Circulating Microparticles as Disease-Specific Biomarkers of Severity of Inflammation in Patients With Hepatitis C or Nonalcoholic Steatohepatitis

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BACKGROUND & AIMS: Microparticles released into the bloodstream upon activation or apoptosis of CD4+ and CD8+ T cells correlate with inflammation as determined by histologic analysis in patients with chronic hepatitis C (CHC). Patients with nonalcoholic fatty liver (NAFL) or nonalcoholic steatohepatitis (NASH) can be differentiated from those with CHC based on activation of distinct sets of immune cells in the liver. METHODS: We compared profiles of circulating microparticles from patients with NAFL and NASH (n = 42), with healthy individuals (controls) using flow cytometry; the profiles were correlated with inflammation grade and fibrosis stage based on histologic analyses. We assessed the ability of the profiles to determine the severity of inflammation and fibrosis based on serologic and histologic analyses. RESULTS: Patients with CHC had increased levels of microparticles from CD4+ and CD8+ T cells; the levels correlated with disease severity based on histologic analysis and levels of alanine aminotransferase. Patients with NAFL or NASH had significant increases in numbers of microparticles from invariant natural killer T cells and macrophages/monocytes (CD14+), which mediated pathogenesis of NASH. Microparticles from CD14+ and invariant natural killer T cells correlated with levels of alanine aminotransferase and severity of NASH (based on histology). Levels of microparticles could differentiate between patients with NAFL or NASH and those with CHC, or either group of patients and controls (area under the receiver operating characteristic curve ranging from 0.56 to 0.99). CONCLUSIONS: Quantification of immune cell microparticles from serum samples can be used to assess the extent and characteristics of hepatic inflammation in patients with chronic liver disease.

Keywords: Noninvasive Assay; Lymphocyte; Serum Assay; Biomarker Assay.

Abbreviations used in this paper: ALT, alanine aminotransferase; AUROC, areas under the receiver operating characteristics; CHC, chronic hepatitis C; FACS, fluorescence-activated cell sorting; HSC, hepatic stellate cells; iNKT, invariant natural killer T; MP, microparticle; NAFL, nonalcoholic fatty liver; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis.

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Vβ11 (invariant natural killer T [iNKT] cells). We found that MP derived from CD14+ and iNKT cells, 2 cell populations that have been implicated as being central to adipose tissue inflammation,\textsuperscript{10,11} were uniquely and characteristically elevated in patients with NAFL and further increased in patients with histologically severe NASH, but not in CHC. We conclude that circulating MP might qualify as a novel tool to quantify the underlying type and extent of inflammation in NAFL/NASH.

Material and Methods

Human Study Cohort

The Committee for Clinical Investigations at the Beth Israel Deaconess Medical Center approved the study, and all patients gave their informed consent before participation. Forty-two patients with CHC, 67 patients with histologically proven NAFL or NASH, and 44 healthy controls were enrolled. Ten of the CHC patients from our earlier study were included to increase the power of this group.\textsuperscript{3} All patients were followed up in the Beth Israel Deaconess Medical Center Liver Center and received physical examinations, regular blood draws, and a diagnostic liver biopsy as part of their standard care. Both patient cohorts had comparable mean age (55 and 49 years, respectively; P > 0.05) and sex (Table 1). Patients with a major second known comorbidity that could affect immune cell activation, such as human immunodeficiency virus infection, autoimmune diseases, or another hepatitis virus infection were excluded. Patients with CHC were characterized as HCV antibody and RNA positive for >6 months, and patients with NAFL/NASH by standard clinical criteria confirmed by liver biopsy with the absence of other liver diseases.\textsuperscript{12} The criteria used for assessing the health of each participant are summarized in Supplementary Table 1.

