Rob Goldin
Centre for Pathology, Imperial College
r.goldin@imperial.ac.uk
STOPAH

1053 patients were available for the primary end-point analysis.

“Pentoxifylline did not improve survival in patients with alcoholic hepatitis. Prednisolone was associated with a reduction in 28-day mortality that did not reach significance and with no improvement in outcomes at 90 days or 1 year.”

Prednisolone or pentoxifylline for alcoholic hepatitis
STOPAH Histology: Background

• In patients with severe alcoholic hepatitis, defined by a Maddrey’s Discriminant Function >= 32, examination of liver histology invariably implies that a transjugular biopsy be performed.

• Facilities for transjugular biopsy are not ubiquitous so need to be sure that histology is useful.

• A recent review of clinical practice suggests that liver biopsy is rarely used in the management of patients with alcoholic hepatitis.
The purpose of this study was therefore to determine the utility of liver histology in patients presenting with a putative clinical diagnosis of severe alcoholic hepatitis by:

1. estimating the proportion of patients in which the histology refutes the clinical diagnosis and

2. assessing the prognostic information provided by histological analysis.
STOPAH Histology

- Two pathologists blinded to the clinical details.
- Biopsies were considered adequate when there were at least 5 portal tracts.
- They were scored using:
  1. Alcoholic Hepatitis Histological Score
  2. NAFLD Activity Score and
  3. Laennec Fibrosis Score
Alcoholic Hepatitis Histological Score (AHHS) for Prognostic Stratification of Alcoholic Hepatitis

<table>
<thead>
<tr>
<th>Fibrosis stage</th>
<th>Points</th>
<th>AHHS categories (0–9 points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None Fibrosis or Portal fibrosis</td>
<td>0</td>
<td>Mild: 0–3</td>
</tr>
<tr>
<td>Expansive fibrosis</td>
<td>0</td>
<td>Intermediate: 4–5</td>
</tr>
<tr>
<td>Bridging fibrosis or Cirrhosis</td>
<td>+3</td>
<td>Severe: 6–9</td>
</tr>
<tr>
<td>Bilirubinostasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hepatocellular only</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Canalicular or ductular</td>
<td>+1</td>
<td></td>
</tr>
<tr>
<td>Canalicular or ductular plus Hepatocellular</td>
<td>+2</td>
<td></td>
</tr>
<tr>
<td>PMN infiltration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No/Mild</td>
<td>+2</td>
<td></td>
</tr>
<tr>
<td>Severe PMN Infiltration</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Megamitochondria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Megamitochondria</td>
<td>+2</td>
<td></td>
</tr>
<tr>
<td>Megamitochondria</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
**NAFLD Activity Score**

<table>
<thead>
<tr>
<th>Steatosis grade</th>
<th>Lobular inflammation</th>
<th>Hepatocellular ballooning</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: &lt;5%</td>
<td>0: None</td>
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</tr>
<tr>
<td>1: 5-33%</td>
<td>1:&lt;2 foci/20x field</td>
<td>1: Mild, few</td>
</tr>
<tr>
<td>2: 34-66%</td>
<td>2: 2-4 foci/20x field</td>
<td>2: Moderate – marked, many</td>
</tr>
<tr>
<td>3: &gt;66%</td>
<td>3: &gt;4 foci/20x field</td>
<td></td>
</tr>
</tbody>
</table>

**NAFLD activity score (NAS): 0-8**

<table>
<thead>
<tr>
<th>Steatosis (0-3) +</th>
<th>Lobular Inflammation (0-3)</th>
<th>+ Ballooning (0-2)</th>
</tr>
</thead>
</table>

*Hepatol 2005;41:1313*
Stage: Laennec Subclassification of Cirrhosis

The need for histological subclassification of cirrhosis: a systematic review and meta-analysis.
Liver Int. 2015 Jul 25.
Laennec Subclassification of Cirrhosis
STOPAH Histology: Prognostic information

• The histological scoring systems: AHHS and NAS were compared with
• clinical scoring systems: DF, MELD, Lille and GAHS for prognostic accuracy using area under the receiver operating characteristic analysis.
STOPAH Histology

- The STOPAH trial recruited 1103 patients with 1053 available for primary endpoint (28 day mortality) analysis.
- Biopsies were taken from 188/1103 (17%) patients during the trial enrolment admission.
- Of these, 25/188 (13%) were inadequate for histological assessment, leaving 163 cases for further evaluation.
STOPAH Histology:
Diagnosis of Alcoholic Hepatitis

• Overall a diagnosis of alcoholic hepatitis was confirmed in 142 of 163 cases (87%).

