Nonalcoholic steatohepatitis: what's new?

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A COLLECTION

OF

THE PUBLISHED WRITINGS

OF THE LATE

THOMAS ADDISON, M.D.,

THE NEW SYDENHAM SOCIETY,
LONDON.

MDCCCLXVIII.

OBSERVATIONS

ON

FATTY DEGENERATION OF THE LIVER.

With respect to the causes of this fatty degeneration of the liver, very little, or alsolutely nothing is known. In most of the cases which I have met with, there has been either positive or strong presumptive evidence that the individuals had indulged in spirit-drinking, and indeed the most exquisite case I ever saw in a young subject, occurred in a female who had for some time subsisted almost exclusively on ardent spirits. On the other hand the extreme frequency of the degeneration in France, where the people are but little given to such indulgences, throws considerable doubt upon such an origin of the complaint, whilst its great frequency there in conjunction with phthisis, would almost lead to a belief that it has some connection with a scrofulous tendency, a belief which, I confess, I am strongly disposed to entertain.

THE LIVER IN OBESITY

A. M. A. ARCHIVES OF INTERNAL MEDICINE

SAMUEL ZELMAN, M.D.

TOPEKA, KAN.

Summary of Argument.—Experimental obesity in animals is uniformly accompanied with hepatic damage. Previous evidence of hepatic damage in human obesity is limited to disturbances of carbohydrate tolerance. The present study indicates the uniform presence of liver damage in human obesity, as manifested by liver-function tests and biopsies. Confirmation is found in the greatly increased incidence of cirrhosis of the liver in the obese, as indicated by insurance statistics. An explanation of the liver damage of obesity is found in (*a*) the high caloric requirement of the obese, constituting a functional overload upon the liver, (*b*) the high-carbohydrate, high-fat, low-protein diet customary among the obese, and (*c*) the increased requirement of choline and vitamins of the B complex imposed by the quantitative and qualitative nature of the obese diet. The reducing program should consist of a high-protein diet supplemented by choline and vitamins of the B complex.

TABLE 4.—*Histologic Changes in Liver in Obesity**

Case No.	Liver Cell Degeneration	Focal Necrosis	Bile Pigment Retention	Fat Vacuolation	Liver Cell Regeneration	Kupffer Cell Mobilization	Periportal Cellular Infiltrate	Periportal Fibrosis	Total Abnormal†
1	2	0	0	2	1	1	2	2	4
2	1	1	2	1	1	0	1	1	1
3	2	1	0	3	2	0	1	1	3
4	1	0	2	0	1	0	1	1	1
5	2	1	2	1	2	1	1	2	4
6	3	1	2	2	2	1	1	1	4
7	2	1	1	2	2	1	3	2	5
8	1	0	1	1	2	1	1	2	2
9	1	0	1	0	2	1	1	0	1
10	2	1	1	3	2	1	1	1	3
11	2	1	0	1	2	2	3	2	5
12	2	1	1	2	2	1	1	1	3
13	2	1	2	1	1	1	2	2	4
14	2	2	1	2	1	2	2	3	6
15	1	0	1	1	1	1	1	1	0
16	..	..	..	..	..	..	..	..	..
17	1	1	2	1	1	1	1	3	2
18	1	1	2	2	1	1	2	1	3
19	1	1	2	2	2	1	1	2	4
20	2	1	2	2	1	1	1	1	3
Total Abnormal †	11	1	9	10	10	2	6	9	3.1 (Average per Case)
% Abnormal	58	5	47	53	53	11	32	47	

REVIEW: Nonalcoholic steatohepatitis

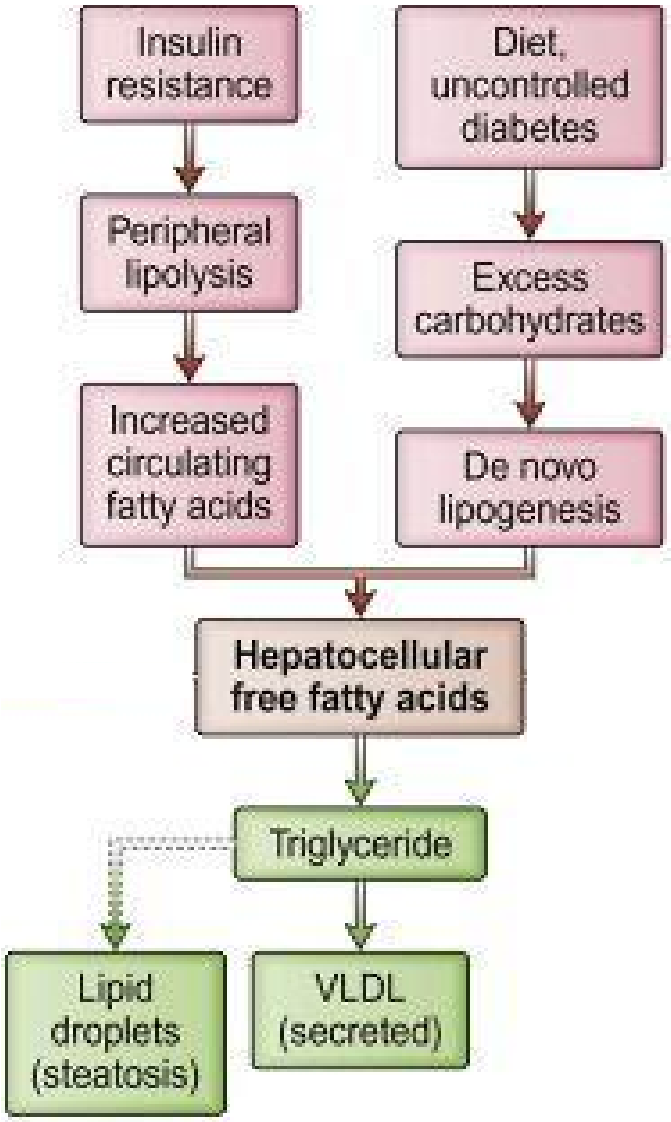
Journal of Gastroenterology and Hepatology (1997) 12, 398–403

JURGEN LUDWIG,* DOUGLAS B McGILL† AND KEITH D LINDOR†

HISTORY OF THE DISEASE

Fat people have fat livers. This has been known for about 40 years; even the appearance of inflammation and fibrosis in some of these fatty livers was described that long ago.[1] However, the finding was largely ignored well into the 1960s. At that time, the condition that we now call nonalcoholic steatohepatitis (NASH) emerged from the spectrum of alcoholic liver diseases. The recognition of this apparently new disease resulted from the then popular intestinal bypass surgery for morbid obesity.[2–9] In many patients who had been treated in this manner, a severe steatohepatitic liver disease appeared as an unexpected and certainly undesired complication. The resemblance between the biopsy features observed in these patients and the well-known findings in alcoholic liver disease was soon noted.[10] The

Triglyceride accumulation leads to steatosis

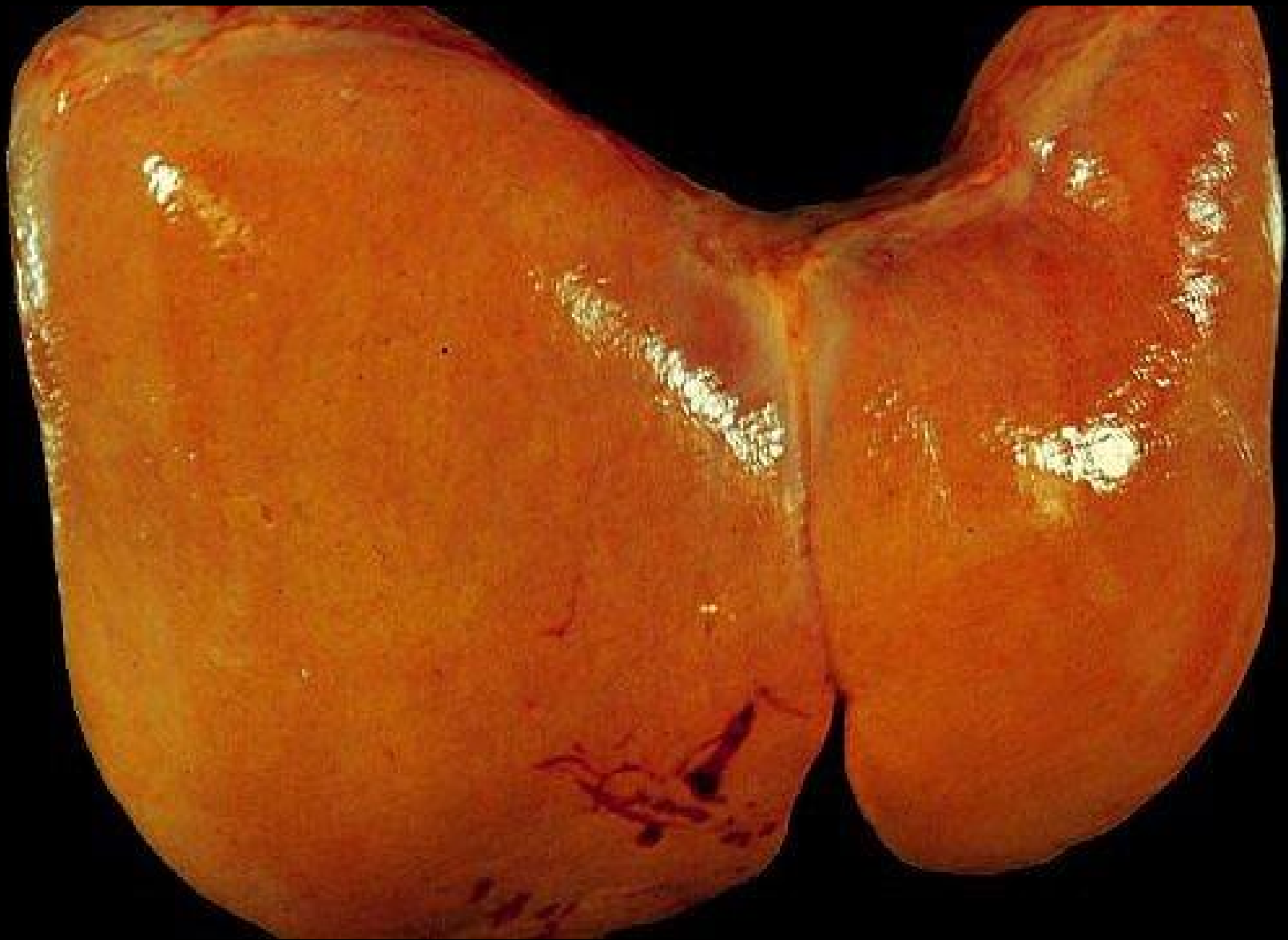


Genetic risk factors for hepatic steatosis

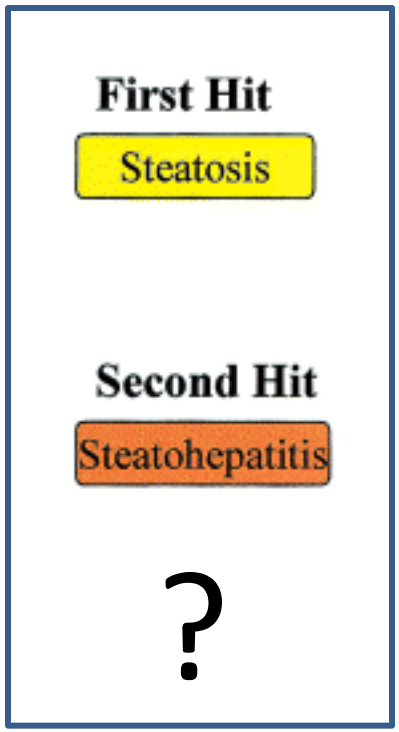
Gene	Protein
PNPLA3	Patatin-like phospholipase domain–containing protein 3
PPP1R3B	Glycogen binding subunit of protein phosphatase 1
NCAN	Neurocan
GCKR	Glucokinase regulatory protein
LYPLAL1	Lysophospholipase-like 1
APOC3	Apolipoprotein C3

MacSween's Pathology of the Liver, 6th Edition
S. Romeo at al. Nat. Genet 40, 1461
E.K Spelites et at PloS Genet. 7, e1001324
KF Petersen et al N Engl. J Med, 362, 1082

Fatty Liver



What Causes Steatohepatitis?



↑ FFA

↓

↑ lipotoxic intermediates

- lysophosphatidylcholines
- ceramides
- phosphatidic acids
- diacylglycerol

↓

Proinflammatory cytokines
- TNF-α
- IL-6

ER stress

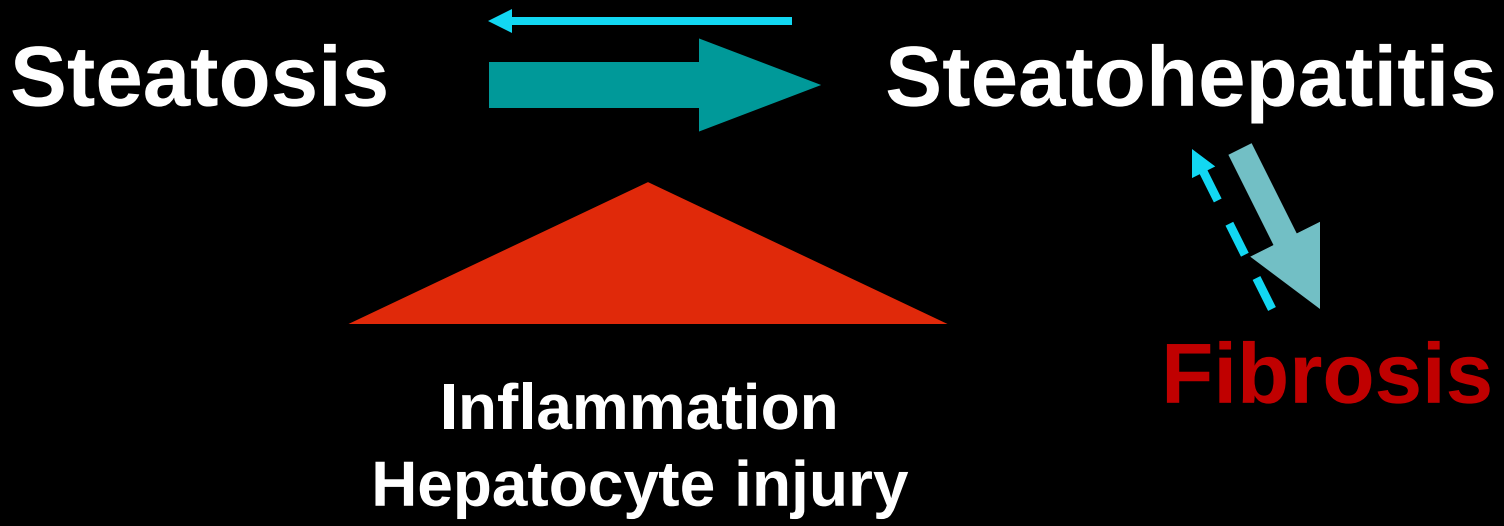
Mitochondria dysfunction

↓

Hepatocellular injury
Inflammation

G.S.Hotamisligil, Cell 140,900 (2011)
A.E. Feldstein. Semin Liver Dis. 30, 391

Non-Alcoholic Fatty Liver Disease (NAFLD)



Most common chronic liver disease in the United States

Why Diagnose NASH?

- **Potential interventions:**
  - **Lifestyle modification**
  - **Several ongoing medication trials**
  - **Bariatric surgery**
- **Prognosis:**
  - **NASH is progressive**
  - **Cirrhosis and its complications in some patients**

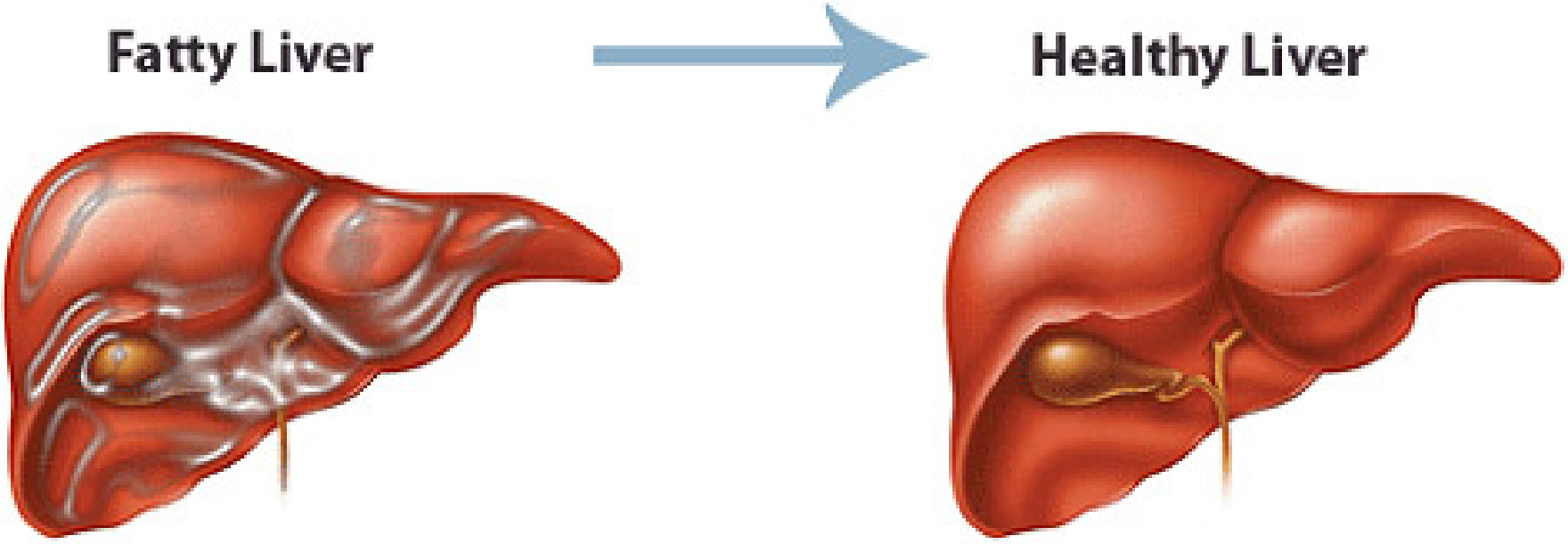
Fatty Liver Treatment Focus Points

Healthy diet

Exercise

Weight loss

Improved diabetic control



Promrat et al., Hepatology 2010.

Cirrhosis?

Jaundice?

Ascites?

TAKE A NAP!

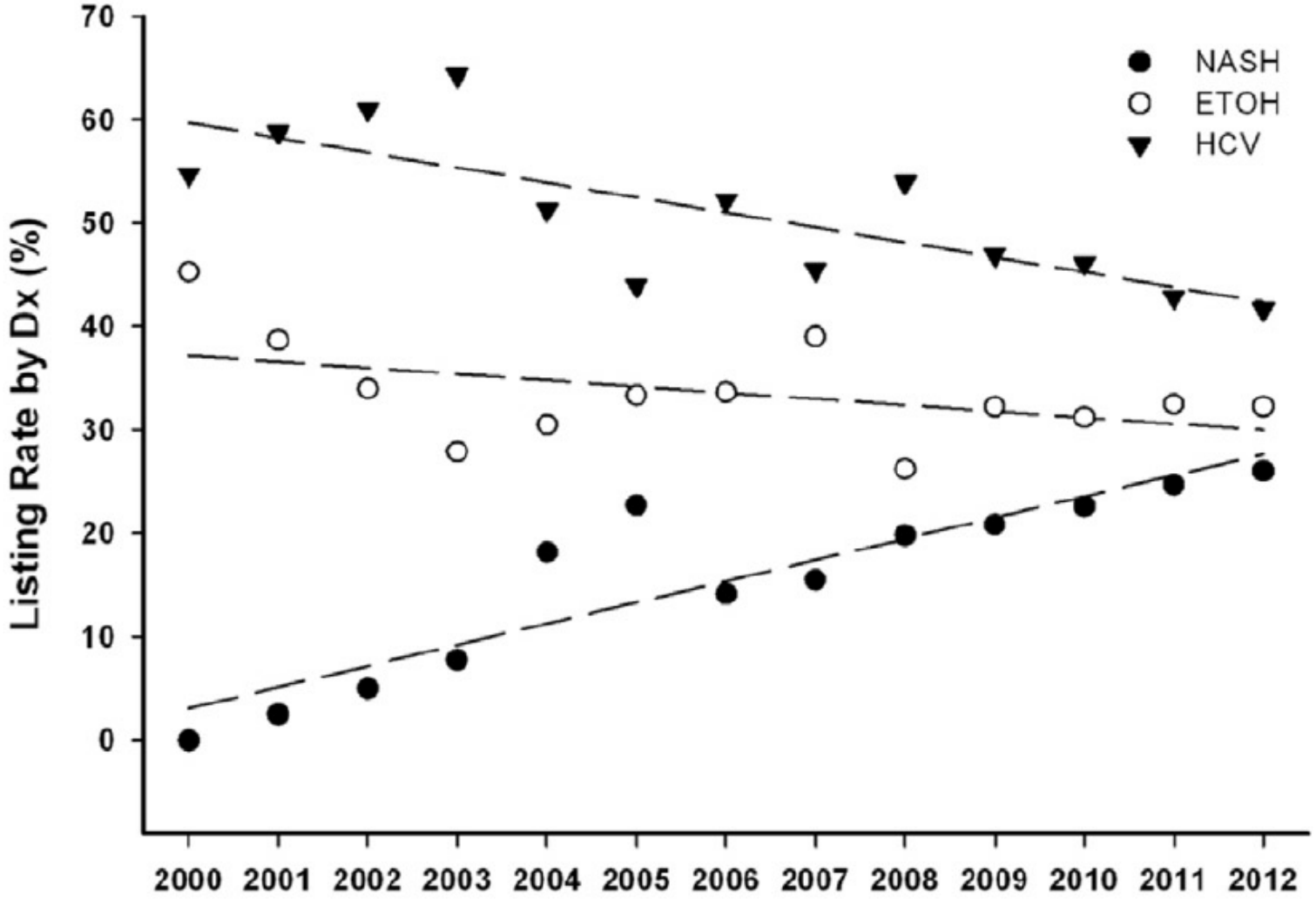
Napping is the recommended medical treatment by 10/10 doctors*

** Liver transplantation also effective in selected cases*

Increasing prevalence of nonalcoholic steatohepatitis as an indication for liver transplantation

(Surgery 2014;156:1049-56.)

R. Cutler Quillin, III, MD, Gregory C. Wilson, MD, Jeffrey M. Sutton, MD, Dennis J. Hanseman,



The Natural History of Nonalcoholic Fatty Liver Disease: A Population-Based Cohort Study

GASTROENTEROLOGY 2005;129:113–121

LEON A. ADAMS,* JAMES F. LYMP,† JENNY ST. SAUVER,§ SCHUYLER O. SANDERSON,‖ KEITH D. LINDOR,* ARIEL FELDSTEIN,* and PAUL ANGULO*

*Division of Gastroenterology and Hepatology, †Division of Biostatistics, §Division of Epidemiology, and ‖Division of Anatomic Pathology, Mayo Clinic College of Medicine, Rochester, Minnesota

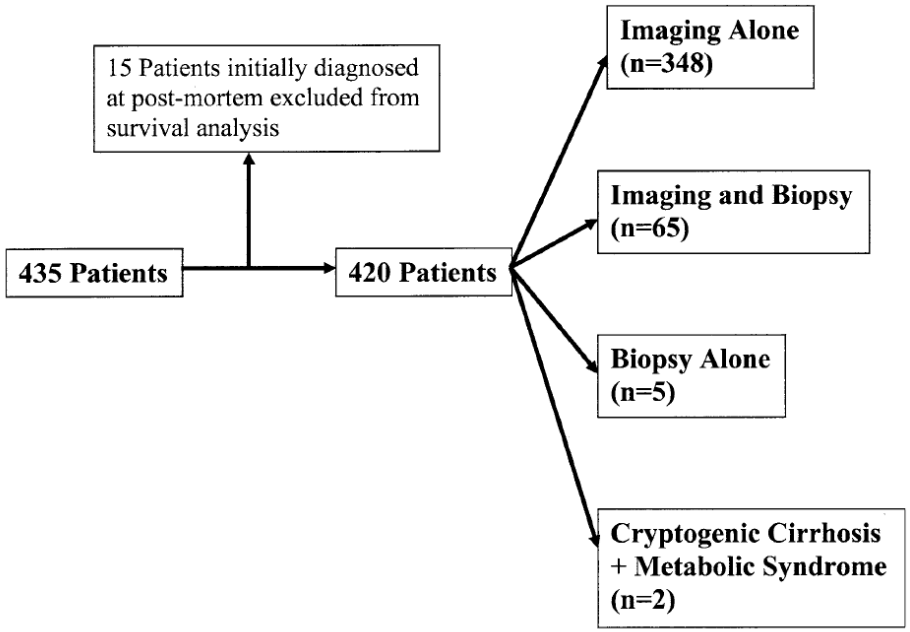


Figure 1. Methods of diagnosis of patients with NAFLD in Olmsted County (1980–2000).

mean follow-up was 7.6 ± 4.0 years

Table 2. Cause of Death

Etiology	N (%) (N = 53)
Malignancy	15 (28%)
Bowel	5 (9%)
Pancreas	3 (8%)
Breast	2 (4%)
Other	5 (9%)
Ischemic heart disease	13 (25%)
Liver disease	7 (13%)
Liver failure	4 (7%)
Variceal hemorrhage	2 (4%)
Hepatocellular carcinoma	1 (2%)
Infection	6 (11%)
Pneumonia	5 (9%)
Sepsis	1 (2%)
Obstructive lung disease	2 (4%)
Congestive cardiac failure	2 (4%)
Cerebrovascular accident	1 (2%)
Gastrointestinal bleed	1 (2%)
Pulmonary embolus	1 (2%)
Aortic aneurysm dissection	1 (2%)
Smoke inhalation	1 (2%)
Retroperitoneal hemorrhage	1 (2%)
Unknown	2 (4%)

