



# AASLD San Francisco 2011

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# Histologic Analysis and Correlation with Clinical Outcomes in the Drug-Induced Liver Injury Network (DILIN)

- To relate histological changes in cases of presumed DILI to short term patient outcome as assessed using the DILIN severity scale.
- Liver biopsies obtained within 6 months of DILI onset in the DILIN prospective study were blindly assessed for 49 separate histological features:
  1. inflammation (11),
  2. fibrosis (2),
  3. steatosis (3),
  4. cholestasis (10),
  5. cellular injury (6),
  6. vascular injury (6) and
  7. other features (11).
- The DILIN Severity Scale (Hepatology 52: 730; 2010), which ranges from 1 (asymptomatic) to 5 (fatal/liver transplant) was used to classify short-term outcome.

# Histologic Analysis and Correlation with Clinical Outcomes in the Drug-Induced Liver Injury Network (DILIN)

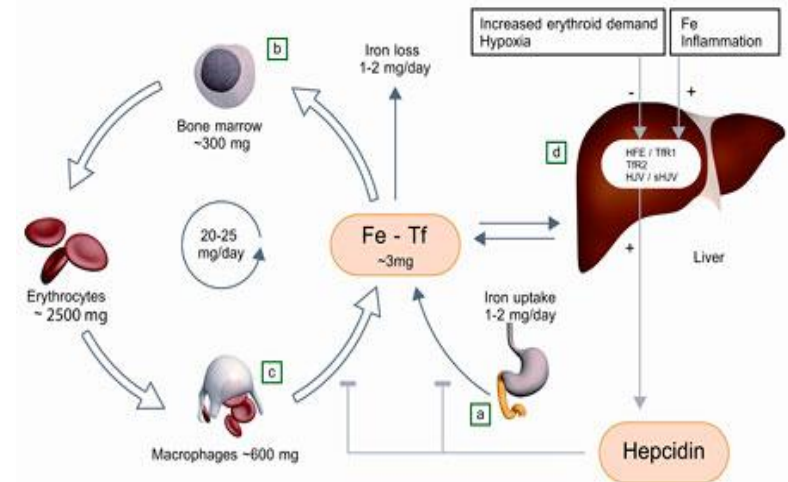
- Adequate biopsies (median 13 portal areas) were available for 253 subjects
- **More severe disease associated with:**
  1. necrosis
  2. fibrosis stage
  3. microvesicular steatosis
  4. cholangiolar cholestasis
- **Milder disease associated with:**
  1. granulomas
  2. increased eosinophils

# Cephalosporin induced liver injury

- 23 patients
- Injury patterns were:
  1. cholestatic (56%)
  2. mixed (26%) and
  3. hepatocellular(17%).
- Liver histology (n =12) showed variable degrees of hepatocyte necrosis and inflammatory infiltrates; ductular proliferation and portal fibrosis were often present.
- The most commonly implicated agent was cefazolin (n=10).
- Among 19 patients with 6-month follow-up, 6 (31%) still had abnormal liver tests.
- Five patients developed DILI after receiving a single IV dose, with mean latency period of 24 days

# Pattern of hepatic iron deposition and the role of hepcidin in the development of NAFLD

- Hepatic iron overload is believed to exacerbate liver injury in non-alcoholic fatty liver disease
- Hepcidin is synthesized in HCs in iron overload and down regulates ferroportin on the surface of macrophages resulting in iron retention within macrophages.



# Pattern of hepatic iron deposition and the role of hepcidin in the development of non - alcoholic fatty liver disease

- **Histological evaluation of NAFLD stage and grade followed that of Brunt:**  
simple steatosis /steatohepatitis = 56/75
- **Stainable hepatic iron was scored according to the pattern of iron deposition (HC = 0-4, REC = 0-2):**  
No stainable iron = 43  
85 patients had HC iron deposition (0/1/2/3/4: n = 46/40/23/21/1),  
58 patients had REC iron deposition (0/1/2; n = 73/31/27);  
mixed HC/REC iron = 55  
HC iron only = 30  
REC iron only = 3

# Pattern of hepatic iron deposition and the role of hepcidin in the development of non - alcoholic fatty liver disease

- The REC iron score, but not the HC iron score, tended to increase as the stage and grade progressed.
- Regression analysis showed that only the HC iron score was independently correlated with the hepcidin mRNA level.

## **Conclusion**

- Iron overload in REC could play an important role in the progression of NAFLD.
- Iron overload in HC may facilitate iron deposition in REC through upregulation of hepcidin.