• Delay in obtaining a liver biopsy resulted in a numerical reduction in the rate of diagnostic confirmation:
  91% for biopsies obtained days 0-2 vs.
  84% for biopsies obtained days 7-28 (n.s.)
## STOPAH Histology

### Prognostic Accuracy

<table>
<thead>
<tr>
<th>Prognostic score</th>
<th>28 day mortality</th>
<th>n</th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DF</td>
<td>28 day mortality</td>
<td>95</td>
<td>0.776</td>
<td>0.632, 0.921</td>
</tr>
<tr>
<td>GAHS</td>
<td></td>
<td>86</td>
<td>0.608</td>
<td>0.409, 0.807</td>
</tr>
<tr>
<td>MELD</td>
<td></td>
<td>96</td>
<td>0.597</td>
<td>0.409, 0.784</td>
</tr>
<tr>
<td>Lille</td>
<td></td>
<td>73</td>
<td>0.812</td>
<td>0.664, 0.961</td>
</tr>
<tr>
<td>AHHS</td>
<td></td>
<td>96</td>
<td>0.693</td>
<td>0.548, 0.838</td>
</tr>
<tr>
<td>NAS</td>
<td></td>
<td>96</td>
<td>0.473</td>
<td>0.315, 0.630</td>
</tr>
</tbody>
</table>
The Alcoholic Hepatitis Histology Score Performs Poorly in a Cohort of Severe Alcoholic Hepatitis in the US

“The AHHS model performed poorly in predicting 90-day mortality or LT in a real-world cohort of severe AH patients in the United States.”
Histological and laboratory correlations

In the 100 selected liver biopsies we sought correlations between haematological and biochemical values at baseline with baseline histological characteristics.

There were strong correlations between:

• serum bilirubin and bilirubinostasis
• prothrombin time and Laennec classification of fibrosis
• serum white blood and neutrophil counts correlated with lobular inflammation
STOPAH Histology

• Liver histology remains a useful tool in patients presenting with features of alcoholic hepatitis but with diagnostic uncertainty.
• Where the diagnosis is made clinically with confidence histology will identify 13% of patients with alternative diagnoses, predominantly that of inactive cirrhosis.
• Provides independent validation of the AHSS
Elevated leukocyte count combined with radiologic evidence of a nodular liver surface in uninfected patients strongly predicts histologic alcoholic hepatitis, obviating the need for liver biopsy.
• The combination of an elevated leukocyte count and a nodular liver surface in the absence of infection retrospectively identified patients with a high likelihood of having histologic AH and suggests that a liver biopsy may not be necessary in this subset.

• For patients with suspected severe AH who do not fulfill these criteria, liver biopsy remains important, as 23% of these patients have no histologic AH.
Limitations: Clinical

• The utility of histology will be greater outside the context of a clinical trial.
Limitations: Pathological

• Number of cases
• Number of stains
What next?

• Complete image analysis
• Put the digitised images up on the web and invite you to score them!
Megamitochondria
Megamitochondria

- Normal, increase with age
- Increased in ALD and may correlate with the amount of alcohol drunk
- May correlate with milder disease.