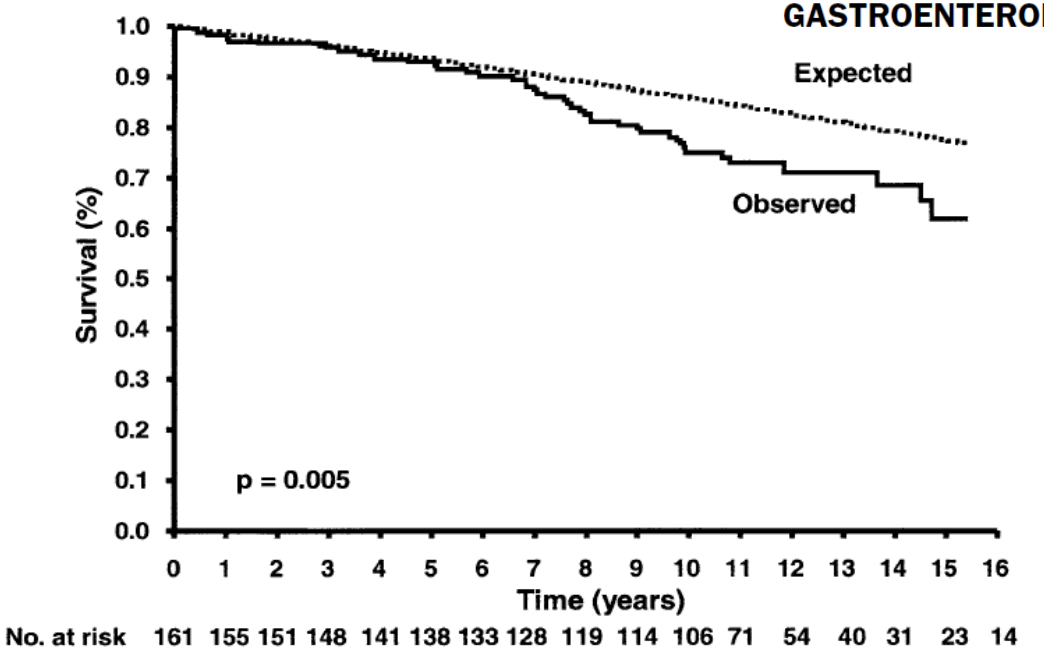
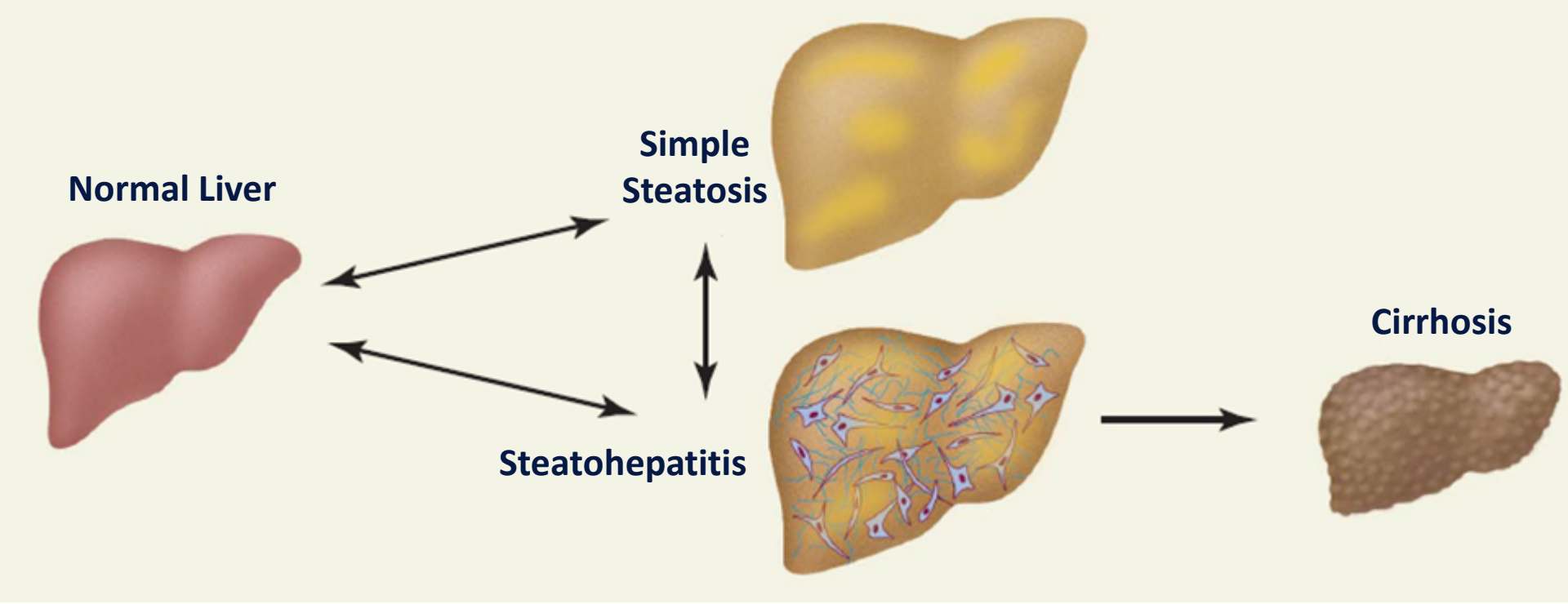


Table 3. Baseline Predictors of Mortality by Multivariate Proportional Hazard Modeling

Variable	Parameter estimate	Standard error	Hazard ratio (95% CI)	*P* value
Age (per decade)	0.08	0.01	2.2 (1.7–2.7)	<.0001
IFG/diabetes	0.97	0.34	2.6 (1.3–5.2)	.005
Cirrhosis at baseline	1.13	0.48	3.1 (1.2–7.8)	.02
Smoking	0.35	0.29	1.4 (0.8–2.5)	.2
Hypertension	−0.32	0.29	0.7 (0.4–1.3)	.3
Ischemic heart disease	0.08	0.37	1.1 (0.5–2.3)	.8



NAFLD patients	N = 132	Cirrhosis (X = 8.3 yrs f/u)
Steatosis	49 (37%)	2 (4%)
Steatosis + lobular inflammation	10 (8%)	0 (0%)
Steatosis + hepatocyte ballooning	19 (14%)	4 (21%)
Steatosis + ballooning + Mallory-Denk or fibrosis	54 (41%)	14 (26%)

Matteoni et al., Gastroenterology 1999.

Meta-analysis: Natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity

Annals of Medicine, 2011; 43: 617–649

GIOVANNI MUSSO[1], ROBERTO GAMBINO[2], MAURIZIO CASSADER[2] & GIANFRANCO PAGANO[2]

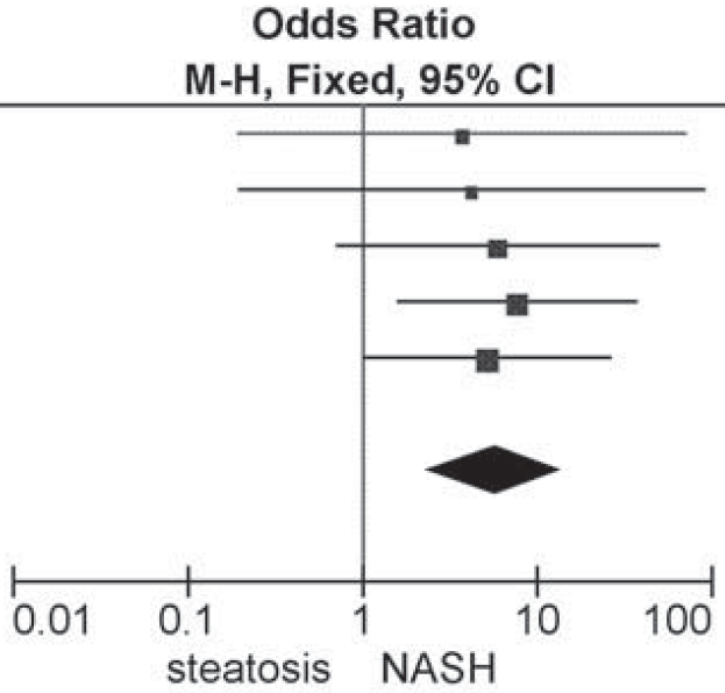
liver-related mortality

Study or Subgroup	Weight	Odds Ratio M-H, Fixed, 95% CI
Adams 2005	13.3%	3.71 [0.20, 70.19]
Ekstedt 2006	10.2%	4.21 [0.20, 89.42]
Matteoni 1999	20.4%	5.91 [0.71, 48.83]
Rafiq 2009	27.5%	7.66 [1.61, 36.52]
Soderberg 2009	28.6%	5.17 [1.03, 26.06]
Total (95% CI)	**100.0%**	**5.71 [2.31, 14.13]**

Total events

Heterogeneity: Chi² = 0.27, df = 4 (P = 0.99); I² = 0%

Test for overall effect: Z = 3.77 (P = 0.0002)



HEPATOLOGY, Vol. 55, No. 6, 2012

The Diagnosis and Management of Non-Alcoholic Fatty Liver Disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association

Naga Chalasani, MD, FACG,[1] Zobair Younossi, MD, FACG,[2] Joel E. Lavine, MD, PhD,[3] Anna Mae Diehl, MD,[4] Elizabeth M. Brunt, MD,[5] Kenneth Cusi, MD,[6] Michael Charlton, MD,[7] and Arun J. Sanyal, MD[8]

Table 4. Risk Factors Associated with NAFLD

Conditions with established association	Conditions with emerging association*
Obesity	Polycystic ovary syndrome
Type 2 diabetes mellitus	Hypothyroidism
Dyslipidemia	Obstructive Sleep apnea
Metabolic syndrome**	Hypopituitarism
	Hypogonadism
	Pancreato-duodenal resection

Correlation of Paired Liver Biopsies in Morbidly Obese Patients With Suspected Nonalcoholic Fatty Liver Disease

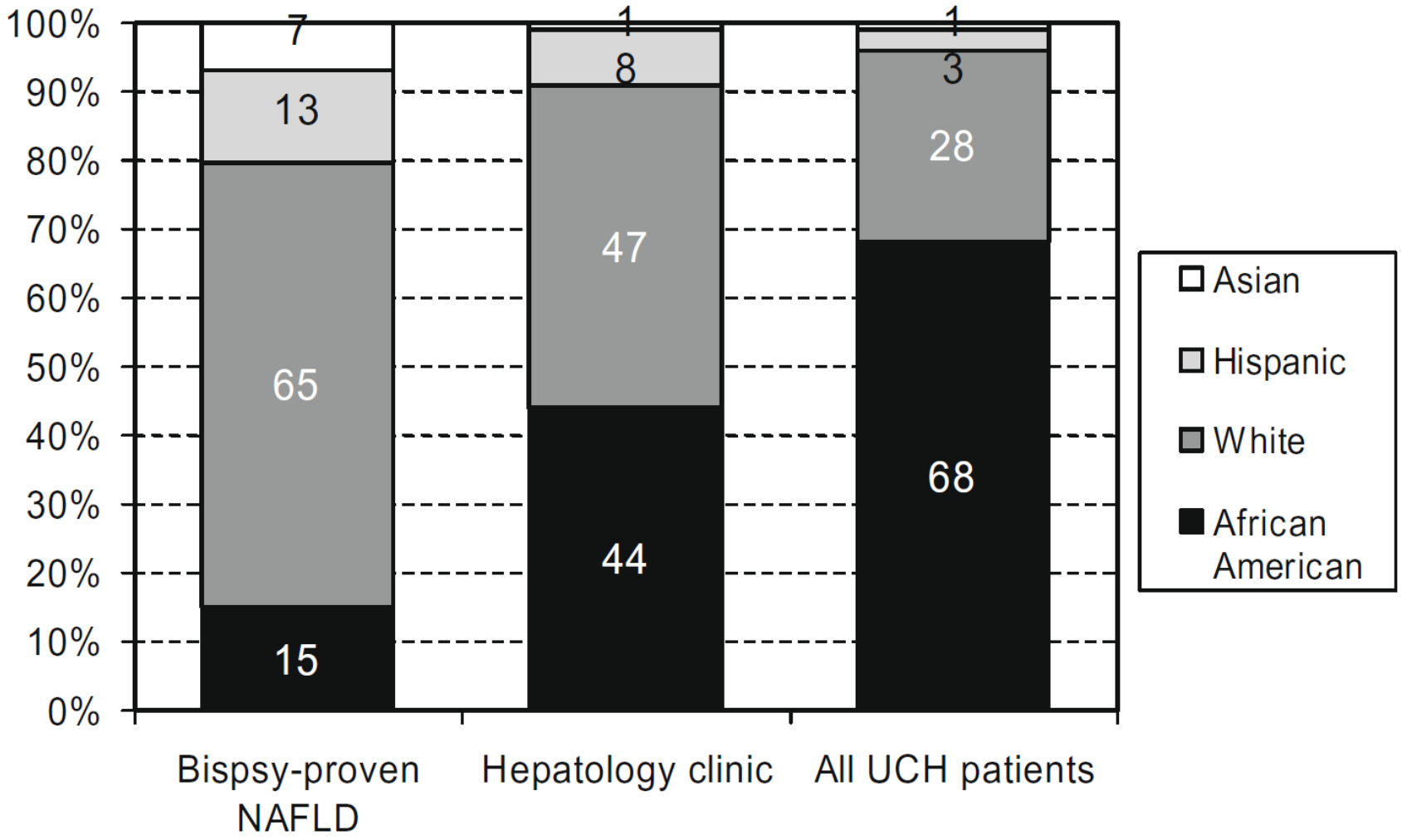
Raphael B. Merriman,[1] Linda D. Ferrell,[2] Marco G. Patti,[3] Shiobhan R. Weston,[1] Mark S. Pabst,[1] Bradley E. Aouizerat,[4] and Nathan M. Bass[1]

- 41 patients undergoing bariatric surgery
- BMI median = 50 (34.5 to 69.8)
- Intra-operative liver biopsy:
  - 43.8% Normal
  - 29.3% Steatosis
  - 26.9% NASH

Influence of ethnicity on histological differences in non-alcoholic fattv liver disease☆

Journal of Hepatology 50 (2009) 797–804

Smruti R. Mohanty[1,*], Tara N. Troy[1], Dezheng Huo[2], Bridget L. O'Brien[3], Donald M. Jensen[1], John Hart[4]



Influence of ethnicity on histological differences in non-alcoholic fattv liver disease☆

Journal of Hepatology 50 (2009) 797–804

Smruti R. Mohanty[1,*], Tara N. Troy[1], Dezheng Huo[2], Bridget L. O'Brien[3], Donald M. Jensen[1], John Hart[4]

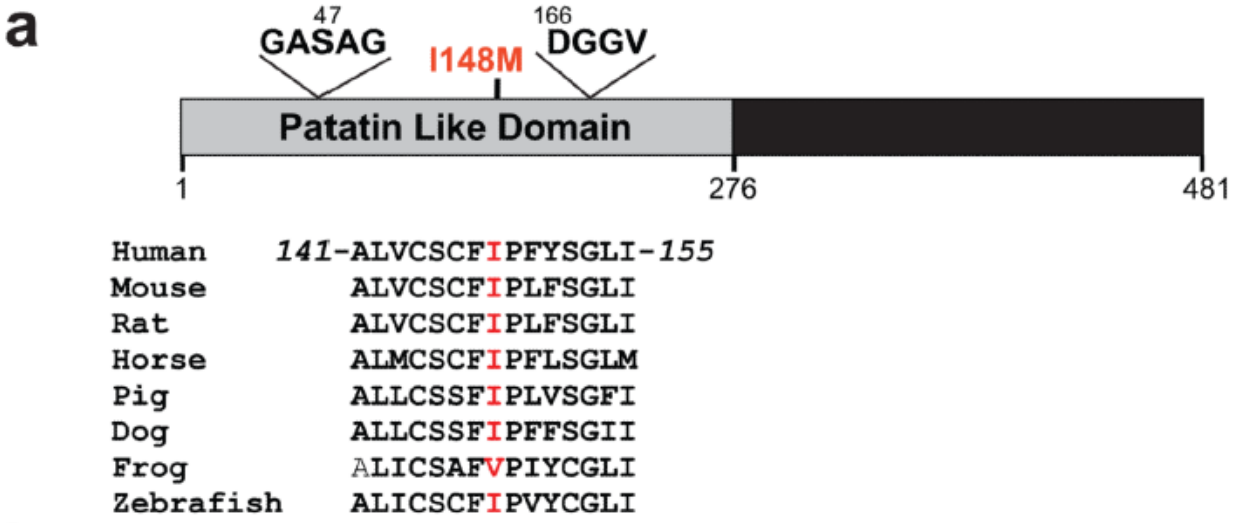
Pathologic findings of NAFLD patients: ***ordinal and binary logistic regressions.***

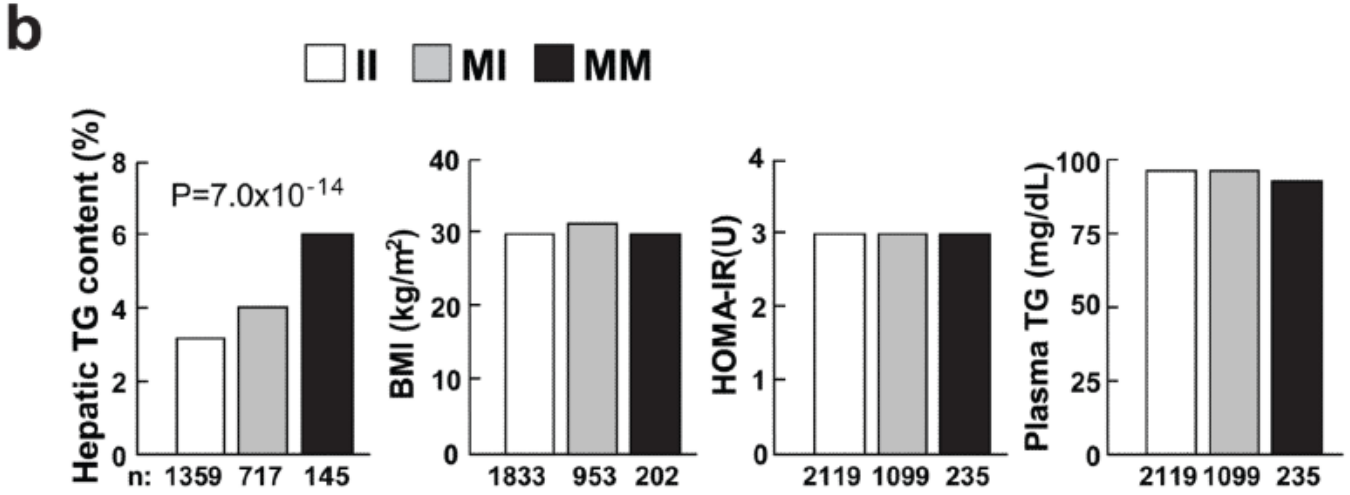
	White ($n = 154$)	African American ($n = 36$)	Hispanic ($n = 32$)	Asian ($n = 16$)
Steatosis				
1	57 (37.0)	20 (55.6)	14 (43.8)	6 (37.5)
2	39 (25.3)	9 (25.0)	7 (21.9)	7 (43.8)
3	58 (37.7)	7 (19.4)	11 (34.4)	3 (18.8)
OR (95% CI)	1.0 (ref.)	0.44 (0.22–0.89)	0.80 (0.39–1.61)	0.69 (0.28–1.69)
Pair-wise p value		0.02	0.54	0.42
Inflammation				
0	99 (64.3)	27 (75.0)	17 (53.1)	6 (37.5)
1	42 (27.3)	7 (19.4)	13 (40.6)	9 (56.3)
2	13 (8.4)	2 (5.6)	2 (6.3)	1 (6.3)
OR (95% CI)	1.0 (ref.)	0.60 (0.26–1.35)	1.44 (0.69–3.02)	2.31 (0.90–5.93)
Pair-wise p value		0.22	0.33	0.08
Fibrosis				
0	88 (57.1)	25 (69.4)	19 (59.4)	5 (31.3)
1	34 (22.1)	7 (19.4)	4 (12.5)	10 (62.5)
2	14 (9.1)	3 (8.3)	3 (9.4)	1 (6.3)
3	9 (5.8)	0	3 (9.4)	0
4	9 (5.8)	1 (2.8)	3 (9.4)	0
OR (95% CI)	1.0 (ref.)	0.56 (0.26–1.19)	1.09 (0.51–2.33)	1.46 (0.62–3.44)
Pair-wise p value		0.13	0.83	0.39
Ballooning				
0	55 (35.7)	16 (44.4)	11 (34.4)	2 (12.5)
1	74 (48.1)	15 (41.7)	13 (40.6)	9 (56.3)
2	25 (16.2)	5 (13.9)	8 (25.0)	5 (31.3)
OR (95% CI)	1.0 (ref.)	0.73 (0.36–1.45)	1.29 (0.62–2.68)	2.67 (1.03–6.93)
Pair-wise p value		0.37	0.50	0.04
Mallory bodies				
No (0)	123 (79.9)	30 (83.3)	20 (62.5)	12 (75.0)
Yes (1)	31 (20.1)	6 (16.7)	12 (37.5)	4 (25.0)
OR (95% CI)	1.0 (ref.)	0.79 (0.30–2.07)	2.38 (1.05–5.39)	1.32 (0.40–4.38)
Pair-wise p value		0.64	0.04	0.65

Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease

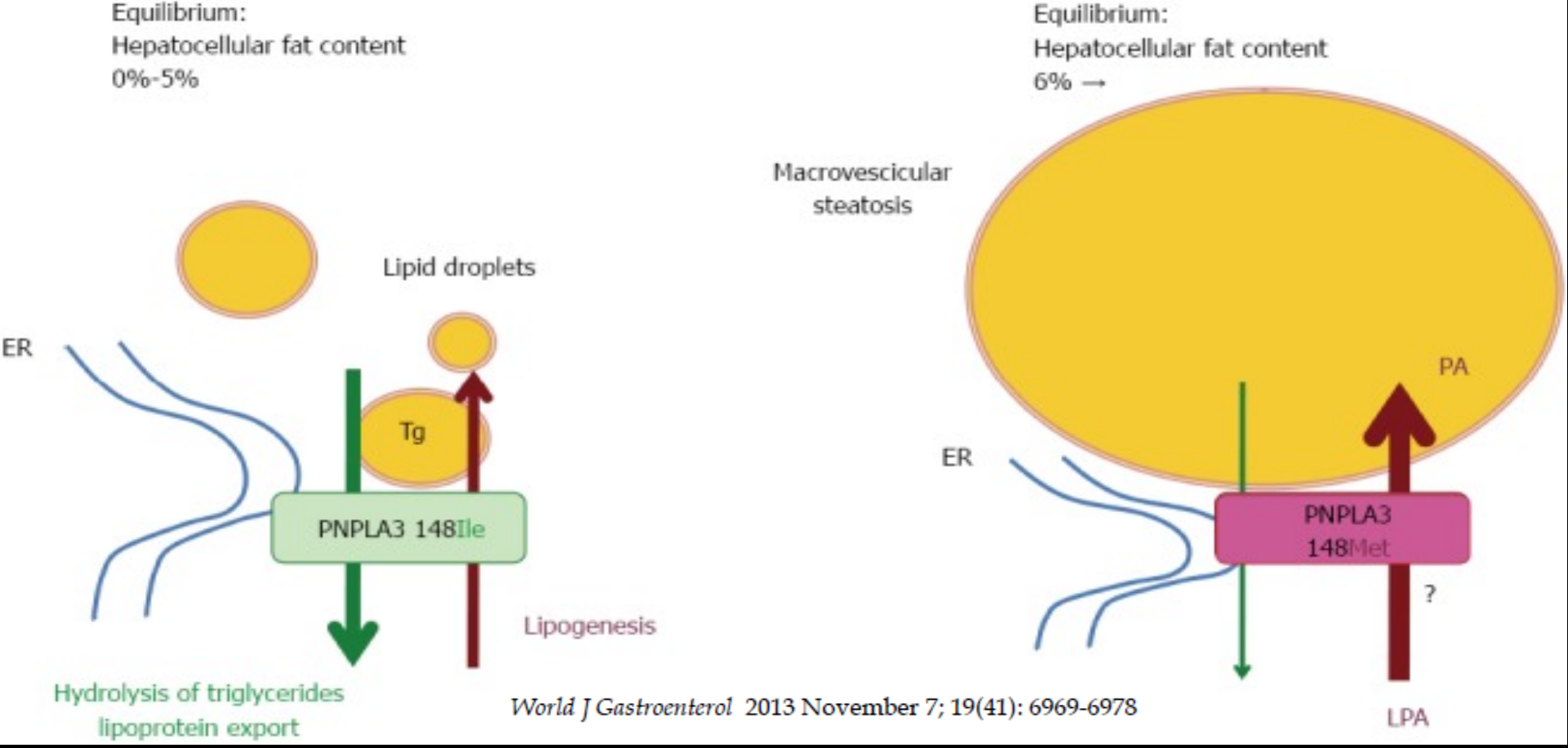
Nat Genet. 2008 December ; 40(12): 1461–1465.

