Coagulopathy in liver disease: complication or therapy?

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Liver cirrhosis

- Major burden of morbidity and mortality worldwide, responsible for 800,000 deaths annually.
- Prevalence and incidence rising in the UK in parallel with epidemic of alcohol abuse and obesity.
- 50,000 living with cirrhosis, 7000 new cases/year
- One of the most complex and multifactorial acquired disorders of haemostasis
Patients with cirrhosis bleed

- “Auto-anticoagulated” – based upon conventional coagulation indices
- 15%-20% mortality
- Many die from haemorrhage
- Liver and GI diseases- largest consumer groups of all blood components in the UK

Hearnshaw et al., Gut 2010; Wells et al, Transfus Med., 2010
Liver cirrhosis is associated with venous thromboembolism among hospitalised patients in a nationwide US study

Wu H, Nyguyen GC
Clin Gastro Hep 2010;8 (9): 800-805

Risk of venous thromboembolism in patients with liver disease; a nationwide population based case-control study

Soggard KK, Horvath-Puho E et al.
Am J Gastroenterol 2009;7: 303-310

Deep Vein Thrombosis and pulmonary embolism in hospitalised patients with cirrhosis

Alim M, Ananthakhrishnan AN et al.
Dig Dis Sci 2011
Limitations of conventional coagulation indices

- **Prothrombin time**
  - Clotting time of a mixture of PPP/TF/CaCL
  - Only measures procoagulant factors
  - Devised to monitor VKAs, NOT any other indication

- Do not predict the risk of bleeding nor outcomes of patients who present with bleeding

- Use engrained in daily practice to guide transfusion e.g. pre-procedurally and to treat bleeding
Global Assays - Thrombin Generation Tests

- Continuously monitors thrombin activity in plasma
- TG triggered by Tissue factor and/or phospholipid
- Fluorescent plate reader
- Used routinely in haematological disorders
Global whole blood assays of coagulation: Rotational thromboelastometry

- Visco-elastic test allowing an assessment in whole blood of the real-time interaction between coagulation factors, platelets, red blood cells, fibrinogen, clot stability and fibrinolysis.
## Results – Thrombin generation in PPP

<table>
<thead>
<tr>
<th>Thrombin generation Parameters</th>
<th>Cirrhotics-compensated N=74</th>
<th>Healthy controls N=30</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lagtime (min)</td>
<td>2.6 (0.9)</td>
<td>3.3 (0.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Peak (nM)</td>
<td>323.4 (91.9)</td>
<td>442.4 (80.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Time to Peak (min)</td>
<td>5.2 (1.7)</td>
<td>6.2 (0.8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ETP (nmol/L)</td>
<td>2036.2 (438)</td>
<td>2665.1 (443)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Jairath et al. Gastroenterology: 2012 (142);5: S-918
Results - ETP Ratio with/without Protac

- Liver
g- GFD

- Variceal

- Bleed

- Control

- Class A

- Class B

- Class C

Patient type

Child Pugh Score
The balance of pro- and anti-coagulant factors

Factor 8

Factor 7

Factor 9

Factor 11

Factor 2
The balance of pro- and anti-coagulant factors

Antithrombin 3

Protein C

Protein S

Tripodi et al., Gastroenterology 2009; Gatt et al., JTH, 2010; Jairath et al. Gastroenterology: 2012 (142);5: S-918
Procoagulant microvesicles in plasma of patients with cirrhosis

- 0.1 - 1.0 μm
- Cell-derived
  - phenotype & function may be cell-type and agonist-dependent
  - Often express membrane proteins from cell of origin
- Variable exposure of phosphatidylserine
- Membrane skeleton may be present
- Nucleus is absent
- Free of nucleic acids
- Lack synthetic capability
Complex Mixture of circulating Microvesicles originating from different tissues

Liver
- Hepatocytes
  [69]
- Cholangiocytes
  [87]
- Myofibroblastic stellate cells
  [87]

Other peripheral tissues
- Placental chorionic villi (trophoblast cells)
  [78]
- Adipocytes (adiposomes)
  [65]
- Microglia
  [72,83]

Immunological system
- Leukocytes
  - Monocytes
    [81]
  - Macrophages
    [67, 82]
  - Dendritic cells
    [86]
  - Neutrophils
    [44, 71]
  - Mast cells
    [84, 50]
  - B & T lymphocytes
    [62, 80]

Other blood cells
- Erythrocytes
  [75]
- Platelets
  [70, 73, 81]
Micro and Nanovesicle function

- coagulation
- communication
- anticoagulation
- transport
- waste management
- inflammation
- cell activation
- angiogenesis
- endothelial dysfunction
Microvesicles and haemostasis

- Microparticles (MVs) key effectors of haemostasis
  - Phosphatidylserine (PS) surface expression and/or
  - Binding sites for procoagulant factors 8, 9, 10
  - Tissue Factor (TF) surface expression