Significance of megamitochondria in alcoholic liver disease.
Gastroenterology. 1986 Jun;90(6):1858-64.
Histopathological agreement

0.65 for fibrosis
0.86 for bilirubinostasis
0.60 for neutrophil infiltration and
0.46 for megamitochondria
Let me know if you are interested:

r.goldin@imperial.ac.uk
“PBC”
• The disease previously known as: ‘Primary Biliary Cirrhosis’

will now be called:

“Primary Biliary Cholangitis”
Fibrosis in PBC

- Absent / few septa 44%
- Numerous septa 31%
- Cirrhosis 25%

Liver International. 2004;24(3)
Primary biliary cholangitis

• **Cons:**
  
  *Tautological*
  
  *Sounds too much like “primary sclerosing cholangitis*

• **Pros:**
  
  *Inaccurate*
  
  *Stigma*

*Am J Gastroenterol* 2015; 110:1536–1538
Extending the Ballooning Score Beyond 2: 
A Proposal for a New Balloon Score
NAFLD Activity Score

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<th>Hepatocellular ballooning</th>
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NAFLD activity score (NAS): 0-8

| Steatosis (0-3) + | Lobular Inflammation (0-3) | + Ballooning (0-2) |

Hepatol 2005;41:1313
• A criticism of the NAFLD Activity Score (NAS) has been that it gives less weight to ballooning than to steatosis or lobular inflammation.

• Beginning in April, 2010 we prospectively classified all biopsies with ballooning as **classical** (CB: distinct, enlarged, with clumped cytoplasm) or **non-classical** (NCB: cytoplasmic changes without distinctive cytomegaly).

• **Severe** ballooning was defined as clusters of CB cells visible at low magnification.

• The NASH CRN new balloon score was created by combining the old balloon score and these new characteristics with a final range from 0 to 4.
## NASH CRN New Balloon Score

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ballooning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>410</td>
<td>194</td>
<td>178</td>
<td>261</td>
<td>188</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean Age (yr)</td>
<td>48.7</td>
<td>50.1</td>
<td>51.4</td>
<td>52.6</td>
<td>52.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% Female</td>
<td>52.9</td>
<td>53.6</td>
<td>62.4</td>
<td>74.7</td>
<td>82.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>33.1</td>
<td>34.2</td>
<td>34.7</td>
<td>35.2</td>
<td>36.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% Diabetes</td>
<td>28.0</td>
<td>35.6</td>
<td>47.2</td>
<td>59.4</td>
<td>62.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% Metabolic Syndrome</td>
<td>66.1</td>
<td>81.4</td>
<td>80.9</td>
<td>83.5</td>
<td>87.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean ALT (U/L)</td>
<td>56.1</td>
<td>59.9</td>
<td>68.7</td>
<td>83.0</td>
<td>85.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean Glucose (mg/dL)</td>
<td>103</td>
<td>105</td>
<td>114</td>
<td>119</td>
<td>127</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean Insulin (mcU/mL)</td>
<td>19.3</td>
<td>23.5</td>
<td>22.0</td>
<td>34.0</td>
<td>34.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean HOMA-IR</td>
<td>5.1</td>
<td>6.3</td>
<td>6.3</td>
<td>10.6</td>
<td>11.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
• Compared to cases without ballooning, NCB cases had more severe histology
• Compared to NCB cases, CB cases had more severe histology
• Severe ballooning cases differed from non-severe ballooning in that they had more severe histology
Renal Biopsy Histopathological Features in Patients with Cirrhosis:
Post Mortem Study of 27 Patients
• Acute tubular necrosis and bile cast formation was semiquantitatively graded as per recent report. (Kidney Int 2013;84:192-97).
• 3+ (numerous bile casts) were considered bile cast nephropathy. Less than 5 casts were not considered significant.
<table>
<thead>
<tr>
<th>Renal histopathology</th>
<th>Total Bilirubin (mg/dL)</th>
<th>S. Creatinine (mg/dL)</th>
<th>Maddrey Discriminant Function</th>
<th>INR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATN (Acute tubular necrosis)</td>
<td>18.2</td>
<td>1.9</td>
<td>91</td>
<td>3.2</td>
</tr>
<tr>
<td>ATN+BCN (Bile cast nephropathy)</td>
<td>27</td>
<td>3.3</td>
<td>83</td>
<td>2.2</td>
</tr>
<tr>
<td>Normal</td>
<td>4.7</td>
<td>1.4</td>
<td>79</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Impact of Hepatic p62 Overexpression on Survival of Patients with Hepatocellular Carcinoma
• p62 is an adaptor or a scaffolding substrate between the ubiquitin–proteasome and the autophagy–lysosome pathways
• The results of the current study indicated that overexpression of p62 was closely associated with overall survival
Differences between radiology and histopathology in HCC transplanted patients: are we judging wrong?
Discrepancy between pathological and radiological findings regarding the number of nodules was found in 103/184 (56%): 78 were underestimated and 25 overestimated by radiology.