Stefano Romeo[1,*], **Julia Kozlitina**[2,3,*], **Chao Xing**[1,2], **Alexander Pertsemlidis**[1], **David Cox**[4], **Len A. Pennacchio**[5], **Eric Boerwinkle**[6], **Jonathan C. Cohen**[1], and **Helen H. Hobbs**[1,7]





Proton magnetic resonance spectroscopy



World J Gastroenterol 2013 November 7; 19(41): 6969-6978

- 53 KD protein (adiponutrin) – lipid acyl hydrolase
- Expressed primarily in hepatocytes
- Associated with NASH and ASH
- Associated with fibrosis in ASH and NASH
- Associated with fibrosis in chronic HCV hepatitis
- Associated with HCC

Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease

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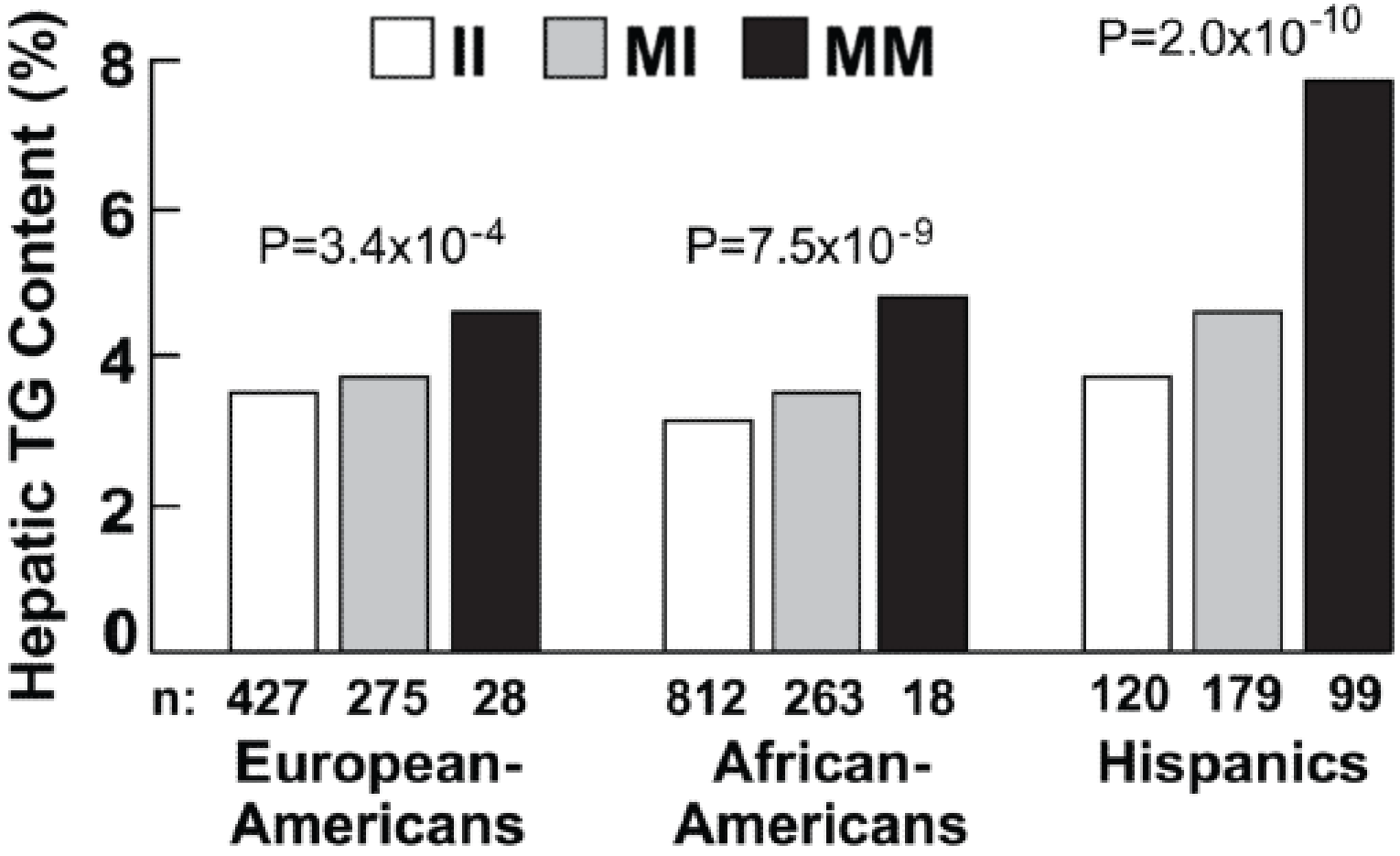
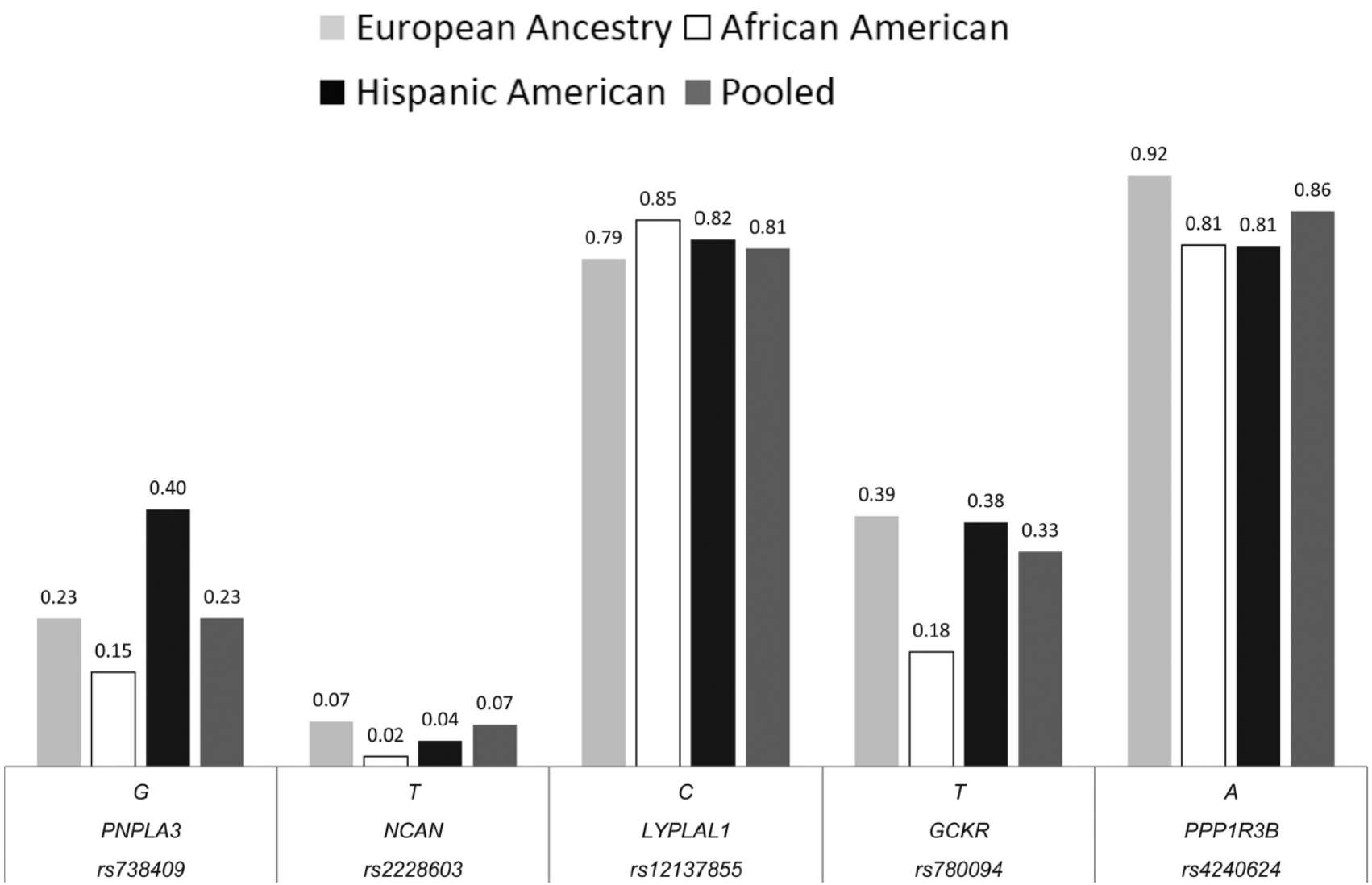
Stefano Romeo[1,*], **Julia Kozlitina**[2,3,*], **Chao Xing**[1,2], **Alexander Pertsemlidis**[1], **David Cox**[4], **Len A. Pennacchio**[5], **Eric Boerwinkle**[6], **Jonathan C. Cohen**[1], and **Helen H. Hobbs**[1,7]



Characterization of European Ancestry Nonalcoholic Fatty Liver Disease-Associated Variants in Individuals of African and Hispanic Descent

(HEPATOLOGY 2013;58:966-975)

Nicholette D. Palmer,[1*] Solomon K. Musani,[2*] Laura M. Yerges-Armstrong,[3*] Mary F. Feitosa,[4*]

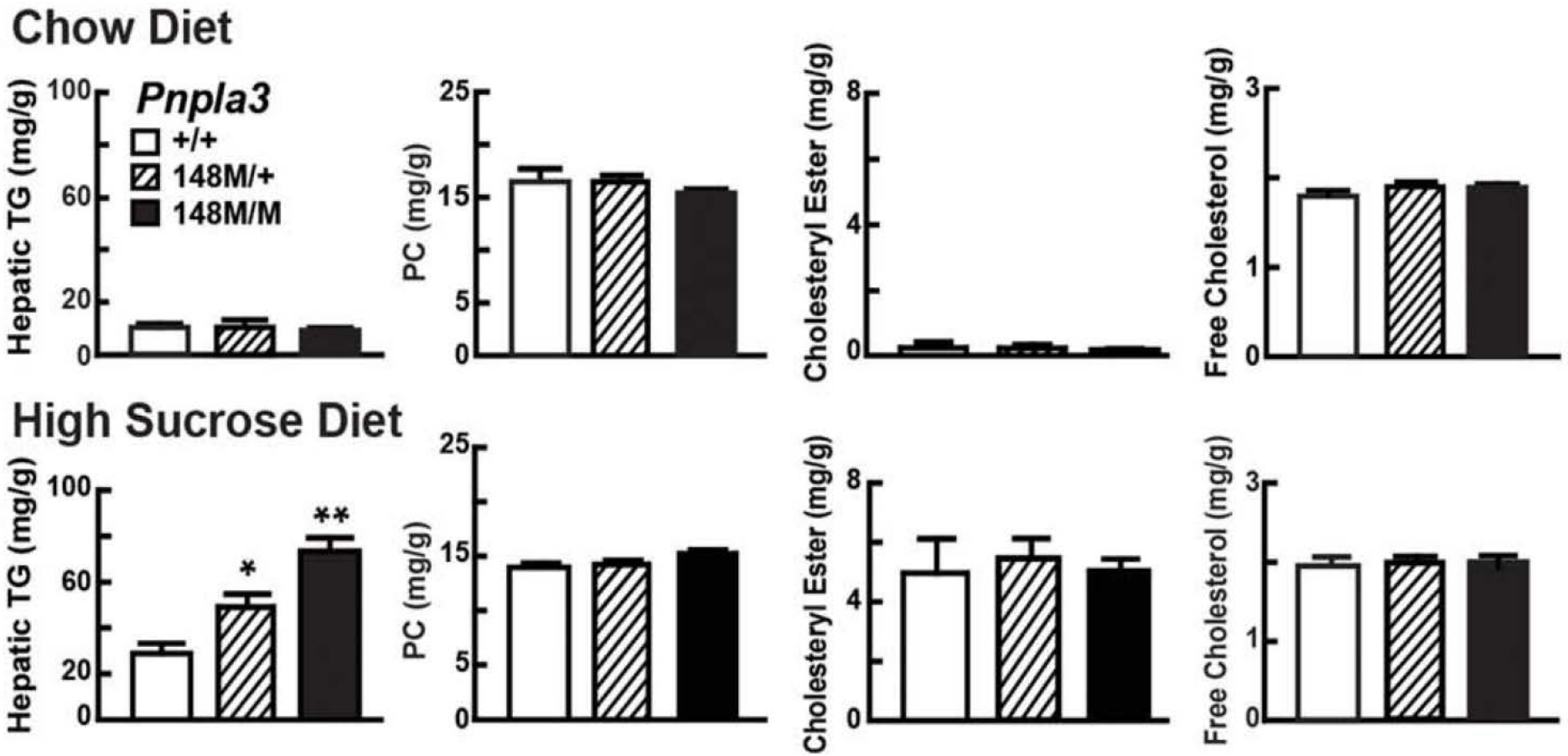


Pnpla3I148M Knockin Mice Accumulate PNPLA3 on Lipid Droplets and Develop Hepatic Steatosis

(HEPATOLOGY 2014;00:000-000)

Eriks Smagris,[1] Soumik BasuRay,[1] John Li,[1] Yongcheng Huang,[1] Ka-man V. Lai,[2] Jesper Gromada,[2] Jonathan C. Cohen,[1] and Helen H. Hobbs[1,3]

A **Catalytically nonfunctional protein**



HEPATOLOGY, Vol. 55, No. 6, 2012

Non-invasive Methods for Assessing NAFLD

Diagnosis of NASH:

1. **Circulating levels of CK18**
2. **Presence of metabolic syndrome**

Presence of advanced fibrosis:

1. **NAFLD Fibrosis Score**
2. **Enhanced Liver Fibrosis Panel**
3. **Transient elastography**

When to obtain a liver biopsy in patients with NAFLD?

Liver biopsy remains the gold standard for characterizing liver histology in patients with NAFLD. However, it is expensive and carries some morbidity and very rare mortality risk. Thus, it should be performed in those who would benefit the most from diagnostic, therapeutic guidance, and prognostic perspectives.

Recommendations

13. Liver biopsy should be considered in patients with NAFLD who are at increased risk to have steatohepatitis and advanced fibrosis. (Strength – 1, Evidence - B)

14. The presence of metabolic syndrome and the NAFLD Fibrosis Score may be used for identifying patients who are at risk for steatohepatitis and advanced fibrosis. (Strength – 1, Evidence - B)

15. Liver biopsy should be considered in patients with suspected NAFLD in whom competing etiologies for hepatic steatosis and co-existing chronic liver diseases cannot be excluded without a liver biopsy. (Strength – 1, Evidence - B)

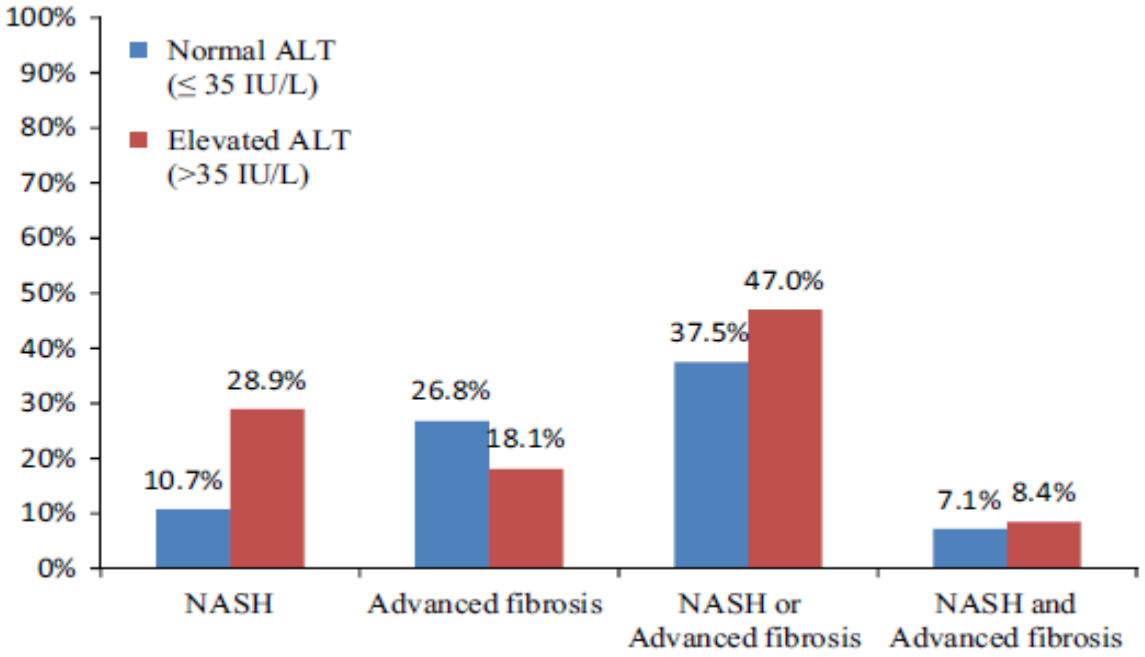
Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD)

Siddharth Verma[1], Donald Jensen[2], John Hart[3] and Smruti R. Mohanty[1]

Liver Int. 2013: 33: 1398–1405

Table 2. Histological characteristics of all patients and patients with normal and elevated alanine aminotransferase (ALT) levels*

Histological finding	All patients (n = 238)	Normal ALT (n = 56)	Elevated ALT (n = 166)	P value
NAS score (mean)	3.2 ± 1.7	2.4 ± 1.5	3.4 ± 1.7	**<0.01**
<5	181 (76)	50 (89.3)	118 (71.1)	
≥5	57 (24)	6 (10.7)	48 (28.9)	
Steatosis ≥2	141 (59.2)	21 (38)	108 (65.1)	**<0.01**
Ballooning ≥1	43 (18.1)	47 (83.9)	134 (80.7)	0.6
Inflammation ≥2	18 (7.6)	1 (1.8)	17 (10.3)	**0.04**
Fibrosis score (mean)	0.8 ± 1.1	1.1 ± 1.4	0.7 ± 1.0	0.19
<2	192 (80.7)	41 (73.2)	136 (81.9)	
≥2	46 (19.3)	15 (26.8)	30 (18.1)	



Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD)

Siddharth Verma[1], Donald Jensen[2], John Hart[3] and Smruti R. Mohanty[1]

Liver Int. 2013: 33: 1398–1405

Table 3. Sensitivity and Specificity of alanine aminotransferase (ALT) for predicting non-alcoholic steatohepatitis (NASH) and advanced fibrosis

	ALT value			
	<35	36–52	53–70	>70
NASH				
Sensitivity (%)	88.9	88.9–72.2	72.2–50	50
Specificity (%)	28.6	28.6–50.6	50.6–60.7	60.7
Advanced fibrosis				
Sensitivity (%)	68.9	68.9–48.9	48.8–40	40
Specificity (%)	22.6	22.6–43.5	43.5–57.6	57.6

Histologic Features of NASH (and ASH)

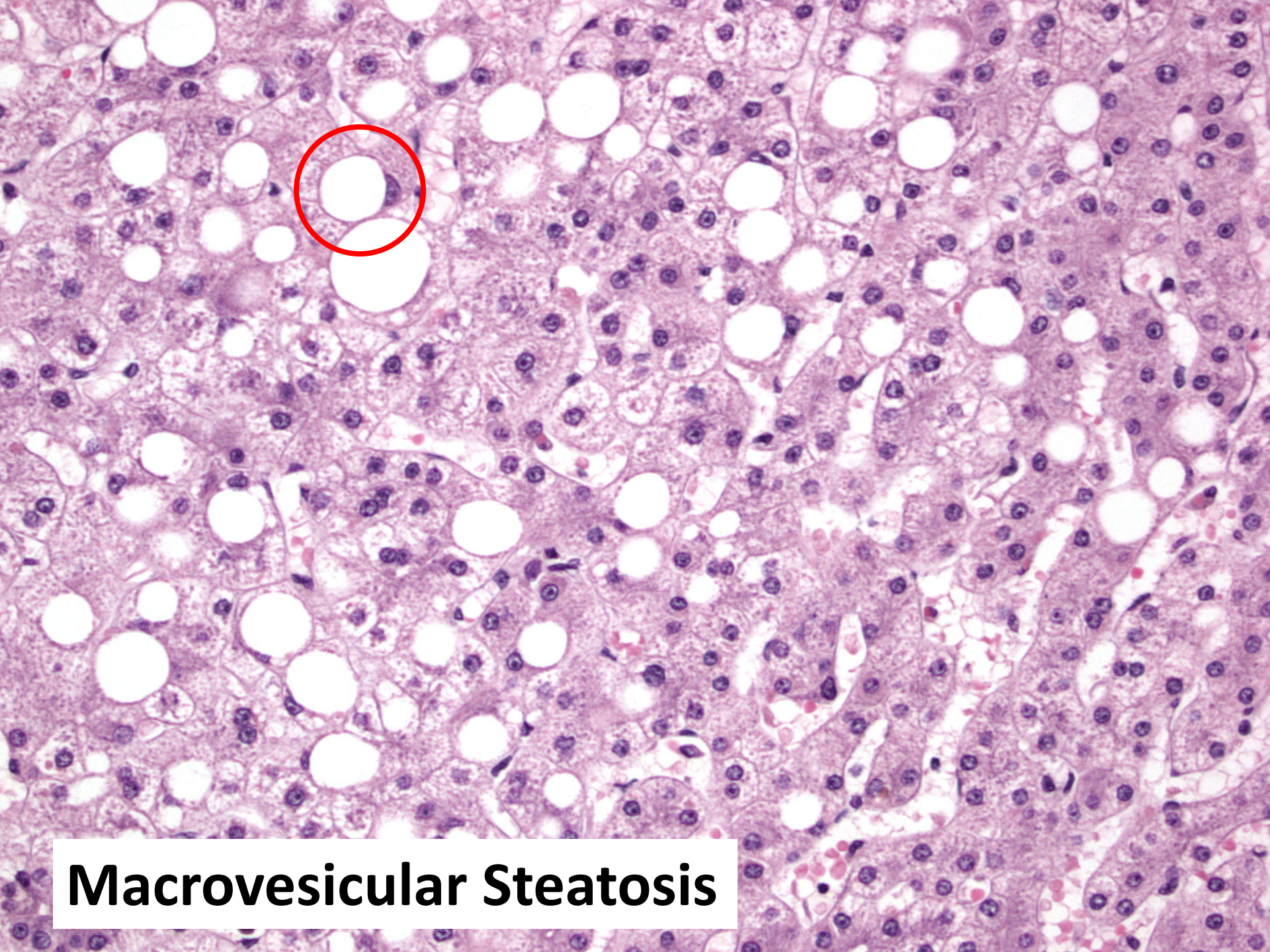
- **Steatosis - predominantly macrovesicular**
- **Inflammation - neutrophilic and lymphocytic**
- **Hepatocyte injury - +/- Mallory-Denk bodies**
- **+ / - fibrosis – centrilobular and/or portal/periportal**

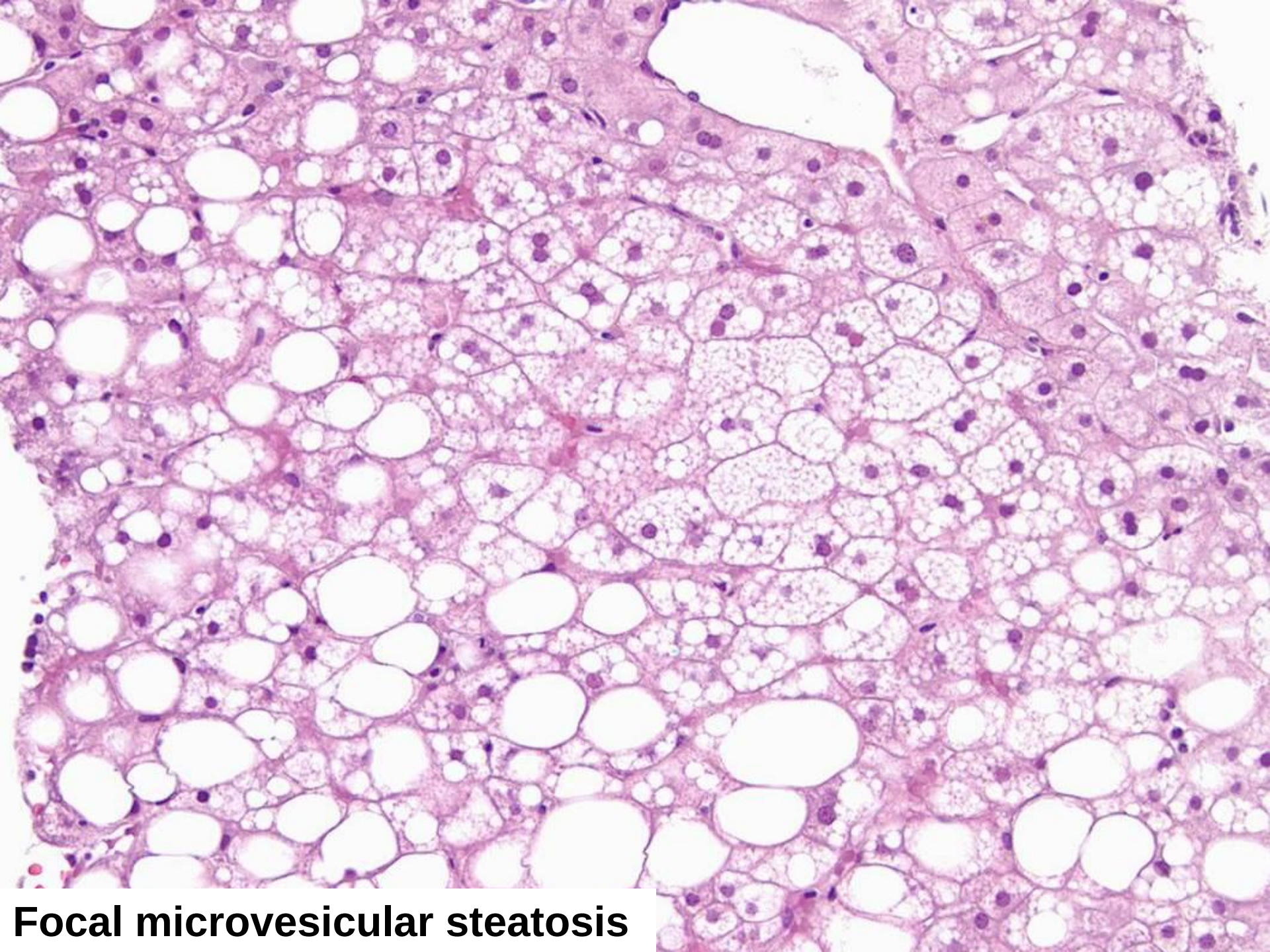
Brunt EM, Clin Liv Dis 2009

Histologic Features of Steatohepatitis

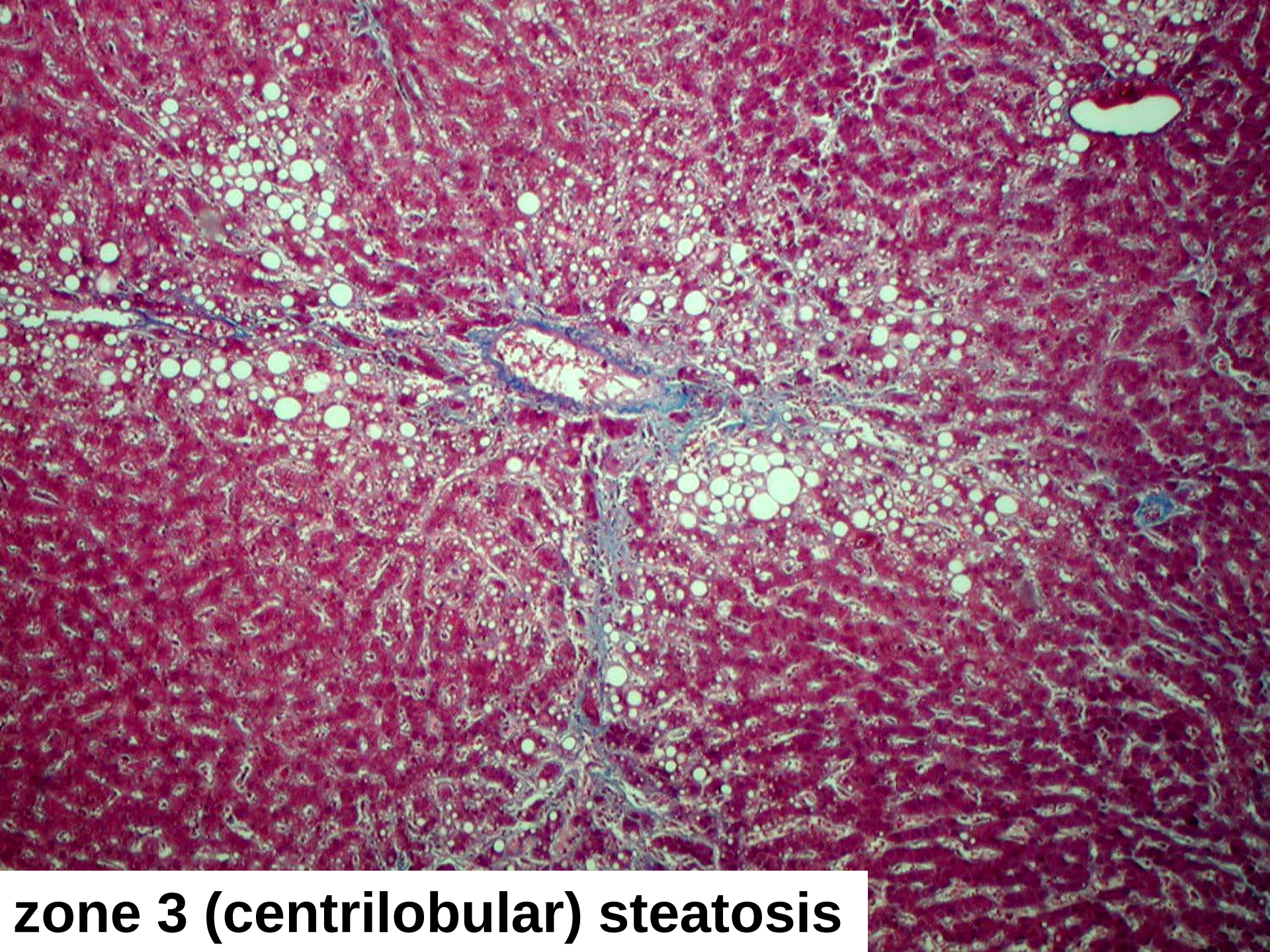
Steatosis

- Extent > 5% (by definition)
- Macrovesicular >> microvesicular:
  - Pure microvesicular steatosis is not a feature in NAFLD
  - Focal microvesicular steatosis is not clinically significant
- Zonal distribution:
  - Often zone 3 predominant in adults
  - Panacinar or a zonal distribution can be seen
  - Can be zone 1 predominant in children

