Measurement of liver fibrosis

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Measurement of liver fibrosis: towards a better characterisation of disease stage

Outline

• Liver disease stage and liver tissue collagen quantification is not the same thing
• There is fibrosis variation within each stage score
• There is a relationship between liver morphology and portal hypertension
• Quantification of hepatic collagen reflects HVPG
• Liver biopsy fibrosis measurement at 1 year post transplantation predicts clinical decompensation in HCV patients
• Liver fibrosis measurement can provide clinically useful prognostic information
What do we mean by “liver fibrosis”?
Differences between morphological appearance, description, stage scoring and liver fibrosis measurement

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Ishak stage: Categorical description</th>
<th>Ishak stage: Categorical assignment</th>
<th>Fibrosis measurement*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fibrosis (normal)</td>
<td>0</td>
<td>1.9%</td>
<td></td>
</tr>
<tr>
<td>Fibrous expansion of some portal areas + short fibrous septa</td>
<td>1</td>
<td>3.0%</td>
<td></td>
</tr>
<tr>
<td>Fibrous expansion of most portal areas + short fibrous septa</td>
<td>2</td>
<td>3.6%</td>
<td></td>
</tr>
<tr>
<td>Fibrous expansion of portal areas with occasional portal to portal (P-P) bridging</td>
<td>3</td>
<td>0.5%</td>
<td></td>
</tr>
<tr>
<td>Fibrous expansion of portal areas with marked bridging (portal to portal (P-P) as well as portal to central (P-C))</td>
<td>4</td>
<td>13.7%</td>
<td></td>
</tr>
<tr>
<td>Marked bridging (P-P and/or P-C), with occasional nodules (incomplete cirrhosis)</td>
<td>5</td>
<td>24.3%</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis, probable or definite</td>
<td>6</td>
<td>27.6%</td>
<td></td>
</tr>
</tbody>
</table>

Standish R et al. An appraisal of the histopathological assessment of liver fibrosis. 
Gut 55;569;2006
Can liver fibrosis be measured as proportion of liver tissue area stained by sirius red in liver biopsies in chronic HCV infection?

<table>
<thead>
<tr>
<th>Ishak stage</th>
<th>N</th>
<th>CPA % Mean Median IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>17</td>
<td>2.8 2.0 1.2-3.4</td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>3.8 3.0 1.9-4.9</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>5.8 5.0 3.7-8.3</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>6.4 6.2 3.8-7.7</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>9.9 8.4 6.7-14</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>14.1 12.5 7.8-19</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>21.7 17.3 15.1-29</td>
</tr>
</tbody>
</table>

Calvaruso V et al.  
Hepatology 49,1236;2009  
(TJ liver biopsies)

<table>
<thead>
<tr>
<th>METAVIR Stage</th>
<th>Area of Fibrosis by Image Analysis (Mean + SEM)</th>
<th>Range of Fibrosis Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>2 ± 0.14</td>
<td>0.7-2.7</td>
</tr>
<tr>
<td>F1</td>
<td>3.4 ± 0.25</td>
<td>2.7-4.6</td>
</tr>
<tr>
<td>F2</td>
<td>5.8 ± 0.7</td>
<td>4.6-10.25</td>
</tr>
<tr>
<td>F3</td>
<td>14.1 ± 0.1/</td>
<td>10.25-19.9</td>
</tr>
<tr>
<td>F4</td>
<td>25.1 ± 1.44</td>
<td>19.9-30.2</td>
</tr>
</tbody>
</table>

Bedossa et al.  
Hepatology 38,1449;2003  
(2x3cm tissue blocks from surgical resections; N=17)
Variability of liver fibrosis within stage scores in chronic hepatitis C

Calvaruso V et al. Hepatology 49,1236;2009 (TJ liver biopsies)

Goodman Z et al. Hepatology 50,1738--;2009 (liver biopsies from HALT-C trial)
Variability of liver fibrosis

- Fibrosis variation within stage scores is a biological reality
- Amount of liver fibrous tissue is a biological continuum as progressive liver disease develops
- It is unreasonable to expect liver fibrous tissue measurements to segregate neatly, with clearly defined cut-offs, into artificial descriptive stage score categories
- Intra stage (within any given descriptive stage “score” definition) differences in the amount of fibrosis may have additional prognostic value: “vive la difference”
Pathophysiology of portal hypertension

Relationship between liver morphology and haemodynamics

The different contributors to increased portal pressure

Bosch J et al. J Hepatol 48 Suppl1,S68;2008
Increased intrahepatic resistance in cirrhosis

**Architectural disturbances**
- Distortion of vascular architecture by fibrosis, scarring, regenerative nodules.
- Thrombosis

*Mechanical Component* ("fixed")
- ~70%

**Functional alterations**
- Active contraction of hepatic stellate cells, vascular smooth cells in the portal venules, and myofibroblasts

