

New insights into fatty liver disease

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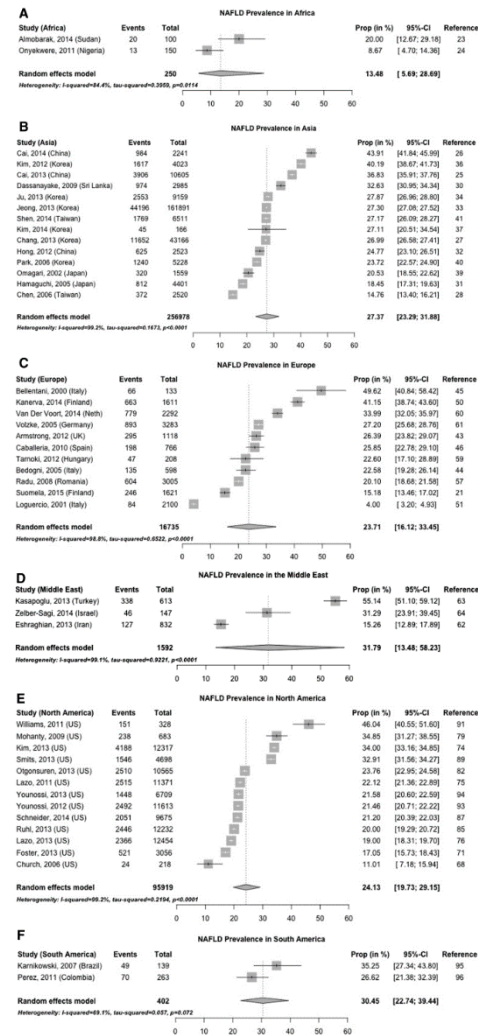
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Prevalence of NASH

Global prevalence of NAFLD is 25% with highest prevalence in the Middle East and South America and lowest in Africa.

Global epidemiology of nonalcoholic fatty liver
[Hepatology](#). 2016 Jul;64(1):73-84.

Global epidemiology of nonalcoholic fatty liver disease —Meta-analytic assessment of prevalence, incidence, and outcomes



Hepatology

Volume 64, Issue 1, pages 73-84, 22 FEB 2016 DOI: 10.1002/hep.28431

<http://onlinelibrary.wiley.com/doi/10.1002/hep.28431/full#hep28431-fig-0003>

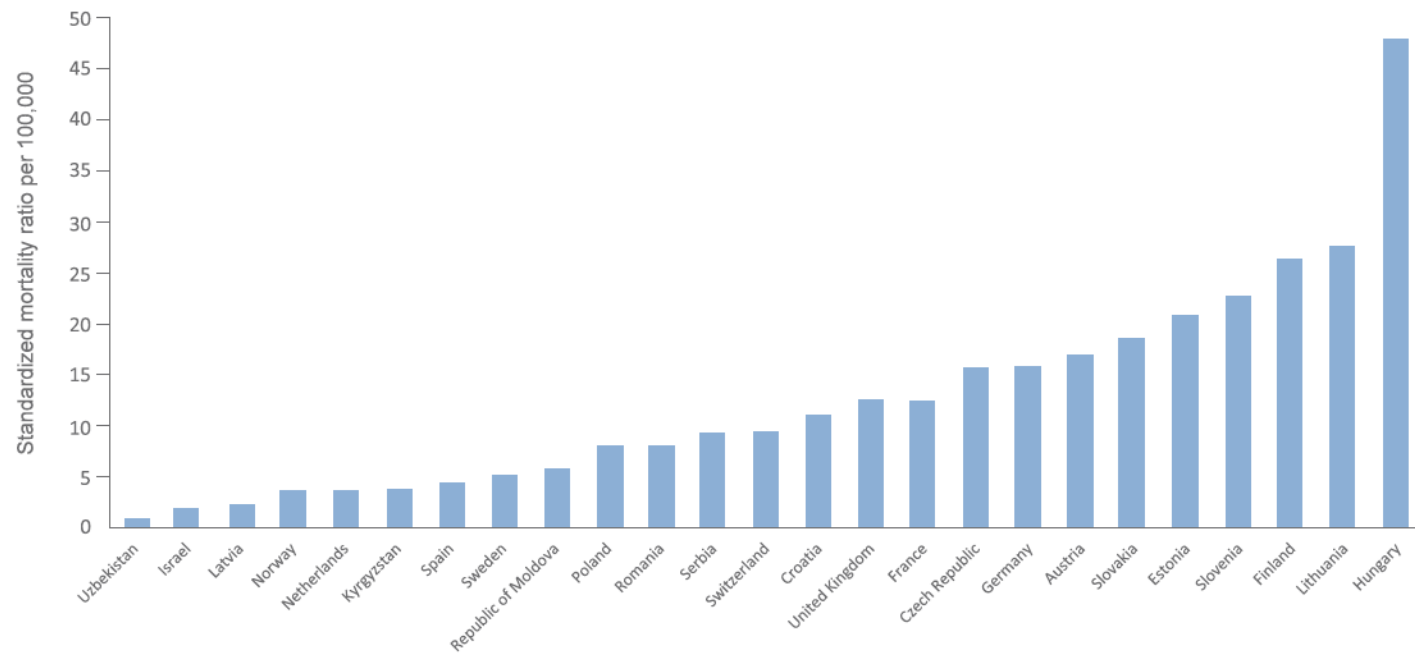


Fig. 9 – Mortality from alcohol-related liver diseases among men in European countries in 2005; WHO 2010 [30].

WHO. European Status Report on Alcohol and Health: World Health Organization. Regional Office for Europe; 2010.