Considering the diameter of the biggest nodule on explant, discordance was observed in 158/181 (87%): 93 were underestimated and 65 overestimated by radiology.
The Clinical-Pathological Spectrum and Outcomes of Liver Injury due to Drugs and Botanicals Associated with Loss of Bile Ducts.
• Out of 461 patients, paucity of intra-lobular bile ducts was found in 6% and was categorized as either:
mild/moderate [25-50% loss] or severe [> 50% loss].
• The likelihood of dying or requiring liver transplantation within 6 months of onset was greater in those with severe bile duct loss.
Cases were attributed to 20 different agents:

3 to amoxicillin/clavulanic acid;
3 to temozolomide;
3 to botanicals [Cactus nopal; Artemisia annua; unknown];
2 to azithromycin; and

1 each due to cefalexin, cefazolin, levofloxacin, moxifloxacin, allopurinol, enalapril, hydralazine, infliximab, lenalidomide, thalidomide, montelukast, olanzapine, quetiapine, lansoprazole, omeprazole, metoclopramide and mesalazine.
Lymph Node Micrometastases are Associated with Worse Survival in Patients with Otherwise Node-Negative Hilar Cholangiocarcinoma
• “To this end, we applied rigorous multiple sectioning of the lymph node tissue blocks to achieve deeper levels, investigated several levels of each lymph node, and increased the sensitivity of tumor cell identification by K19 immunolabeling. “
Using this technique, micrometastases were detected in 5% of the collected lymph nodes, which related to 12% of the 91 patients who were initially characterized as pN0 on the basis of conventional histologic examination of the surgical resection specimen with H&E staining.
RIP3-dependent hepatocyte necroptosis in human and experimental non-alcoholic steatohepatitis
But now there is another type of programmed cell death!
• In chronic liver disease patients, RIP3 levels were significantly increased and correlated with steatohepatitis histological severity.

• Necroptosis is increased in the liver of NAFLD patients (and in experimental models of NASH).
Histology predicts the need for liver transplantation in patients with acute severe autoimmune hepatitis
• In patients with severe acute autoimmune hepatitis histological assessment has a key prognostic role as the absence of chronic hepatitis predicts the need of liver transplantation, despite steroids therapy.
Long term Effects of Bariatric Surgery In Patients with Nonalcoholic Fatty Liver Disease: A Literature Review and Meta-Analysis
• Our search identified 2588 citations with 5 trials, having 4399 patients, meeting our eligibility criteria.

• We identified 29 of uncontrolled trials reporting changes in histology after bariatric surgery.
• 20 studies reported a decrease in the prevalence of hepatic steatosis (from 84.5% to 36.1%),
• 6 studied reported a decrease in the prevalence of NASH (from 51.45% to 9.76%),
• 9 reported a change in fibrosis score.
• In this meta-analysis, patients with NAFLD who underwent bariatric surgery showed significant reduction of all-cause mortality, weight loss, fibrosis score and ALT.
Concomitant Non-Alcoholic Fatty Liver Disease Drives Progression of Overall Liver Disease More Than Chronic Hepatitis B Alone in Chinese-Americans
• Despite the proven efficacy of current antiviral therapy for chronic hepatitis B (CHB), up to 20% of patients on long term oral antiviral treatment who have complete HBV suppression have persistently elevated serum alanine transferase levels.

• It has been previously shown that non-alcoholic fatty liver disease (NAFLD) is a likely culprit.

• Asians may be especially affected given endemic CHB and significant prevalence of metabolic syndrome and NAFLD despite lower body mass index and rate of obesity compared to other ethnicities.
• Of the 148 patients, 41 were NAFLD+: 38 with steatosis, 18 with steatohepatitis, 13 with steatofibrosis
• There is a significantly higher combined necro-inflammatory activity and overall combined staging in patients in the NAFLD+ group.