*Dynamic Component* (modifiable by drugs)
- ~30%

Marra F. Pathophysiology of portal hypertension
EASL Postgraduate course 2008
Relationship between liver disease and haemodynamics

- van Leeuwen D et al. Scand J Gastroenterol. 24,65;1989
- “Wedged hepatic venous pressure (HVWP) recording and venography for the assessment of pre-cirrhotic and cirrhotic liver disease.”
  - One of the first papers to explore relationship between liver histopathology and HVWP
- The four main groups and the means of the pressure gradients (WHVP - FHVP) with their 95% confidence limits were
  - Near-normal liver (n = 8), 3.4 mm Hg (2.2-4.6)
  - Chronic active hepatitis (n = 12), 6 mm Hg (4.35-7.65)
  - Chronic hepatitis in transition to cirrhosis (n = 9), 10.3 mm Hg (6.6-14.1)
  - Cirrhosis (n = 8), 15.4 mm Hg (9.4-21.4)
- A pressure gradient of more than 5 mm Hg was always associated with significant liver disease on liver biopsy
Relationship between liver morphology and haemodynamics
liver disease stage reflects hepatic venous pressure gradient
(post transplantation HCV liver disease)

- Carrion JA et al. Liv Transpl 12,1791;2006
- 169 liver biopsies (66 percutaneous, 103 transjugular) from 124 HCV-infected liver transplant recipients with determination of hepatic venous pressure gradient (HVPG).
- “Close correlation between liver stiffness measurement and HVPG (Pearson coefficient, 0.84; P < 0.001).”
- “There was a significant positive relationship between the fibrosis stage and HVPG (Kruskal-Wallis P<0.001).”
Relationship between liver morphology and haemodynamics
liver disease stage reflects hepatic venous pressure gradient
(post transplantation HCV liver disease)

- 90 consecutive HCV OLT patients underwent 170 hepatic venous pressure gradient (HVPG) measurements concomitant with transjugular liver biopsy
- HVPG correlated with Ishak stage ($r = 0.73$, $P < 0.001$) for mild (0-3) and severe fibrosis (4-6)
Amount of fibrosis within cirrhosis correlates with HVPG

• “The Laennec grading system for assessment of hepatic fibrosis: validation by correlation with wedged hepatic vein pressure and clinical features”
  - Laennec system (“modified from Metavir system”) categorises cirrhosis as mild (thin septa, large nodules), moderate, severe (broad septa, “minute” nodules)
  - 302 transjugular biopsies (presumably including a range of aetiologies)
  - Fibrosis “scores” correlated with HVPG, ascites, INR, bilirubin and inversely with albumin; as well as Child-Pugh class
Small nodules and thick septa (ie amount of fibrosis) reflects HVPG in cirrhotic patients (various aetiologies; mainly HCV and alcohol)

- “Histological-hemodynamic correlation in cirrhosis - a histological classification of the severity of cirrhosis”
  - Nagula S et al. J Hepatol 44:111;2006
  - 43 cirrhotic patients
  - (34 transjugular, 9 percutaneous) liver biopsies and hepatic venous pressure gradient (HVPG) measurements were performed within 6 mths of each other
  - Sinusoidal fibrosis, septal thickness, loss of portal tracts and central veins, nodule size, inflammation, steatosis and iron were analyzed
  - Small nodule size (<1.0 mm) and thick septa (>0.4 mm) had the highest independent predictive value for clinically significant portal hypertension (HVPG ≥10 mmHg)
Small nodules and thick septa (ie amount of fibrosis) indicate higher hepatic venous pressure gradients
Nagula S et al. J Hepatol 44:111;2006
Royal Free computer-assisted image analysis of liver collagen

- Sirius red stained sections
- Grey level thresholding and detection of biopsy area
- Computer assisted RGB detection (green overlay) of sirius red areas
- Structural collagen (e.g., septal portal tract and large blood vessel, i.e., unrelated to HCV disease fibrosis) is edited manually and removed from CPA
  - No editing CPA = 12.5%
  - After editing CPA = 5.7%
Relationship between liver morphology and haemodynamics
Liver biopsy fibrosis measurement reflects hepatic venous pressure gradient

- 225 liver biopsies from 115 patients
- Hepatic venous pressure gradient (HVPG) measured at the time of transjugular liver biopsy
- Liver collagen was expressed as a proportion of liver biopsy tissue area (collagen proportionate area, CPA) and compared with the HVPG
- Significant correlation between CPA and HVPG (Spearman’s r=0.61; p<0.001)
  - Calvaruso V et al. Hepatology 49,1236;2009
Collagen proportionate area reflects hepatic venous pressure gradient (HVPG) better than Ishak stage (or grade)