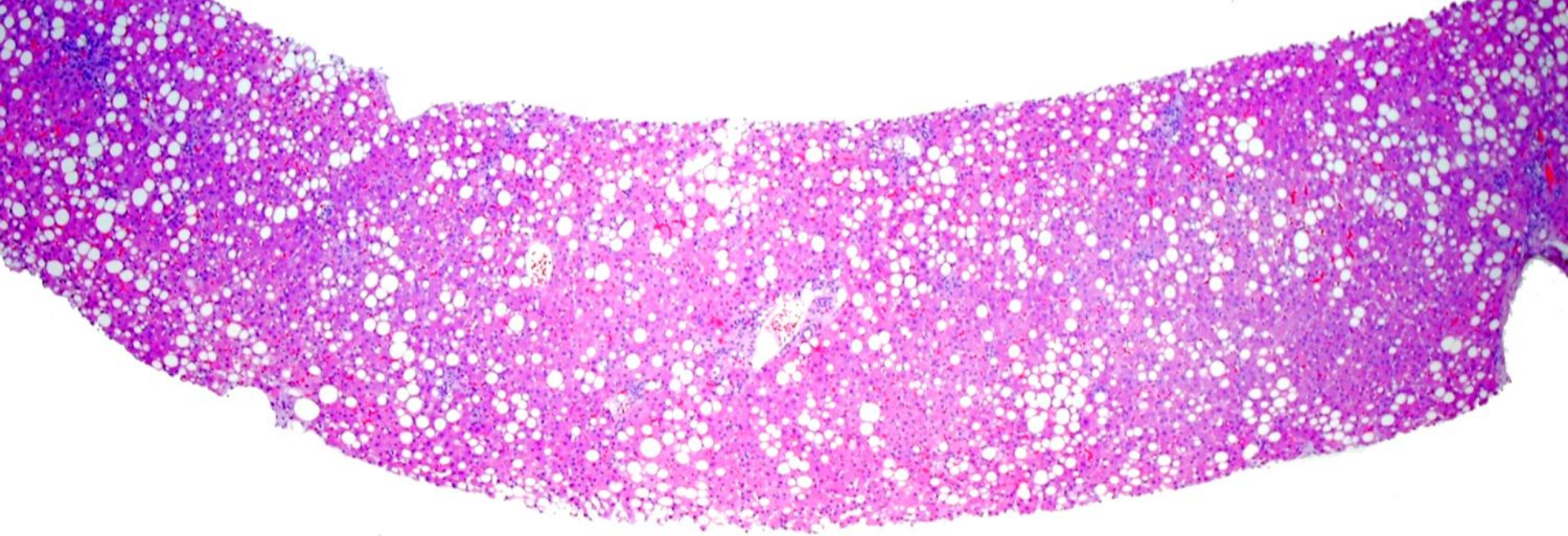

Macrovesicular Steatosis



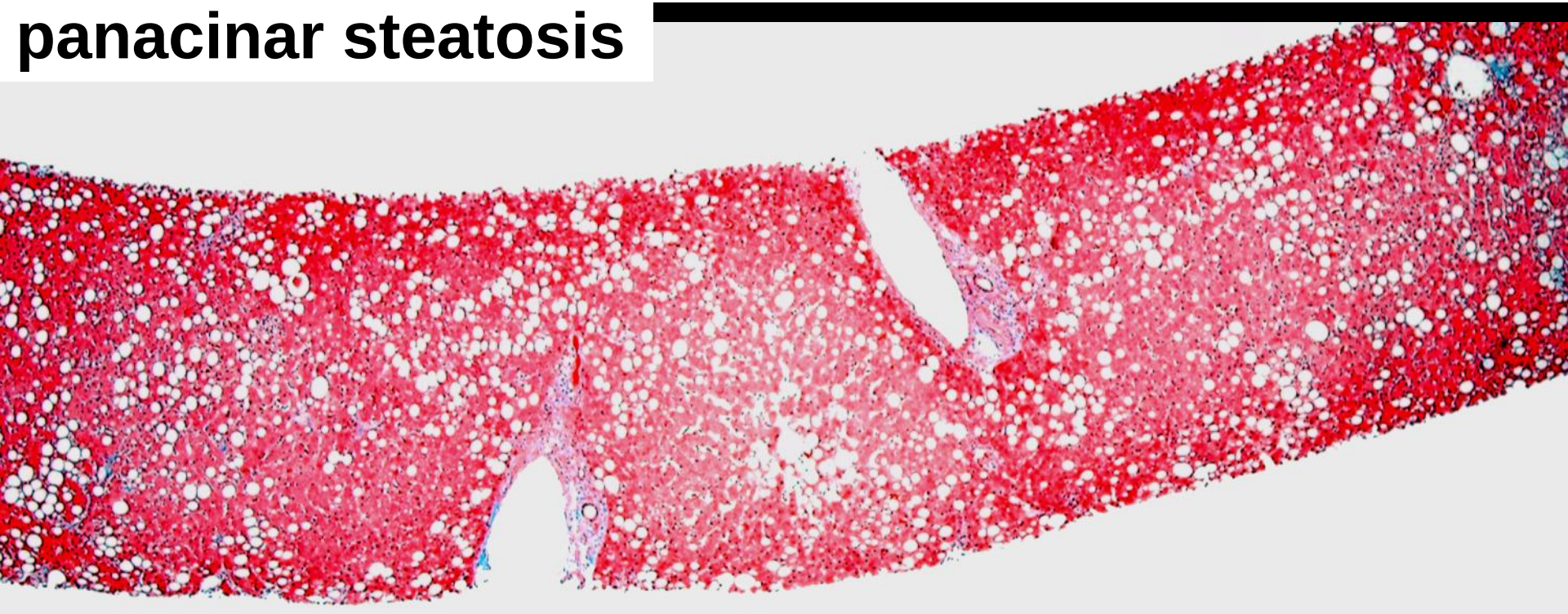
Focal microvesicular steatosis

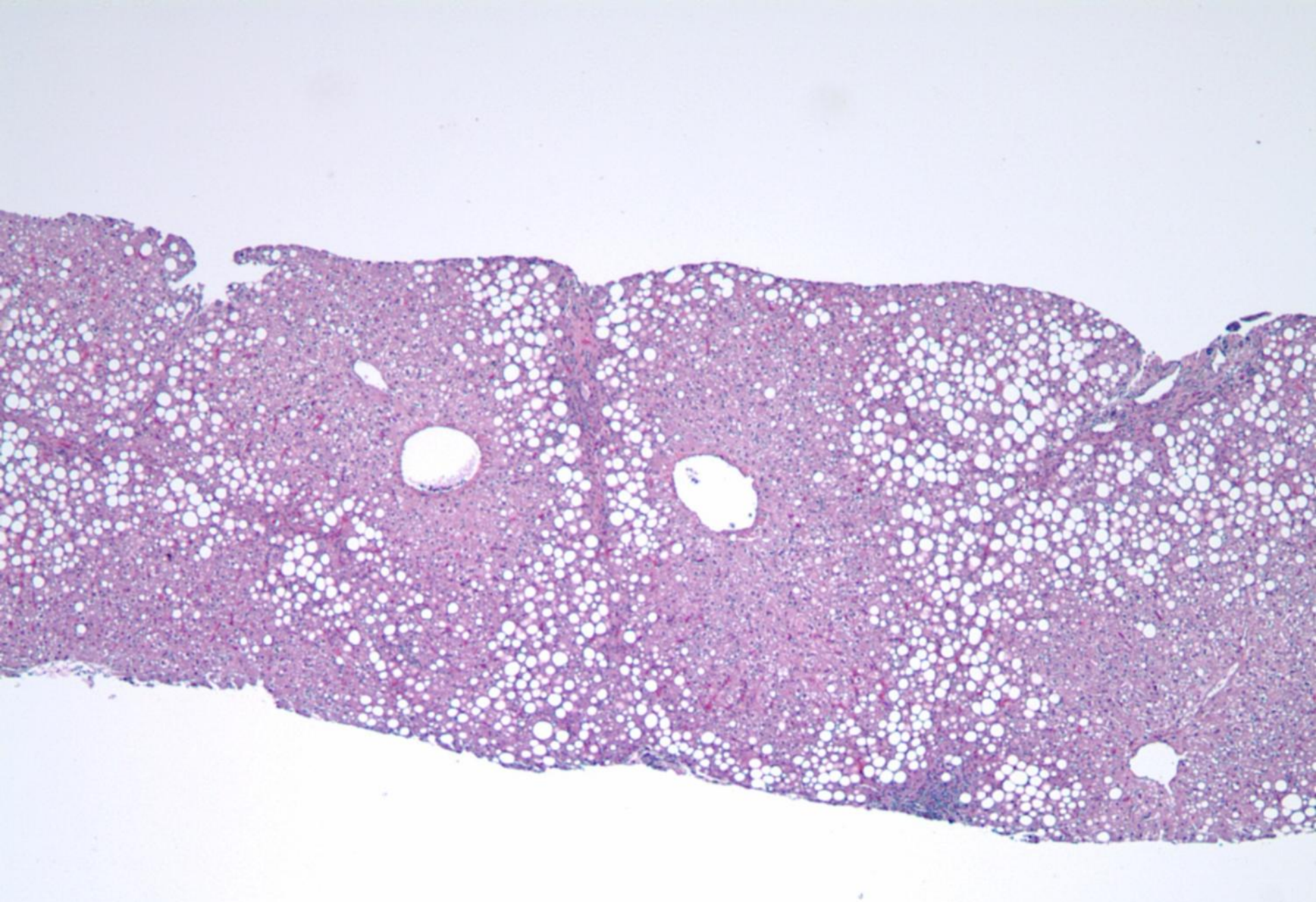


zone 3 (centrilobular) steatosis

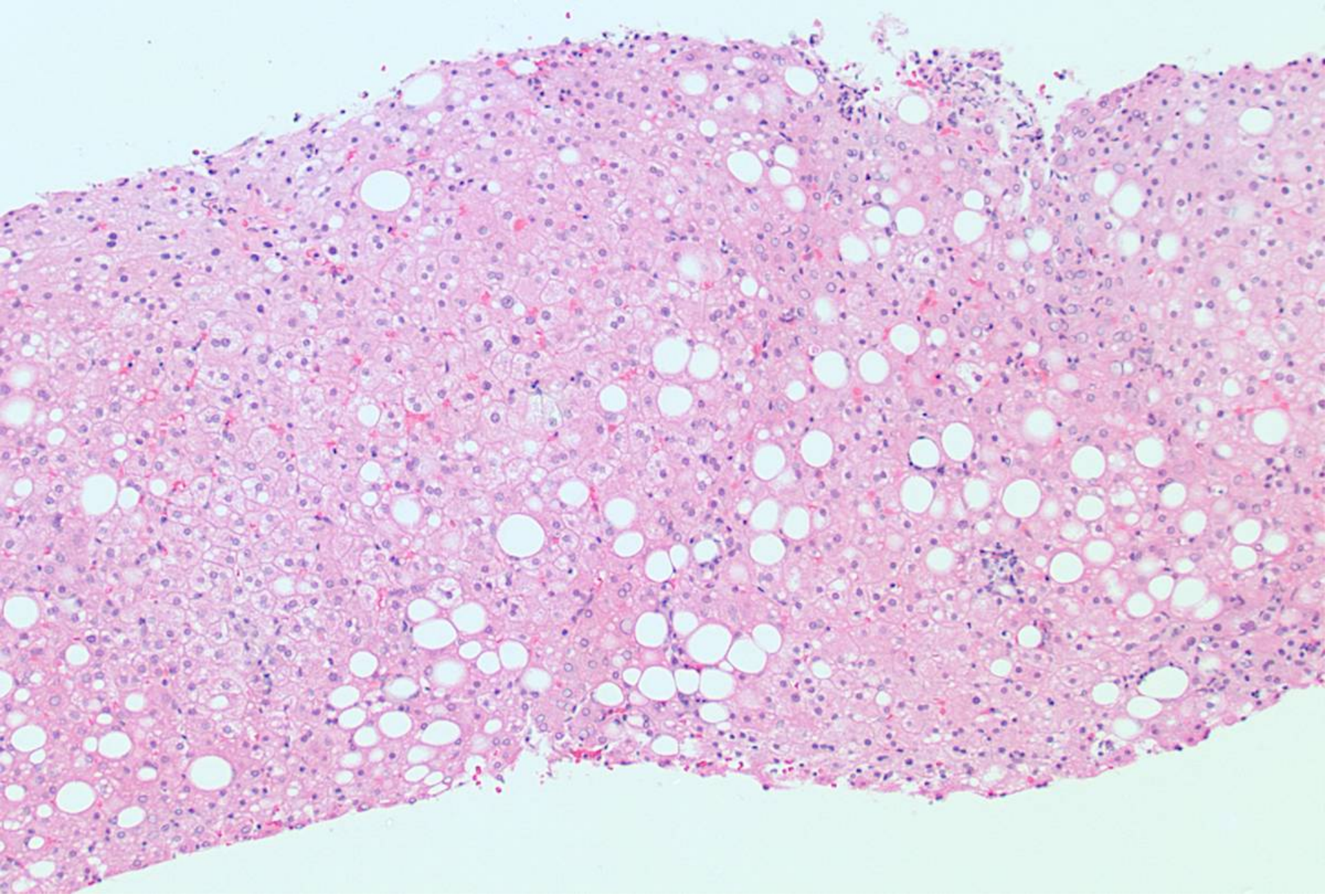


panacinar steatosis





zone 1 (periportal) steatosis in pediatric NAFLD

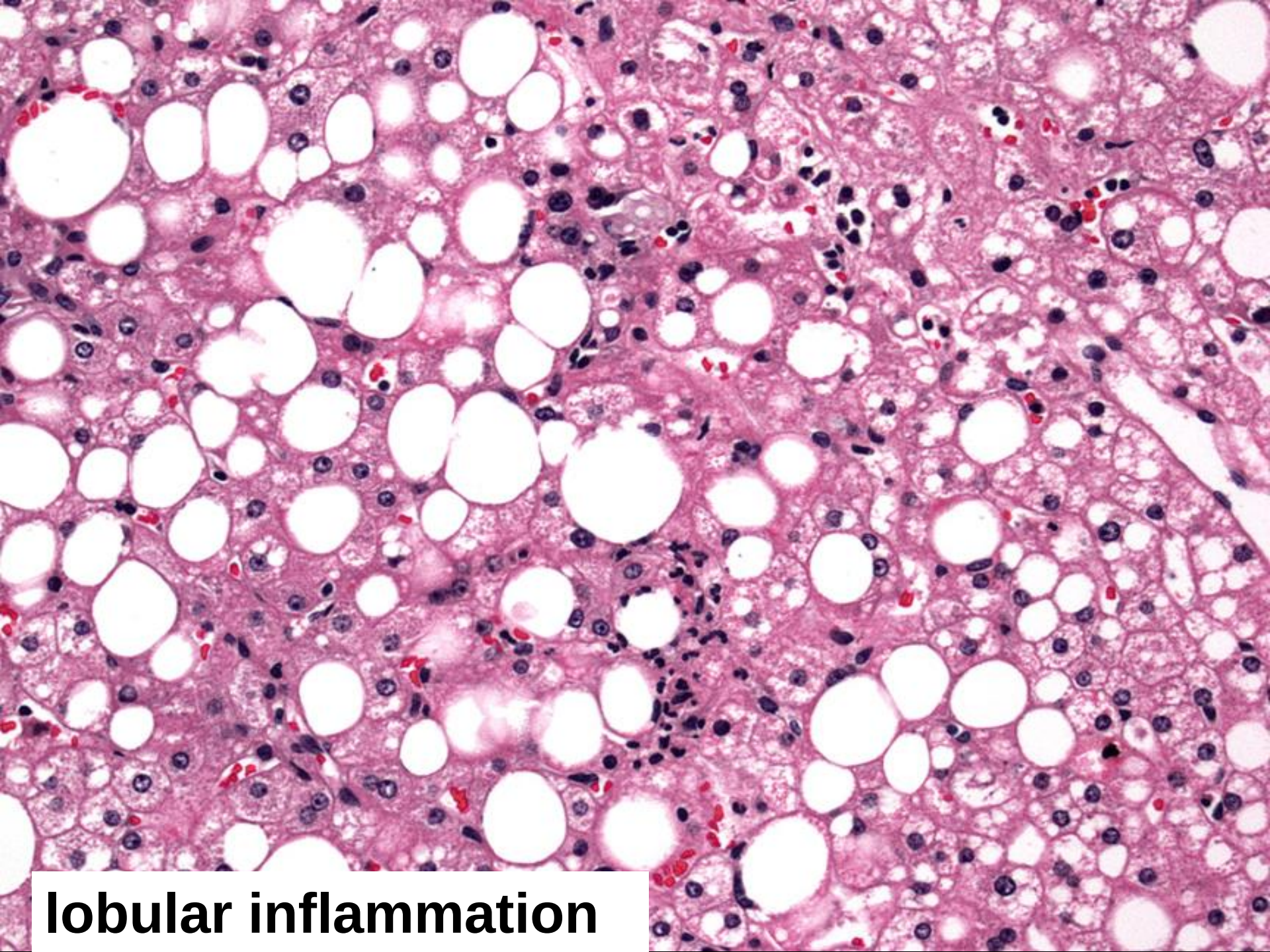


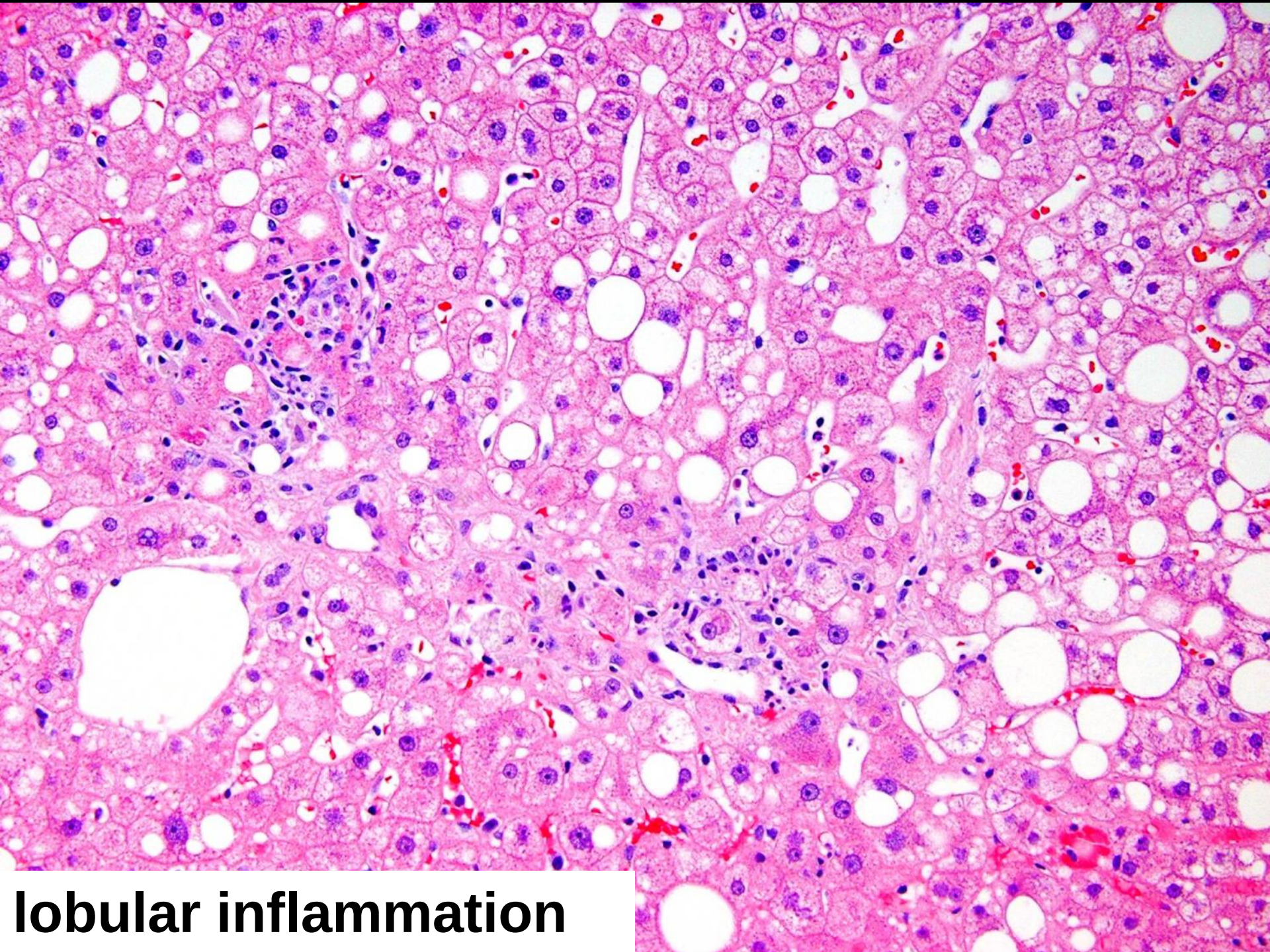
azonal steatosis

Histologic Features of Steatohepatitis

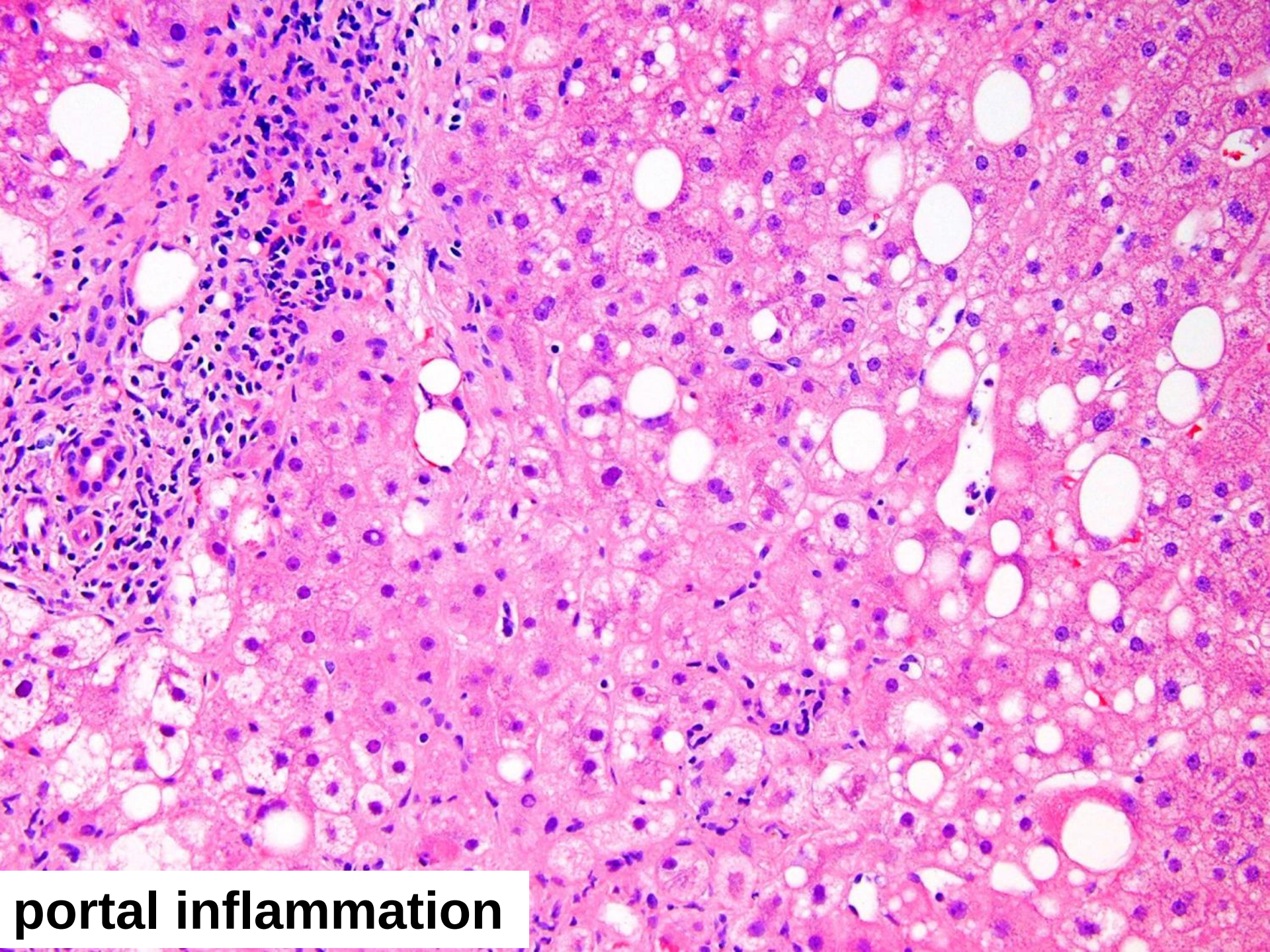
Inflammation

- **Lobular inflammation:**
  - Clusters of neutrophils, esp. surrounding Mallory-Denk bodies
  - Clusters of lymphocytes
  - Clusters of macrophages / Kupffer cells (microgranulomas)
- **Portal inflammation:**
  - Mostly seen in pediatric NAFLD, resolving NASH, and in severe disease
  - Dense inflammation suggests superimposed AIH / chronic viral hepatitis
  - Autoantibodies (ANA, SMA) present in 40% of patients with NAFLD


