



## Fatty Liver Disease

Diagnostic Challenges and Updates

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## Obesity in Antiquity

Obesity Treatment  
Brisk walking  
Wrestling



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## Definitions

- **NAFLD** – Fat ( $\geq 5\%$ ) in the liver (imaging or histology) in a patient without secondary fat accumulation.
- **NASH**-NAFLD with histologic evidence of liver injury in the form of ballooned hepatocytes and inflammation +/- fibrosis.
- **NAFL** – NAFLD without the above histologic findings associated with NASH.

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## Secondary Hepatic Fat

- Macrovesicular
  - Excess alcohol
  - HCV
  - Wilson Disease
  - Starvation/TPN
  - Medications (amiodarone, methotrexate, tamoxifen, corticosteroids)
- Microvesicular

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## Secondary Hepatic Fat

- Macrovesicular
- Microvesicular
  - Reye Syndrome
  - Acute Fatty Liver of pregnancy
  - Medications (e.g. antiretrovirals, valproate)

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## Natural History

- **NASH**- Can progress to cirrhosis and liver failure (and rarely hepatocellular carcinoma)
- **NAFL** – Risk of progression to cirrhosis and liver failure is considered *minimal (with increased risk associated with NAFL with inflammation)*

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## Practice Guidance from the AASLD



Naga Chalasani, Zohar Younossi, Joel E. Lavine, Michael Charlton, Kenneth Cook, Mary Rinella, Stephen A. Harrison, Elizabeth M. Brunt, and Arun J. Sanyal. The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance From the American Association for the Study of Liver Diseases. Hepatology 67(1), 2018.

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**TABLE 3. Risk Factors Associated With NAFLD**

Common Conditions With Established Association	Other Conditions Associated With NAFLD
Obesity	Hypothyroidism
T2DM	Obstructive sleep apnea
Dyslipidemia	Hypopituitarism
MetS*	Hypogonadism
Polycystic ovary syndrome	Pancreatoduodenal resection
	Psoriasis

\*The Adult Treatment Panel III clinical definition of MetS requires the presence of three or more of the following features: (1) waist circumference greater than 102 cm in men or greater than 88 cm in women; (2) TG level 150 mg/dL or greater; (3) HDL cholesterol level less than 40 mg/dL in men and less than 50 mg/dL in women; (4) systolic blood pressure 130 mm Hg or greater or diastolic pressure 85 mm Hg or greater; and (5) fasting plasma glucose level 110 mg/dL or greater.<sup>(287)</sup>

Naga Chalasani, Zohar Younossi, Joel E. Lavine, Michael Charlton, Kenneth Cook, Mary Rinella, Stephen A. Harrison, Elizabeth M. Brunt, and Arun J. Sanyal. The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance From the American Association for the Study of Liver Diseases. Hepatology 67(1), 2018.

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## Genetic Factors

- *PNPLA3* – encodes adiponutrin. A SNP at position 148 is associated with hepatic steatosis, NASH, and increased fibrosis stage (as well as incidence of HCC)
- *TM6SF2* – a SNP at position 167 has similar associations as *PNPLA3* SNP

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## Scoring Systems

- **NAS**- Unweighted composite of steatosis, lobular inflammation, and ballooning scores. Useful to measure changes in biopsies in clinical trials. Fibrosis is scored separately
- **SAF score** – Semiquantitative score consisting of steatosis amount, activity (lobular inflammation and ballooning) and fibrosis

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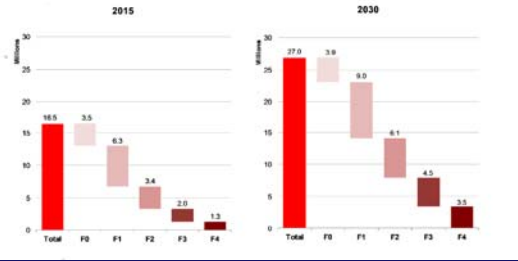
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Figure 3b. Distribution of NASH Population by Fibrosis Stage – US, 2015 & 2030



Estes C., Razavi H., Loomba R., Younossi Z., Sanyal A.J. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology, 2017 in press (10.1002/hep.29466)

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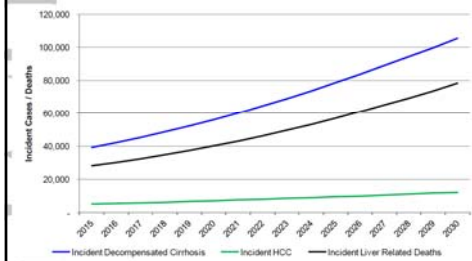
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Figure 5. Incident Decompensated Cirrhosis, HCC and Liver-Related Deaths among Prevalent NAFLD Population – US, 2015-2030



Estes C., Razavi H., Loomba R., Younossi Z., Sanyal A.J. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology, 2017 in press (10.1002/hep.29466)

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## Conclusions

- With continued high rates of adult obesity and diabetes, in an aging population, **NAFLD related liver disease and mortality will increase in the US**
- Strategies to slow growth of NAFLD and **therapeutic options are necessary to mitigate disease burden**

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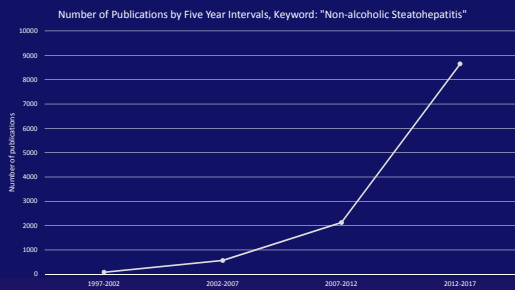
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## Response to the Crisis



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## Outline

1. Essential histologic criteria for diagnosis of steatohepatitis
2. Centrizonal arteries
3. Aggressive NASH
4. Diagnostic pitfalls
5. Revisiting the NAS

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## Steatohepatitis: Essential Features

### AASLD and NASH Clinical Research Network

- Steatosis ( $\geq 5\%$ )
- Inflammation (lobular)
- Hepatocellular injury  
    Ballooned hepatocytes

+/- Pericellular fibrosis

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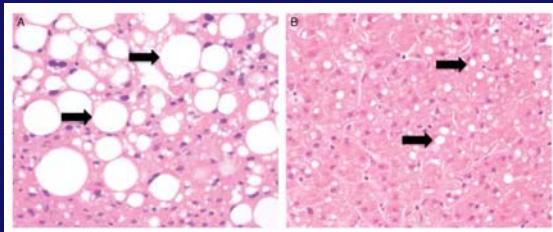
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## Large or Small Droplet Macrovesicular Steatosis



Choi WT, Jen KY, Wang D, Tavakoli M, Roberts JP, Gill RM. Donor Liver Small Droplet Macrovesicular Steatosis Is Associated With Increased Risk for Recipient Allograft Rejection. *Am J Surg Pathol*. 2017 Mar;41(3):365-373. doi: 10.1027/PAS.0000000000000902. PubMed PMID: 28259835.