# Extrahepatic iron deposition in cirrhosis: a mimic of genetic hemochromatosis?

- Hepatic iron deposition unrelated to genetic haemochromatosis is common in cirrhosis.
- Given that GH can cause iron loading of multiple organs, the aim of this study was to determine whether hepatic haemosiderosis occurring in the context of cirrhosis is associated with iron deposition in extrahepatic tissues.
- 111 cases of cirrhosis
- Liver, spleen, pancreas and heart were stained for iron
- Hepatic iron deposition was graded using a standard system (0-4+)
- In other tissues, the percentage of cells with stainable iron was estimated
- Genotyping for the HFE C282Y mutation was performed using fixed liver tissue in all cases with 2+ or greater liver iron scores or with significant cardiac or pancreatic iron.

## **Liver**

- 26/110 cases (24%) had increased hepatocyte iron:
  - 2+ in 11 cases,
  - 3+ in 10 cases and
  - 4+ in 5.
- This group was predominantly male (81%)
- The main aetiologies of cirrhosis were: alcohol, hepatitis C and/or NASH.

# Extrahepatic iron deposition in cirrhosis: a mimic of genetic hemochromatosis?

## **Heart**

- Cardiac iron deposition involving 10-95% of cardiomyocytes was present in 7 cases (6%).
- All subjects with cardiac iron had heavy liver and pancreatic iron deposition;
- 5/7 had undergone echocardiography, with normal results.

## **Pancreas:**

- Pancreatic iron was present in 27 cases (25%).
- Most subjects with pancreatic iron deposition also had heavy hepatic iron deposition but in 1/3 cases, significant pancreatic iron was observed with only minimal hepatic iron.

## **Spleen:**

- Splenic iron was present in 60 cases (55%); the amount was highly variable and showed no clear relationship to hepatic iron grade.

## **Conclusion:**

- This is the first description of iron deposition in multiple organs in cirrhosis unrelated to GH.
- It is a heterogenous phenomenon that can occur in the absence of significant hepatic haemosiderosis.
- A minority of cirrhotic patients exhibit a pattern of iron deposition that can mimic GH.
- Additional studies will be needed to determine the mechanisms and assess the functional significance of extrahepatic secondary iron deposition.

# Hans Popper Basic Science State-of-the-Art Lecture

*Heart Attacks and Other Liver Diseases*



Michael Brown

# Direct hyperbilirubinemia in sickle cell disease is a marker of intrinsic liver pathology and a predictor of mortality

- Elevated direct bilirubin ( $>0.4$  g/dL) has is associated with reduced survival in patients with sickle cell disease
- **Hyperbilirubinaemia**  
increased haemolysis or impaired liver function.
- 80 patients were evaluated including 48 with liver biopsies.
- Direct bilirubin was 0.5 g/dL and above in 45 patients.

# Direct hyperbilirubinemia in sickle cell disease is a marker of intrinsic liver pathology and a predictor of mortality

## Liver biopsy:

- mild inflammation (median Ishak 3) and fibrosis (median Ishak 0)
- sickled red blood cells and pigmented macrophages are common
- **Regenerative changes:**

Seen in 54%, ranging from mild (17%) to established nodular regenerative hyperplasia (in 7%).

CK7 staining of hepatocytes near the central vein, indicating the presence of hepatocyte progenitor cells, was positive in 19 of the 22 (86%) biopsy specimens with other regenerative changes.

- **Other histological changes:**
  - sinusoidal dilation (73%),
  - sinusoidal fibrosis (62%)
  - portal venopathy (22%)
  - central venopathy was not seen

# Direct hyperbilirubinemia in sickle cell disease is a marker of intrinsic liver pathology and a predictor of mortality

- **Conclusions**

**Direct bilirubin correlates with :**

1. Levels of primary bile acids
2. Specific markers of hepatic pathology

Direct bilirubin elevation, a predictor of mortality in SCD, is associated with primary liver injury.

# Histological Evolution of Hepatitis C Virus Infection After Renal Transplantation

- There is no study evaluating liver biopsies from the same patients before and after renal transplantation.
- 22 HCV-infected patients submitted to liver biopsy pre- and post-RTx were included.

**Time post-transplant was  $4 \pm 2$  years and time between biopsies was  $5 \pm 2$  years:**

- Fibrosis progression was observed in 11 (50%) of the patients and
- Necro-inflammatory activity worsening was observed in 7 (32%) of patients.
- The histological progression occurred even among those without significant histological lesions in pre-transplant biopsy.