Calvaruso V et al. Hepatology 49, 1236; 2009

Predictors of portal hypertension (ie HVPG ≥ 6mmHg)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Multivariate Analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Ishak grading score</td>
<td>1.214 (0.940 – 1.567)</td>
<td>0.138</td>
</tr>
<tr>
<td>Ishak staging score</td>
<td>1.372 (0.979 – 1.923)</td>
<td>0.067</td>
</tr>
<tr>
<td>Collagen Proportionate Area(%)</td>
<td>1.206 (1.094 – 1.331)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Predictors of “clinically significant” portal hypertension (ie HVPG ≥ 10mmHg)

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<td>P value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ishak grading score</td>
<td>1.126 (0.812 – 1.561)</td>
<td>0.477</td>
</tr>
<tr>
<td>Ishak staging score</td>
<td>1.577 (1.000 – 2.482)</td>
<td>0.05</td>
</tr>
<tr>
<td>Collagen Proportionate Area(%)</td>
<td>1.105 (1.026 – 1.191)</td>
<td>0.009</td>
</tr>
</tbody>
</table>
CPA adds value to the liver biopsy assessment of liver disease stage

- Measurement of CPA (as a HVPG surrogate) improves the histological description of liver disease stage
- A relationship between the amount of liver collagen and portal pressure could be useful to stratify prognostic groups
- Histological disease stage, CPA and HVPG are surrogates for patient outcome measures
  - Correlation with prognosis and treatment outcomes is necessary
Computer-assisted image analysis of liver collagen at one year biopsy can predict clinical outcome in HCV post liver transplant patients
Manousou P et al. Hepatology 50(S4),302A;2009

• Biopsies : one year post LT (12-15m); >15mm in length)

• Decompensation : the first occurrence of
  – ascites, hydrothorax
  – variceal bleeding
  – jaundice (≥3mg/dl – in the absence of other causes)
  – encephalopathy
Computer-assisted image analysis of liver collagen at one year biopsy can predict clinical outcome in HCV post liver transplant patients
Manousou P et al. Hepatology 50(S4),302A;2009

• Variables analysed (associated with HCV fibrosis in the literature):
  – Age/gender recipient/donor
  – HCC / ALD as associated aetiologies
  – Cold/warm ischemic time
  – Blood group compatibility and HLA disparity
  – Histological acute hepatitis
  – Rejection episodes and treatment
  – CMV viraemia, bacterial infections/antibiotics
  – Diabetes mellitus pre/post-transplant
  – Genotype, viral load pre/post-Tx, antiviral treatment/SVR
  – Initial/1 year – immunosuppression,
  – HVPG, Ishak stage and CPA at one year biopsy
ROC curve of CPA, HVPG and stage at 12m post LT for the prediction of clinical decompensation

- CPA AUROC 0.962
  - 95% CI: 0.921 – 1.000
- Stage AUROC 0.877
  - 95% CI: 0.781 – 0.972
- HVPG AUROC 0.874
  - 95% CI: 0.790 – 0.959

In Cox regression analysis, only CPA could predict clinical decompensation (p=0.0001, Exp(B)=1.158, 95%CI 1.102 – 1.217)
Computer-assisted image analysis of liver collagen at one year biopsy can predict clinical outcome in HCV post liver transplant patients
Manousou P et al. Hepatology 50(S4),302A;2009

All patients decompensated when both values were >6

<table>
<thead>
<tr>
<th></th>
<th>CPA≤6%</th>
<th>CPA&gt;6%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>62</td>
<td>5 (19%)</td>
</tr>
<tr>
<td>Decomp.</td>
<td>1 (1.5%)</td>
<td>21</td>
</tr>
</tbody>
</table>

CPA and HVPG (89 patients)
Computer-assisted image analysis of liver collagen at one year biopsy can predict clinical outcome in HCV post liver transplant patients
Manousou P et al. Hepatology 50(S4),302A;2009

Time (mths) to first episode of decompensation with a CPA cut off value of 6%

89 patients

p<0.001, Chi-square 54.2
Computer-assisted image analysis of liver collagen at one year biopsy can predict clinical outcome in HCV post liver transplant patients
Manousou P et al. Hepatology 50(S4),302A;2009

• CPA measurement at 1 year post LT was predictive of clinical decompensation in HCV patients:
  – CPA had good sensitivity and specificity
  – CPA was a better predictor than Ishak stage and HVPG
  – CPA combined with HVPG had better precision
Measurement of liver fibrosis: towards a better characterisation of disease stage

Conclusion

• The morphological definitions of liver disease stages have been useful
• Other clinically relevant aspects such as portal hypertension should be reconsidered from the histopathological perspective
• Liver biopsy fibrosis measurement reflects HVPG and improves prognostic evaluation
• Liver biopsy fibrosis measurement at one year post transplantation predicts clinical decompensation in HCV patients