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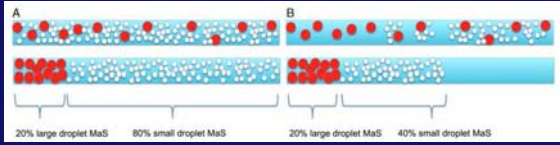
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## Estimation of Steatosis



Choi WT, Jen KY, Wang D, Tavakoli M, Roberts JP, Gill RM. Donor Liver Small Droplet Macrovesicular Steatosis Is Associated With Increased Risk for Recipient Allograft Rejection. *Am J Surg Pathol*. 2017 Mar;41(3):365-373. doi: 10.1097/PAS.0000000000000802. PubMed PMID: 28059836.

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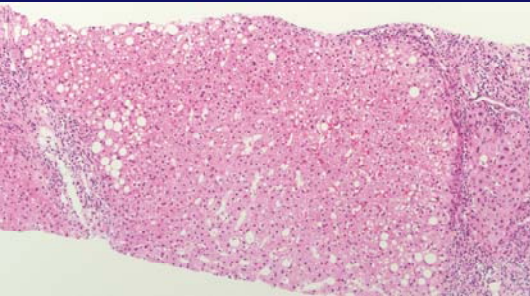
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## Mild Steatosis (Grade 1, scale 0-3)



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, *Surgical Pathology Clinics*, Volume 6, Issue 2, Pages 227-257, June 2013), adapted with permission from Elsevier.

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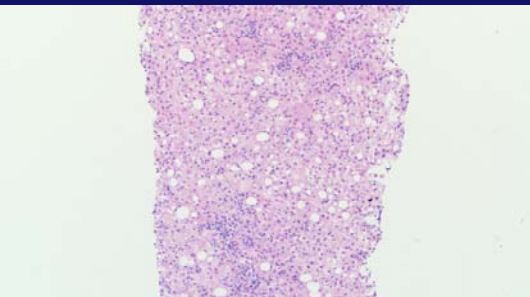
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## Moderate Steatosis (Grade 2, scale 0-3)



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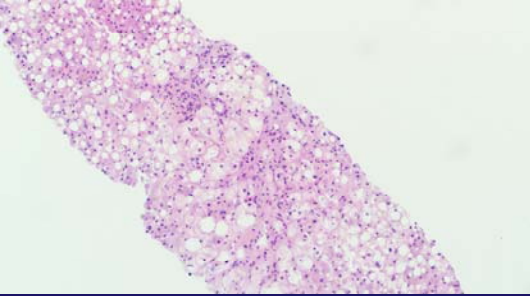
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### Severe Steatosis (Grade 3, scale 0-3)



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### Steatohepatitis: Essential Features

#### AASLD and NASH Clinical Research Network

- Steatosis (>5%)
- Inflammation (lobular)
- Hepatocellular injury  
Ballooned hepatocytes

+/- Pericellular fibrosis

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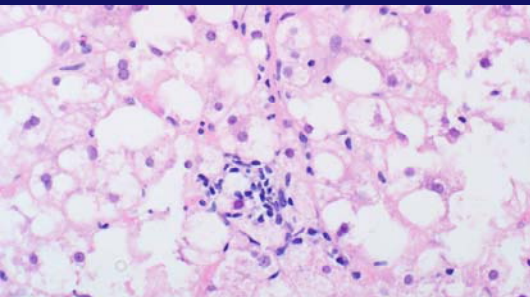
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### Lobular Inflammation in NASH



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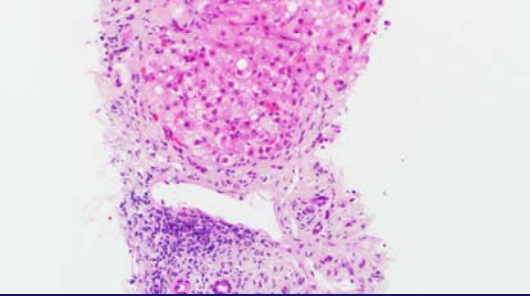
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## Portal Inflammation in NASH



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## Steatohepatitis: Essential Features

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- Steatosis (>5%)
  - Inflammation (lobular)
  - Hepatocellular injury
- Ballooned hepatocytes**

+/- Pericellular fibrosis

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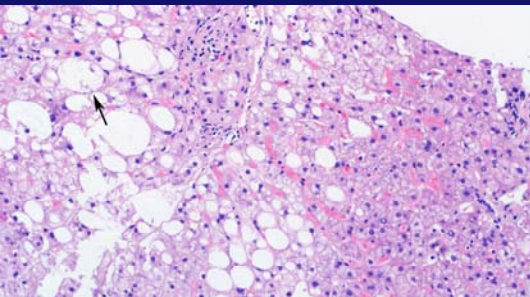
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## Ballooned Hepatocyte



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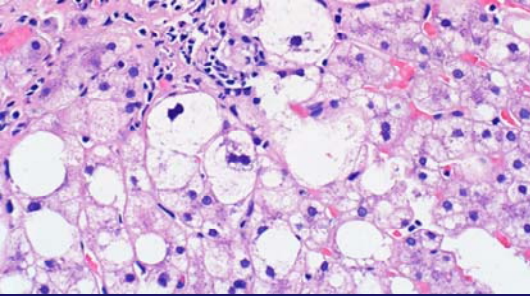
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## Multiple Ballooned Hepatocytes