## **Conclusion:**

- The practice of starting treatment in the pre-transplant phase based on histological severity should be revised
- As interferon cannot be used safely after RTx, treatment is indicated for all ESRD patients with hepatitis C.

# The prediction of liver related outcomes using histological tools as an endpoint for studies evaluating anti-fibrotic therapies

- Liver related outcomes (LRO) represent a meaningful end-point for future anti-fibrotic therapies.
- Staging of liver fibrosis on histology is a surrogate for these outcomes but may not be an ideal tool.
- Our initial aim was to determine liver related outcomes and survival from the individual stages of significant fibrosis.
- Our second aim was to assess the value of morphometric collagen quantification within cirrhosis to predict clinical outcomes.
- The study cohort was selected from a single centre within the Trent HCV study, a prospective cohort which began in 1991 to address the natural history of chronic hepatitis C.

# The prediction of liver related outcomes using histological tools as an endpoint for studies evaluating anti - fibrotic therapies

## **Inclusion criteria for this study were:**

- significant fibrosis ( $\geq$  Ishak Stage (IS) 3 on biopsy) and
- at least 3 years follow up post biopsy.

## **LRO was defined as:**

- decompensation (variceal bleeding, ascites, encephalopathy),
  - HCC,
  - liver transplant and
  - liver-related death.
- 
- Automated morphometry was performed to measure the Collagen Area Fraction (CAF).
  - Survival at 3, 5 and 7 years respectively was evaluated.

# The prediction of liver related outcomes using histological tools as an endpoint for studies evaluating anti-fibrotic therapies

- The study cohort comprised 155 patients
- The median follow up time was 78 months.
- A LRO occurred in 48 patients (31.0%, estimated annual incidence 5.2%)
  
- The median CAF was calculated for each Ishak stage:
  - IS 3: median CAF 3.7%, IQR 1.5-5.1
  - IS 4: median CAF 5.2%, IQR 2.8-7.4
  - IS 5: median CAF 6.8%, IQR 3.4-9.5
  - IS 6: median CAF 9.9%, IQR 6.2-15.7
  
- Within Ishak stage 6: the median CAF scores predicting LRO were higher (13.89, 12.48 and 12.69%) compared to median CAF scores in patients with no outcomes (8.59, 8.32 and 8.10%) at years 3, 5 and 7 respectively.

# Histological and clinical C and persistently normal ALT levels characteristics of patients with chronic hepatitis

25-30% of patients with hepatitis C virus (HCV) infection have persistently normal ALT (PNALT) levels, although the definition of PNALT differs by study.

Limited data are available regarding the clinical and histological correlates of newer, more stringent ALT thresholds (Ann Hepatol 2010;9 Suppl:72-9).

Review of an HCV database identified patients with an ALT measurement at the time of liver biopsy, at least one additional measurement over the next 12 months, and liver biopsy slides available for review.

Patients with end stage renal disease, transplant recipients, and those on antiviral therapy were excluded.

All liver biopsies were assigned a **Histological Activity Index (HAI)** by two hepatologists

**Stringent (S)PNALT** was defined as <19 for women and <29 for men (Ann Intern Med 2002;137:1-10).

**Liberal (L)PNALT** was defined as <30 (Gastroenterology 2004;127:1724-32).

# Histological and clinical characteristics of patients with chronic hepatitis C and persistently normal ALT levels

## **(S)PNALT in 7.5% (18/238)**

- More likely to be male and to have cirrhosis compared to patients with elevated ALT.
- There was no difference in the HAI inflammatory score between the two groups

## **(L)PNALT in 18% (42/238)**

- (L)PNALT patients were more likely to be female and to have cirrhosis compared to those with elevated ALT.
- HAI inflammatory scores were similar between the two groups

## **Conclusion:**

- PNALT did not distinguish patients with more mild liver disease whether the stringent or liberal criteria were applied.
- A relatively high proportion of PNALT patients had histological evidence of cirrhosis

# Ductular reaction in untreated autoimmune hepatitis is associated with inflammation and fibrosis

- Fibrosis following chronic hepatocellular damage in hepatitis C and NASH has been associated with ductular reaction (DR).
- DR is not regarded as a feature of AIH but its prevalence in AIH has not been well documented.
- The aim of this study is to determine the prevalence and grade of DR in newly diagnosed, untreated AIH patients and its association with inflammation and fibrosis.
- Pretreatment liver biopsies from 33 patients who fulfilled the simplified criteria for AIH were included and stained for cytokeratin 7.
- DR was semi-quantitatively scored:
  - 0: none
  - 1+ : patchy DR on the circumference of portal tracts
  - 2+ : DR covering at least 50% of the portal tract and
  - 3+ : DR surrounding the portal tracts
- DR was present in 94% of cases, the majority in mild and moderate degrees.
- DR was correlated with fibrosis and extent of inflammation.