Isolation of T-Cell MP From Human Serum and Plasma

From controls, both plasma and serum were drawn at the same time point, in order to compare results for MP isolation between the 2 methods. Additionally, to assess changes in MP profiles in short-term follow-up, healthy controls were subjected to serial blood sampling 7 days apart. For plasma, blood was collected in citrate-containing tubes and for serum, blood was collected in standard Vacutainers (both BD Vacutainer; BD, Franklin Lakes, NJ, USA) and left for 1 hour at 37°C to allow clot, followed by centrifugation at 4000 rpm for 20 min at 4°C. Clot palettes were carefully separated and plasma or serum supernatants were stored at –80°C for further MP isolation. MP were isolated by differential centrifugation between 10,000g and 100,000g as described,\textsuperscript{3} and S100-MP sedimenting at 100,000g were characterized by fluorescence-activated cell sorter (FACS) using staining for Annexin V, CD1c, CD3, CD4, CD8, CD14, CD16, CD15, CD41, CD147, Vα24/Vβ11 (eBioscience, San Diego, CA; BioLegend; Becton Dickinson, San Jose, CA). Notably, these surface markers were not described on exosomes,\textsuperscript{13} another class of membrane-coated vesicles. All antibodies were titrated against the matching isotype control before use on patient’s samples, as shown in detail in Supplementary Figure 1. MP preparations were characterized on an LSR2 FACS sorter (Becton Dickinson), and cytometric data were analyzed with FlowJo 8.8.6 software for MAC OSX (Tree Star, Inc, Ashland, OR). MP were gated on forward and sideward scatter. A detailed overview of our gating strategy is shown in Supplementary Figure 2. To avoid nonspecific antibody binding, Fc receptors on MP and target cells were blocked with FcR Blocking Reagent (Miltenyi Biotec, Auburn, CA). Antibody solutions were centrifuged before FACS to avoid artifacts due to aggregation.

Liver Histology

Liver biopsies were performed with an 18-gauge Menghini needle for clinical indications and encompassed at least 8 portal tracts. Biopsy specimens were formalin-fixed, paraffin-embedded, sectioned, and stained with H&E, Masson’s trichrome, reticulin, and periodic acid–Schiff stains. Two experienced histopathologists graded and staged the liver samples according to Metavir (CHC and NAFL/NASH)\textsuperscript{14} and NAS score (NAFL/NASH).\textsuperscript{15} Only specimens predating the MP isolation from the patients’ serum by no more than 12 months were used for comparisons.

Statistical Analysis

All data are arithmetic means with SD. Differences between independent experimental groups (NAFL/NASH, CHC, NAS score 0–3, NAS score 4–8

<table>
<thead>
<tr>
<th>Patients, n\textsuperscript{a}</th>
<th>CHC</th>
<th>NAFL, total</th>
<th>NAS score 0–3</th>
<th>NAS score 4–8</th>
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</thead>
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<tr>
<td>Patients, n\textsuperscript{a}</td>
<td>42</td>
<td>67</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>Female, %\textsuperscript{b}</td>
<td>31 (13)</td>
<td>38.8 (26)</td>
<td>36.4 (12)</td>
<td>41.2 (14)</td>
</tr>
<tr>
<td>Male, %\textsuperscript{b}</td>
<td>69 (29)</td>
<td>61.2 (41)</td>
<td>64.6 (21)</td>
<td>58.8 (20)</td>
</tr>
<tr>
<td>Age, y, mean (range)</td>
<td>55.1 (30–81)</td>
<td>48.7 (28–73)</td>
<td>47.9 (28–73)</td>
<td>49.4 (31–68)</td>
</tr>
<tr>
<td>ALT, IU/L, mean (range)</td>
<td>89.7 (16–291)</td>
<td>70.1 (13–201)</td>
<td>64.3 (13–201)</td>
<td>75.8 (36–165)</td>
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<tr>
<td>Biopsy grade, mean (range)</td>
<td>1.63 (0–3)</td>
<td>48 (0–4)</td>
<td>26 (0–2)</td>
<td>22 (1–4)</td>
</tr>
<tr>
<td>Biopsy stage, mean (range)</td>
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<td>1.07 (0–4)</td>
<td>0.39 (0–4)</td>
<td>1.76 (0–4)</td>
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<td>4.5 (0–8)</td>
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<td>61.2</td>
<td>76.5</td>
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<td>2.4</td>
<td>6.2</td>
<td>12.9</td>
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</table>

NOTE. NAFL/NASH patients were subdivided according to their NAS score as indicated.
NA, not applicable.
\textsuperscript{a}Total number of patients in each cohort.
\textsuperscript{b}Percentage of sex distribution (absolute numbers in parentheses).
and controls) were characterized using the Kruskal–Wallis test with subsequent pairwise comparisons and adjustment for multiple comparisons using Dunn’s Multiple Comparison Test. Differences between plasma and serum MP levels were analyzed using the Mann–Whitney test. A pairwise Pearson algorithm was used for correlation analysis of MP levels with blood cells, alanine aminotransferase (ALT), histological grade and stage, and the NAS score. Scatterplots of the pairwise data are presented with corresponding linear regression lines. To assess the predictive ability of the 6 MP populations (CD4⁺/H11001, CD8⁺/H11001, CD14⁺/H11001, CD15⁺/H11001, CD41⁺/H11001, and iNKT) for discriminating between individuals with CHC and NAFL/NASH, we calculated sensitivity, specificity, and areas under the receiver operating characteristics (AUROC) curve. All calculations were done with Prism 5 software (GraphPad Software, Inc, La Jolla, CA). An error level \( P < .05 \) was considered significant.