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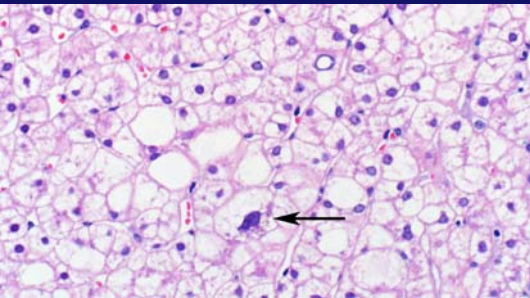
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## BH Mimic – Small Droplet Fat



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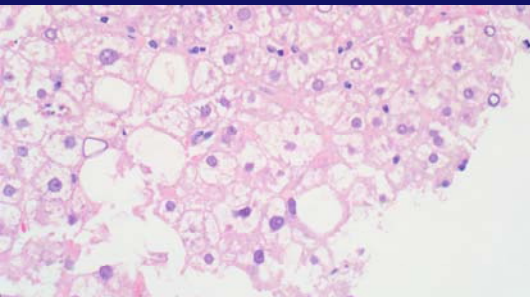
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## BH Mimic - Glycogenosis



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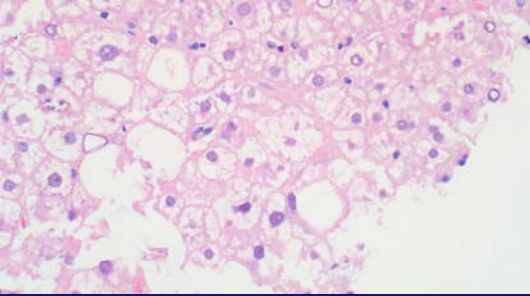
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## BH Mimic - Processing



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## Steatohepatitis: Essential Features

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- Steatosis (>5%)
  - Inflammation (lobular)
  - Hepatocellular injury
- Ballooned hepatocytes

+/- Pericellular fibrosis

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## Staging - Modified Brunt Method

Stage 1A	Pericentral/sinusoidal Fibrosis – Delicate
Stage 1B	Pericentral/sinusoidal Fibrosis – Dense
Stage 1C	Periportal Fibrosis
Stage 2	Pericentral/sinusoidal and Periportal Fibrosis
Stage 3	Bridging Fibrosis
Stage 4	Cirrhosis

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Stage 1

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Stage 2

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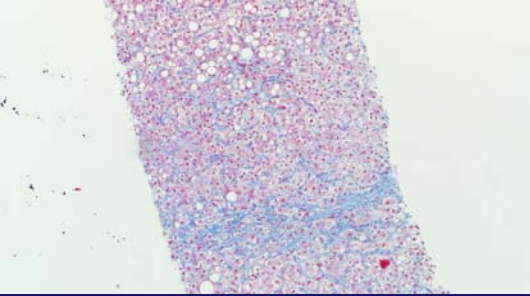
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### Stage 3



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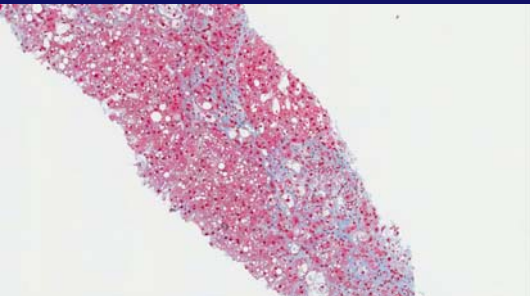
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### Stage 3



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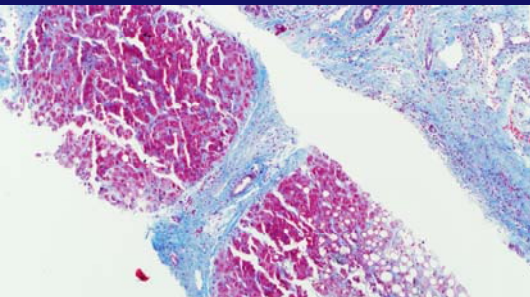
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### Stage 4



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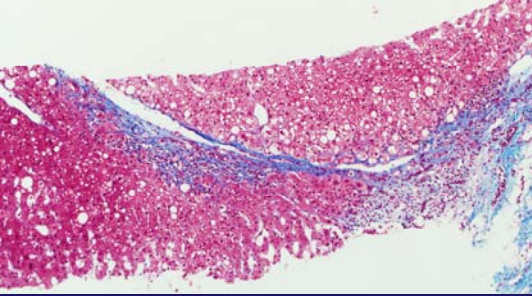
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## Fibrosis Pitfall – Tangential



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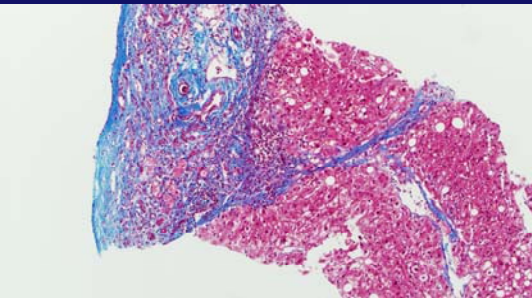
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## Fibrosis Pitfall - Subcapsular



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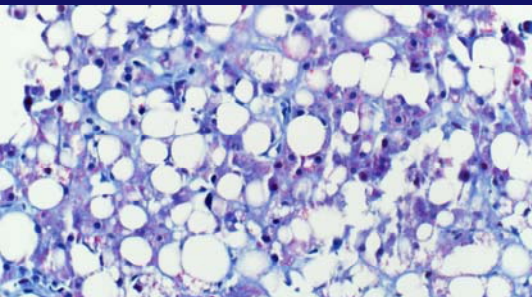
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## Fibrosis Pitfall – Overstained



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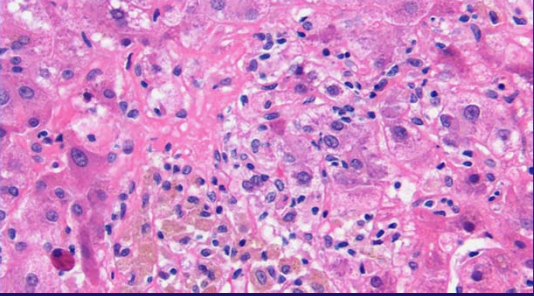
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Fibrosis Pitfall – Histiocyte  
Aggregate



