Mechanism of Thrombocytopenia in Hepatitis C as Evaluated by the Immature Platelet Fraction

Marjorie L. Zucker MD

Hepatitis C and Thrombocytopenia

- Chronic hepatitis C affects 170 million people worldwide, 3.2 million in USA
- Prevalence of thrombocytopenia in chronic hepatitis C is at least 24%
- Serological evidence of hepatitis C in 30% of subjects with chronic ITP
Possible Mechanisms of Thrombocytopenia in Hepatitis C

Peripheral platelet destruction
- Immune-mediated
- Hypersplenism

Decreased platelet production
- Reduced thrombopoietin (TPO) synthesis
- Viral effects on megakaryocytes
- Interferon therapy

Reticulated (Immature) Platelets
- Immature platelets released into peripheral blood, contain residual RNA
- Measured by flow cytometry using RNA binding dyes (thiazole orange)
- Used as index of bone marrow thrombopoietic activity (analogous to RBC retic. count)
- Useful in differentiating decreased platelet production from peripheral platelet destruction
Immature Platelet Fraction (IPF) on Sysmex XE-2100

- Automated method quantifying immature RNA-containing platelets, available on a standard blood cell analyzer, reported with CBC.
- Fluorescent flow cytometry using a polymethine dye with Sysmex IPF Master software.
- IPF is proportion of platelets with highest 3% fluorescent intensity

### RBC fragments

**Fluorescence**

**Erythrocytes**

**Reticulocytes**

**Forward Scatter**

**IPF**

**Fluorescent Optical Platelet Count**

**Fluorescence**

**RETICULOCYTE CHANNEL**
IPF in Evaluation of Thrombocytopenia

Differentiation between decreased platelet production and peripheral platelet destruction

Briggs et al, 2004:

- Increased IPF in 73% patients with ITP, 100% with platelets <50 th/uL, 100% patients with TTP
- Low IPF in 100% patients with thrombocytopenia due to chemotherapy
- In typical ITP may avoid need for bone marrow examination

Mean Days to Recovery Following HPC Transplantation

Zucker M et al, Lab Hematol. 2006; 12;125-130
Clinical Use of IPF

- Evaluation of thrombocytopenia - differentiation between decreased platelet production and peripheral platelet destruction
- Predictor of hematopoietic recovery following stem cell transplantation

Aim of Study

Investigate mechanisms of thrombocytopenia in chronic hepatitis C utilizing
- immature platelet fraction (IPF %) as an index of platelet production
- assays of thrombopoietin (TPO)
Regulation of TPO Levels

SYNTHESIS

Other sources (kidney)

Uptake by TPO Receptors (megas, platelets)

Increased receptors (ITP, TTP) \( \downarrow \) TPO level

Decreased receptors (AA, chemo) \( \uparrow \) TPO level

Study Design

- 47 patients with chronic hepatitis C
  - 29 with thrombocytopenia
  - 18 without thrombocytopenia
  - 6 in each group were taking interferon

- Clinical data
  - Cirrhosis, portal hypertension, splenomegaly
  - Medications
  - Results of lab tests (within 3 months) – albumin, INR, LFT's, hepatitis C viral load
Study Design

- Blood samples
  - CBC, platelet count, IPF(%) - Sysmex XE-2100
  - Thrombopoietin (TPO) assay – Quantikine immunoassay

Findings in Patients with Hepatitis C

(mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Low platelets n=29</th>
<th>Normal platelets n=18</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>51 ± 6.2</td>
<td>50 ± 6.2</td>
<td>N/S</td>
</tr>
<tr>
<td>M/F</td>
<td>20/9</td>
<td>12/6</td>
<td>N/S</td>
</tr>
<tr>
<td>Platelets (th/uL)</td>
<td>68 ± 25</td>
<td>233 ± 105</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IPF (%)</td>
<td>9.0 ± 4.8</td>
<td>4.7 ± 2.4</td>
<td>&lt;.001</td>
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<tr>
<td>TPO (pg/mL)</td>
<td>110 ± 107</td>
<td>132 ± 124</td>
<td>N/S</td>
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Findings in Patients with Hepatitis C

- IPF elevated in
  - 17/29 (59%) with thrombocytopenia
  - 2/18 (11%) with normal platelet counts

- In thrombocytopenic patients IPF elevated in
  - 11/19 (58%) with splenomegaly
  - 6/10 (60%) without splenomegaly

### Findings in Patients with Hepatitis C (mean ± SD)

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<tr>
<td>Albumin (g/dL)</td>
<td>3.0 ± 0.5</td>
<td>3.8 ± 0.4</td>
<td>&lt;.001</td>
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<tr>
<td>INR</td>
<td>1.3 ±0.2</td>
<td>1.1 ± 0.2</td>
<td>&lt;.001</td>
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<td>LFT's</td>
<td>27 (93%)</td>
<td>10 (56%)</td>
<td>&lt;.005</td>
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<td>Cirrhosis</td>
<td>26 (90%)</td>
<td>4 (22%)</td>
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<td>Portal HT</td>
<td>23 (79%)</td>
<td>4 (22%)</td>
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<tr>
<td>Enlarged spleen</td>
<td>19 (66%)</td>
<td>1 (6%)</td>
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### Correlation of Parameters in All Subjects with Hepatitis C