• Clinically, alanine transaminase, body mass index, and weight in pounds are significantly higher in the NAFLD+ group.
Systemic Disease Associated With Noncirrhotic Portal Hypertension

Clinical Liver Disease, Vol 6, No 4, October 2015
Pathophysiological Mechanisms Leading to NCPH in Systemic Diseases

• **Sinusoidal compression** (e.g. sarcoidosis)
• **Sinusoidal occlusion/infiltration** (e.g. metastatic disease)
• **Vascular remodeling in nodular regenerative hyperplasia** (e.g. systemic lupus erythematosus)
• **Defenestration of sinusoidal lining** (e.g. early alcoholic disease)
• **Arterio-portal shunting** (e.g. hereditary hemorrhagic telangiectasia)
Diagnostic Criteria for Idiopathic Noncirrhotic Portal Hypertension

1) Clinical signs of portal hypertension  
e.g. Splenomegaly/hypersplenism
2) Exclusion of cirrhosis on liver biopsy
3) Exclusion of chronic liver disease  
e.g. chronic viral hepatitis
4) Exclusion of conditions causing other forms of noncirrhotic portal hypertension  
e.g. Congenital liver fibrosis, Sarcoidosis, Schistosomiasis
5) Patent portal and hepatic veins (Doppler ultrasound or computed tomography scanning)

*All five criteria must be fulfilled to diagnose idiopathic noncirrhotic
Repeated biopsies within 23 years showed increased frequency of HCV infections and suggested a 3-year median monitoring to allocate fibrosis progressions.
• Fibrosis progression rate becomes important for low fibrosis groups, since they are non-prioritized for the new direct acting retrovirals in most countries.
• Therefore, to target a strategy of fibrosis monitoring within low fibrosis population, based on studied intervals is needed.
• Fibrosis progression in the absence of SVR significantly affects the long-term natural history of this process even in low fibrosis groups

• Median monitoring intervals of 3 years is suggested to allocate fibrosis and is crucial in non-SVR & non-transplant patients with F0-F2.
Integrator complex subunit 6 (INTS6) is a prognostic marker for hepatocellular carcinoma
• Integrator complex subunit 6 (INTS6), previously known as the gene encoding deleted in cancer cells 1 (DICE1) was found to play a tumor suppressive role in certain types of solid tumors just like prostate cancer and cervical cancer.
Image 1 Immunohistochemical staining of INTS6 in HCC. INTS6 protein expression localized mainly to the nuclei of cancer cells. Different INTS6 staining intensities [negative 0, weak 1, moderate 2, and strong 3, respectively] are indicated in micrographs.
• The results of our study showed that down-regulated INTS6 expression was associated with poorer prognosis in HCC patients.
Intraductal papillary neoplasm of bile duct is a heterogeneous disease with respect to its histopathologic similarities to pancreatic intraductal papillary mucinous neoplasm.
• Intraductal papillary neoplasm of bile duct (IPNB) is characterized by papillary tumor covered by well-differentiated neoplastic epithelium with fine fibrovascular cores in the dilated bile ducts.

• IPNB reportedly resembles intraductal papillary mucinous neoplasm (IPMN) of the pancreas
• IPNB cases were classifiable into four groups with their histopathological similarities to IPMN:
  group A (identical to IPMN)
  group B (similar but a little different from IPMN),
  group C (focally or vaguely similar to IPMN) and
  group D (different from IPMN, 10 cases).
Liver Fibrosis is Present in One-Third of Adults with Alpha-1 Antitrypsin Deficiency Without Overt Liver Disease
• The primary aim of this study is to define the prevalence and histologic spectrum of liver disease in adults with AATD.
• Adults with confirmed AATD Pi*ZZ and other rare AATD alleles
• Clinically significant liver fibrosis defined by Ishak fibrosis score ≥2.
In this cross section of older AATD subjects, over one-third had clinically significant liver fibrosis confirmed by biopsy.

This is much higher than our previously reported prevalence of 7.9%.

We also confirmed our previous finding that ALT and GGT for affected subjects fall within normal range, which limits clinical utility.

The histologic spectrum was varied both in fibrosis and qualitative presence of AATD globules.
That’s all Folks!