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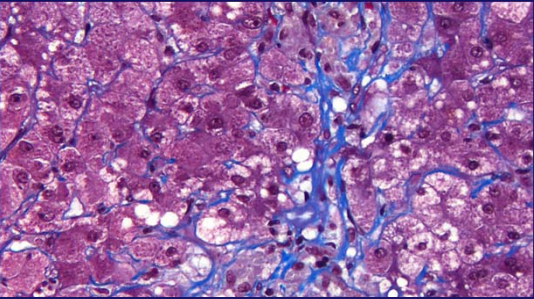
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Fibrosis Pitfall – Histiocyte  
Aggregate



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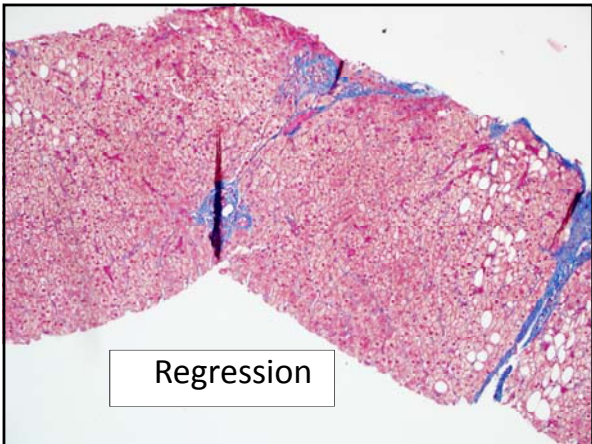
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## Steatohepatitis: Non-essential Features

- Mallory hyaline in Zone 3
- Mild iron deposits in hepatocytes or sinusoidal cells
- Megamitochondria
- Glycogenated nuclei
- Lipogranulomas
- Acidophil bodies (occasional)
- Centrizonal arteries

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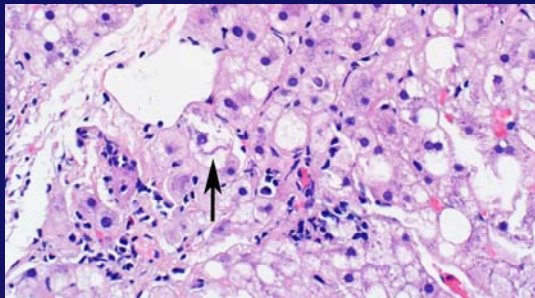
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## Mallory Hyaline



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## Histologic Variation

### **PATTERN 1: CLASSIC STEATOHEPATITIS**

Steatosis with mild inflammation, hepatocellular ballooning, and pericellular fibrosis

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## Histologic Variation

### **PATTERN 2: STEATOSIS WITHOUT HEPATOCELLULAR INJURY**

Steatosis without hepatocyte ballooning or pericellular fibrosis is insufficient for a diagnosis of steatohepatitis and represents NAFL

Low rate of progression (~5%) to significant fibrosis

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## Histologic Variation

### **PATTERN 3: STEATOSIS WITH SWOLLEN HEPATOCYTES/NON-CLASSIC BALLOONED HEPATOCYTES**

Borderline for steatohepatitis; if clinical risk factors are present, it is best to manage the patient as appropriate for steatohepatitis

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## Histologic Variation

### **PATTERN 4: BALLOONED HEPATOCYTES OR PERICELLULAR FIBROSIS WITHOUT STEATOSIS**

Uncommon in patients with metabolic risk factors

Ballooned Hepatocytes Only	Pericellular Fibrosis Only
Recent cessation of Alcohol	Chronic venous outflow obstruction
Amiodarone	Remote CZ injury

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## Histologic Variation

### **PATTERN 5: STEATOSIS WITH PERICELLULAR FIBROSIS, BUT NO BALLOONED HEPATOCYTES**

Borderline for steatohepatitis in the appropriate clinical context

Other considerations: chronic venous outflow obstruction, drug (e.g. oxaliplatin), remote parenchymal rejection (post-transplant)

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## Histologic Variation

### **PATTERN 6: CIRRHOSIS WITH STEATOSIS AND/OR BALLOONED HEPATOCYTES**

Cirrhosis with histologic features of NAFLD is best considered NASH cirrhosis. Some cases may show residual pericellular fibrosis.

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ORIGINAL ARTICLE

### Centrizonal Arteries and Microvessels in Nonalcoholic Steatohepatitis

Ryan M. Gill, MD, PhD,\* Patricia Belt, BS,† Laura Wilson, ScM,‡ Nathan M. Bass, MD, PhD,‡ and Linda D. Ferrell, MD\*

(Am J Surg Pathol 2011;35:1400-1404)

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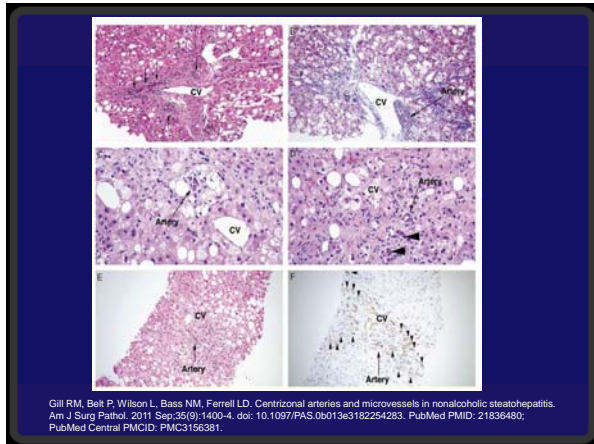
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**TABLE 1. NASH Fibrosis Stage Versus Prevalence of Centrifugal Arteries (Grade)\***

	Grade 0	Grade 1	Grade 2	Grade 3	Total (%)
Stage 1b/1c	17	2	0	0	2/19 (11%)
Stage 2	25	7	1	1	9/34 (27%)
Stage 3	15	8	10	3	21/36 (58%)
Stage 4	3	1	2	5	8/11 (73%)
Total	60	18	13	9	40/100 (40%)

Artery grades: 0, no central zones with artery; 1, 1 to 2 central zones with artery/biopsy; 2, > 2 and < 50% of central zones with artery; 3, ≥ 50% of central zones with artery. Definitions of stages: 1b - centrilobular fibrosis only, without the use of trichrome stain (ie, readily discernible on H&E stain), 1c - periportal fibrosis only, 2 - centrilobular and periportal fibrosis, 3 - bridging fibrosis, 4 - cirrhosis.  
\*P < 0.001 using univariate ordinal logistic regression.