**COFFEE TIME?**

# Oral administration of 1,7 - dimethylxanthine attenuates the formation of hepatic fibrosis in the cholestatic rat liver

- Several epidemiological studies suggest a beneficial effect of coffee consumption on the formation and progression of fibrogenic diseases, particularly of the liver.
- Recent data point to a modulation of TGF- $\beta$  signalling by the methylxanthine caffeine, and even more potent, its primary demethylated metabolite paraxanthine (1,7-dimethylxanthine [1,7-DMX]).
- Sprague Dawley rats were subjected to prolonged bile duct ligation or sham operated with or without concomitant oral 1,7-DMX (1mM) application.
- Oral 1,7-DMX application (1mM) attenuated cholestatic hepatocellular injury in vivo as determined by biochemical serum analysis and reduced intercellular collagen deposition in the cholestatic rat liver as seen in HE- and Sirius-red staining.



# Genetic factors associated with histologic features and treatment outcome in patients with chronic hepatitis C.

**In chronic hepatitis C, steatosis is associated with:**

- fibrosis and
- impaired response to antiviral therapy.

**Recently several SNPs were identified in patients with NAFL/NASH which may causally link to hepatic fat accumulation and progression to fibrosis are SNP in :**

- rs738409 in the adiponutrin gene (PNPLA3) and
- rs2645424 of farnesyl diphosphate farnesyl transferase 1

**437 patients:**

- homozygous G allele carriers of rs738409 had a higher rate of moderate/severe steatosis and advanced fibrosis than patients carrying the C allele
- genetic polymorphism of FDFT1 had no impact on steatosis, and fibrosis progression

# Etiologic, serologic, and outcome characterization of inpatients with liver pathology at a large tertiary care facility in East Africa

- “To better describe the poorly understood burden of liver pathology in East Africa, we performed an etiologic, serologic and outcome characterization of inpatients at a large tertiary care facility in Uganda.”
- **193 patients:**
  - Hepatitis B surface antigen in 31%,
  - Hepatitis C antibody in 3%
  - HIV mono-infection in 32%,
  - HBV-HIV co-infection in 10%.
- Despite high prevalence of viral hepatitis in East Africa, more than 2/3 of patients had no evidence of hepatitis B or C.

**EXPERIMENTAL HEPATITIS C DRUG  
CURES 100% OF PATIENTS IN TRIAL!**

# Experimental Hepatitis C Drug Cures 100% Of Patients In Trial.

- Pharmasset's PSI-7977 is a nucleotide analogue that directly targets viral polymerase
- All 40 patients who received PSI-7977 drug responded after 12 weeks.
- About half the patients were followed up to 24 weeks, and they were all cured. There were no significant adverse events.
- The drug was tested in combination with ribavirin in patients with hepatitis C genotypes 2 and 3.
- There were four groups in the study: Those who were treated with no interferon and those who received 4 weeks, 8 and 12 weeks of it
- Pharmasset has begun a Phase 3 clinical trial of the drug in combination with ribavirin, without interferon.

# Evolution of inflammation, ballooning and fibrosis in patients with NASH after bariatric surgery

890 morbidly obese treated with bariatric surgery were prospectively included in a study recording at 0, 1 and 5 years, with consecutive liver biopsies

**At baseline, NASH patients (NAS  $\geq 3$ , n=261) were significantly higher than in non-NASH patients (NAS < 3, n=629):**

- age (43 vs 40)
- fibrosis (0.8 vs 0.3)
- ballooning (0.6 vs 0.02)
- inflammation (0.8 vs 0.1)
- steatosis (61 vs 21%,)
- IR (3.3 vs 3.1)

**In a multivariate analysis, IR was an independent predictive factor of:**

- NASH, ballooning, inflammation and fibrosis

# Evolution of inflammation, ballooning and fibrosis in patients with NASH after bariatric surgery

**In patients with NAS $\geq$ 3, between 0 and 1 years, the following improved:**

- IR (3.3 to 2.9)
- ballooning (0.7 to 0.2)
- inflammation (0.8 to 0.4)
- steatosis (66 to 24%)
- fibrosis (0.9 to 0.75)

There was no change between 1 and 5 years.

