marrow failure syndromes (eg, congenital amegakaryocytic thrombocytopenia), splenectomy will not result in clinically significant improvement. However, it is possible that for certain individuals a small change in circulating platelet number may improve hemostasis. For example, affected males with GATA1 mutations in one family reported diminished frequency and severity of bleeding episodes in adulthood despite persistent thrombocytopenia. It is not clear whether this change was due to splenectomy. I believe that splenectomy should be reserved for those rare individuals for whom hematopoietic stem cell transplantation is unavailable and the potential impact of a small absolute increase in circulating platelets is clinically significant.

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References

To the editor:

Association of HCV-related mixed cryoglobulinemia with specific mutational pattern of the HCV E2 protein and CD81 expression on peripheral B lymphocytes

Interaction of the hepatitis C virus (HCV) envelope (E) 2 protein with the cellular receptor CD81 leads to B lymphoproliferation in vitro, a major characteristic of mixed cryoglobulinemia (MC). Within E2, 2 CD81 binding sites have been described in vitro comprising the hypervariable region 1 (HVR1) and HVR2. In patients with chronic hepatitis C, CD81 expression on peripheral B lymphocytes is increased. We hypothesize, that specific amino acid (aa) sequences within E2 and CD81 expression on B lymphocytes may act as determinants for the development of MC in vivo.

There were 58 consecutive patients with chronic hepatitis C tested for MC. Cryoprecipitates were detectable in 14 (24.0%) of patients with chronic hepatitis C, tested for MC. Cryoprecipitates were detectable in 14 (24.0%) of patients with chronic hepatitis C, tested for MC.
58 patients and immunofixation showed MC type II in all patients. MC-positive patients did not differ from those without MC regarding age, alanine aminotransferase level, baseline viremia, and estimated duration of infection.

The E2 regions including HVR1 and HVR2/CD81 binding sites were sequenced and the obtained E2 aa sequences were analyzed according to physicochemical properties. The hydrophobic pattern of the E2 regions was widely conserved and allowed no differentiation between patients with and without MC. Analyses of the solvent accessibility showed that the majority of mutations were located within the HVR1 and HVR2/CD81 binding sites at residues predicted on the surface of E2 (exposed), underlining the functional relevance of these regions (Figure 1A). Furthermore, we evaluated the predictability of MC based on the E2 sequences and used adjusted classification algorithms composed of statistical learning procedures, probabilistic models, and empiric likelihood ratio tests. These classification algorithms account for mutational frequencies and dependencies between different aa positions. Classification rates (CRs) and Matthews correlation coefficients (MCCs) were calculated using a nested cross-validation procedure, and the importance of individual aa positions was expressed as differences in MCCs in comparison with random permutations at this position. There were 2 HVR1 positions (389 and 398; CR, 67%; MCC, 0.32) and 3 HVR2/CD81-binding site positions (474, 493, and 497; CR, 72%; MCC, 0.29) correlated with the presence of MC (Figure 1A). The identified positions give reason for site-directed mutagenesis studies to evaluate their influence of the E2 binding affinity to CD81.

Fluorescence-activated cell sorter (FACS) analyses showed that CD81 expression on CD19+ B lymphocytes, representing the vast majority of B lymphocytes, was significantly higher in patients with MC (923.7 ± 264.8) compared with those without MC (774.5 ± 147.3; P = .045, one-tailed), patients chronically infected with hepatitis B virus (665.4 ± 132.3; P = .010), and healthy controls (476.0 ± 100.5; P < .001; Figure 1B). These results underline the concept that CD81 exerts a specific role in the host interaction with HCV. Higher CD81 expression was notably found in MC-positive patients infected with HCV-1a isolates possessing high binding affinity to CD81 in vitro (P = .024, data not shown). B lymphoproliferation is thought to be driven by HCV-specific antigen in vitro. Accordingly, HCV core antigen levels of peripheral blood mononuclear cells (PBMCs) were higher in MC-positive patients (4.86 ± 2.41 pg/mL) compared with those without MC (3.63 ± 1.46 pg/mL; P = .047; Figure 1C).

In conclusion, classification algorithms provide information about specific E2 positions correlated with MC. CD81 expression and HCV core antigen levels in PBMCs are increased in patients with MC.

To the editor:

**Induction of imatinib metabolism by hypericum perforatum**

Hypericum perforatum (St John Wort) is an herbal preparation commonly used by cancer patients for mood elevation and is known to induce intestinal and hepatic cytochrome P450 3A4 (CYP3A4) and P-glycoprotein. CYP3A4 is the major isoenzyme responsible for imatinib metabolism, making imatinib potentially susceptible to drug interactions with numerous compounds, including hypericum perforatum.

We evaluated the impact of hypericum perforatum on imatinib pharmacokinetics in an open label, fixed sequence, complete crossover study. There were 10 volunteers who received single doses of imatinib (400 mg) prior to and following 2 weeks of hypericum perforatum (300 mg) thrice daily. Imatinib plasma concentrations were determined at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours after dosing by liquid chromatography (LC)–tandem mass spectrometry (MS/MS). Free imatinib concentrations were measured at each 3- and 24-hour sample by ultrafiltration.

Imatinib pharmacokinetics were significantly altered by hypericum perforatum (Table 1). The median area under the curve (AUC0-24) decreased by 32% (28.9 vs 19.7 μg·h/mL), with maximum concentration (Cmax) and half-life reduced by 29% and 21%, respectively. Imatinib was approximately 95% protein bound.

### Table 1. Pharmacokinetics of imatinib when administered alone and with hypericum perforatum in 10 subjects

<table>
<thead>
<tr>
<th>Period</th>
<th>Tmax, h</th>
<th>Cmax, μg/mL</th>
<th>Half-life, h</th>
<th>AUC0-24, μg·h/mL</th>
<th>Cmax, μg/mL</th>
<th>Protein binding, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib</td>
<td>2.5 (1-4)</td>
<td>1.80 (0.88-2.6)</td>
<td>13.5 (10.8-18.1)</td>
<td>28.9 (13.4-36.7)</td>
<td>0.11 (0.05-0.17)</td>
<td>95.0 (90.3-97.7)</td>
</tr>
<tr>
<td>Imatinib + hypericum perforatum</td>
<td>2.5 (1-6)</td>
<td>1.28 (0.92-2.0)</td>
<td>10.7 (9.1-13.5)</td>
<td>19.7 (10.7-25.0)</td>
<td>0.05 (0.03-0.12)</td>
<td>95.1 (90.9-97.3)</td>
</tr>
</tbody>
</table>

Data presented as median (range). Tmax indicates time of Cmax; Cmax, imatinib concentration at 48 hours.

*P < .005 by paired t test on logarithmically transformed data.
Association of HCV-related mixed cryoglobulinemia with specific mutational pattern of the HCV E2 protein and CD81 expression on peripheral B lymphocytes

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