Gill RM, Belt P, Wilson L, Bass NM, Ferrell LD. Centrifugal arteries and microvessels in nonalcoholic steatohepatitis. Am J Surg Pathol. 2011 Sep;35(9):1400-4. doi: 10.1097/PAS.0b013e3182254283. PubMed PMID: 21836480; PubMed Central PMCID: PMC3156381.

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Monika Sarkar et al. / *Journal of Hepatology* 58 (2013) 101–107

101

**Aggressive non-alcoholic steatohepatitis following rapid weight loss and/or malnutrition**

Jia-Huei Tsai<sup>1,2</sup>, Linda D Ferrell<sup>3</sup>, Vivian Tan<sup>4</sup>, Matthew M Yeh<sup>5</sup>, Monika Sarkar<sup>6</sup> and Ryan M Gill<sup>3</sup>

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## Aggressive NASH

- NASH presenting as ALF
- We described 6 patients who developed ALF following rapid loss or malnutrition
- 4 patients either died or required urgent liver transplant
- Pathologic findings similar to advanced alcoholic steatohepatitis

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## Pathologic Features

- Extensive/circumferential centrizonal pericellular fibrosis
- Central scar with perivenular sclerosis/veno-occlusion with superimposed hepatocellular dropout
- Abundant/prominent hepatocellular balloons, and numerous Mallory-Denk bodies
- Centrizonal arteries often prominent

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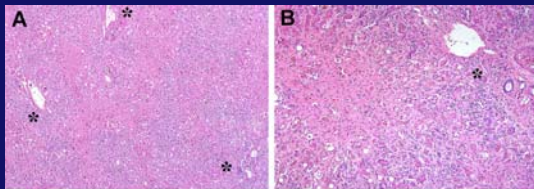
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## Severe Centrizonal Scarring



Tsai JH, Ferrell LD, Tan V, Yeh MM, Sarkar M, Gill RM. Aggressive non-alcoholic steatohepatitis following rapid weight loss and/or malnutrition. *Mod Pathol*. 2017 Jun;30(6):834-842. doi: 10.1038/modpathol.2017.13. Epub 2017 Mar 3. PubMed PMID: 28256569.

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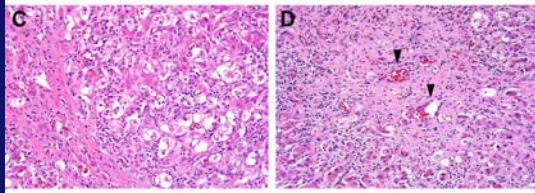
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## Prominent BH and Centrizonal Arteries



Tsai JH, Ferrell LD, Tan V, Yeh MM, Sarkar M, Gill RM. Aggressive non-alcoholic steatohepatitis following rapid weight loss and/or malnutrition. *Mod Pathol.* 2017 Jun;30(6):834-842. doi: 10.1038/modpathol.2017.13. Epub 2017 Mar 3. PubMed PMID: 28256569.

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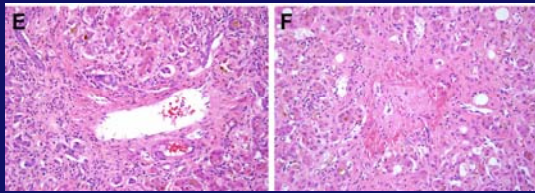
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## Ductular Reaction, Cholestasis, and Central Vein Occlusion



Tsai JH, Ferrell LD, Tan V, Yeh MM, Sarkar M, Gill RM. Aggressive non-alcoholic steatohepatitis following rapid weight loss and/or malnutrition. *Mod Pathol.* 2017 Jun;30(6):834-842. doi: 10.1038/modpathol.2017.13. Epub 2017 Mar 3. PubMed PMID: 28256568.

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## Diagnostic Challenges

1. Alcoholic steatohepatitis
2. Burnt out NASH cirrhosis
3. Drug induced steatohepatitis
4. Hereditary hemochromatosis
5. Metabolic disorders
6. Microvesicular steatosis
7. More than mild portal inflammation

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## Alcoholic Steatohepatitis

- Alcoholic steatohepatitis can not be definitively distinguished from NASH by histology

	NASH	ASH
Steatosis	++	+
Ballooned hepatocytes	+	++
Lobular inflammation	+	++
Mallory hyaline	+	++
Neutrophil infiltrate	+	++
Cholestasis	+/-	+
Obliterated CV	+/-	+

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## Burnt-out NASH Cirrhosis

- Typical steatohepatitis features regress with progression of fibrosis and may be lost with cirrhosis
- Many cases labeled as cryptogenic cirrhosis; since this population has a high incidence of type 2 DM, NASH is considered to be the most likely etiology
- Rule out other etiologies and correlate with NASH risk factors

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## Drug Induced Steatohepatitis

- Histologic changes identical to NASH have been identified in patients without NASH risk factors exposed to certain drugs

Definite Association	Possible Association
Amiodarone	Tamoxifen
Irinotecan	Steroids
Methotrexate	Estrogen
Perhexiline	Diethylstilbestrol
Maleate/Diethylaminoethoxyhexesterol	

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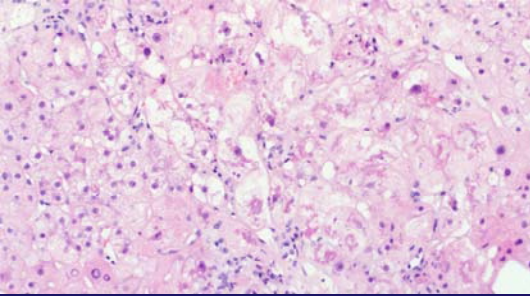
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## Amiodarone Toxicity



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.

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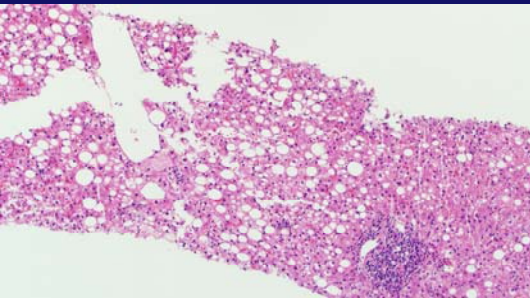
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## Methotrexate



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.