**Results**

**MP Isolated From Plasma and Serum Yield Comparable Results**

We previously described MP from plasma.³ In order to test if (stored) serum could be used for MP quantification as well, we compared matched plasma-serum pairs from a subset of patients and healthy controls. When comparing relative levels of S100-MP (in the following simply termed MP) populations in serum vs plasma, both serum CD15⁺ (neutrophil) and CD41⁺ (platelet-derived) MP were reduced, the latter reported previously by others,¹⁶ CD4⁺ MP were slightly decreased and CD8⁺ MP were significantly increased, whereas CD14⁺ and iNKT S100-MP remained unchanged (Figure 1). At present, we cannot explain these minor to moderate differences of certain MP populations in serum vs plasma, but the profiling of those MP turned out not to be relevant for the present study on patients with NAFL and NASH, or their differentiation from CHC patients. We also found some variation in percentages of MP in some individuals, but overall this was not significant (Supplementary Figure 3A). Such changes can result from slight alterations of immune activation in individual patients, eg, due to minor (subclinical) infection or by physical activity.¹⁷–¹⁹

Additionally, we did not observe a significant correlation between blood cell counts and corresponding MP percentages, as demonstrated for monocytes (CD14⁺), neutrophils (CD15⁺), or platelets (CD41⁺) (see Supplementary Figure 3B). These results are in line with our previous findings in patients with CHC, and support the hypothesis that it is activated cells within affected organs (such as the liver) that are the source of plasma membrane MP and not the majority of nonactivated circulating blood cells.³,¹⁸

**Patients With CHC and NAFL/NASH Show Characteristic MP Profiles**

Sixty-seven and 42 patients with NASH/NAFL and CHC, respectively, were included in the study (Table 1). MP results are summarized in Figure 2A and Supplementary Table 2, and AUROC curves are shown in Figure 2B and Supplementary Figure 4A and B. CHC and NAFL/NASH were associated with increased percentages of CD4⁺ MP compared to healthy controls (40% and 29%, respectively). Similarly, levels of CD8⁺ MP were significantly higher in NAFL/NASH (56%) and in CHC (26%) compared to healthy controls. However, CD4⁺ and CD8⁺ S100-MP did not discriminate between CHC and NAFL/NASH, with low AUROC values of 0.71 and 0.59, respec-

![Figure 1. Comparison of S100-MP determinations from serum and plasma. FACS analysis revealed that both fresh plasma and stored serum samples can be used reliably to determine the levels of CD4⁺, CD14⁺, and iNKT MP. In agreement with previous reports, levels of platelet-derived MP (CD41⁺) were significantly decreased in serum.¹⁶ n, number of serum/plasma pairs; the bold number is the difference (in percent) between the means (not bold) of the measured MP population in serum vs plasma. Differences (percent in bold) between serum and plasma were calculated using the following formula: (mean plasma MP – mean serum MP)/mean serum MP.](image-url)
Figure 2. Gross overview/profile of the percentages of different S100-MP populations in patients with NAFL, CHC, and in healthy controls. (A) MP were isolated by differential centrifugation and analyzed by FACS as described in Materials and Methods. The overall P value for each MP population for the Kruskal–Wallis test was set at \( P < .0001 \) before assessing pairwise relationships by the post-hoc Dunn’s multiple comparisons approach to compare the 3 study cohorts (CHC, NAFL/NASH, and healthy controls). (B) AUROC curves were created using those cut-off values that yielded the highest likelihood to differentiate between CHC, NAFL, and healthy controls (see also Supplementary Table 1). n, number of patients in each MP analysis. Occasional missing data points are due to limitation of serum. Ten additional patients with CHC for whom only CD4\(^{+}\) and CD8\(^{+}\) MP were available were included (see Table 1).
tively (Supplementary Table 2). In contrast, the exclusive elevation of CD14+ and iNKT MP in NAFL/NASH compared to CHC led to AUROC values of >0.99 and 0.97, respectively. At cutoffs of 9.7% and 3.6%, respectively, we observed sensitivity and specificity values >87% for differentiation from CHC. Of, note these 2 cell populations have recently been implicated as being central to NAFL/NASH pathogenesis.11,20 In NAFL/NASH CD15+ and CD41+, MP levels were reduced significantly by 42% and 32%, respectively, compared to CHC. This reduction for CD15+ and CD41+ MP percentages was also associated with a high specificity score, 96%, but with lower sensitivity scores of 78% and 18%, respectively.