Distinction of alcoholic liver disease from non-alcoholic liver disease

- Clinical features

Alcohol intake: less than 10g/day in women, 20 g/day in men

- Blood tests

- Imaging

- Histology

Alcohol production in NASH

- Dysbiosis might increase intestinal ethanol production; for example, 1 g of *Escherichia coli* can produce 0.8 g of ethanol per hour in anaerobic conditions
- The *ob/ob* mice that develop NASH have higher early-morning breath alcohol content compared with their lean littermates
- Elevated blood ethanol levels have also been observed in patients with NASH
- Ethanol produced in the gut might contribute to liver injury by increasing intestinal permeability and portal LPS levels

The role of the gut microbiota in NAFLD

Nature Reviews Gastroenterology & Hepatology 13, 412–425 (2016)

Histological features common to ASH and NASH

- Large droplet fatty change
- Inflammation, lobular or portal
- Ballooning +/- Mallory–Denk bodies
- Fibrosis
- Apoptotic hepatocytes
- Megamitochondria

Differences between ASH and NASH

- The unique zone 1 borderline injury pattern of pediatric fatty liver disease does not have a corresponding injury pattern in alcoholic liver disease.
- The following are commoner in ASH:
 - diffuse microvesicular steatosis,
 - cholestasis
 - neutrophilic satellitosis of Mallory–Denk body-containing balloon cells
 - dense networks of perisinusoidal fibrosis
- Only ASH shows a significant incidence of veno-occlusive lesions and sclerosing hyaline necrosis.

When is it NAFLD and when is it ALD?

Nuclear Vacuolation

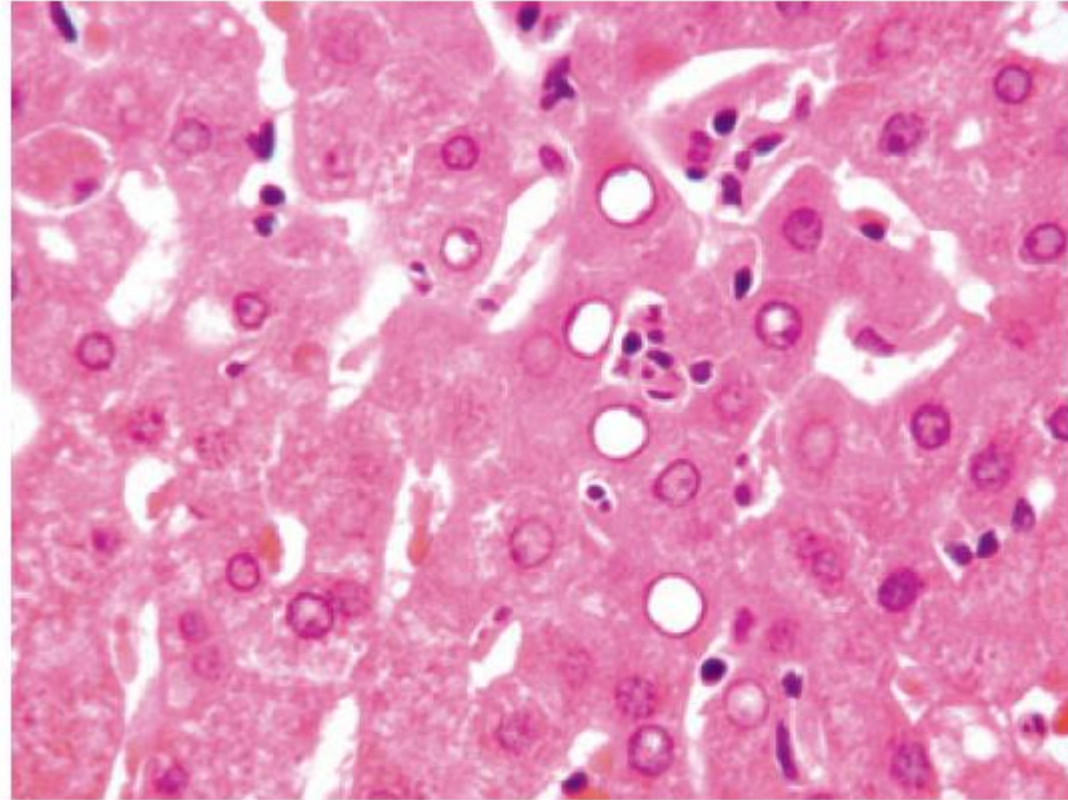


Figure 1. Nuclear vacuolation, but no fatty change, in a patient with chronic hepatitis B who was aged 25 with normal body mass index and no history of excessive alcohol intake. (H&E-stained section.)

Physiological hepatic nuclear vacuolation is common in the 20s and persists into the 30s

- Physiological hepatic nuclear vacuolation-how long does it persist?
Histopathology. 2010 Mar;56(4):426-9.

