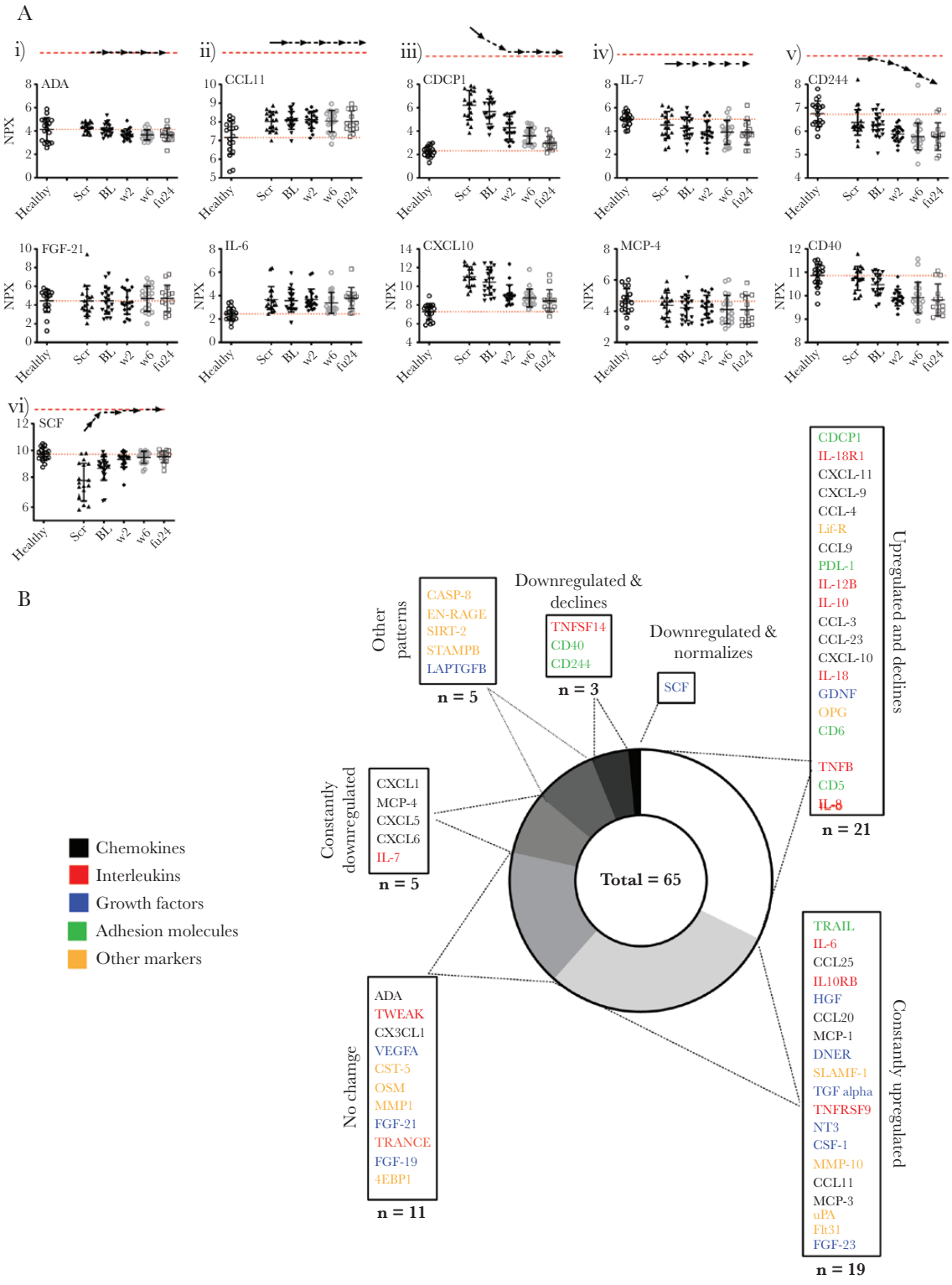


**Figure 4.** Different kinetics of longitudinal soluble inflammatory mediator (SIM) expression before, during, and after treatment of acute hepatitis C. (A) The soluble inflammatory milieu does not normalize upon successful direct-acting antiviral treatment for the majority of markers. The plots indicate the duration for which the analysis was performed starting from screen, baseline, during therapy, end of treatment, and 24 weeks of follow-up. The plots indicate 2 representative SIMs for each pattern. The arrows over the plot indicate the expression pattern of the SIM during the course of treatment until follow-up. The red dotted lines indicate the threshold values. These are the normalized protein expression values of the healthy donors for the respective marker. Six different kinetics were observed as shown by arrows on top of each plot: (i) no change to healthy, (ii) constantly upregulated, (iii) upregulated and stepwise decline, (iv) constantly downregulated, (v) downregulated and decline, and (vi) downregulated and normalizes. (B) Pie chart summarizing the distribution of the various markers within each kinetic as shown above. The markers are color coded according to their function.

either remained elevated or declined to some extent; however, they never normalized to the level of healthy controls. Among the markers that elevated at screen, were followed by either a steep (eg, CDCP1, IL-18) or a stepwise decline (eg, CXCL-10, IL-12B). Although CXCL-10 remained elevated, we did observe a rapid reduction. This was in accordance with a recent study, in which a similar decline in CXCL-10 was observed [22]. Although no marker normalized within this group, 2 markers, namely, CXCL-11 and CD6, declined even below levels found in healthy controls at follow-up week 24. In addition, there were a few markers that were downregulated and either remained suppressed (eg, IL-7, CXCL-5) or declined