Figure 3A shows an MP analysis for the extremes of ALT values of the 2 liver disease cohorts focusing on T cell and iNKT MP. Here, we confirmed our earlier finding that patients with CHC and an ALT >100 IU/L (termed active) had significantly elevated levels of circulating CD4+ MP as compared to CHC patients with ALT <40 IU/L (termed mild) and to healthy controls. In contrast, NAFL/NASH patients with both low and high ALT levels were characterized by only a minor elevation of CD4+ MP (28%) compared to healthy controls, although this was statistically significant. CD8+ MP were also increased in NAFL/NASH, especially in the high ALT group, similar to serologically active vs inactive CHC.

There was only a nonsignificant (P > .05) increase of iNKT MP in CHC, either with low or with high ALT values, vs healthy controls. However, in all patients with NAFL/NASH, iNKT MP were strikingly elevated (NAFL/NASH with high ALT [>100 IU/L]: by 124% vs normal controls; NAFL/NASH with ALT <100 IU/L: by 114% vs CHC with high ALT).

**CD14+ MP Subgroup Analysis in NASH**

Recently, a link between chronic liver disease progression and CD14+CD16- and CD14+CD16+ cells was reported.21 In a representative cohort of NAFL/NASH patients (ALT range, 31–109 IU/L), we found comparable percentages of MP from CD14+CD16- (classical), CD14+CD16+ (nonclassical, proinflammatory) monocytes, and CD14+CD1c+ myeloid dendritic cells (Figure 3B).

**Correlation Between ALT and MP in Patients With NAFL/NASH and CHC**

Correlations are shown in Figure 4. As expected, CD4+ and CD8+ MP correlated slightly better with ALT levels in patients with CHC (Figure 4A) as compared to...

**Figure 3.** MP levels in relation to normal and high ALT values and biopsy stage, and analysis CD14+ MP subsets. (A) MP levels in CHC or NAFL/NASH patients with normal ALT values (<40 IU/mL, numbers as indicated) or high ALT values (>100 IU/mL, numbers as indicated) as a surrogate of hepatic inflammation, and in comparison to S100-MP populations from healthy controls. The overall P value for each MP population for the Kruskal–Wallis test was set at P < .0001 before assessing pairwise relationships by the post-hoc Dunn’s multiple comparisons approach to compare the 3 study cohorts (CHC, NAFL/NASH, and healthy controls). (B) Analysis of CD14+ S100-MP subpopulations in a representative cohort of NAFL/NASH patients. CD14+CD16-: “classically activated” monocytes; CD14+CD16+: “nonclassically activated, inflammatory” monocytes, CD14+CD1c+: myeloid DCs. (C) In CHC CD4+ and CD8+, MP correlated well with fibrosis stage as we described previously in a smaller cohort (r = 0.63; P < .0001; r = 0.59; P = .0002, respectively). However, CD4+, CD8+, CD14+, and iNKT MP levels did not correlate with stage in NAFL/NASH (data not shown). Variations in numbers are due to limitation of serum.
However, the good correlations for all MP subpopulations with ALT in NAFL/NASH were lost when patients with ALT >80 IU/mL were included (data not shown) and improved with ALT <80 IU/L. The best correlations were found between ALT levels of patients with NAFL/NASH and their circulating CD14 and iNKT MP ($r = 0.63; P = .0001; r = 0.59; P = .0001$). Interestingly, for CD8$, CD15$, and CD41$ MP, no clinically relevant correlations were found ($r < 0.5$), although the correlations for CD$^8$ and CD$^{15}$ MP were statistically significant. Subanalysis ruled out a sex effect for all correlations (data not shown).