lobular inflammation



lobular inflammation

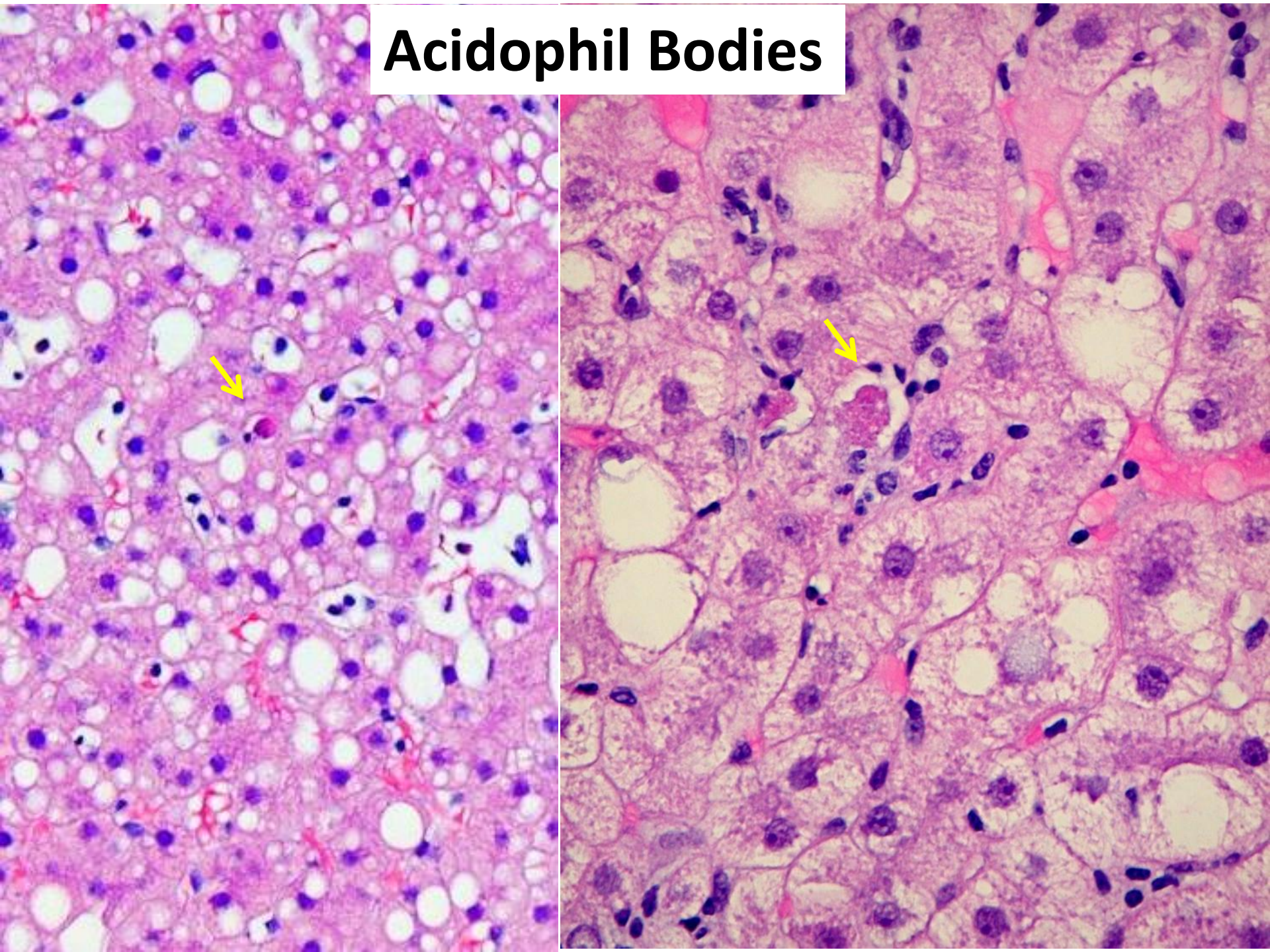

portal inflammation

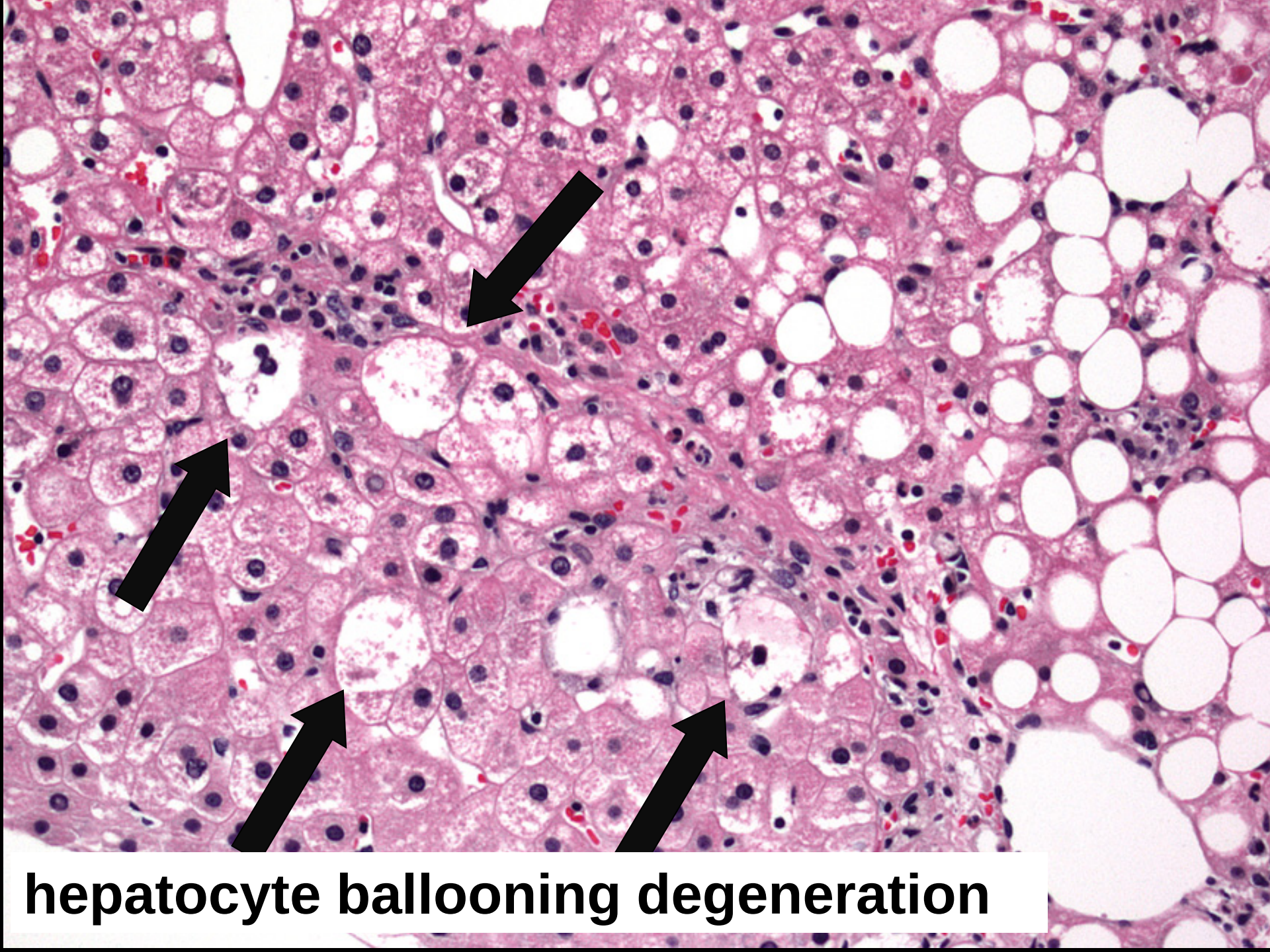
Histologic Features of Steatohepatitis

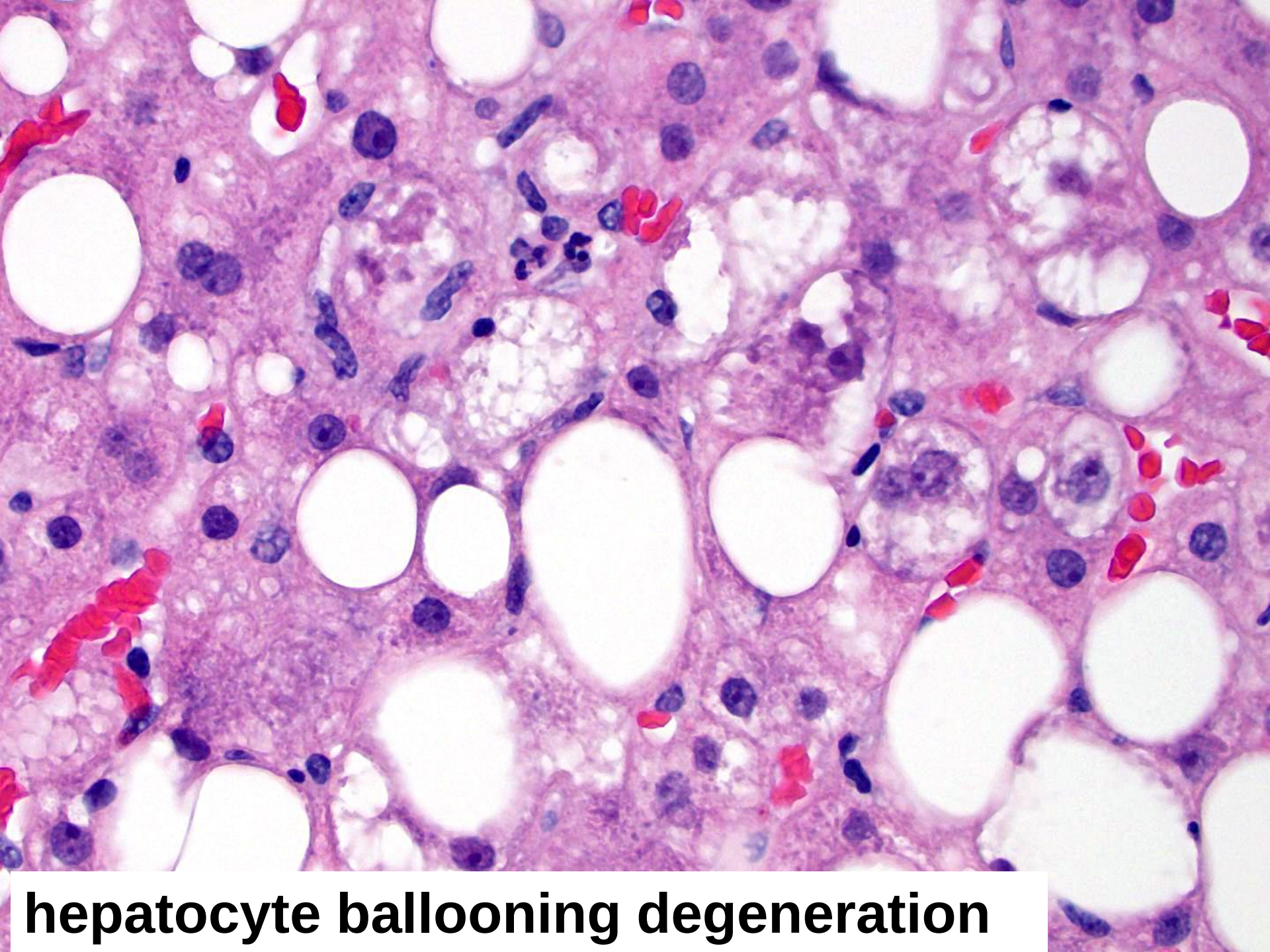
Hepatocyte injury

- **Hepatocyte ballooning degeneration:**
  - Most difficult and subjective feature of steatohepatitis
  - Enlarged hepatocytes with wispy or clumped cytoplasm and a centrally placed nucleus
  - Most prominent in zone 3 in areas of perisinusoidal fibrosis
  - Can contain Mallory-Denk bodies
  - Loss of cytokeratin 8/18 IHC can aid identification
  - Not common in pediatric NAFLD
- **Acidophil bodies**

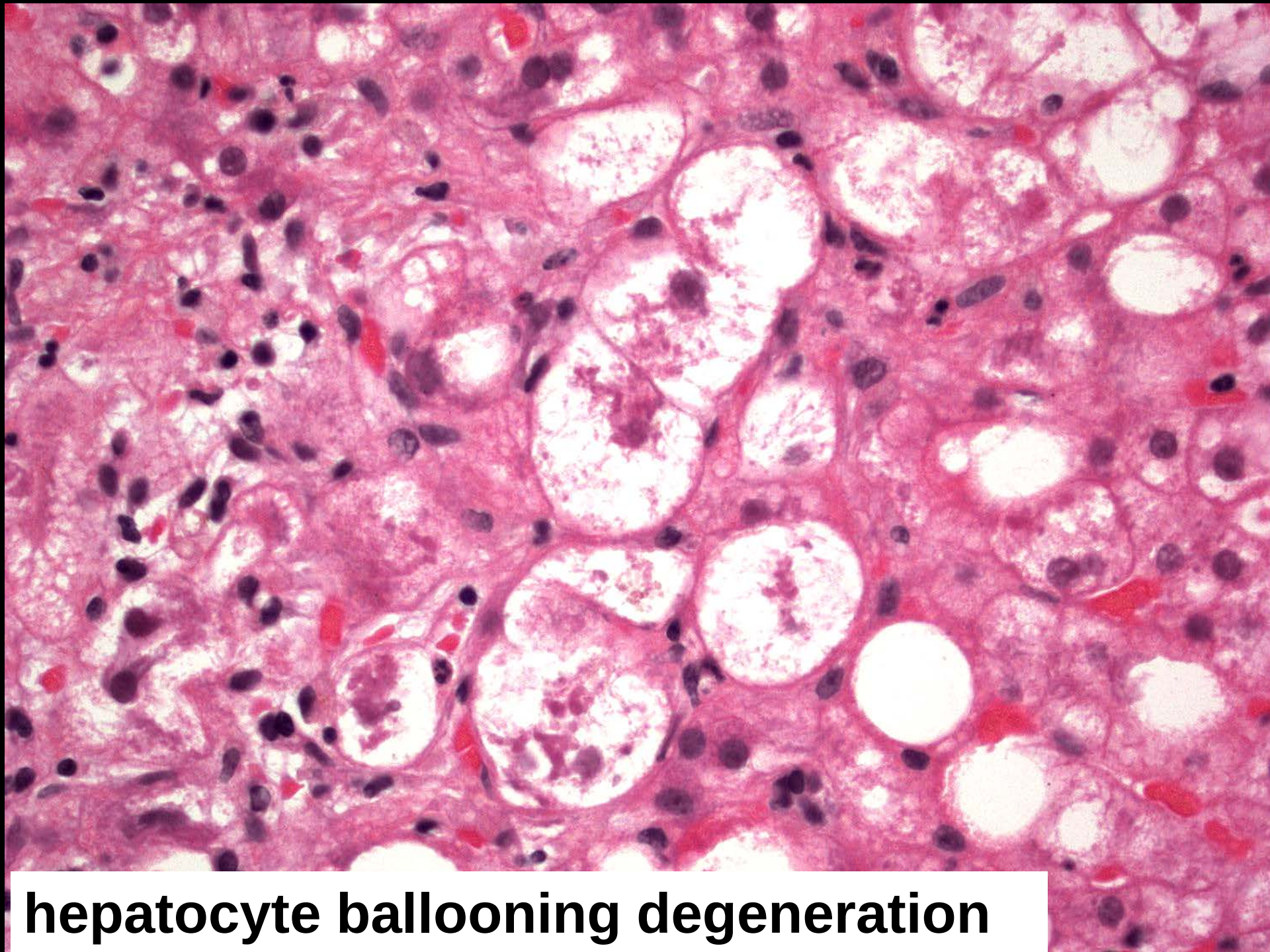
Acidophil Bodies




hepatocyte ballooning degeneration



hepatocyte ballooning degeneration



hepatocyte ballooning degeneration

Histologic Features of Steaohepatitis

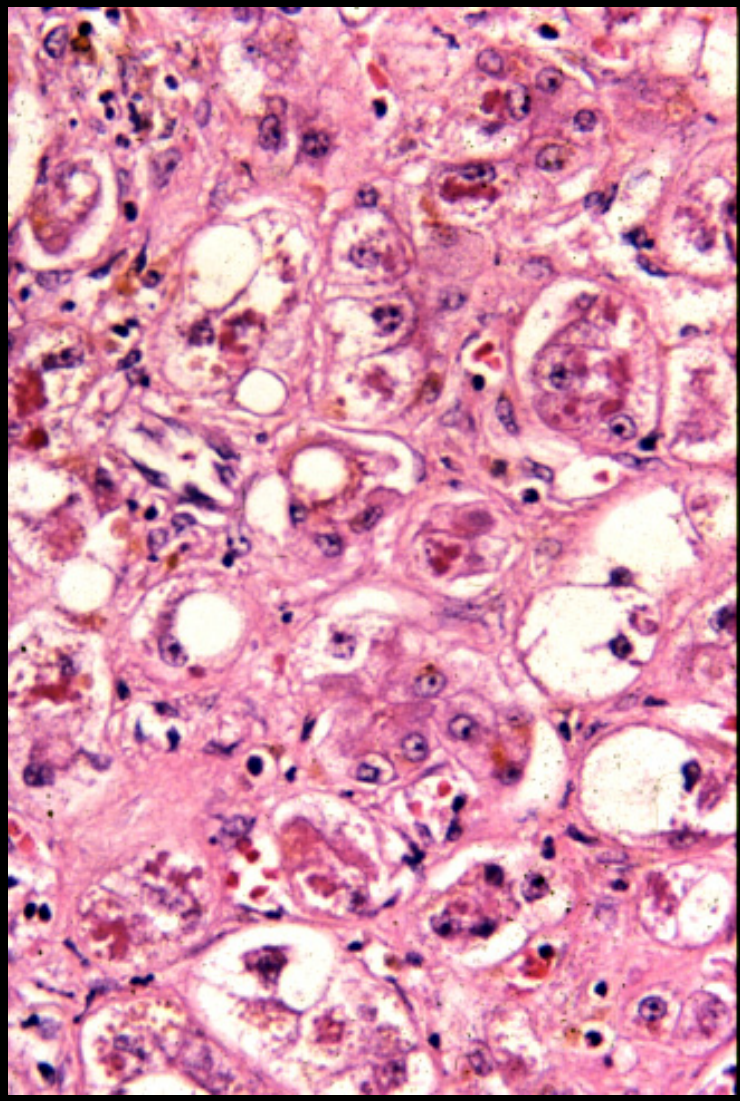
Often present, but not required

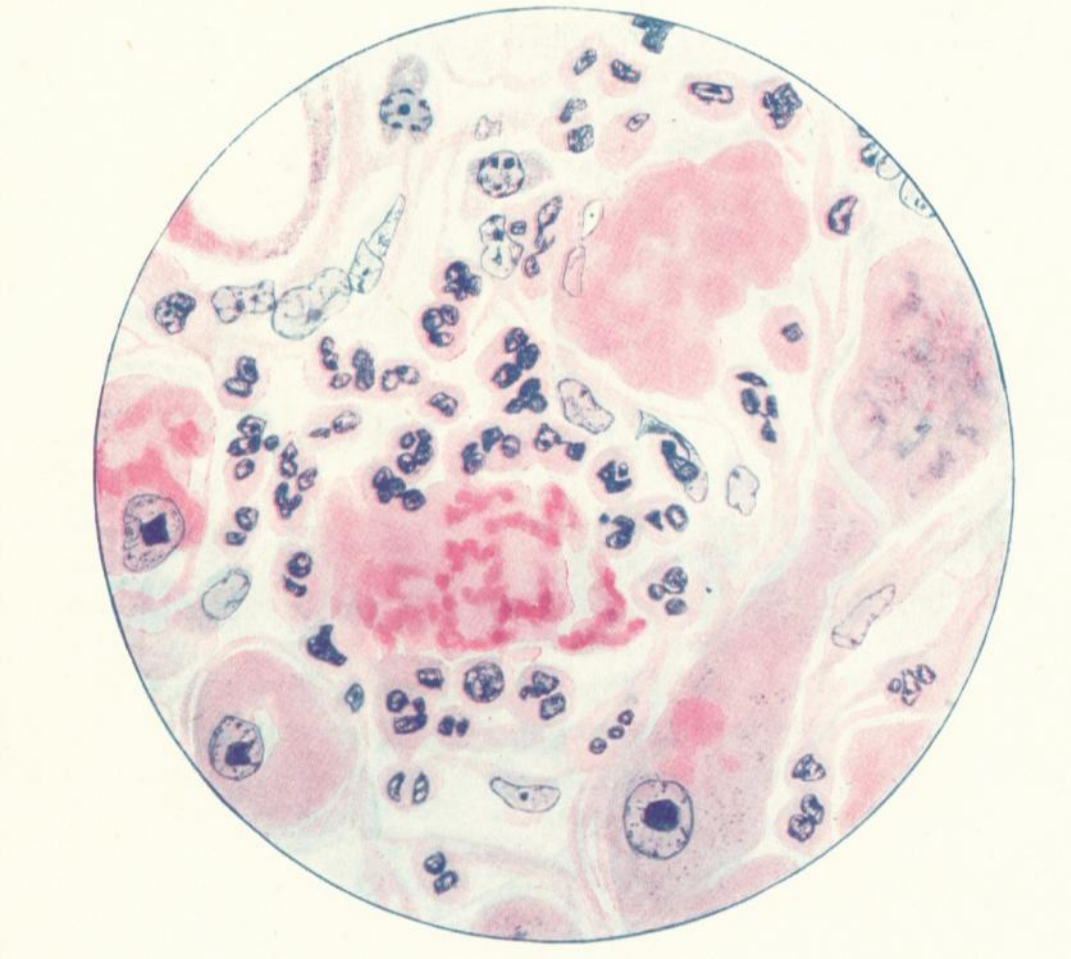
- Mallory-Denk bodies
- Glycogenated hepatocyte nuclei
- Lobular lipogranulomas

Brunt Clin Liver Dis 2009

Mallory-Denk Bodies

- Located in zone 3
- Denatured cytokeratin filaments
- Associated with ubiquitin
- Occur in ballooned hepatocytes
- CK7, CK18, CK19 +
- Sometimes cuffed by neutrophils
- Also seen in zone 3 in ASH and amiodarone toxicity





Mallory Body

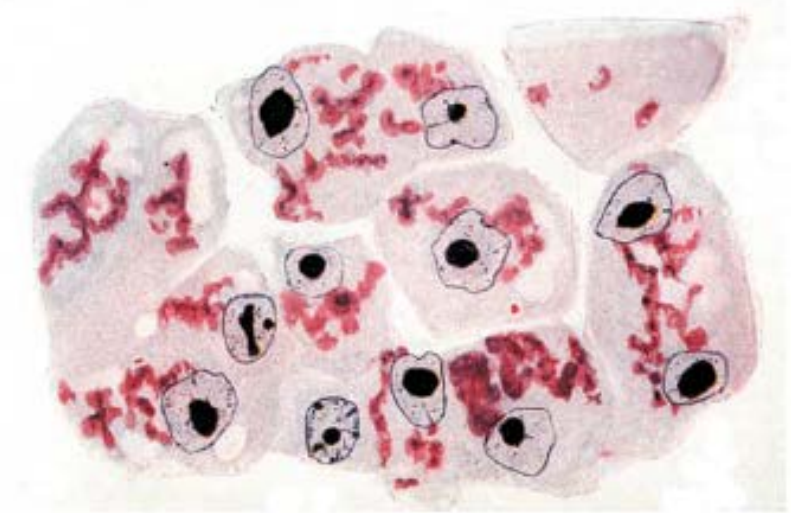
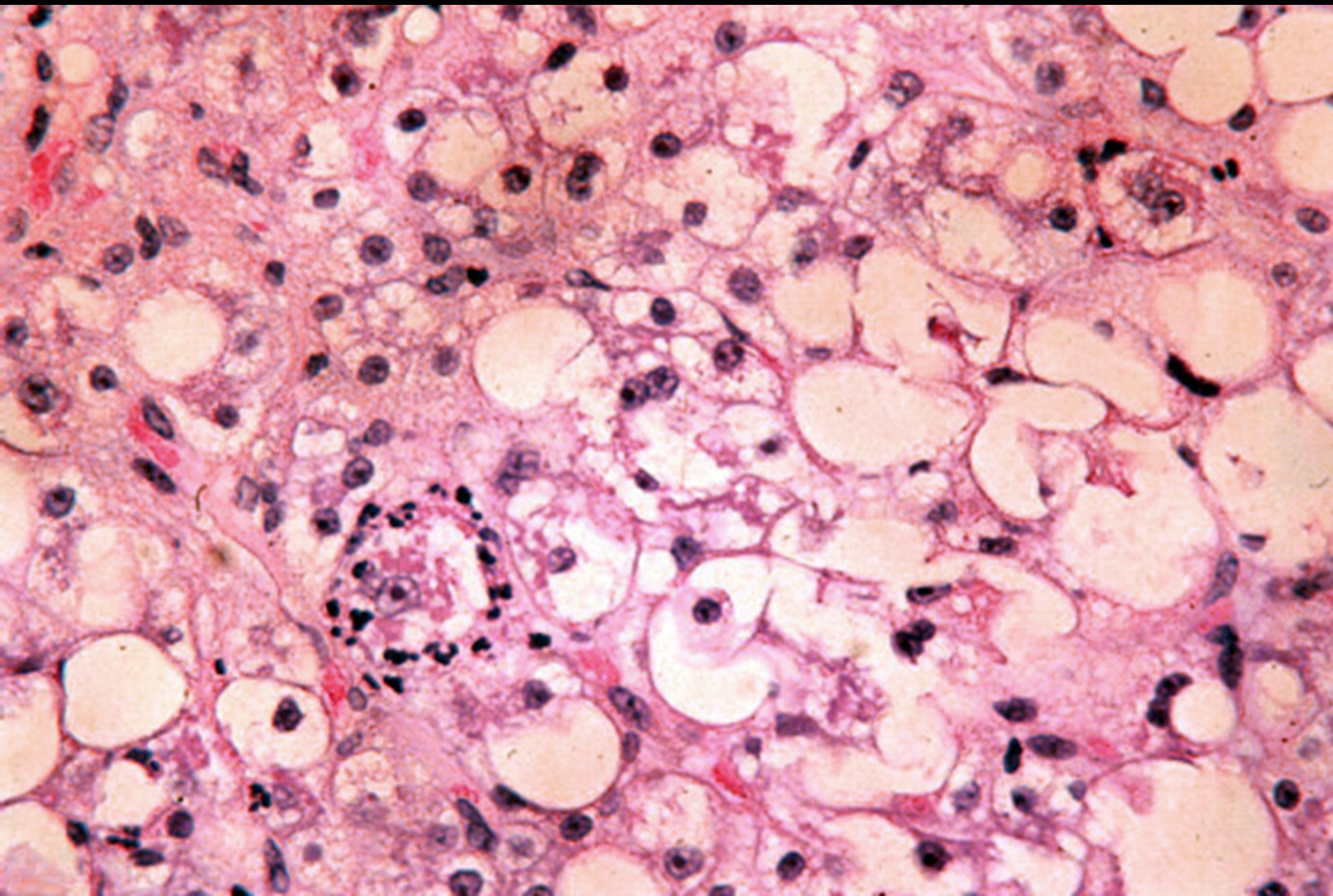
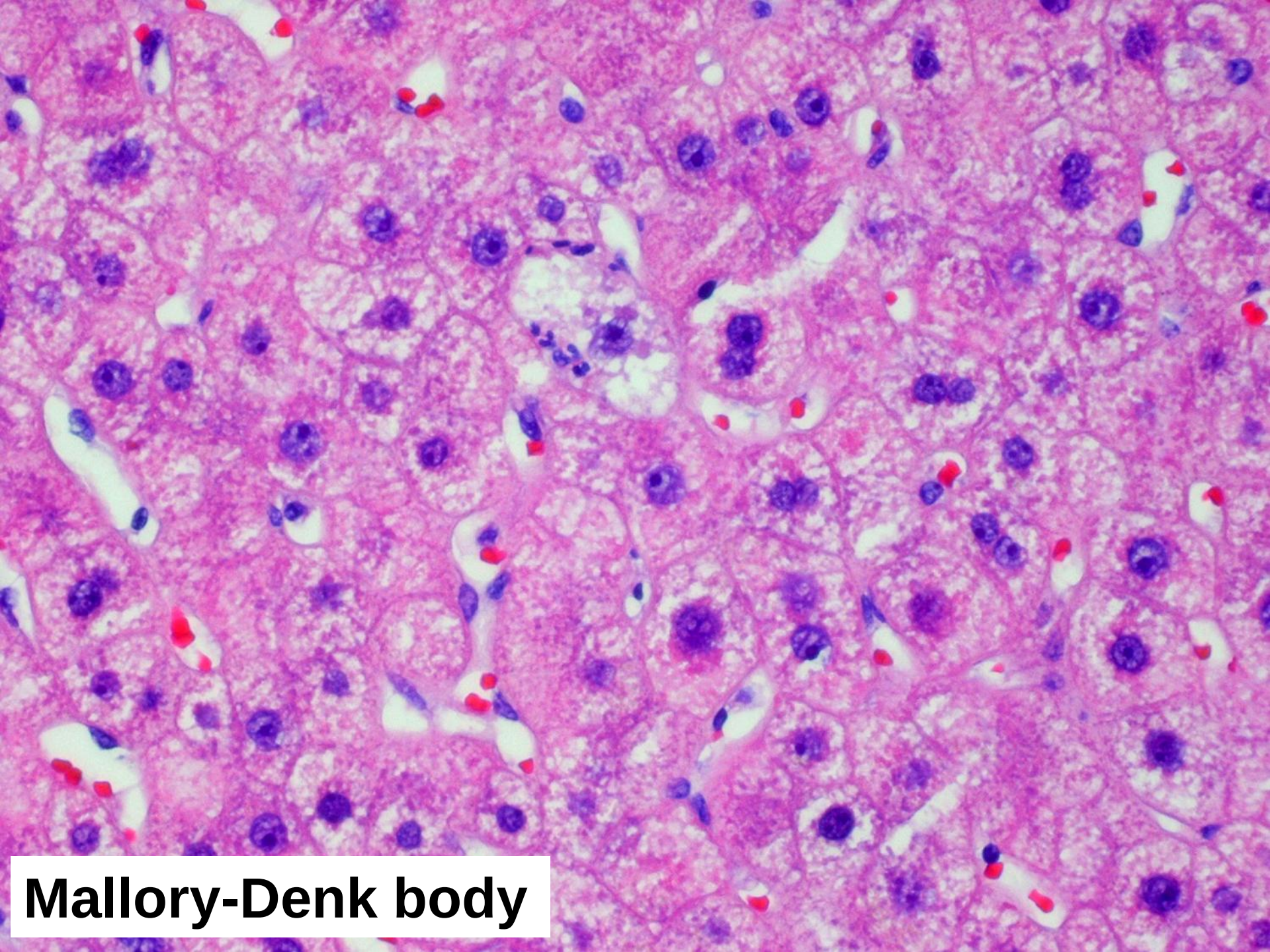