further (CD244, CD40). This pattern is in line with our previous findings in chronic hepatitis C in which suppressed parameters also did not recover despite viral clearance [13]. Of note, 1e marker, namely, SCF, was suppressed before the start of treatment but reached a normal expression level at week 24. Stem cell factor is present in a membranous as well as in a soluble form. Its receptor, c-kit, belongs to the family of platelet-derived growth factor receptors (PDGF-R) [38]. It is produced by fibroblasts, epithelial cells, and mast cells. In addition to regulating hemopoietic stem cells homing and proliferation, SCF has proliferative effects on hepatocytes and may be involved in liver regeneration [39]. The low amounts of SCF before therapy could be attributed to its excessive use during the acute infection. This eventually restores to a normal level with antivirals during follow-up. In addition, a few other markers showed a slight decline towards normalization at week 24 (follow-up), that is, MCP-2 and TNF-B. It is interesting to note that high interindividual variability was observed for some other parameters, for example, LATGF-B, STAMPB, CASP-8, SIRT-2, and EN-RAGE. This is in line with our previously published study wherein we addressed the subject of chronic HCV infection leading to increased donor-to-donor expression variation of NK-cell receptors within an individual [15, 40]. In earlier studies on acute HCV, markers that have shown similar expression kinetics compared with the present study are CXCL-10, IL-18, VEGF, MCP-4, and CCL4 [19, 41, 42].

Overall, and more importantly, even upon early treatment of acute hepatitis C, we did not observe complete SIM restoration. This is similar to what has been observed in chronic hepatitis C [13]. This observation could be of importance in case of HCV reinfection, and it may also explain some level of persistence of extrahepatic manifestations despite viral clearance [43]. Future