### Circulating MP as Predictors of Histological Grade in CHC and NAFL/NASH

We confirmed our earlier findings of a good correlation between circulating CD$^4$ and CD$^8$ MP and histological inflammation grade in CHC. In addition,
iNKT MP correlated even better with histological grade in patients with CHC than CD4+ and CD8+ MP \((r = 0.76; P < .0001, \text{Figure 5A})\). Although CD14+, CD15+, and CD41+ MP did statistically correlate with grade in CHC, this was considered clinically irrelevant \((r < 0.5)\). In NAFL/NASH, iNKT MP correlated well with histological grade \((r = 0.58; P < .0001)\), and this correlation must be considered as clinically relevant \((\text{Figure 5B})\). Other correlations, even when statistically significant, turned out to be clinically irrelevant \((r < 0.5)\).

**Correlation Between Biopsy Stage and MP in Patients With NAFL/NASH and CHC**

In CHC, CD4+ and CD8+ MP correlated well with fibrosis stage, as described by us earlier in a smaller cohort \((r = 0.63; P < .0001; r = 0.59; P = .0002, \text{respectively})\).
tively, Figure 3C). However, CD4⁺, CD8⁺, CD14⁺, and iNKT MP levels did not correlate well with stage in NAFL/NASH (data not shown). In addition, no correlations for either CHC or NAFL/NASH were found with circulating CD15⁺ and CD41⁺ MP (data not shown).

**Circulating MP as Predictors of the Histological Severity in NAFL/NASH**

MP were correlated with the NAFLD activity score (NAS), currently considered the gold standard for the assessment of the severity of inflammation and apoptosis in NAFL/NASH (Figure 6A). Although statistically significant, CD4⁺ and CD8⁺ MP correlated only weakly (therefore lacking clinical relevance) with the NAS score ($r = 0.42; P = .0004$; $r = 0.38; P = .0016$, respectively), as did iNKT MP ($r = 0.47; P = .0006$). As previously noted, CD14⁺ MP, derived from cells that play a particular role in NASH pathogenesis correlated best with the NAS score ($r = 0.60; P < .0001$).

Notably, CD14⁺ MP correlated strongly with iNKT MP in all patients with NAFL/NASH ($r = 0.7; P < .0001$) (Figure 6B), far exceeding correlations between all other combinations, and supporting the hypothesis that these 2 MP populations are not only uniquely released in NAFL, but also linked in pathogenesis.

**Discussion**

Cell-derived MP are recently discovered vehicles of intercellular communication and emerging tools to quantify cell-specific pathological processes. Several publications describe MP shedding in inflammatory conditions, such as malaria (platelet-, red blood cell-, and monocyte-derived MP), heart failure (endothelium-derived MP), arthritis, human immunodeficiency virus infection, end-stage renal failure, in coagulation disorders (platelet-derived MP), and even after moderate exercise. We recently showed that T-cell S100-MP, which are found at...
increased levels in the plasma of patients with CHC, can fuse with HSC plasma membranes via intercellular adhesion molecule 1 and consequently up-regulate HSC fibrolytic gene expression by transfer of CD147, suggesting a possible functional role of inflammatory cell MP in chronic liver diseases.1

The aim of this study was to explore if 2 prevalent but mechanistically different chronic liver diseases, CHC and NAFL/NASH, can be distinguished by their S100-MP profiles, if these profiles are biologically plausible, and if they could serve as novel plasma or serum biomarkers of disease activity.

Typically, hepatic fibrosis progression and acute hepatitis require activation of various immunological competent cells, such as T cells, NK/T cells, dendritic cells, and macrophages (Kupffer cells).25 Because activation or early apoptosis of cells can result in shedding of MP, levels of certain MP originating from different cell types can be measured by FACS in healthy subjects and in patients with different diseases.1,3,18

By analyzing serum MP for 6 cell surface markers representing major immune cell populations that are involved in hepatic inflammation and fibrogenesis (CD4+/8+ T cells, CD14+ monocytes/macrophages/ dendritic cells, CD15+ neutrophils, CD41+ platelets, and Vo24/V11-positive iNKT cells3,7,9,18), we could identify S100-MP profiles that are highly characteristic for either NAFL/NASH or CHC. CHC was dominated by CD8+ and CD41+ MP, and NAFL/NASH patients showed a unique elevation of CD14+ and iNKT MP and a decrease in CD15+ and CD41+ MP, irrespective of ALT levels or histological markers of disease activity. The S100-MP patterns allowed an almost complete separation of patients with CHC or NASH and healthy controls.