Non-Alcoholic Liver Disease

NAFLD Activity Score

| Steatosis grade | Lobular inflammation | Hepatocellular ballooning |
|--|-----------------------------|----------------------------------|
| 0: <5% | 0: None | 0: None |
| 1: 5-33% | 1: <2 foci/20x field | 1: Mild, few |
| 2: 34-66% | 2: 2-4 foci/20x field | 2: Moderate – marked, |
| 3: >66% | 3: >4 foci/20x field | many |
| NAFLD activity score (NAS): 0-8 | | |
| Steatosis (0-3) + | Lobular Inflammation (0-3) | + Ballooning (0-2) |

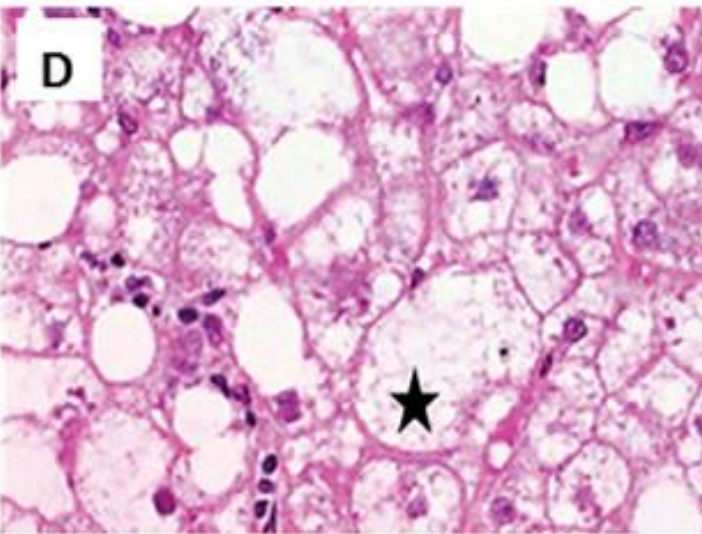
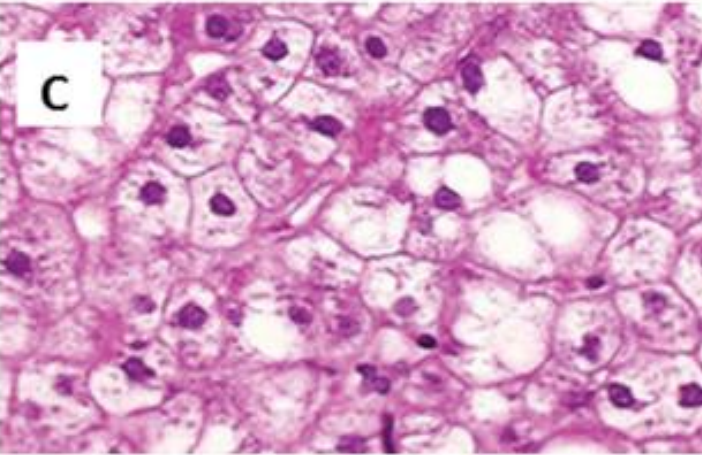
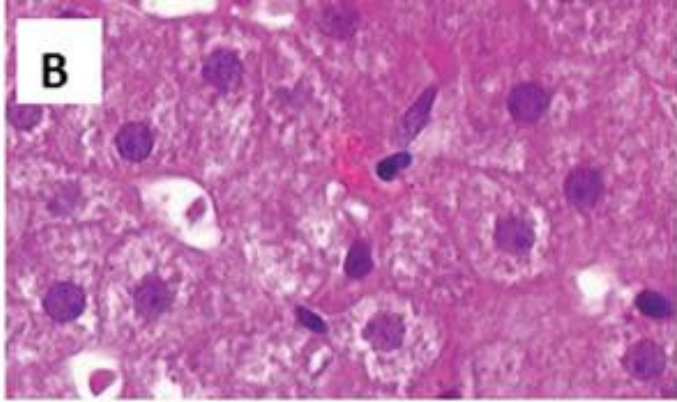
The NAS and the Histopathologic Diagnosis in NAFLD: Distinct Clinicopathologic Meanings. Hepatology. 2011 Mar; 53(3): 810–820.

NAFLD Activity Score

- “The diagnosis of nonalcoholic steatohepatitis (NASH) is defined by the presence and pattern of specific histological abnormalities on liver biopsy.
- A separate system of scoring the features of nonalcoholic fatty liver disease called **the NAFLD Activity Score (NAS) was developed as a tool to measure changes in NAFLD during therapeutic trials.**
- However, some studies have used threshold values of the NAS, specifically $NAS \geq 5$, as a surrogate for the histologic diagnosis of NASH. “

The NAS and the Histopathologic Diagnosis in NAFLD: Distinct Clinicopathologic Meanings. *Hepatology*. 2011 Mar; 53(3): 810–820.

What is ballooning?



Ballooning and lobular inflammation grading.
B) Normal hepatocytes, ballooning, grade 0. Cytoplasm is pink and granular and liver cells have **sharp angles**. H&E, X 40.

(C) Ballooning, grade 1. Hepatocytes have **rounded contours with clear reticular cytoplasm**. Size is quite similar to that of normal hepatocytes, H&E, X 40.

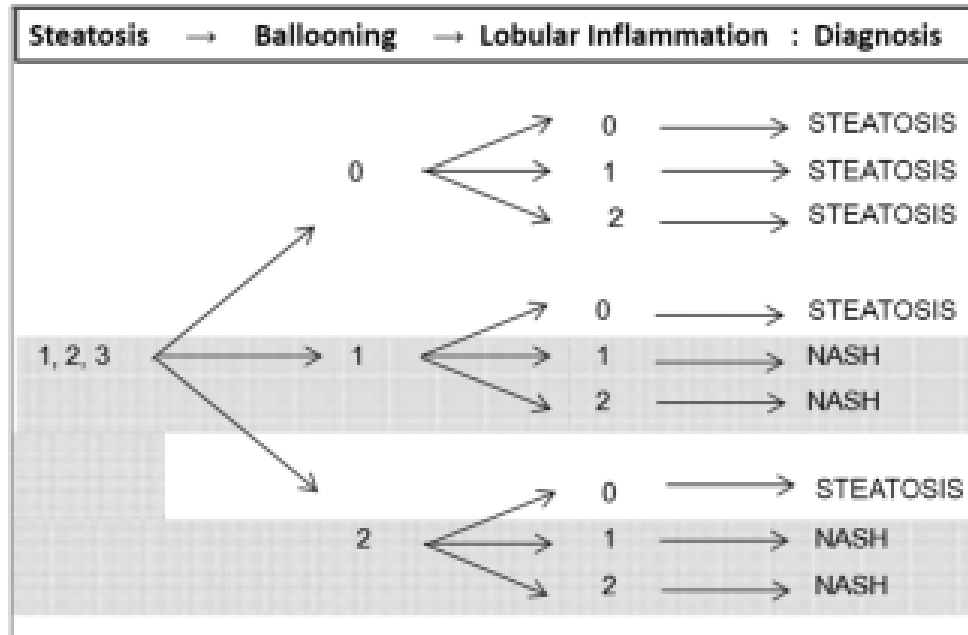
(D) Ballooning, grade 2. Cells are **rounded with clear cytoplasm and twice as large as normal hepatocytes** (star), H&E, X 40

Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. Hepatology. 2012 Nov;56(5):1751-9

Steatosis, Activity, Fibrosis Score

- Activity grade (A, from 0-4) was the unweighted addition of hepatocyte ballooning (0-2) and lobular inflammation (0-2):
- Cases with A_0 ($A = 0$) had no activity, A_1 ($A = 1$), mild activity, A_2 ($A = 2$), moderate activity, A_3 ($A \geq 3$) severe activity.
- Stage of fibrosis (F) was assessed using the score described by NASH-CRN

Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease



Improves interobserver variation.

Utility and appropriateness of the Fatty Liver Inhibition of Progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. *Hepatology*. 2014; 60: 565–575

Does the SAF predict natural history?

After adjustment for fibrosis, the SAF score was not associated with increased mortality in NAFLD.

SAF score and mortality in NAFLD after up to 41 years of follow-up.

Scand J Gastroenterol. 2016 Sep 10:1-5.

Extending the Ballooning Score Beyond 2: A Proposal for a New Balloon Score

AASLD Abstract 2015

- 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** (distinct, enlarged, with clumped cytoplasm) or **non-classical** (cytoplasmic changes without distinctive cytomegaly).
- **Severe** ballooning was defined as clusters of classical ballooned 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.

Conclusions:

- Compared to cases without ballooning, non-classical ballooning cases had more severe histology
- Compared to non-classical ballooning cases, classical ballooning cases had more severe histology
- Severe ballooning cases differed from non-severe ballooning in that they had more severe histology

Although distinguishing NASH from NAFLD may still have relevance for clinical practice, we suggest that using this binary classification in clinical research is somewhat artificial.

Liver histology and clinical trials for nonalcoholic steatohepatitis- perspectives from 2 pathologists. [Gastroenterology](#). 2015 Nov;149(6).

Progression of fibrosis in NAFLD

- A meta-analysis of studies of paired liver biopsy studies.
- The annual fibrosis progression rate in patients with NAFL who had stage 0 fibrosis at baseline was 0.07 stages compared with 0.14 stages in patients with NASH.
- These findings correspond to 1 stage of progression over 14.3 years for patients with NAFLD and 7.1 years for patients with NASH.
- Liver fibrosis progresses in patients with NAFLD and NASH.

Fibrosis Progression in Nonalcoholic Fatty Liver vs Nonalcoholic Steatohepatitis.

Clinical Gastroenterology and Hepatology 2015;13:643–654

Prognosis of NAFLD

NAFLD patients have increased risk of death, with a high risk of death from cardiovascular disease and liver-related disease.

The NAS was not able to predict overall mortality, whereas fibrosis stage predicted both overall and disease-specific mortality.

Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. [Hepatology](#). 2015 May;61(5):1547-54.

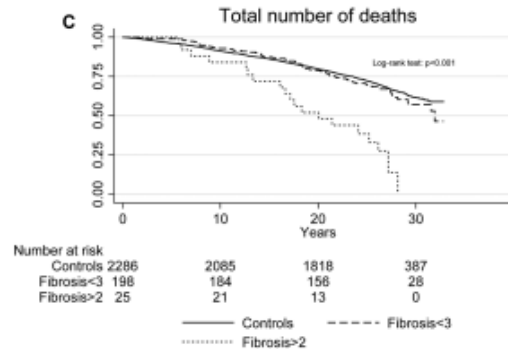
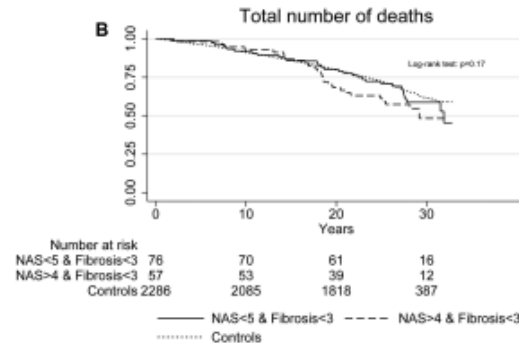
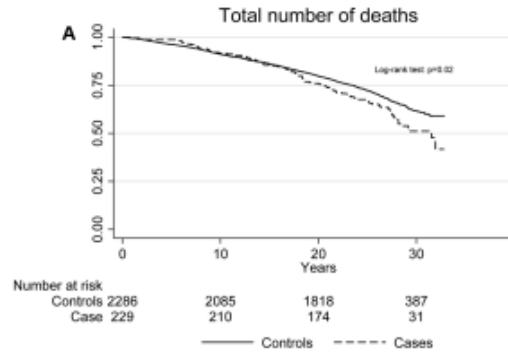
Factors determining the prognosis of NAFLD

- “Patients with fibrosis, regardless of steatohepatitis or NAFLD activity score, had shorter survival times than patients without fibrosis.”