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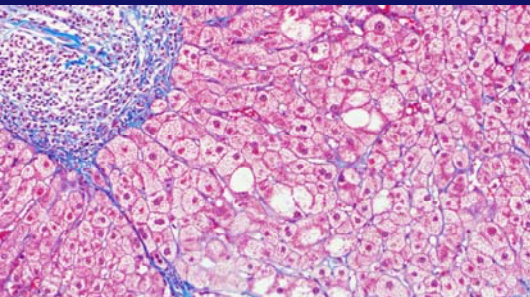
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## Methotrexate with Portal Fibrosis



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.

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## Hereditary Hemochromatosis

- A mild to moderate hepatocyte siderosis (generally nonzonal) and/or Kupffer cell siderosis is seen in ~20% of NAFLD patients
- Serum ferritin is an acute phase reactant that is commonly increased in NAFLD patients
- Increased iron saturation would more strongly suggest hereditary hemochromatosis
- C282Y *HFE* mutation in an established NASH patient may warrant biopsy to evaluate iron overload

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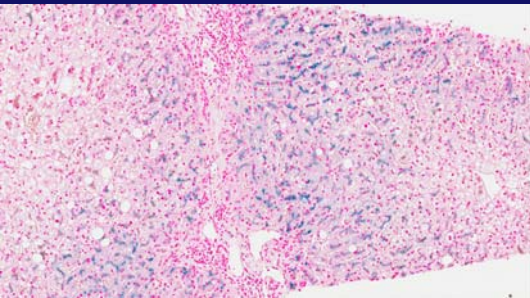
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## Periportal Siderosis in HH



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, *Surgical Pathology Clinics*, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.

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## Metabolic Disorders

- **Glycogenic hepatopathy**
  - Type 1 DM with poor glycemic control
  - Glycogenosis, minimal fat, and abundant megamitochondria
- **Diabetic hepatosclerosis**
  - Non-zonal perisinusoidal fibrosis and BM deposition in patients with long standing insulin dependent DM, minimal steatosis, no ballooning
- **Wilson disease**
  - Steatosis (non-zonal), glycogenated nuclei, Mallory hyaline, swollen hepatocytes, portal inflammation and fibrosis

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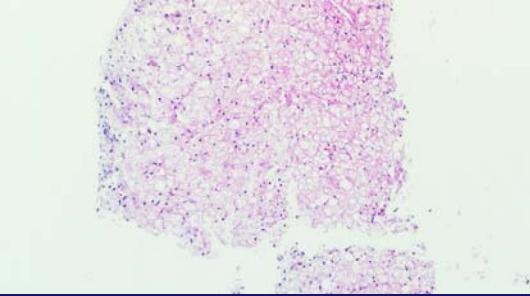
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## Glycogenic Hepatopathy



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.

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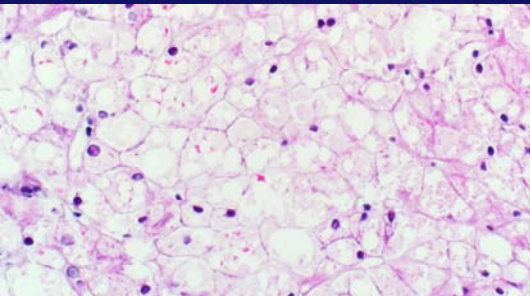
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## Glycogenic Hepatopathy



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.

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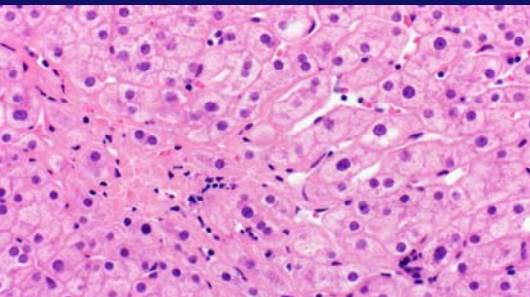
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## Diabetic Hepatosclerosis



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.

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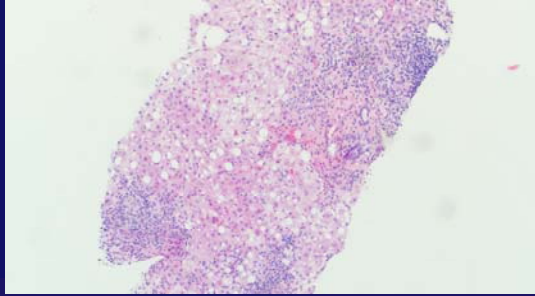
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### Steatosis and Portal Inflammation in Wilson Disease



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013), adapted with permission from Elsevier.

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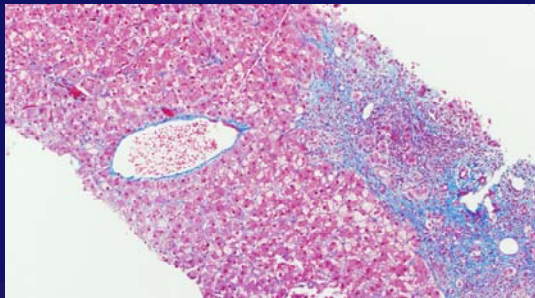
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### Periportal Fibrosis in Wilson Disease



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013), adapted with permission from Elsevier.

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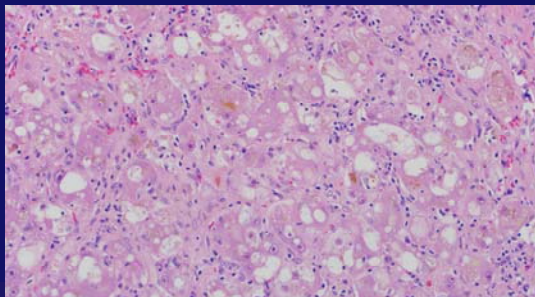
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### Wilson Disease with Swollen Hepatocytes



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013), adapted with permission from Elsevier.