These findings are not only in excellent agreement with the pathophysiology of both diseases, but also reveal novel insights into disease pathogenesis, as also published in recent studies. First, the homing of circulating immune cells to the liver and their turnover increases during hepatic inflammation, likely increasing circulating MP released during their activation or apoptosis.1 Second, CD8+>CD4+ T cells,6 as well as NKT cell populations27 including iNKT cells,28 are major immune effectors in CHC, although histological inflammation of the liver appears to be better reflected by iNKT MP than by blood iNKT cells.27,28 Third, iNKT cells have been implicated as major drivers of inflammation and fibrosis progression in NASH.20,29 Fourth, CD14+ macrophages>monocytes appear to play a prominent role in peripheral adipose tissue inflammation, the associated metabolic syndrome,30,31 and hepatic necroinflammation in NASH.32 In the latter context, inflammatory CD14+ CD16+ monocytes are linked to disease progression and fibrogenic activation of HSCs,31 as has been shown for a variety of inflammatory diseases, including rheumatoid arthritis, diabetes, atherosclerosis, and bacterial infections.33 Our CD14+ S100-MP subgroup analysis revealed that CD14+ S100-MP in NAFL/NASH originated in equal proportions from CD16- (classical monocytes or macrophages), CD1c+ (myeloid dendritic cells), and CD16+ (inflammatory monocytes or macrophages). Because the latter cell population is lower in normal subjects (7.5%), the relative quantity of elevated count of CD14+CD16+ S100-MP in patients with NAFL/NASH indicates activation of inflammatory monocytes/macrophages.21 In addition, there was an excellent correlation between CD14+ and iNKT MP in patients with NAFL/NASH, which underscores a functional link between activation of these immune cells in patients with fatty liver and NASH.

Although inflammatory cell S100-MP correlated with ALT levels and histological staging/grading, these correlations were limited. This is expected, because ALT is a suboptimal surrogate of hepatic inflammation, apoptosis, or necrosis,34 and biopsy is a tarnished gold standard due to high sampling variability.35–37 In CHC, the disease with the lowest expected sampling variability (~25% and 33% for 1 Metavir grade and stage difference, respectively37), there was a good correlation of CD41+ or CD8+ MP with ALT and biopsy grade and stage. In NAFL/NASH, which incurs a higher biopsy sampling variability (~40% for 1 Metavir grade or stage39), CD14+ and iNKT MP only correlated well with ALT up to 80 IU/L, but poorly with fibrosis stage. The histological NAS score, considered the gold standard for the diagnosis of NASH, showed only a modest correlation with CD14+ and iNKT MP. Although a positive NAS score >4 renders the best evidence for NASH, a borderline or negative score does not exclude its presence because this score, which consists of 3 subscores for hepatic steatosis, lobular inflammation, and ballooning, is affected by significant sampling variability, especially for detection of hepatocellular ballooning,39 which is missed in at least 50% of biopsies.38 Additionally, due to its multicomponent character, the NAS score is influenced by the skill and experience of the reading pathologist, more than other histological scores, and is prone to intra- and inter-observer variability.15,40 In contrast, a quantitative diagnostic test that can measure overall activation of a certain immune cell subset, such as circulating MP, might circumvent biopsy sampling and observer variabilities. In addition, it might rather reflect current disease activity compared with a more static picture as reflected by biopsy assessment, permitting, for example, the “real-time” monitoring of disease-specific anti-inflammatory therapies. However, at present we cannot present evidence that S100-MP quantification is superior to state of the art diagnostics for NAFL/NASH, which will require future large prospective and follow up studies. It will be particularly interesting to follow up MP profiles in patients with NASH who undergo treatment with, for example, insulin sensitizers or antioxidants, or who begin to favorably change their lifestyle.41–43

In conclusion, by analyzing circulating S100-MP, a systemic profile of immune cell subsets that are prominently involved in CHC or NAFL/NASH can be obtained. MP profiling corroborates the in vivo significance of pathophysiological hypotheses on immune-mediated liver dis-
eases; as shown here for CHC and NAFL/NASH, S100-MP appear to represent a novel diagnostic tool to assess overall disease severity and especially activity, with the advantage of being specific, noninvasive, and quantitative.

**Supplementary Material**

Note: To access the supplementary material accompanying this article, visit the online version of Gastroenterology at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2012.04.031.

**References**


**CIRCULATING MICROPARTICLES IN HEPATITIS C AND NASH**


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Conflicts of interest
The authors disclose no conflicts.

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