Liver fibrosis, but no other histologic features, associates with long-term outcomes of patients with nonalcoholic fatty liver disease.

[Gastroenterology August 2015](#) Volume 149, Issue 2, Pages 389–397

Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up



Hepatology

Volume 61, Issue 5, pages 1547-1554, 23 MAR 2015 DOI: 10.1002/hep.27368

<http://onlinelibrary.wiley.com/doi/10.1002/hep.27368/full#hep27368-fig-0002>

NASH and cardiovascular disease

NAFLD is an **independent** risk factor for atherosclerosis and cardiovascular disease

- **Fatty liver as an independent predictor of early carotid atherosclerosis: results from a large transversal and long-term follow-up** J Hepatol, 65 (2016), pp. 95–102
- **Nonalcoholic fatty liver disease and vascular function: cross-sectional analysis in the Framingham heart study** Arterioscler Thromb Vasc Biol, 35 (2015), pp. 1284–1291

Liver Histology and Clinical Trials for Nonalcoholic Steatohepatitis I

- Fibrosis even, in the earliest recognizable stage, is a more powerful predictor of long-term outcome than other histologic features or diagnostic categorization.
- Fibrosis is easy to assess histologically and highly reproducible between pathologists. It is a robust criterion provided that the biopsy is adequate (recommended 2- cm core from at least a 16-G needle) and has obvious clinical prognostic value.

Liver histology and clinical trials for nonalcoholic steatohepatitis-perspectives from 2 pathologists. [Gastroenterology](#). 2015 Nov;149(6).

Liver Histology and Clinical Trials for Nonalcoholic Steatohepatitis II

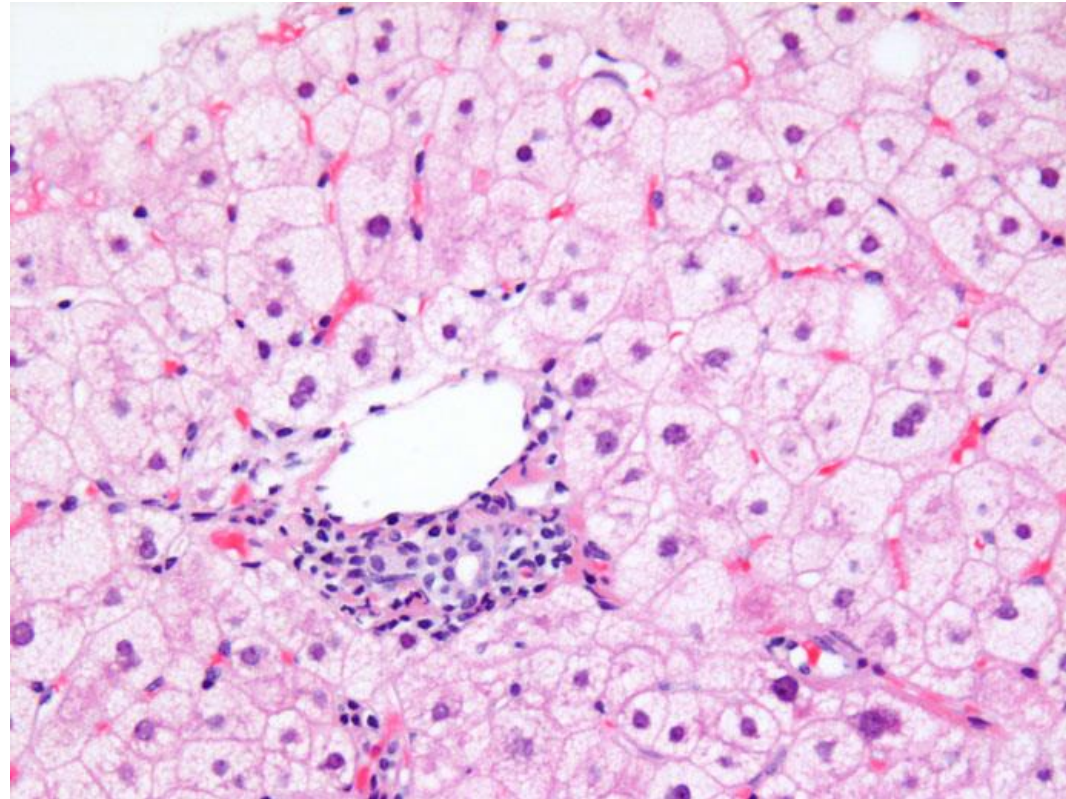
- Fibrosis regression might, therefore, be the best surrogate marker for assessing benefit of a drug.
- The drawback is that fibrosis regression may need more time to be observed than regression of other inflammatory or hepatocellular lesions and probably would need a larger clinical sample. It is an achievable goal because fibrosis regression was observed in the recent FLINT study over a 72-week period.