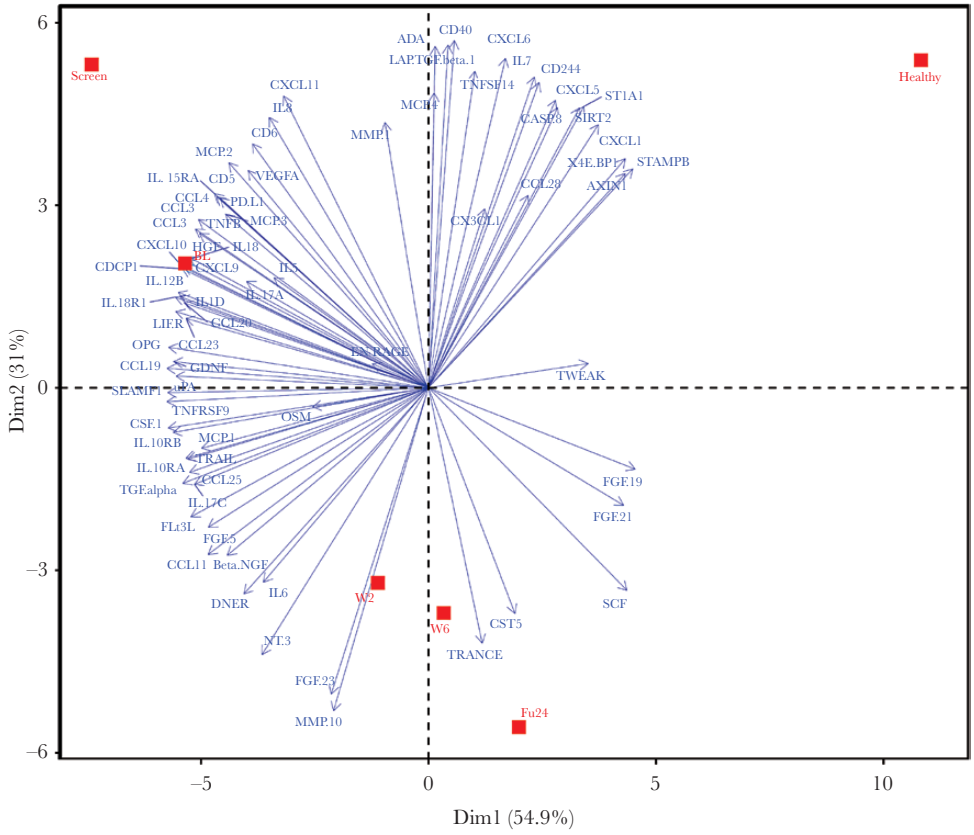


**Figure 5.** Cluster analysis depicts dynamic changes over time with no restoration after hepatitis C virus (HCV) cure in acute HCV. Principal component analysis (PCA) was performed with the median values of measured soluble factors in acute HCV-infected patients at screen (scr), baseline (BL), during treatment (W2), end of treatment (W6), and follow-up (Fu 24). These values were compared with healthy controls. Dots indicate the included time points/subgroups, and each vector represents an individual soluble inflammatory mediator with length and direction equaling to the contribution to PCA and the difference between the groups, respectively.

long-term studies are required to determine the overall clinical relevance of this finding. For other infections, it also has been shown that distinct alterations may persist after clearance. Suppression of viral replication with antivirals does not lead to major functional restoration of hepatitis B virus (HBV) and human immunodeficiency virus (HIV)-specific T cells [44–46]. With respect to NK cells, they are activated and exhausted in HBV and hepatitis D virus infection [10, 47]. Likewise, MAIT cells are affected by both chronic HBV and hepatitis D virus infections as well as by HIV infection, with severely reduced cell numbers in circulation [48–50].

This study has obvious strengths and limitations that can be summarized as follows. First and foremost, the study included a well characterized cohort of patients, with only 6 weeks of exposure to DAAs, derived from a multicenter study. Because the treatment regimen was exclusively composed of DAAs, the effects of IFN and ribavirin, which could have altered the immune responses, were ruled out. In addition, all of the patients enrolled in this study achieved an SVR. The technological method used in this study identified a large number of parameters through a relatively sensitive high-throughput assay. On the other hand, there were few limitations of the study. The first limitation is the relatively short follow-up (week 24) and the lack of immune cell population analyses. In addition, it would also have been relevant to compare our studied patients with a cohort of patients who receive protease inhibitors without sofosbuvir as well as with patients who spontaneously cleared, because some of the patients treated in this study might have cleared the virus spontaneously.

## CONCLUSIONS

In conclusion, early antiviral treatment and viral clearance of acute hepatitis C is associated with marked changes in the soluble inflammatory milieu. However, early treatment of very recent infection does not completely normalize altered SIM patterns. Thus, HCV-induced imprints on the immune system may persist even after a short duration of viremia.

### Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases online*. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

### Notes

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