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Summary of Findings

- **Thrombocytopenic group**
  - ↑ IPF
  - ↓ albumin, ↑ INR, LFT's
  - ↑ cirrhosis, portal HT, splenomegaly

- **Platelet count correlates with**
  - IPF, INR (inverse); albumin (direct)

- **Log viral load correlates with**
  - TPO, albumin (inverse); INR (direct)

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**Multivariable Analysis**

**Relative Risk of Thrombocytopenia (95% C)**

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<tr>
<th>Factor</th>
<th>Ratio (95% CI)</th>
<th>P Value</th>
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<tr>
<td>Interferon therapy vs. not</td>
<td>1.60 (0.79, 2.97)</td>
<td>NS</td>
</tr>
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<td>TPO (100 pg/mL increase)</td>
<td>0.69 (0.47, 1.12)</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (0.5 g/dL decrease)</td>
<td>1.33 (1.12, 1.58)</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>TPO % (2% increase)</td>
<td>1.09 (1.02, 1.17)</td>
<td>&lt; 0.05</td>
</tr>
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<td>Spleen enlarged vs. not</td>
<td>1.91 (1.15, 3.19)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Log viral load (5 point increase)</td>
<td>1.08 (0.95, 1.21)</td>
<td>NS</td>
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Peripheral platelet destruction or sequestration

A major mechanism of thrombocytopenia in chronic hepatitis C

- IPF significantly increased in thrombocytopenic patients
- IPF shows significant inverse correlation with platelet count

Splenomegaly

Platelet sequestration by an enlarged spleen is a major factor in thrombocytopenia in chronic hepatitis C

- Significantly more common in thrombocytopenic patients (10X higher incidence)
- Associated with highest relative risk of thrombocytopenia (RR=1.9)
Other Mechanisms

- In thrombocytopenic patients IPF elevated in a similar proportion of those with and without splenomegaly (58% vs 60%)

- Hypersplenism is not the only mechanism for peripheral platelet destruction

- Other likely mechanisms include antiplatelet antibodies and immune complexes

Defect in Platelet Production

- Small subgroup of thrombocytopenics without splenomegaly and with a normal IPF value (4/29, 14%)

- Possible suppression of thrombopoiesis by
  - Antiplatelet antibodies
  - CD8 positive T cells
  - HCV infection of megakaryocytes
  - Cytokines
  - Bone marrow disorder
**Decreased TPO synthesis?**

- No difference in TPO levels between thrombocytopenics and non-thrombocytopenics and no correlation between TPO levels and platelet count
- TPO levels not related to relative risk of thrombocytopenia
- Complex interactions between TPO synthesis and degradation - TPO levels in chronic liver disease may not reflect rate of TPO production

**Hepatitis C Viral Load**

- The only factor to show a significant (inverse) correlation with TPO
- Significant correlation with albumin (inverse) and INR (direct)
- No correlation with platelet count (on multivariable analysis)

High viral load in blood likely corresponds to high level of virus in hepatocytes - suggests inhibition of protein synthesis
Summary of Findings in 29 Thrombocytopenic Patients

<table>
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<tr>
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<th>IPF Increased</th>
<th>IPF Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Splenomegaly</strong></td>
<td>Platelet sequestration in spleen (n=11, 38%)</td>
<td>Hypersplenism plus failure of thrombopoiesis to compensate (n=8, 27%)</td>
</tr>
<tr>
<td><strong>No Splenomegaly</strong></td>
<td>Immunologic destruction of platelets (n=6, 21%)</td>
<td>Decreased platelet production (n=4, 14%)</td>
</tr>
</tbody>
</table>

Possible Clinical Application

- Major mechanism of thrombocytopenia is peripheral platelet destruction, but defect in platelet production may contribute in some cases, especially where IPF is normal.
- Further testing may be warranted in the subgroup with normal IPF - includes a bone marrow to rule out bone marrow defect which may be associated with hepatitis C (eg lymphoproliferative disorder).
Summary

- Peripheral sequestration or destruction of platelets is the major mechanism for thrombocytopenia in chronic hepatitis C; hypersplenism is the most notable cause
- Failure of thrombopoiesis to compensate is most likely a contributory mechanism

Summary (contd)

- Factors associated with liver disease in general are associated with thrombocytopenia in chronic hepatitis C
- Superimposed on this are factors specific to hepatitis C (immunologic and virus-mediated)
- Low TPO levels were not related to the occurrence of thrombocytopenia in this study.