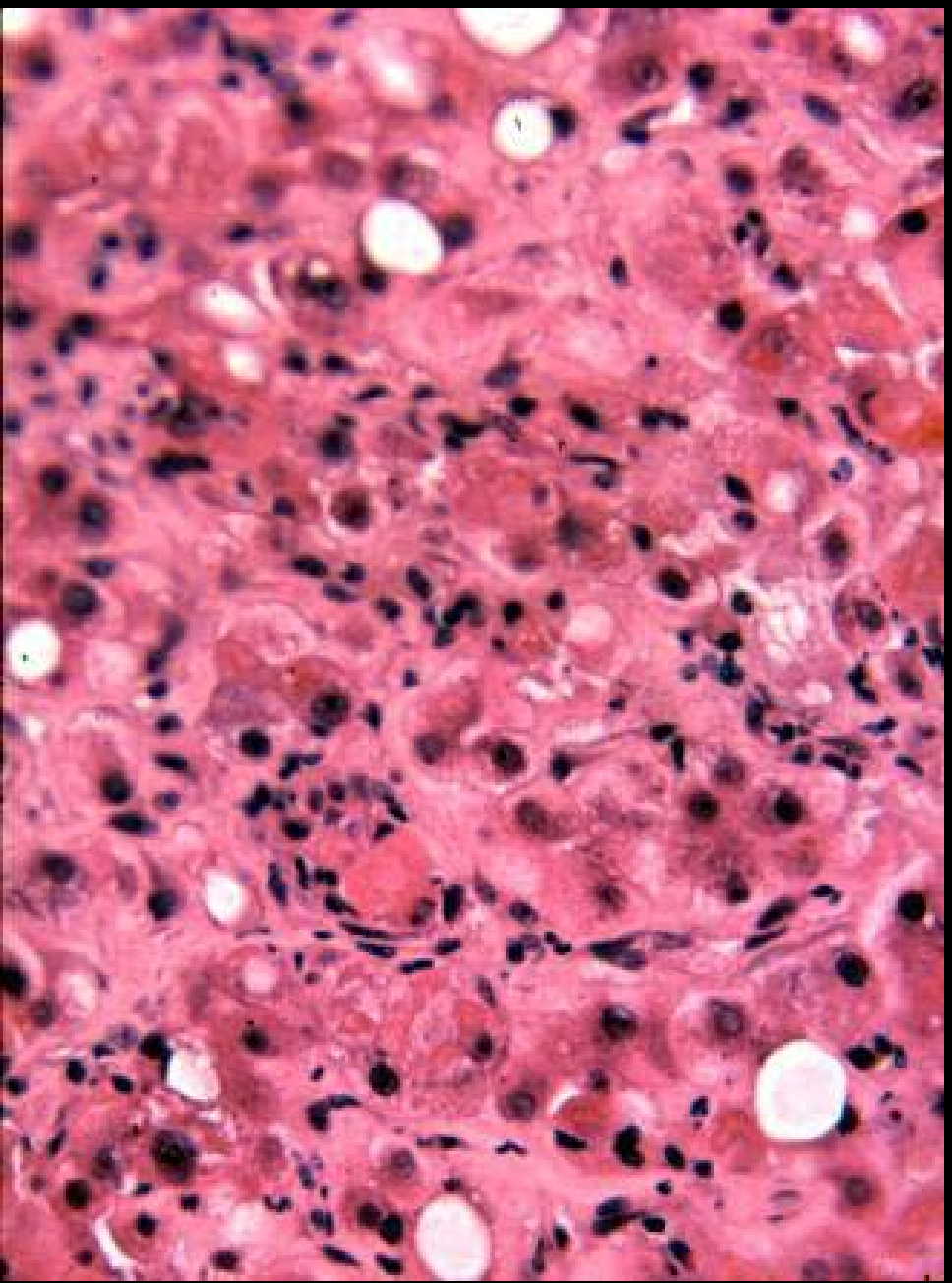
Fig. 1. **Top:** "The Chief" at his microscope, from an illustration on the cover of the October 2001 issue of the *American Journal of Pathology* celebrating its centennial (maybe[3]) taken from a photograph in the 1933 *Journal* supplement[4] that was dedicated to FBM, in honor of his 70th birthday and the opening of the Mallory Institute of Pathology of Boston City Hospital. **Bottom:** Hyaline droplets appearing in the cytoplasm of liver cells and fusing together, from the liver biopsy of a patient with alcoholic cirrhosis. Reproduced with permission from an illustration by Miss Etta R. Piotti in FB Mallory's 1914 textbook *Principles of Pathologic Histology*.[16]

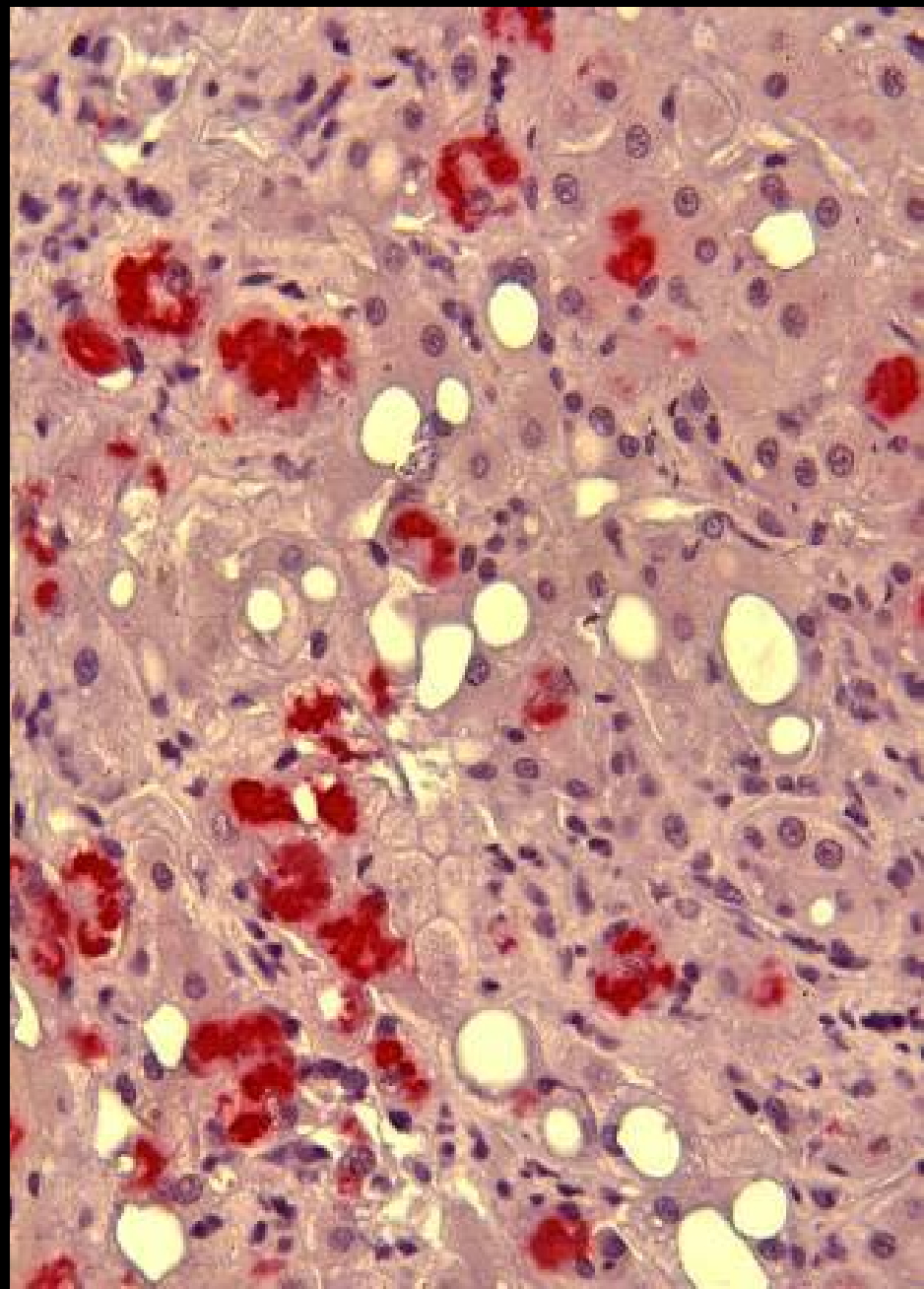
Mallory-Denk Body Ringed by Neutrophils




Mallory-Denk body

Mallory-Denk Bodies - Ubiquitin Immunostain





Histologic Features Unusual for Steatohepatitis
Consider other liver diseases

- Pure or predominant microvesicular steatosis
- Portal > lobular inflammation*
- Portal > centrilobular fibrosis*
- Prominent hepatocyte ballooning with minimal steatosis (consider amiodarone toxicity)
- Epithelioid granulomas
- Conspicuous plasma cells
- Chronic cholestatic features

* Except pediatric NASH

ON

DISEASES OF THE LIVER.

BY

GEORGE BUDD, M.D., F.R.S.,

PROFESSOR OF MEDICINE IN KING'S COLLEGE, LONDON; LATE FELLOW OF CAIUS COLLEGE, CAMBRIDGE.

With Colored Plates and Wood-cuts.

THIRD AMERICAN,

FROM THE THIRD AND REVISED LONDON EDITION.

PHILADELPHIA:
BLANCHARD AND LEA.
1857.

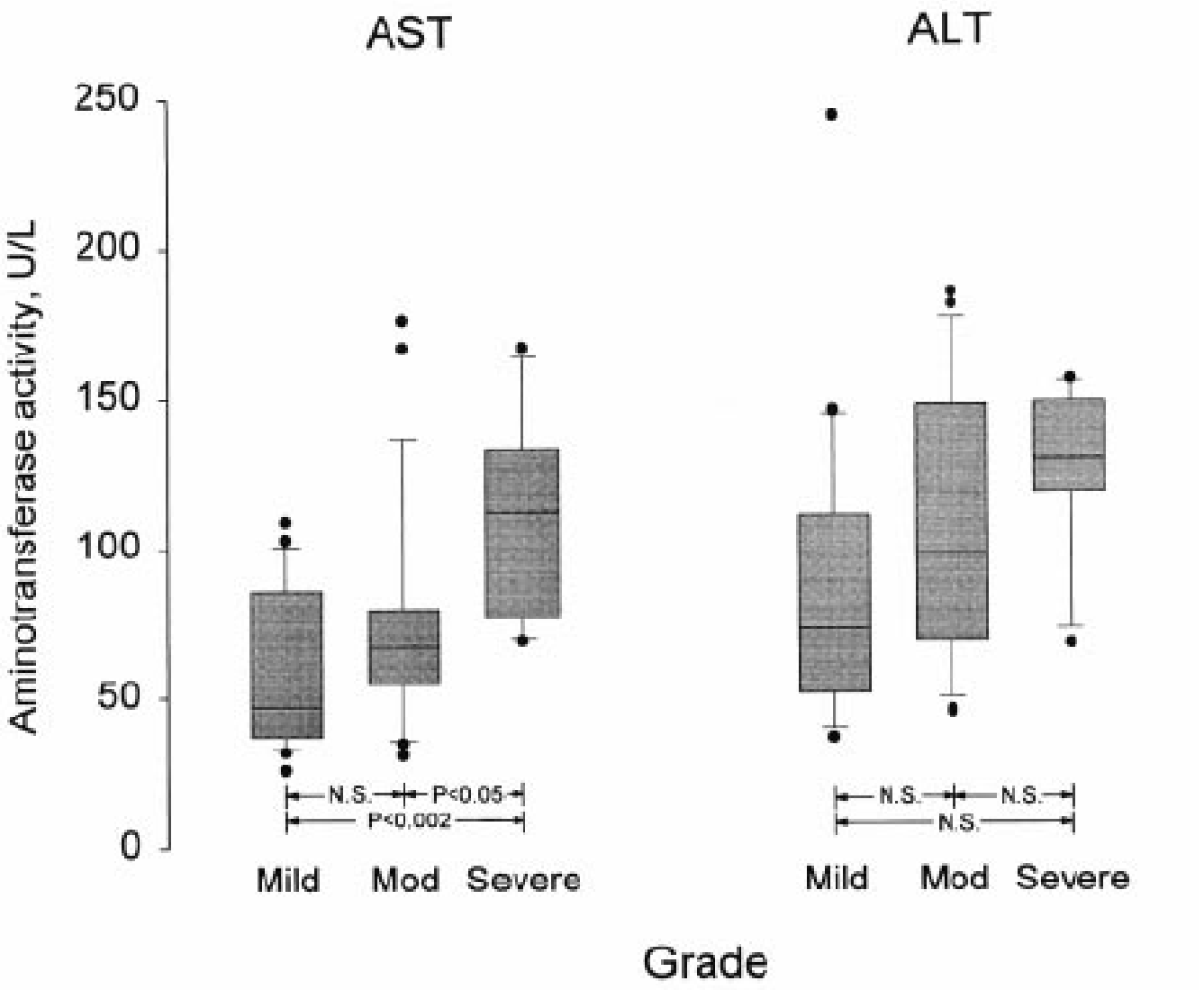
A liver still more remarkable for the large amount of fat it contained fell under my observation in King's College Hospital, in the spring of 1850. It was taken from a drunkard, and was in a state of cirrhosis, as well as of fatty degeneration, and in consequence presented a very remarkable "hob-nailed" appearance, from the nodules of cirrhosis being enlarged by the accumulation of oil. A portion of it blazed when thrown into the fire, and a particle from the lobular substance had under the microscope almost the appearance of ordinary fatty tissue, from the number and size of the oil-globules it contained. Dr. L. S. Beale made an analysis of a portion of it for me, and found that 65 parts in 100—about six-sevenths of all the *solid* matter in the liver—consisted of fat.

Grading of NASH*

EM Brunt. Sem Liver Dis 2001; 21:3-16.

GRADE	Steatosis	Hepatocyte Ballooning	Lobular Inflammation	Portal Inflammation
MILD	Up to 66%; mostly macrovesic.	Zone 3; occasional cells	Scattered polys and mononuclear cells	None or mild
MOD	Up to 66%; usually mixed	Zone 3; obvious	Polys with ballooned cells & areas of pericellular fibrosis	Mild to Moderate
SEVERE	> 66%; usually mixed	Predom. Zone 3; marked	Polys with ballooned cells & areas of pericellular fibrosis	Mild to Moderate

*modified version



American Journal of Gastroenterology (1999) **94**, 2467–2474;

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

(HEPATOLOGY 2005;41:1313-1321.)

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

- Nine study pathologists (NIDDK consortium)
- 32 adult and 18 pediatric biopsies
- 14 histologic features scored:
  - Degree of macrovesicular steatosis (0-3)
  - Degree of lobular inflammation (0-3)
  - Degree of hepatocyte ballooning (0-2)
  - Degree of fibrosis (0-4)
- NAFLD Activity Score (NAS):
  - Score ≥ 5 correlated with diagnosis of NASH
  - Score < 3 correlated with diagnosis of not NASH

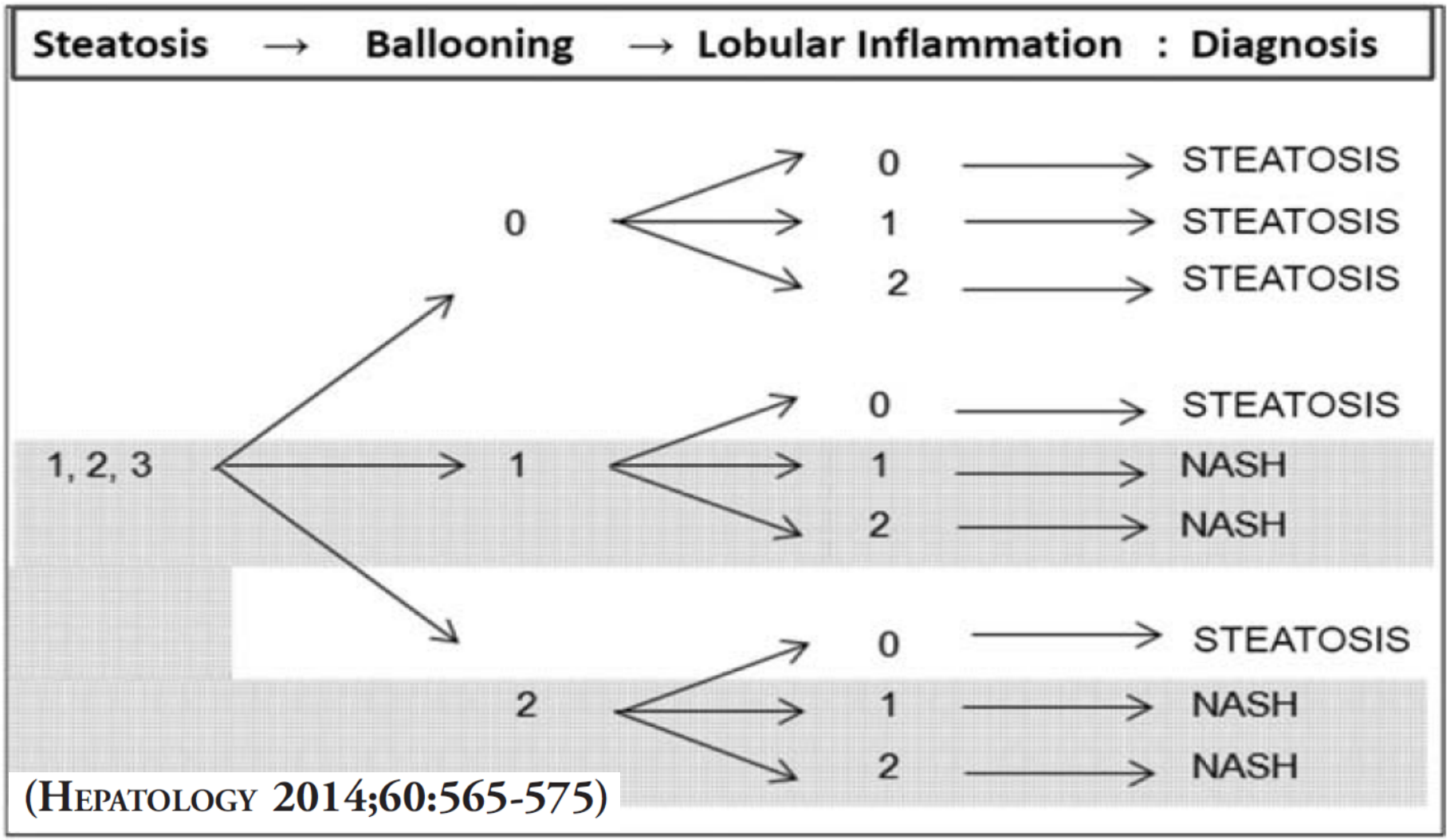
in fatty liver disease.[22,23] Each of the major features (steatosis grade, lobular inflammation, ballooning, and fibrosis) showed independent correlation with a diagnosis of steatohepatitis. Based on this observation and the reproducibility studies, we defined a NAS for evaluating histological changes after therapeutic intervention trials. It is important to note that the primary purpose of the NAS is to assess overall histological change; it is not intended that numeric values replace the pathologist's diagnostic determination of steatohepatitis. The NAS has also not been studied as a measure of the rapidity of disease progression, nor should it be taken as an absolute severity scale.

Table 2. Inter- and Intra-rater Variability

Item	Agreement (Kappa Score)		
	Intra-rater	Interrater	
	Adult Cases (32 cases, 9 raters)	Adult Cases (32 cases, 9 raters)	Pediatric Cases (18 cases, 9 raters)
Steatosis, grade	0.83	0.79	0.64
Steatosis, location	0.39	0.31	0.39
Microvesicular steatosis	0.37	0.34	0.02
Fibrosis	0.85	0.84	0.62
Lobular inflammation	0.60	0.45	0.28
Microgranulomas	0.40	0.18	0.15
Lipogranulomas	0.38	0.26	0.00
Portal inflammation	0.55	0.45	0.42
Ballooning	0.66	0.56	0.22
Acidophil bodies	0.28	0.19	0.27
Pigmented macrophages	0.38	0.15	0.06
Megamitochondria	0.28	0.16	−0.03
Mallory's hyaline	0.64	0.58	0.69
Glycogenated nuclei	0.66	0.58	0.32
Diagnostic classification	0.66	0.61	0.32

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

Pierre Bedossa and the FLIP Pathology Consortium*



(Hepatology 2014;60:565-575)

(Hepatology 2014;60:565-575)

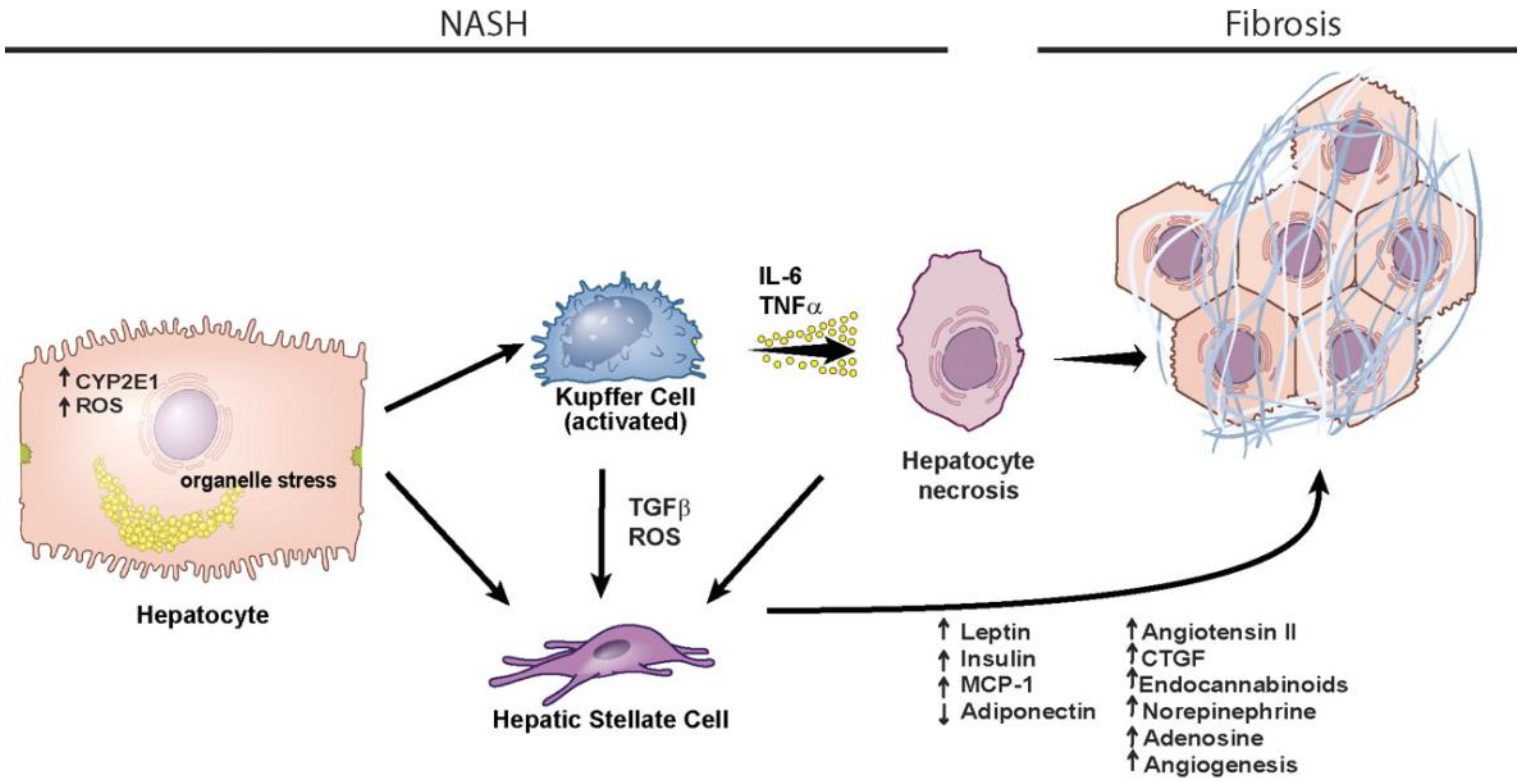
Table 2. Agreement for Diagnosis Before and After Use of Algorithm in the Two Groups of Pathologists

		κ Score	Biopsy With Full Agreement Between Pathologists (%)	Biopsy With Agreement With Reference Diagnosis (%)
Group 1	Baseline classification	0.54	26/40 (65%)	31/40 (77%)
	Algorithmic classification	0.66	34/40 (85%)	39/40 (97%)
Group 2	Baseline classification	0.35	18/40 (45%)	17/40 (42%)
	Algorithmic classification	0.61	28/40 (70%)	30/40 (75%)

Why Grade and Stage NASH?