# Evolution of inflammation, ballooning and fibrosis in patients with NASH after bariatric surgery

Between 0 and 5 years, NASH significantly decreased from 28.2 to 14.9%.

The disappearance of NASH occurred mainly at 1 year without significant differences between 1 and 5 years.

Patients with persistence of NASH had higher IR at 1 year (3.1 vs 2.8,) but no longer at 5 years (2.93 vs 2.98) than those with disappearance of NASH. The persistence of steatosis is still associated with higher IR index at 5 years.

This result suggests that persistence at long term of NASH seems to involve other pathways.

# Clinical and Histological Determinants of NASH and Advanced Fibrosis in the Elderly

There are limited data on clinical and histological features of nonalcoholic fatty liver disease in the elderly.

**Subjects were divided into 2 groups:**

- $\geq 65$  years (elderly) and
- 18-65 years.

**796 patients with biopsy-proven NAFLD:**

- 61 in the elderly group and
- 735 in the non-elderly group.

Table 1. Histologic differences between elderly versus non-elderly NAFLD patients

Histology	Non-elderly (N=735)	Elderly (N=61)	P-value
<b>Lobular inflammation:</b>			<b>0.009</b>
< 2 foci	392 (53%)	22 (36%)	
≥ 2 foci	343 (47%)	39 (64%)	
<b>Ballooning (any)</b>	484 (66%)	46 (75%)	0.13
<b>NAFLD Activity Score ≥5</b>	351 (48%)	34 (56%)	0.23
<b>Presence of definite NASH</b>	412 (56%)	44 (72%)	<b>0.02</b>
<b>Fibrosis stage:</b>			<b>0.001</b>
Bridging or Cirrhosis	183 (25%)	27 (44%)	
<b>Mallory bodies (any)</b>	541 (74%)	32 (52%)	<b>&lt;0.001</b>

# Clinical and Histological Determinants of NASH and Advanced Fibrosis in the Elderly

## **Comparing the 2 groups:**

- The elderly were more likely to have NASH and advanced fibrosis
- Steatosis and portal inflammation were not different.

## **Histological differences between patients with NASH vs non-NASH among the elderly included:**

- more ballooning (100% vs 12%),
  - more Mallory bodies (72% vs 0%), and
  - more advanced fibrosis (52% vs. 24%).
- 
- 24% of elderly patients have fibrosis without NASH