Increasing procoagulant activity
# Microvesicle phenotype in Cirrhosis

<table>
<thead>
<tr>
<th>Microvesicles /µL Plasma Mean (sd)</th>
<th>Cirrhotics- compensated (N=28)</th>
<th>Healthy controls (N=10)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total PS positive (AV)</strong></td>
<td>1413.5 (1985)</td>
<td>278.7 (259)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Platelet derived (CD41+)</td>
<td>233.5 (215)</td>
<td>147.3 (190)</td>
<td>0.17</td>
</tr>
<tr>
<td>Red cell derived (CD235+)</td>
<td>225.9 (384)</td>
<td>196.6 (352)</td>
<td>0.86</td>
</tr>
<tr>
<td>Endothelial derived (CD31+/CD41-)</td>
<td>48.8 (52)</td>
<td>20.1 (13)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Total TF positive (CD142)</strong></td>
<td>71.9 (70)</td>
<td>19.0 (8)</td>
<td>0.11</td>
</tr>
<tr>
<td>Platelet derived (CD41+)</td>
<td>16.9 (8.6)</td>
<td>11.9 (7.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>White cell derived (CD45+)</td>
<td>19.8 (7.6)</td>
<td>9.9 (4.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Monocyte derived (CD14+)</td>
<td>18.1 (6.9)</td>
<td>10.5 (2.6)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Jairath et al. Gastroenterology: 2012 (142);5: S-951
The haemostatic balance in compensated cirrhosis: normal-hypercoagulable

- Procoagulant factors
- Platelets number
- Platelet function
- Fibrinogen
- Fibrinolysis
- Red cell mass

- Anticoagulant factors
- Von-Willebrand factor
- ADAMTS 13
- Factor 8
- Exosomes and MVs

Endothelial dysfunction
- Portal Hypertension
- Uraemia
- Sepsis

Hospitalisation

Tripodi et al., Gastroenterology 2009; Lisman and Porte, Blood 2010
Acute and Chronic Changes in the Microcirculation of the Liver in Inbred Strains of Mice Following Infection with Mouse Hepatitis Virus Type 3

Peggy Macphee, Vincent Dindzans, Lai-Sun Fung and Gary Levy

Focal necrosis at 24 hours-H&E
Necrotic Areas at Day 3-H&E
Micro-thrombi and ischaemic Hepatocytes- Day 3 microscopy

Hepatology 1985 (5):4;649-660
Hepatic and Portal Vein Thrombosis in Cirrhosis: Possible Role in Development of Parenchymal Extinction and Portal Hypertension

Ian Wanless, Florence Wong, Lawrence Blendis, Paul Greig, Jenny Heathcote, Gary Levy

- Histological studies on small and medium sized intra-hepatic veins and sinusoids in 61 explanted human livers
- Entire spectrum of post-thrombotic changes in these vessels
  - Acute thrombosis and intimal fibrosis
  - Partial recannalisation
  - Multiple layers of fibrosis – recurrent thromboses
  - Severe obliteration in the smallest veins
- Irreversible loss of hepatocytes from a region and replacement by fibrous tissue
Aimed to assess the effect of anticoagulation with warfarin on hepatic fibrogenesis in a mouse model

Two strains of mice – CCl4 vs. CCl4 + Warfarin

Warfarin treated mice had 1/3 reduction fibrosis scores than mice given CCl4 and no warfarin

Change in fibrosis correlated with HSC activation

Anticoagulation slowed hepatic fibrogenesis
  • May be through thrombin signalling via HSC
  • Provides rationale for anticoagulation as a therapy
Anticoagulation has the potential to reduce hepatic fibrogenesis

TF+VIIa → Xa+Va → IIa (thrombin) → Platelets aggregation → Initiation
- TF/FVIIa
- FIXa/FVIIa
- Warfarin, UFH, LMWH, FXals

Endothelial activation → Inflammatory cells recruitment → Propagation
- FXa/FVa
- Thrombin
- Warfarin, UFH, LMWH, DTIs
Moving from the laboratory to the patient – RCTs of anticoagulation in cirrhosis

- **Population** – 70 patients with Child score 7-10
- **Intervention/comparator** – Enoxaparin vs. Placebo
- **Outcomes** – PVT, decompensation, survival, bleeding
- **Study Design** - randomised, open label

<table>
<thead>
<tr>
<th></th>
<th>LMWH % (n)</th>
<th>Placebo % (n)</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVT</td>
<td>0 (0/34)</td>
<td>17 (6/36)</td>
<td>0.01</td>
</tr>
<tr>
<td>Decompensation</td>
<td>12 (4/34)</td>
<td>61 (22/36)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Bleeding</td>
<td>0 (0/34)</td>
<td>0 (0/36)</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>24 (8/34)</td>
<td>36 (13/36)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Moving from the laboratory to the patient – RCTs of anticoagulation in cirrhosis

- **Population** – 90 patients with HCV and transplant within previous 4 months

- **Intervention/comparator** – Warfarin (INR 2-3) vs. standard care

- **Outcomes** – Stage of fibrosis at 24 months; no. HSC per high power field; non-invasive markers of fibrosis

- **Study design** – randomised, open-label, stratified by centre/gender
Conclusions

- Hypercoagulation of CLD appears to be associated with hepatic fibrogenesis.

- Modulating coagulation may be a relevant therapeutic target for development of novel anti-fibrotics.

- Aforementioned trials are proof of concept and will provide some pilot data for possible larger phase 3 trials.

- Patient selection will be key and global tests of coagulation could play a role in personalising treatment.
Acknowledgements

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