Liver Histology and Clinical Trials for Nonalcoholic Steatohepatitis III

- An expanded semiquantitative fibrosis staging system or a sensitive technique such as morphometry may help to quantify fibrosis regression in even shorter trials, but the amount of collagen loss needed to define a clinically relevant has yet to be defined

Alcoholic Liver Disease

Small droplet fatty change/
Foamy degeneration



Alcoholic foamy degeneration

21 Japanese patients

Presented with jaundice and hepatomegaly

Reversible

Alcoholic foamy degeneration - a pattern of acute alcoholic injury of the liver.
Gastroenterology. 1983 Apr;84(4):683-92

Alcoholic Foamy Degeneration and Alcoholic Fatty Liver With Jaundice: Often Overlooked Causes of Jaundice and Hepatic Decompensation That Can Mimic Alcoholic Hepatitis Clinical. Liver Disease, Vol 6, No 6, December 2015

A Histologic Scoring System for Prognosis of Patients With Alcoholic Hepatitis

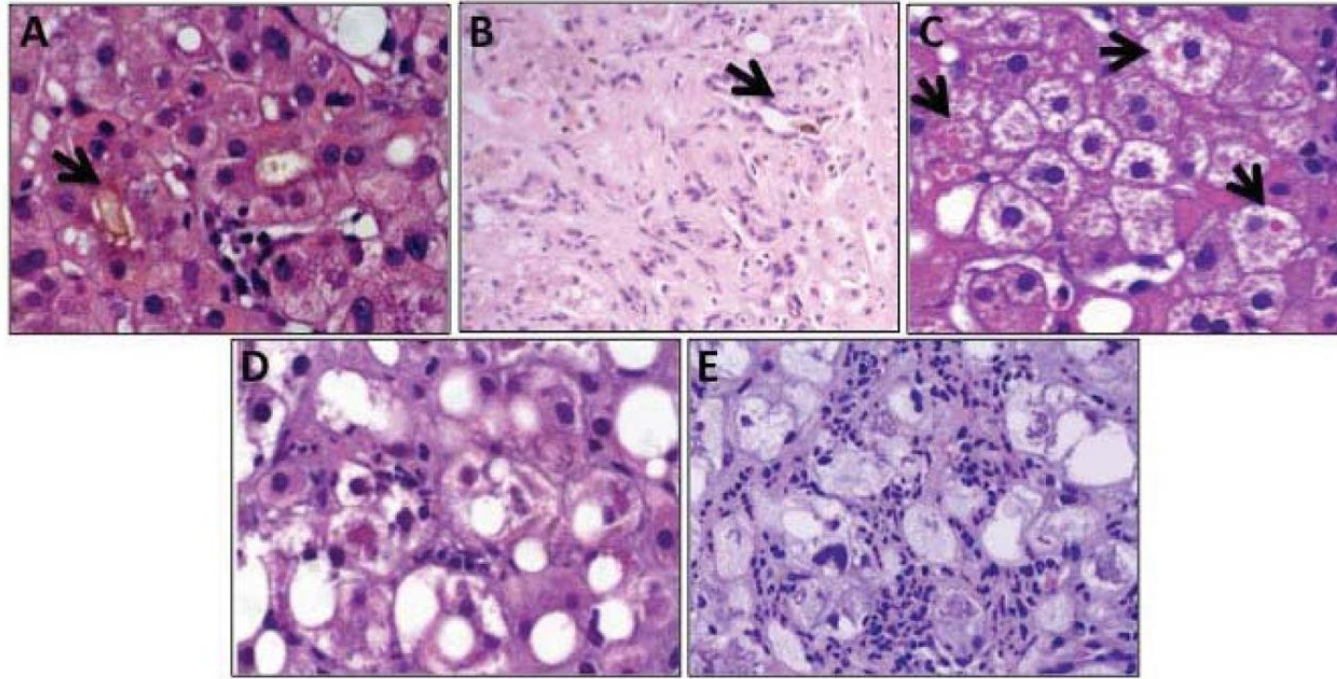
Gastroenterology. 2014 May; 146(5): 1231–1239.

Development of the scoring system

- The system was developed in a group of 217 patients and a semi-quantitative scoring system was developed, called the alcoholic hepatitis histologic score (AHHS).
- The system was validated in an independent set of 109 patients.

Alcoholic Hepatitis Histological Score (AHHS) for Prognostic Stratification of Alcoholic Hepatitis

| | Points | |
|---|---------------|-------------------------------------|
| Fibrosis stage | | AHHS categories (0–9 points) |
| None Fibrosis or Portal fibrosis | 0 | Mild : 0–3 |
| Expansive fibrosis | 0 | Intermediate: 4–5 |
| Bridging fibrosis or Cirrhosis | +3 | Severe: 6–9 |
| Bilirubinostasis | | |
| No | 0 | |
| Hepatocellular only | 0 | |
| Canalicular or ductular | +1 | |
| Canalicular or ductular plus Hepatocellular | +2 | |
| PMN infiltration | | |
| No/Mild | +2 | |
| Severe PMN Infiltration | 0 | |
| Megamitochondria | | |
| No Megamitochondria | +2 | |
| Megamitochondria | 0 | |



Histological Features Independently Associated with 90-day survival:

A) Hepatocellular and canalicular bilirubinostasis

B) Ductular bilirubinostasis

C) Megamitochondria

D and E) Mild and severe PMN infiltration

Histopathological agreement: Kappa values

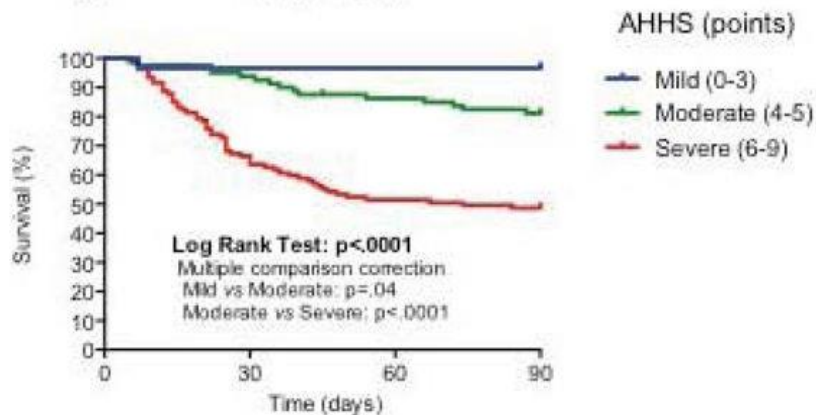
0.65 for fibrosis

0.86 for bilirubinostasis

0.60 for neutrophil infiltration and

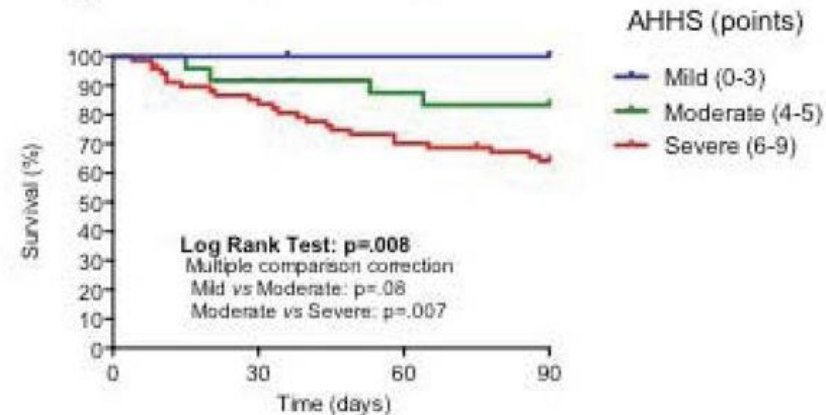
0.46 for megamitochondria.

a) Study Cohort



| | | | | |
|----------------|-----|----|----|----|
| Mild (0-3) | 30 | 29 | 29 | 29 |
| Moderate (4-5) | 80 | 75 | 68 | 65 |
| Severe (6-9) | 107 | 68 | 55 | 52 |

b) Validation Cohort



| | | | | |
|----------------|----|----|----|----|
| Mild (0-3) | 17 | 17 | 17 | 17 |
| Moderate (4-5) | 24 | 22 | 21 | 20 |
| Severe (6-9) | 68 | 57 | 48 | 44 |

Points to note

- Neutrophil infiltration is associated with a more favorable outcome “shifts the paradigm” and the authors suggest “that patients with active inflammation probably have more regenerative potential”
- Nearly half of the patients with canalicular/ductular bilirubinostasis or hepatocellular plus canalicular/ductular bilirubinostasis developed a bacterial infection during hospitalization

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
N Engl J Med. 2015 Apr 23;372(17):1619-28.

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 the Alcoholic Hepatitis Histological Score

STOPAH Histology: Diagnosis of Alcoholic Hepatitis

- Overall a diagnosis of alcoholic hepatitis was confirmed in 87% of biopsies, overall, 91% obtained within 2 days of starting treatment.

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

Other things which have been be done and perhaps we could/ should do:

Senescence markers

- Hepatocyte Expression of the Senescence Marker p21 Is Linked to Fibrosis and an Adverse Liver-Related Outcome in Alcohol-Related Liver Disease. PLOS One September 2013, Volume 8, Issue 9, e72904

Progenitor markers

- Liver progenitor cell markers correlate with liver damage and predict short-term mortality in patients with alcoholic hepatitis. Hepatology. 2012 Jun;55(6):1931-41.

Chemokine expression

- Hepatic Expression of CXC Chemokines Predicts Portal Hypertension and Survival in Patients With Alcoholic Hepatitis. Gastroenterology. 2009 May;136(5):1639-50.

Apoptosis Markers

- Hepatocyte apoptosis is a pathologic feature of human alcoholic hepatitis. Journal of Hepatology 34 (2001) 248±253

Proliferation makers

- Hepatic cell proliferation plays a pivotal role in the prognosis of alcoholic hepatitis. Journal of Hepatology 2015 vol. 63 j 609–621
- Progenitor cell expansion and impaired hepatocyte regeneration in explanted livers from alcoholic hepatitis. Gut 2015;64:1949–1960.

Fat and liver tumours

HCC in patients with NAFLD

- “In noncirrhotic HCC patients, histological steatosis was frequently present, whereas overt steatohepatitis did not occur.”