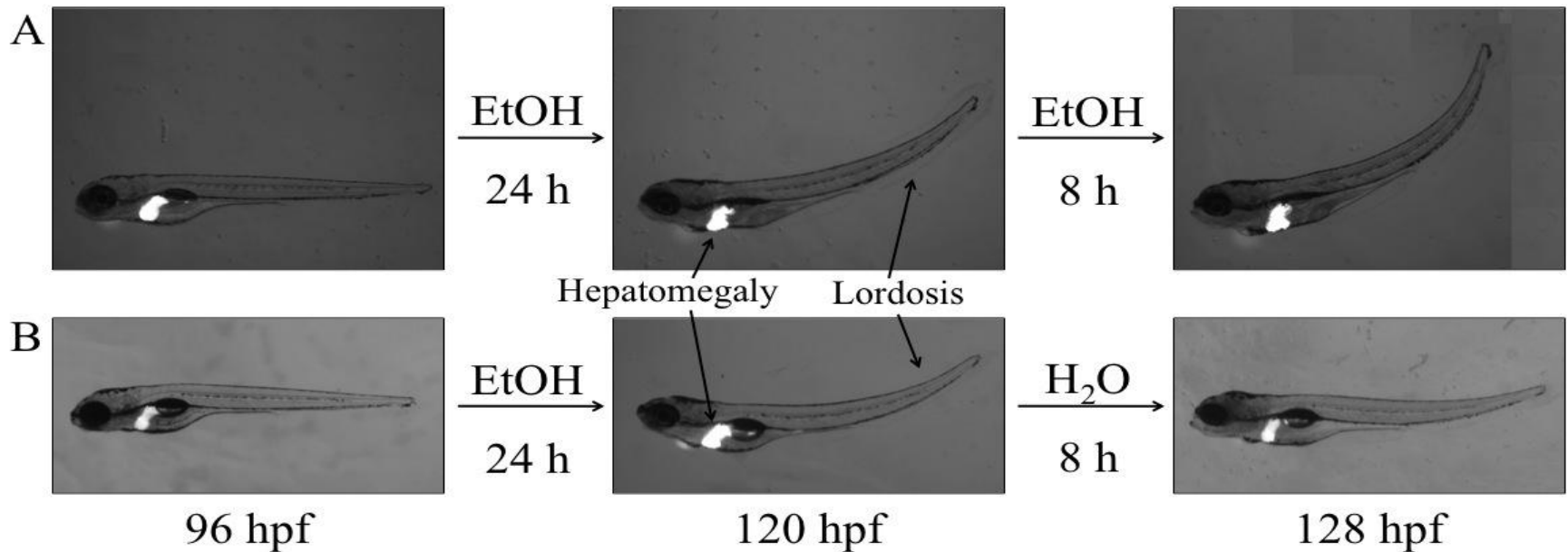
## **Conclusions:**

The prevalence of fibrosis is substantial even in the absence of NASH in the elderly.

NASH and advanced fibrosis are more common in elderly patients with NAFLD

Liver biopsy should be considered to find candidates for treatment to help prevent progression of NASH

# Remarkable reversal of phenotypic effects in zebrafish acute alcoholic liver disease model



Acute alcohol treatment causes severe phenotypic and hepatic abnormalities in zebrafish and these effects are reversible upon abstinence.

# Development and Validation of a Novel Histological Classification with Prognostic Value for Alcoholic Hepatitis.

Alcoholic hepatitis (AH) is the most severe form of alcoholic liver disease and carries a high short-term mortality.

The aim of this study was to generate a new histological scoring system capable of predicting the mortality of patients with AH.

116 patients with biopsy-proven AH were prospectively included at the Hospital Clinic Barcelona

All biopsies were blindly assessed by a central pathologist and extensive histological parameters were evaluated.

Univariate and multivariate logistic regression analysis were used to evaluate the impact of histological parameters on short-term survival (90 days).

A predictive semi-quantitative histological score (alcoholic hepatitis histological score AHHS) was built using the histological parameters that were independently associated with short-term survival.

The resulting score was validated in 105 patients from 2 independent cohorts from Europe and USA

# Development and Validation of a Novel Histological Classification with Prognostic Value for Alcoholic Hepatitis.

## **Fibrosis stage:**

1. No fibrosis / portal fibrosis
2. Expansive fibrosis
3. Bridging fibrosis/Cirrhosis

## **Lobular fibrosis:**

1. No fibrosis / zone 3 (central)
2. Zone 2 and 3(midzonal)
3. Panlobular

## **Bilirubinostasis**

1. None
2. Hepatocellular
3. Canalicular/ductal
4. Hepatocellular and canalicular/ductal

## **Megamitochondria**

1. None
2. Present

## **Fat**

1. Mild
2. Moderate
3. Severe

## **PMN infiltration**

1. None/mild
2. Severe

## **Mallory bodies**

1. No/occasional
2. Marked

## **Ballooning**

1. Occasional
2. Marked

# Development and Validation of a Novel Histological Classification with Prognostic Value for Alcoholic Hepatitis.

## **Fibrosis stage:**

- 1 No fibrosis / portal fibrosis
- 2 Expansive fibrosis
- 3 Bridging fibrosis/Cirrhosis

## **PMN infiltration**

- 0 None/mild
- 1 Severe

## **Bilirubinostasis**

- 1 None
- 2 Hepatocellular
- 3 Canalicular/ductal
- 4 Hepatocellular and canalicular/ductal

## **Megamitochondria**

- 0 None
- 1 Present

## **Histological Score: 0-7**

- Mild: 0-2
- Moderate: 3-5
- Severe: 6-7

# Development and Validation of a Novel Histological Classification with Prognostic Value for Alcoholic Hepatitis

Overall 90-day mortality was 28%.

On multivariate analysis, the histological score predicted 90-day survival.

The generated AHHS showed:

- an AUROC of 0.75 (95%CI 0.65-0.84) in predicting 90-day survival.
- Kaplan-Meier analysis using cut-off values of 2 and 5 points was able to stratify patients with high, intermediate and low risk of death (63%, 23% and 0%, mortality rate respectively)

The AHHS was validated in the 2 confirmatory cohorts and the AUROC was 0.82 (95% CI 0.73-0.90)

“We developed and validated a novel histological scoring system that may be useful for clinical decision making in this severe medical condition.”

# Steroids or Pentoxifyline for Alcoholic Hepatitis (STOPAH) Trial

AASLD, Boston:  
Friday, November 9<sup>th</sup>, 2012



But .....

# American Presidential Election: Tuesday, November 6<sup>th</sup>, 2012