- Staging provides information about current degree of chronic damage (i.e., fibrosis) in the liver
- Grading is supposed to:
  - Correlate with liver chemistry test elevations????
  - Predict future risk of fibrosis

Necroinflammatory Activity Leads to Fibrosis

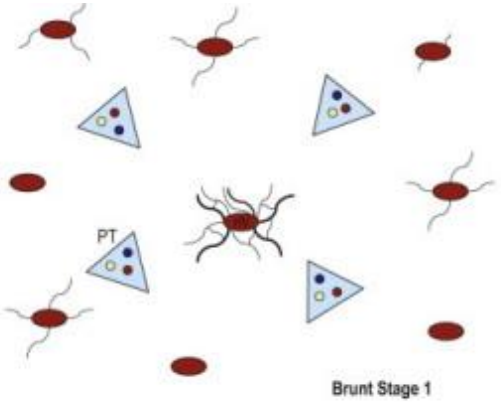


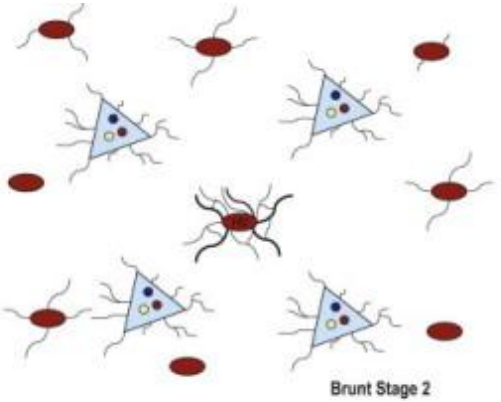
American Journal of Physiology - Gastrointestinal and Liver Physiology May 2011Vol.

Staging of NASH

EM Brunt. Sem Liver Dis 2001; 21:3-16.

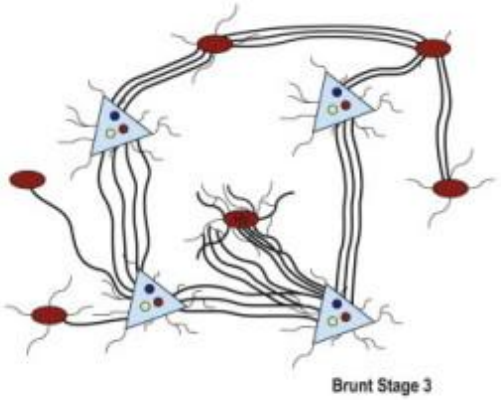
Stage 1:
zone 3
(perivenular)
pericellular

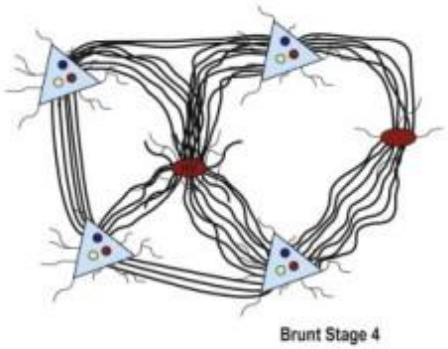




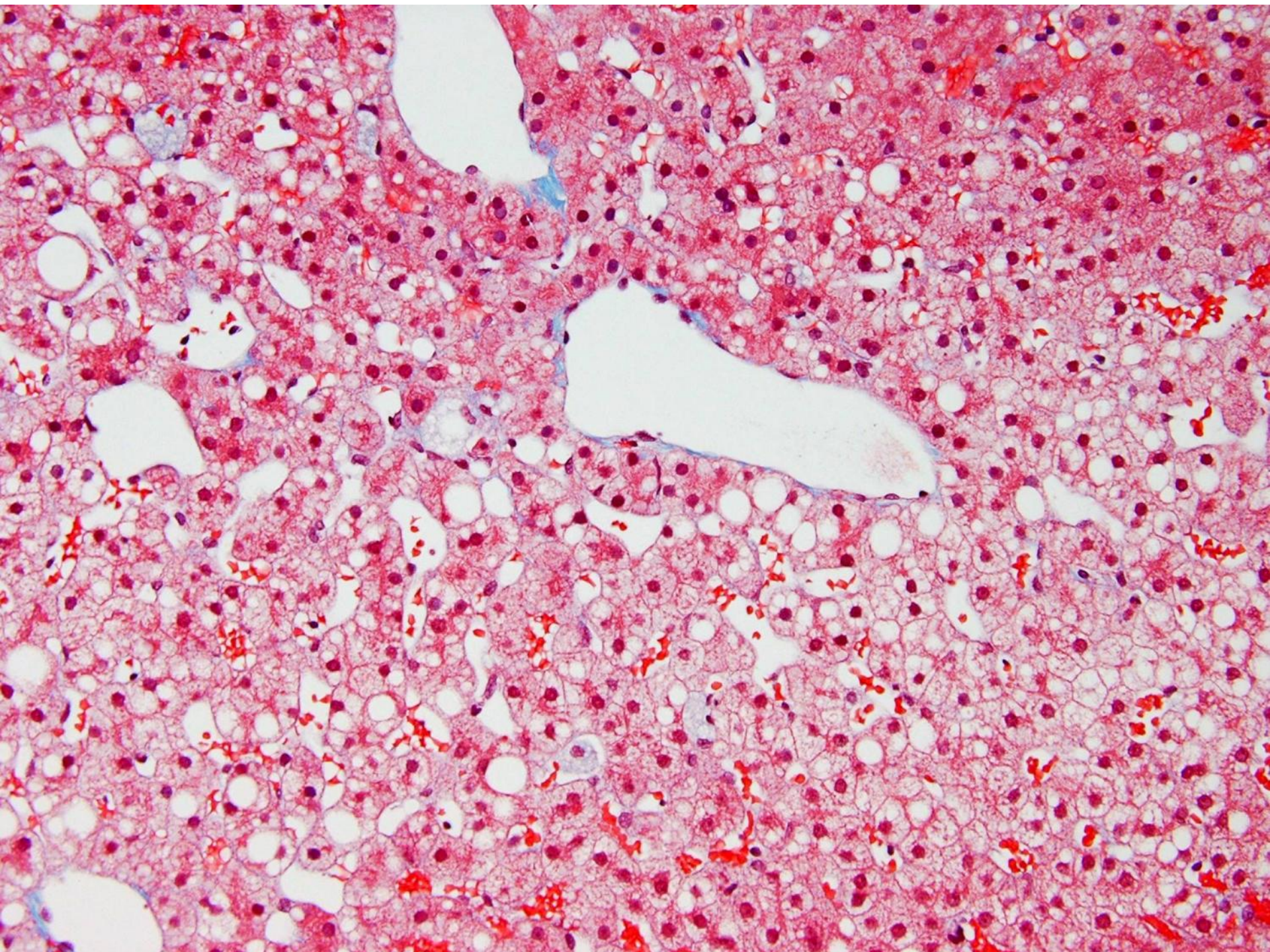
Stage 2:
As in Stage I
+
portal /
periportal
fibrosis