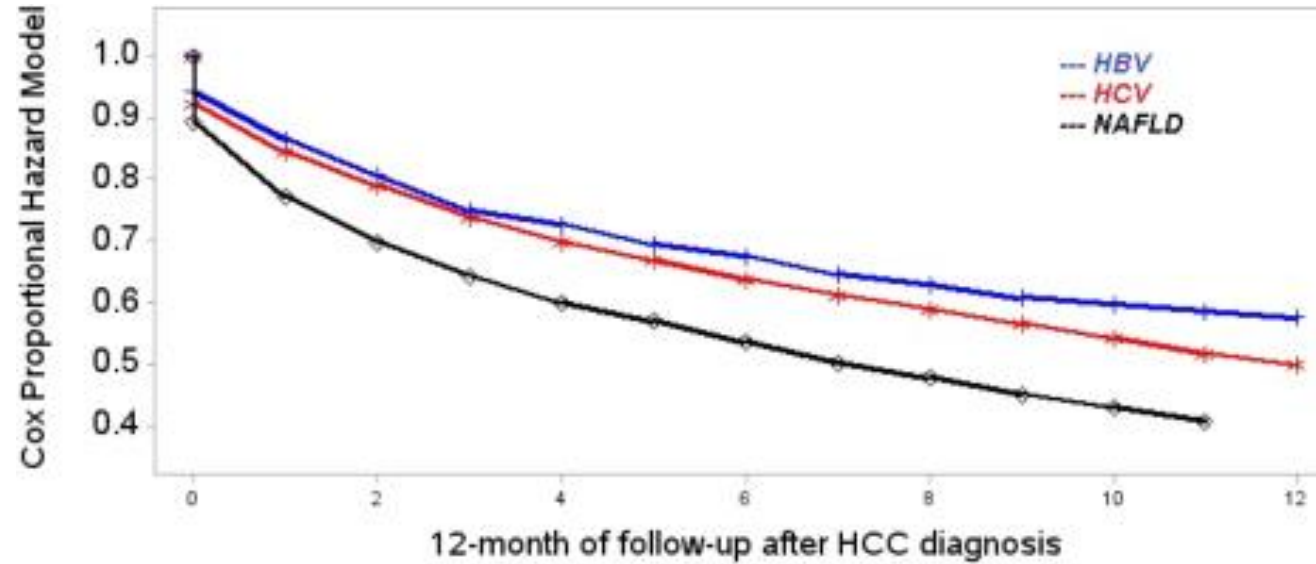
Hepatocellular carcinoma in noncirrhotic livers is associated with steatosis rather than steatohepatitis. *Eur J Gastroenterol Hepatol.* 2016 Aug;28(8):955-62.

NAFLD-HCC

- could arise also in the absence of cirrhosis
- more often detected at a later tumor stage
- but after patient matching, it has a similar survival rate compared to HCV infection

- HEPATOLOGY 2016;63:827–838

Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009



* Adjusted for age (years) at HCC diagnosis and tumor stage;
Source: SEER-Medicare, 2004 - 2009

Hepatology

Volume 62, Issue 6, pages 1723-1730, 24 OCT 2015 DOI: 10.1002/hep.28123
<http://onlinelibrary.wiley.com/doi/10.1002/hep.28123/full#hep28123-fig-0003>

Hepatocytes “tumours” that contain intracellular fat

- multifocal hepatic steatosis,
- focal nodular hyperplasia (22-85%)
- adenomas
- hepatocellular carcinoma

Fatty liver deposition and sparing: a pictorial review

Insights Imaging. 2011 Oct; 2(5): 533–538.

Fatty change in hepatic adenoma

- **HNF1 α mutated adenoma:**

characteristic: diffuse and homogeneous

- inflammatory adenoma:

11% of cases: focal and heterogeneous.

- β -catenin mutated adenoma

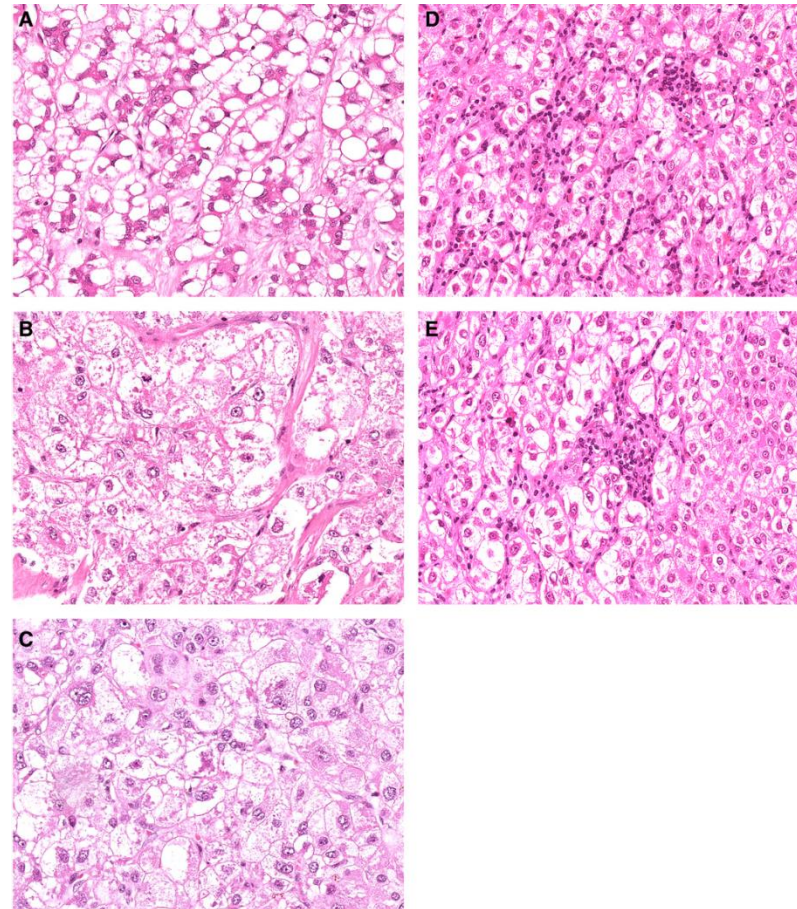
21% of cases: diffuse but non-homogeneous

Steatotic HCC

- Steatotic HCC is a common histological variant of HCC with distinct association with underlying fatty liver, steatohepatitis and metabolic risks.
- Despite more favourable baseline tumour features, it was associated with late tumour relapse.

Steatotic hepatocellular carcinoma: a variant associated with metabolic factors and late tumour relapse. *Histopathology* 2016

Steatotic hepatocellular carcinoma: a variant associated with metabolic factors and late tumour relapse



Histopathology

9 SEP 2016 DOI: 10.1111/his.13029

<http://onlinelibrary.wiley.com/doi/10.1111/his.13029/full#his13029-fig-0001>