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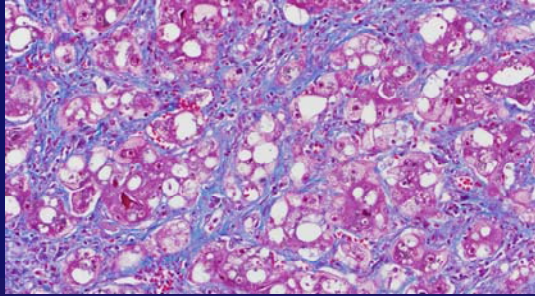
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### Wilson Disease with Pericellular Fibrosis



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013), adapted with permission from Elsevier.

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### Microvesicular Steatosis

- Pure microvesicular steatosis does not occur in NASH and indicates severe mitochondrial injury
- Reye syndrome, acute fatty liver of pregnancy, alcoholic foamy liver degeneration, drug (cocaine, tetracycline, valproic acid, zidovudine), and rare genetic disorders.
- Many NAFLD cases will have a minor component of microvesicular fat

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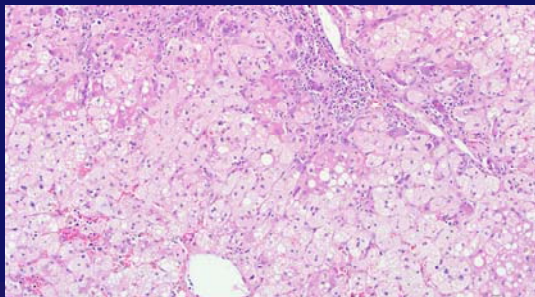
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### Diffuse Microvesicular Steatosis



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013), adapted with permission from Elsevier.

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## More than Mild Portal Inflammation

- NASH portal inflammation is typically mild
- Prominent portal inflammation raises consideration of other causes (HBV, HCV, AIH, PBC, Wilson disease)
- If other etiologies are excluded, this can be considered NASH with prominent portal inflammation
- May be associated with a higher degree of fibrosis

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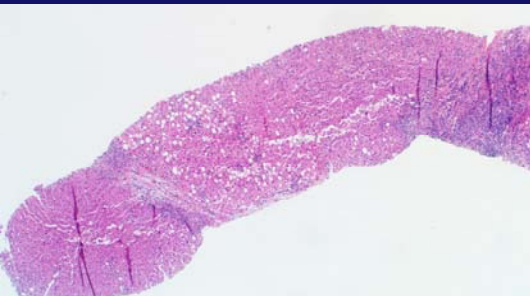
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## More than Mild Portal Inflammation



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.

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## Pediatric NASH

- NASH cirrhosis seen as young as 8 years of age
- AST/ALT screening has been considered for obese children starting at age 10
- Type 1 pediatric NASH: Identical to adult type NASH
- Type 2 pediatric NASH: Severe panacinar steatosis, no ballooned hepatocytes, early portal based fibrosis (stage 1C)
- Children younger than age 2 with fatty liver should be evaluated for rare genetic disorders

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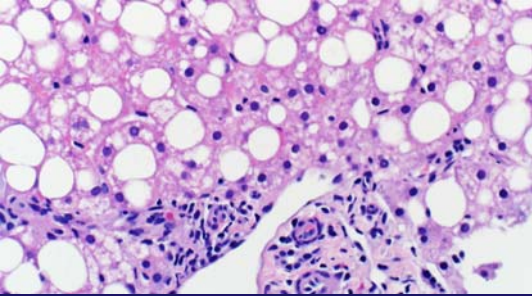
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## Severe Pan-acinar Steatosis



Gill R. M. and Kakar S. Non-alcoholic steatohepatitis: an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.

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## NASH CLINICAL RESEARCH NETWORK (CRN)

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### NIDDK Workshop on Fatty Liver Disease 1998

- No good estimates of disease prevalence or severity (but suspected that this was a big problem)
- Little information on the natural history
- No non-invasive diagnostic tests
- No standard methods for evaluating liver biopsy
- No approved therapies

Courtesy of Dr. David Kleiner, NIH



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## NASH Clinical Research Network

- Sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases
- 18 Participating Academic Clinical Centers (8 Adult, 10 Pediatric), 1 Data Coordination Center, and the NIDDK Project Scientists
- Established to focus on the etiology, contributing factors, natural history, complications, and therapy of nonalcoholic steatohepatitis

Courtesy of Dr. David Kleiner, NIH NASH CRN

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## NASH CRN Studies

**Foundation for trials:**

- Pathology standardization → NAFLD Activity Score ("NAS")
- Utility of laboratory ALT reference ranges
- Impact of TZDs on mitochondrial ultrastructure

**Primary Goal of the Pathology Committee**

Create a scoring system for evaluating liver biopsies that could be used for clinical trials and natural history studies

Courtesy of Dr. David Kleiner, NIH NASH CRN

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## Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease

David E. Kleiner,<sup>1</sup> Elizabeth M. Brunt,<sup>2</sup> Mark Van Natta,<sup>3</sup> Cynthia Behling,<sup>4</sup> Melissa J. Contos,<sup>5</sup> Oscar W. Cummings,<sup>6</sup> Linda D. Ferrell,<sup>7</sup> Yao-Chang Lin,<sup>8</sup> Michael S. Torbenson,<sup>9</sup> Ayur Unalp-Arida,<sup>4</sup> Matthew Yeh,<sup>10</sup> Arthur J. McCullough,<sup>11</sup> and Arun J. Sanyal<sup>12</sup> for the Nonalcoholic Steatohepatitis Clinical Research Network<sup>13</sup>

- H&E and Trichrome only
- 9 pathologists, 2 independent reads
- Scoring system included features for grading/staging plus other findings
- Defined an "Activity Score" for use in clinical trials to objectively measure composite histologic change
- Score based on results of multivariable analysis
- Excluded fibrosis to avoid mixing "stage" with "grade"

**NAFLD Activity Score (NAS) = Steatosis (0-3) + Lob. Inf. (0-3) + Ballooning (0-2)**

Hepatology 41: 1313; 2005 Courtesy of Dr. David Kleiner, NIH NASH CRN

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THE NEW ENGLAND JOURNAL of MEDICINE NASH CRN

ORIGINAL ARTICLE

N Engl J Med 2010;362:1675-85.

Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis

**Effect of Vitamin E or Metformin for Treatment of Nonalcoholic Fatty Liver Disease in Children and Adolescents**  
The TONIC Randomized Controlled Trial JAMA, April 27, 2011—Vol 305, No. 16 1659

**Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial**

www.thelancet.com Published online November 7, 2014 [http://dx.doi.org/10.1016/S0140-6736\(14\)61933-4](http://dx.doi.org/10.1016/S0140-6736(14)61933-4)

Courtesy of Dr. David Kleiner, NIH

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**Ballooning is Associated with Long Term Survival, Whereas Steatosis is Not**

Angulo et al., Gastroenterology 149: 389; 2015

**Steatosis**

Survival free of liver transplantation  
P = 0.607

**Ballooning**

Survival free of liver transplantation  
P < 0.001

Courtesy of Dr. David Kleiner, NIH NASH CRN

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**Problem**

Steatosis accounts for more weight in the NAS than Ballooning

**Possible Solutions**

- Drop Steatosis from the score
- Extend the Ballooning Scale

Courtesy of Dr. David Kleiner, NIH NASH CRN

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### NASH CRN Studies

Background development  
RFA writing  
Ray Monto, M.D.  
Patricia Roberts, Ph.D., M.P.H.

Funding, 1<sup>st</sup> mtg

RFA release

TONIC en. TONIC fu.  
PIVENS en. PIVENS fu.  
"Database" Enrollment, n = 1136  
Database Follow up

FLINT en. FLINT fu.  
Database-2 Enrollment and Follow-Up, n=2372

2000 01 02 03 04 05 06 07 08 09 10 11 12 13 14

Pathology committee discussions on better characterization of ballooning  
Defined two new concepts for prospective evaluation:

- Classical vs Non-Classical Ballooning
- Severe vs Not Severe Ballooning

New definitions implemented with the first case in DB2

Courtesy of Dr. David Kleiner, NIH

NASH CRN

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### Classical vs Non-Classical

- **Classical ballooning**
  - Enlarged (>1.5x normal)
  - Cytoplasmic clearing
  - Cytoplasmic clumping
  - May have Mallory-Denk bodies
- **Non-Classical ballooning**
  - Typically in zone 3, perivenular
  - Smaller
  - Same cytoplasmic alterations
  - Lack Mallory-Denk bodies

NASH CRN

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### Non-Classic Ballooned Hepatocyte

Gill R. M. and Kakar S. Non-velocytic steatohepatitis: an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013), adapted with permission from Elsevier.

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## Classical vs. Non-Classical HB

Substantial agreement (weight kappa 0.76 (95% CI=0.64, 0.88))

NASH CRN

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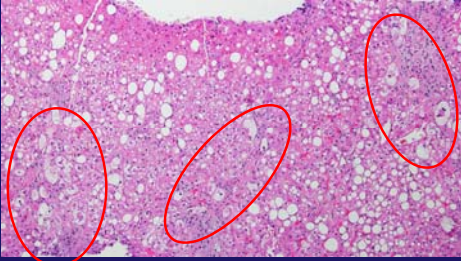
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## Severe Hepatocyte Balloons

- Several foci of classic hepatocyte balloons immediately apparent at low magnification (4x)



Courtesy of Dr. David Kleiner, NIH

NASH CRN

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

David E. Kleiner<sup>1</sup>, Elizabeth M. Brunt<sup>2</sup>, Patricia H. Bell<sup>2</sup>, Cynthia A. Behling<sup>4</sup>, Ryan M. Gill<sup>5</sup>, Cynthia D. Guy<sup>6</sup>, Brent A. Neuschwander-Tetri<sup>7</sup>, Arun J. Sanyal<sup>8</sup>, Mark L. Van Natta<sup>2</sup>; <sup>1</sup>Laboratory of Pathology, National Cancer Institute, Bethesda, MD; <sup>2</sup>Johns Hopkins School of Public Health, Baltimore, MD; <sup>3</sup>Washington University, St. Louis, MO; <sup>4</sup>Sharp Memorial Hospital, San Diego, CA; <sup>5</sup>University of California, San Francisco, San Francisco, CA; <sup>6</sup>Duke University Medical Center, Durham, NC; <sup>7</sup>Saint Louis University, St. Louis, MO; <sup>8</sup>Virginia Commonwealth University, Richmond, VA

- 1226 biopsies
- Demographic, anthropometric, laboratory data within 6 months of biopsy extracted

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## Proposed Modified Hepatocyte Balloon Score

Old Ballooning Score	Classical?	Severe?	New Ballooning Score	Description
0 - None	No	No	0	No ballooning
1 - Few or 2 - Many	No	No	1	Only Non-classical
1 - Few	Yes	No	2	Few Classical
2 - Many	Yes	No	3	Many Classical
2 - Many	Yes	Yes	4	Severe, Many Classical

- Reduces effect of many "non-classical" hepatocyte balloons when no classical ballooning seen
- Gives more weight to ballooning
- Better correlation with diagnosis

NASH CRN

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## Highlights presented at AASLD

1. Diagnosis
2. Fibrosis
3. Age and gender associations
4. Diabetes and metabolic syndrome
5. Liver enzymes

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## Summary and Conclusions

- We have proposed a new ballooning score based on careful morphological characterization of the range of ballooned hepatocytes
- The new balloon score doubles the dynamic range of the current balloon score
- The score shows excellent correlation with clinical disease features, as well as with patient demographics

NASH CRN

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## Acknowledgments

### \*Writing Group Members

- Pathologists
  - David Kleiner (NCI)
  - Elizabeth Brunt (Wash U)
  - Cynthia Behling (UCSD)
  - Melissa Contos (VCU)
  - Bill Cummings (IU)
  - Ryan M Gill (UCSF)
  - Cynthia Guy (DUKE)
  - Rish Pai (CWRU)
  - Danielle Allende (CWRU)
  - Michael Torbenson (JHU)
  - Matthew Yeh (UW)
- NIDDK Project Scientists
  - Ed Doo
  - Averell Sherker
- Clinical Centers and Principal Investigators
  - CWRU: Art McCullough
  - DUKE: Anna Mae Diehl
  - IU: Naga Chalasani
  - SLU: Brent Neuschwander-Tetri
  - UCSD: Rohit Loomba
  - UCSF: Norah Terrault
  - VMHC: Kris Kowdley
  - VCU: Arun Sanyal
- DCC (JH School of Public Health)
  - James Tonascia
  - Mark Van Natta
  - Pat Belt
  - Laura Wilson

And the many other investigators, research assistants, nurses and patients of the NASH CRN



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