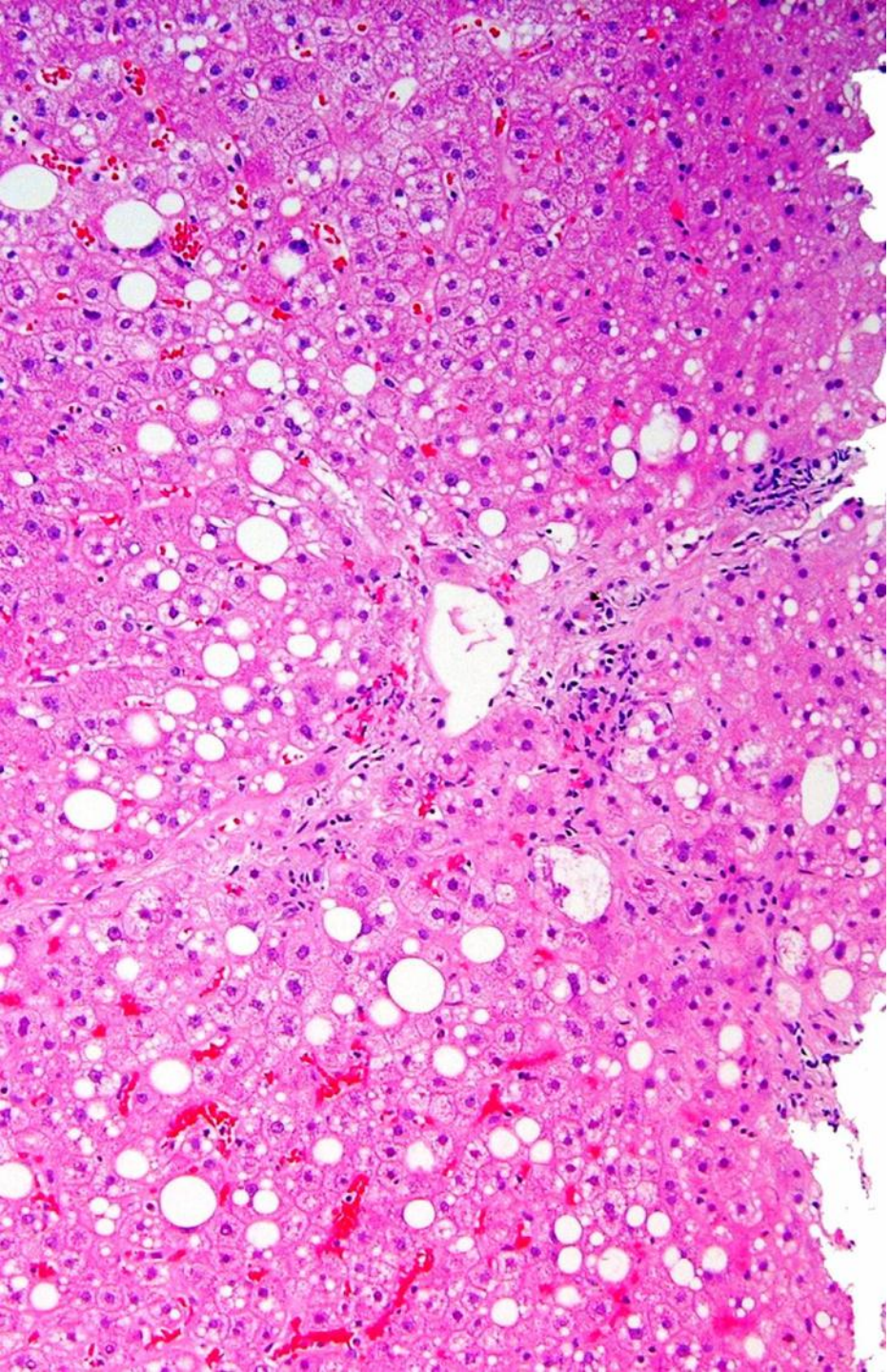
Stage 3:
bridging
fibrosis

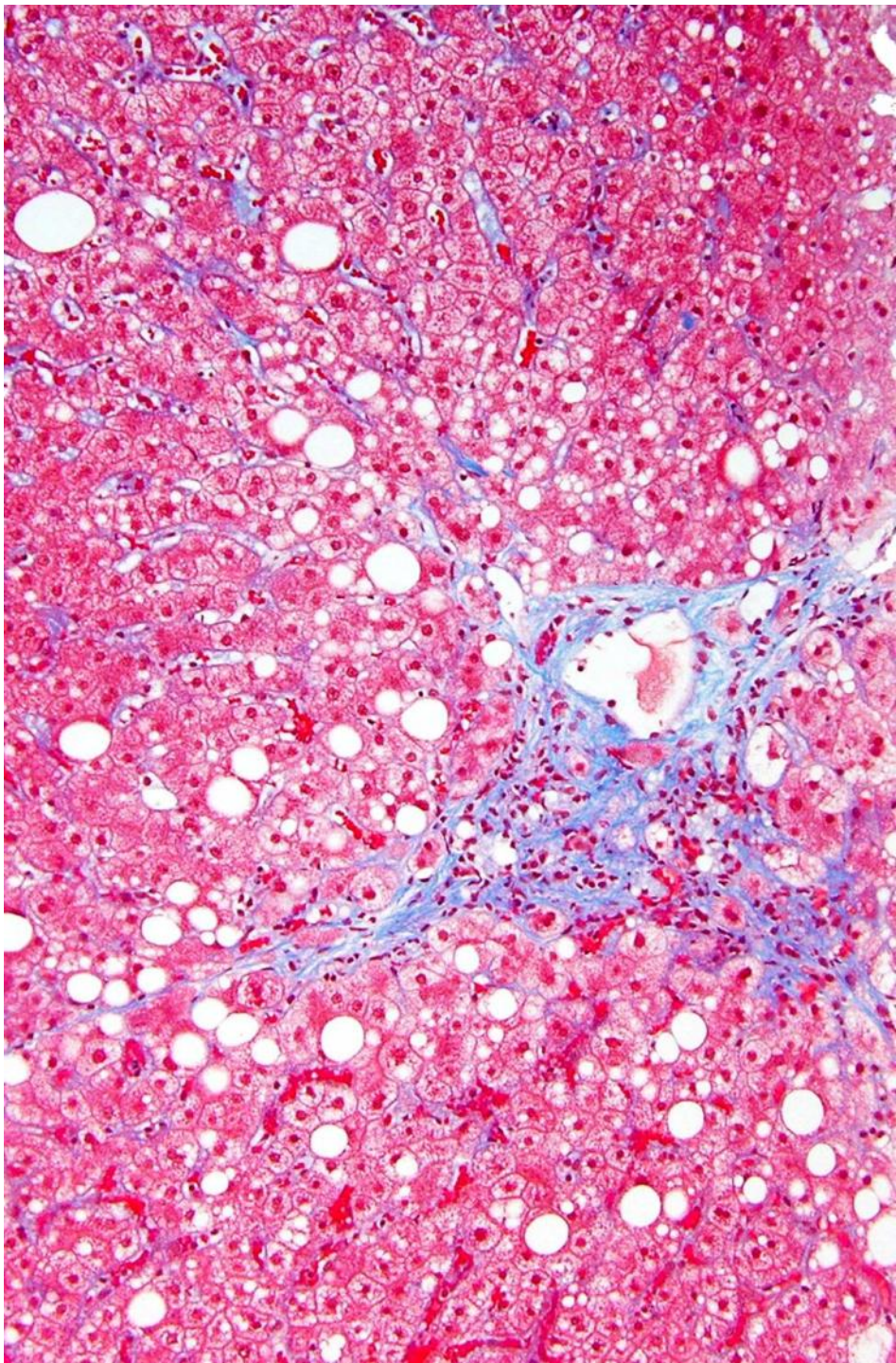


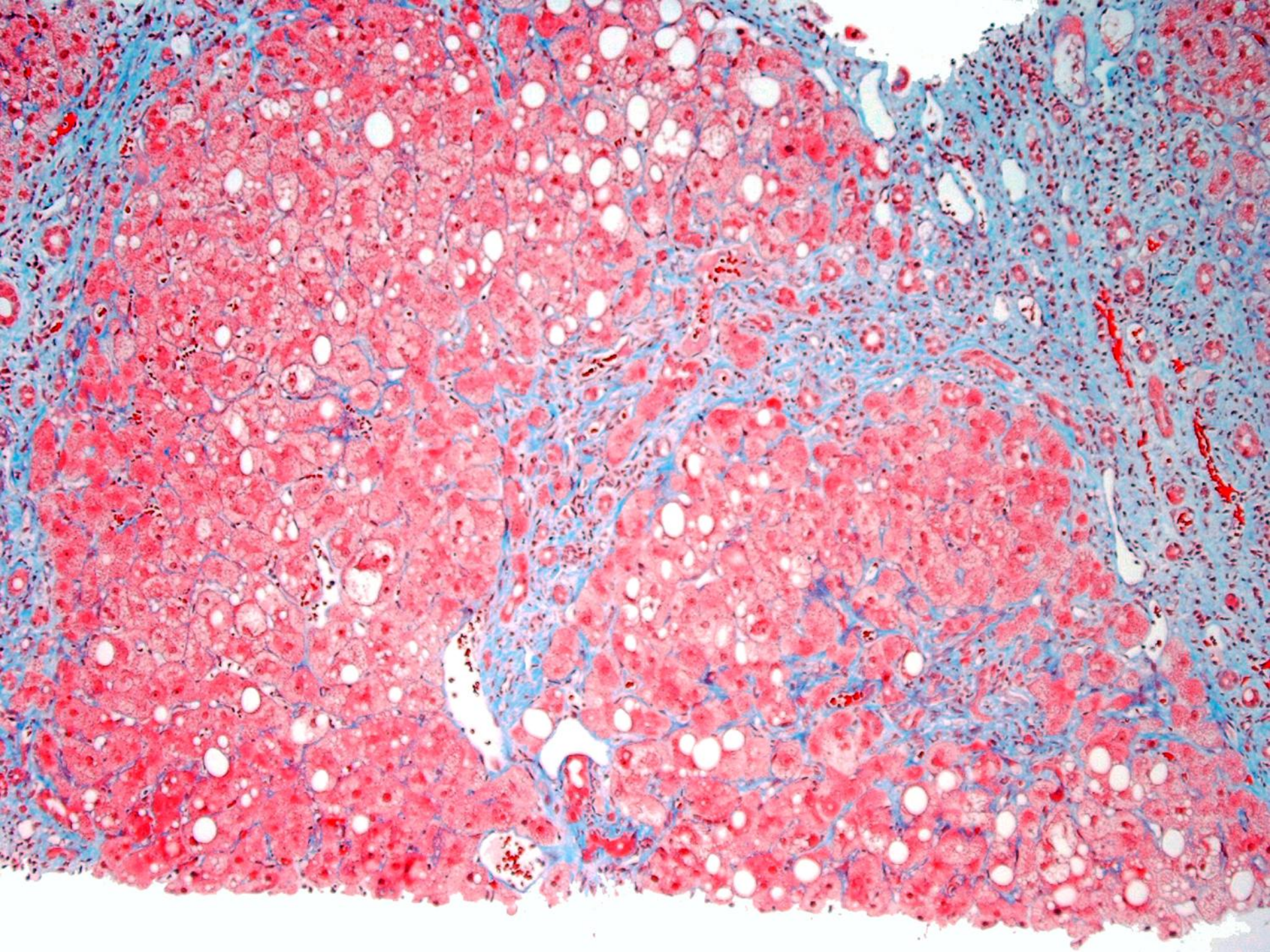


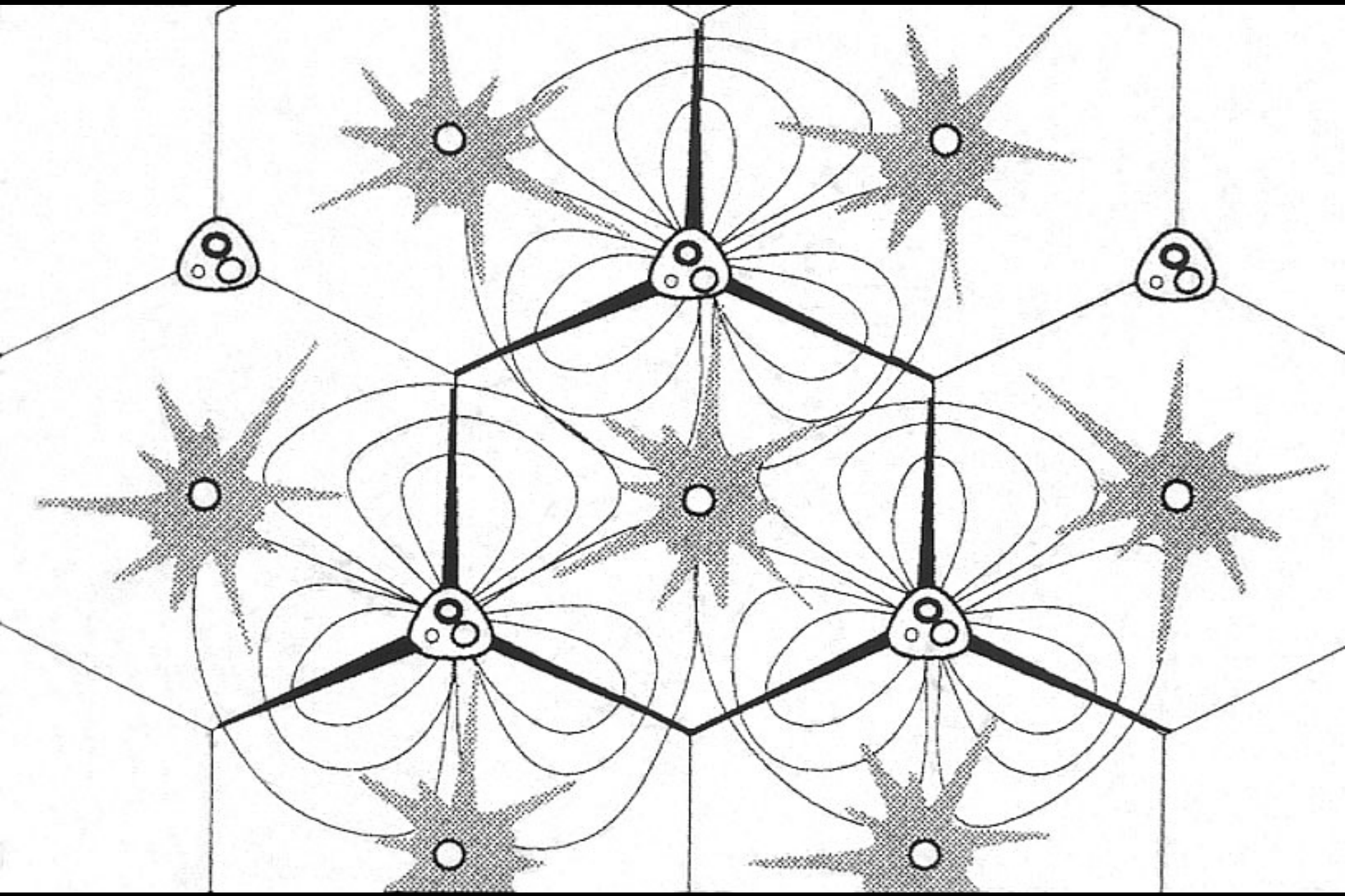
Stage 4:
Cirrhosis

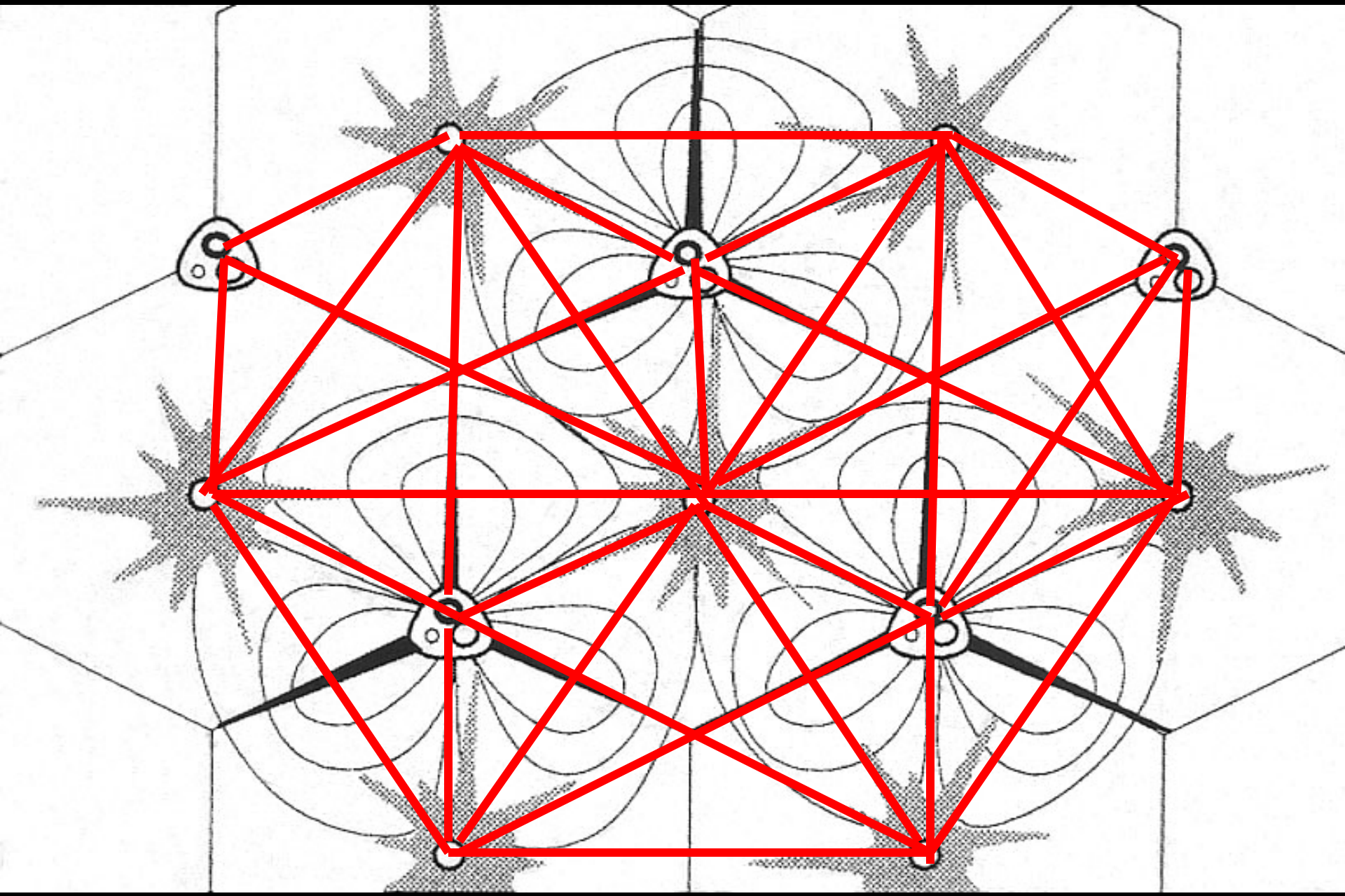


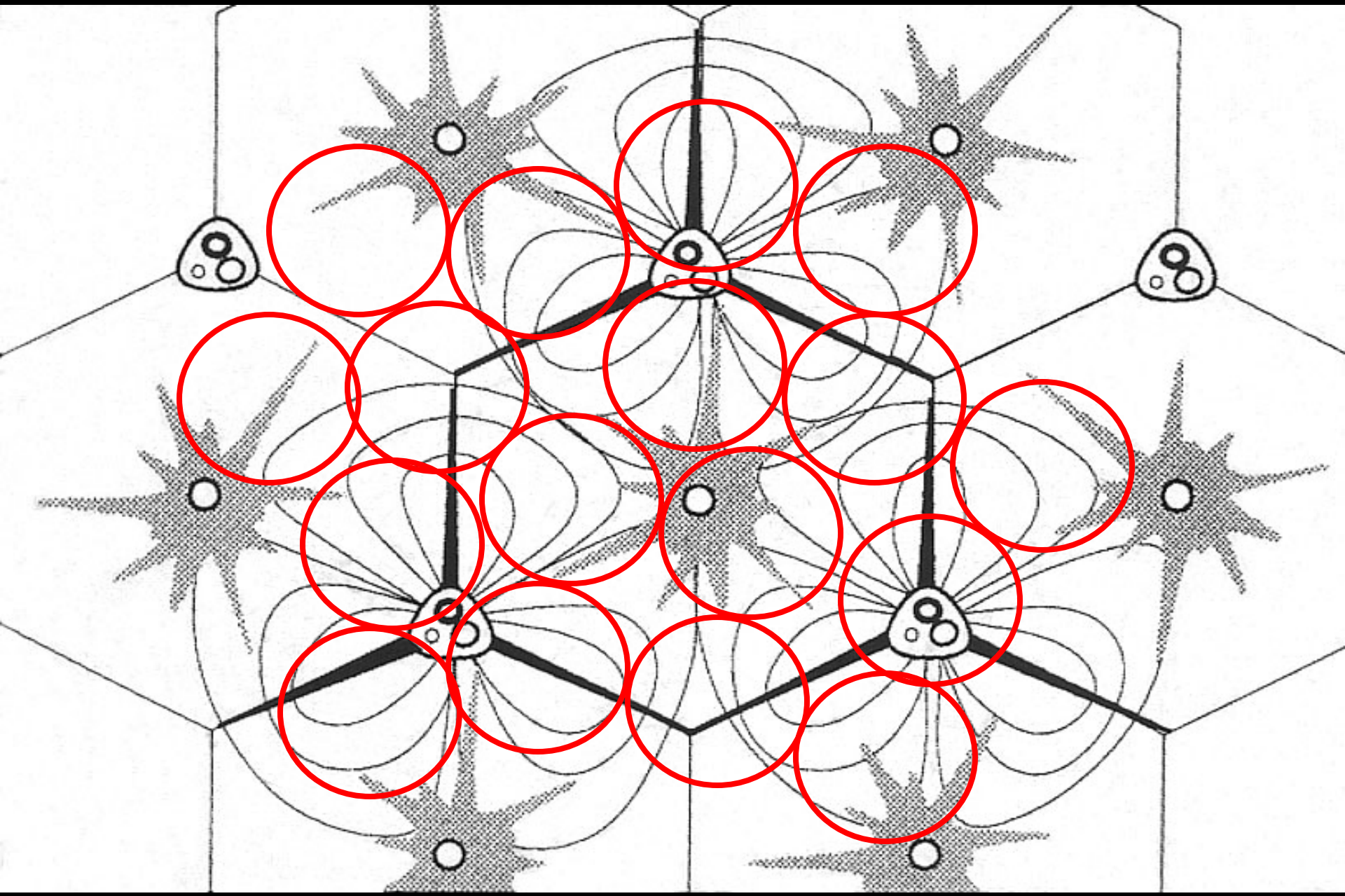


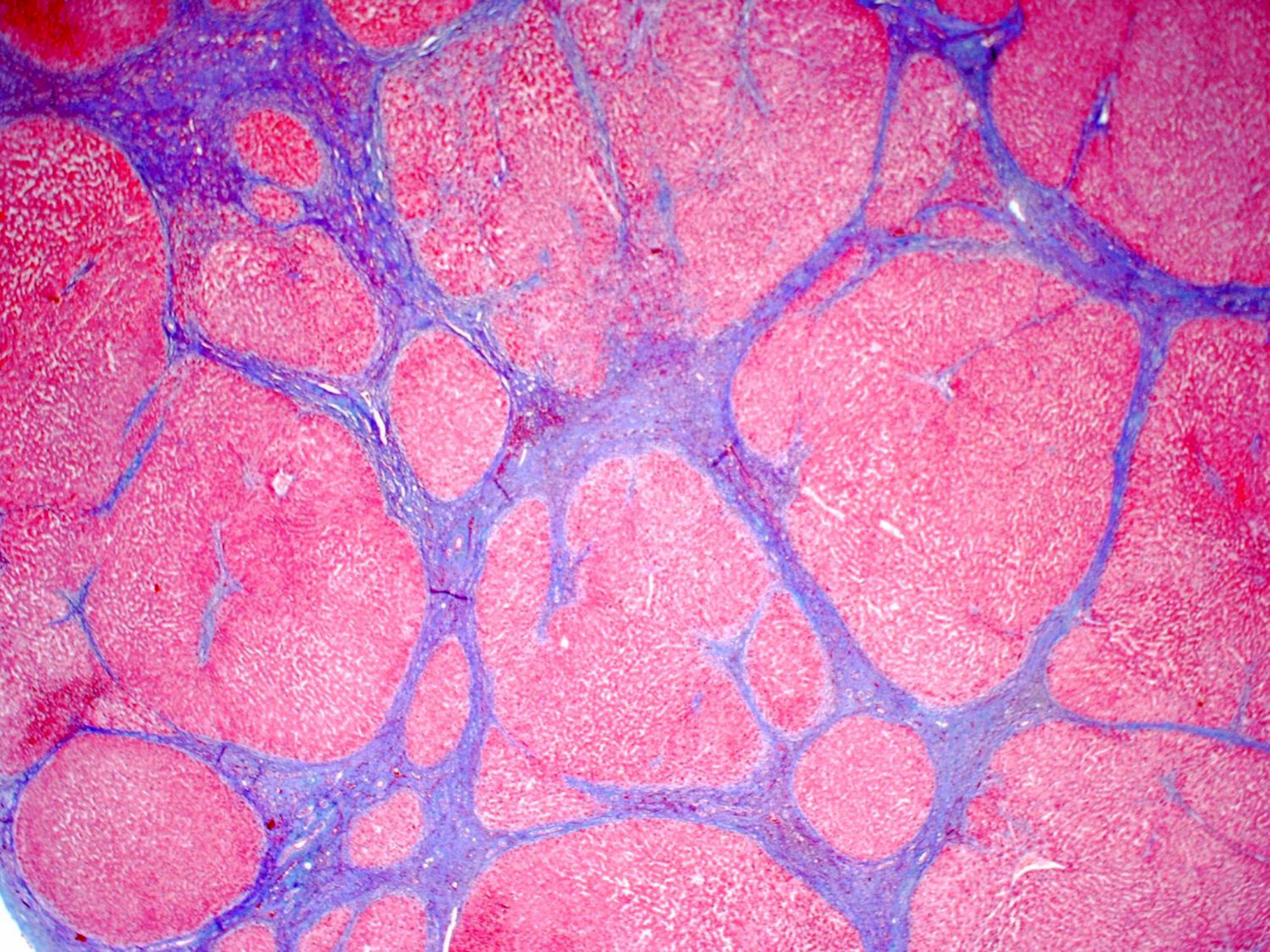


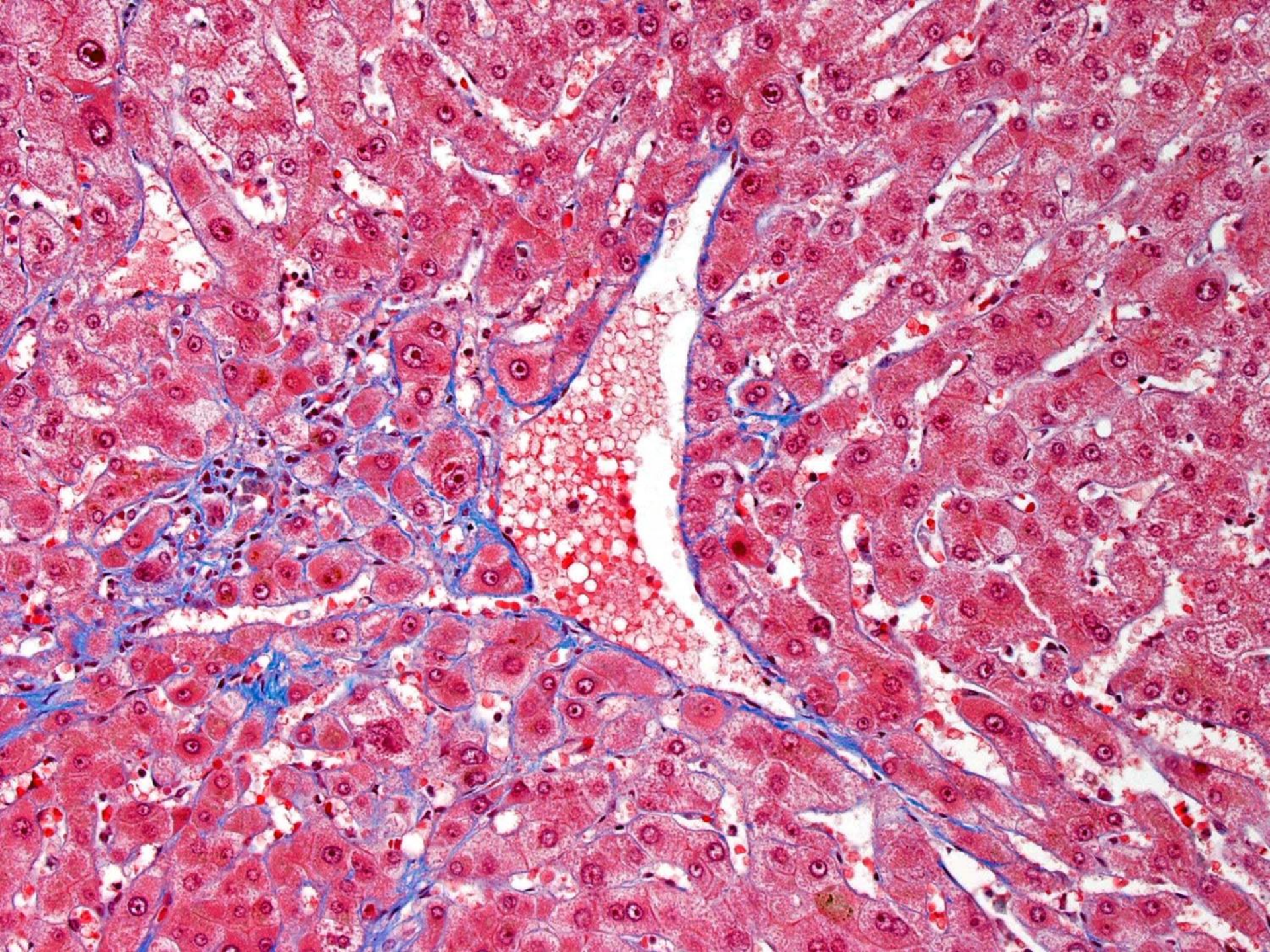


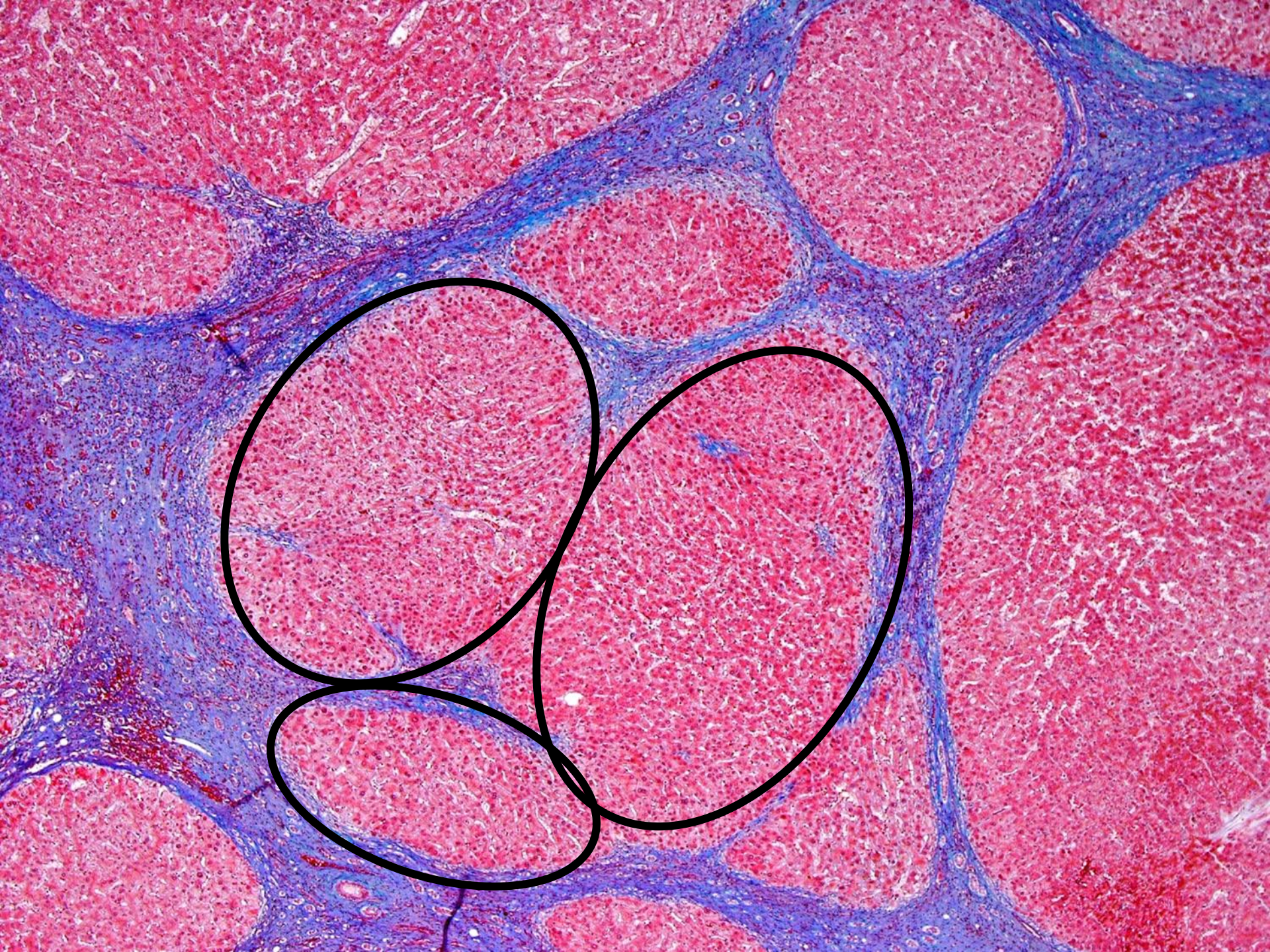












Can NASH grade predict progression of fibrosis?

Long-Term Follow-up of Patients With NAFLD and Elevated Liver Enzymes

Hepatology 2006; 44(4):865-73.

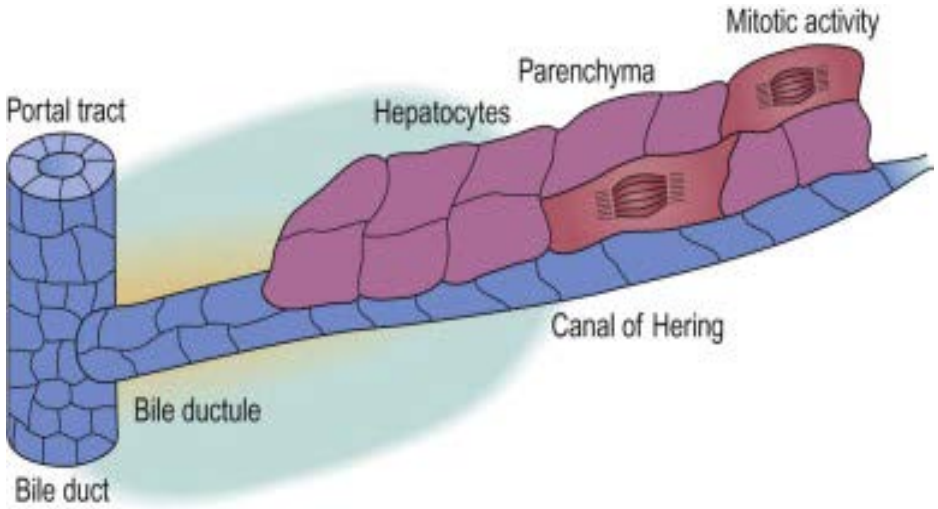
Mattias Ekstedt,[1] Lennart E. Franzén,[2] Ulrik L. Mathiesen,[3] Lars Thorelius,[4] Marika Holmqvist,[5] Göran Bodemar,[1] and Stergios Kechagias[6]

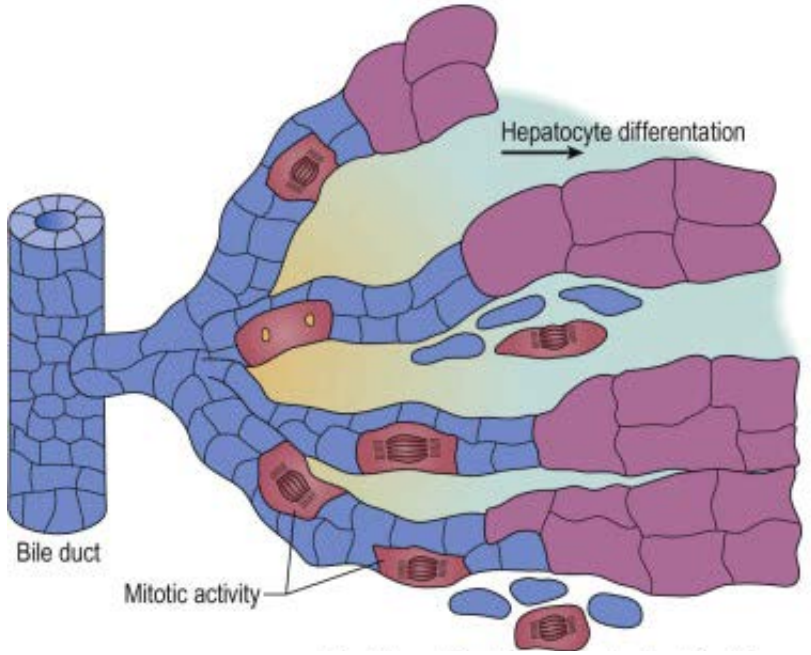
- 129 patients
- 71 NASH, 46 steatosis, 12 steatosis with unspecific inflammation
- mean follow-up (SD) was 13.7 (1.3) years
- 41% NASH patients demonstrated progression of fibrosis

Histology at Baseline Versus Progression in Fibrosis Stage

Necroinflammation	Progressive Fibrosis (n = 29)	Nonprogressive Fibrosis (n = 41)	*P*
Lobular inflammation	4 (14%)	1 (3%)	NS
Portal inflammation	9 (31%)	7 (17%)	NS
Periportal inflammation	4 (14%)	3 (7%)	NS
Hepatocellular ballooning	3 (10%)	2 (5%)	NS
Mallory's hyaline	1 (3%)	1 (2%)	NS

Mechanisms of Hepatic Regeneration

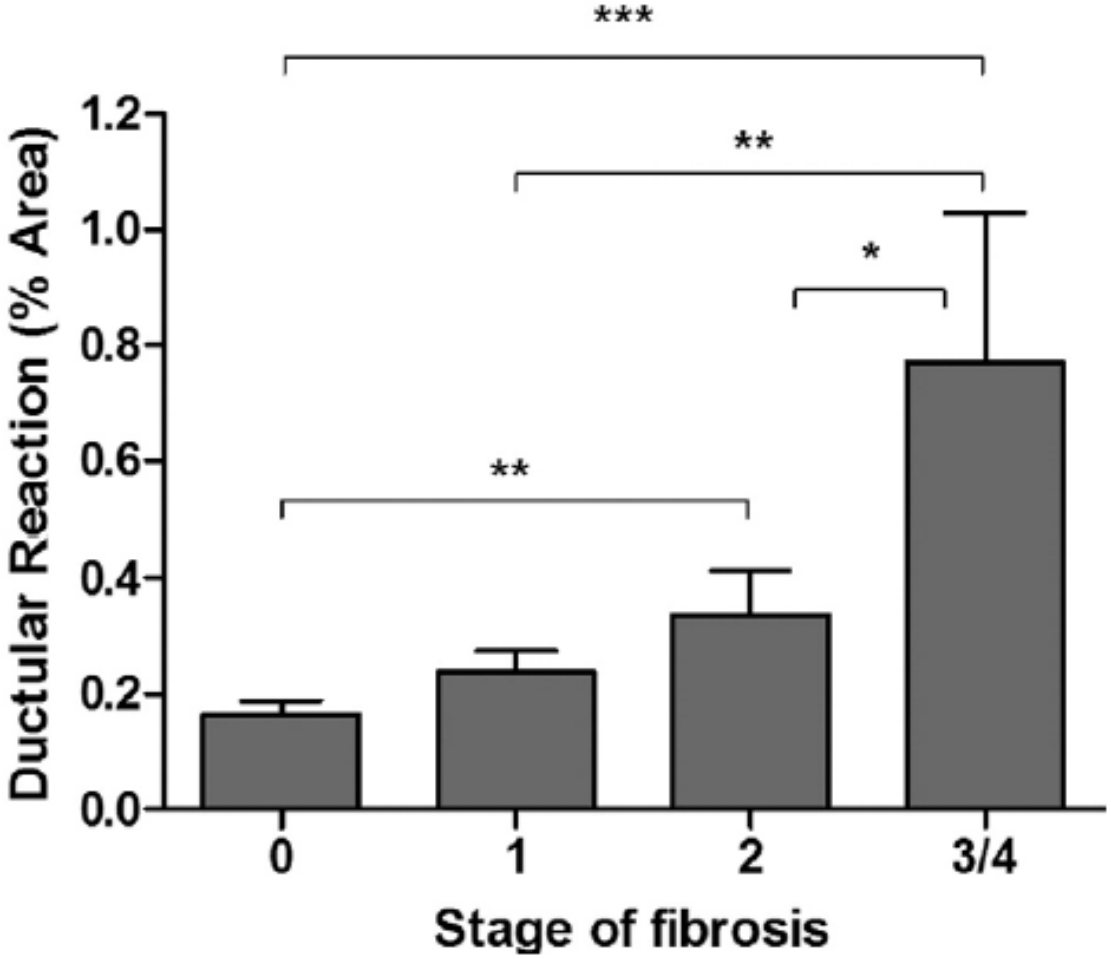


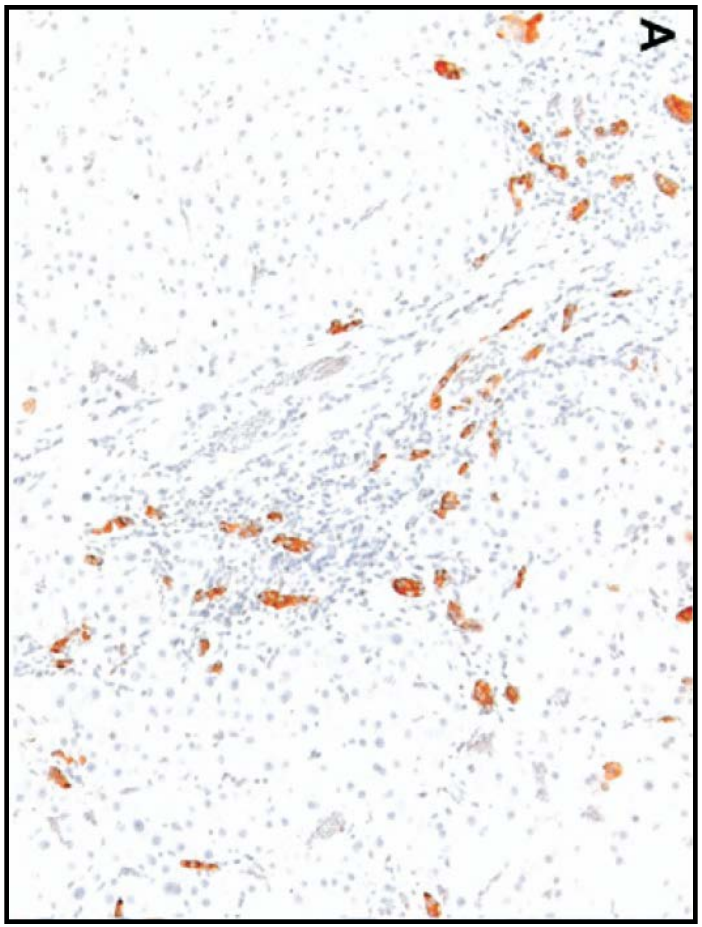


GASTROENTEROLOGY 2007;133:80–90

Progressive Fibrosis in Nonalcoholic Steatohepatitis: Association With Altered Regeneration and a Ductular Reaction

MICHELLE M. RICHARDSON,* JULIE R. JONSSON,* ELIZABETH E. POWELL,* ELIZABETH M. BRUNT,‡ BRENT A. NEUSCHWANDER–TETRI,‡ PRITHI S. BHATHAL,§ JOHN B. DIXON,‖ MARTIN D. WELTMAN,¶ HERBERT TILG,# ALEXANDER R. MOSCHEN,# DAVID M. PURDIE,** ANTHONY J. DEMETRIS,‡‡ and ANDREW D. CLOUSTON*





Aberrant expression of cytokeratin 7 in perivenular hepatocytes correlates with a cholestatic chemistry profile in patients with heart failure

Rish K Pai and John A Hart[1]

MODERN PATHOLOGY (2010) 23, 1650–1656

Aberrant Centrizonal Features in Chronic Hepatic Venous Outflow Obstruction

Centrilobular Mimicry of Portal-based Disease

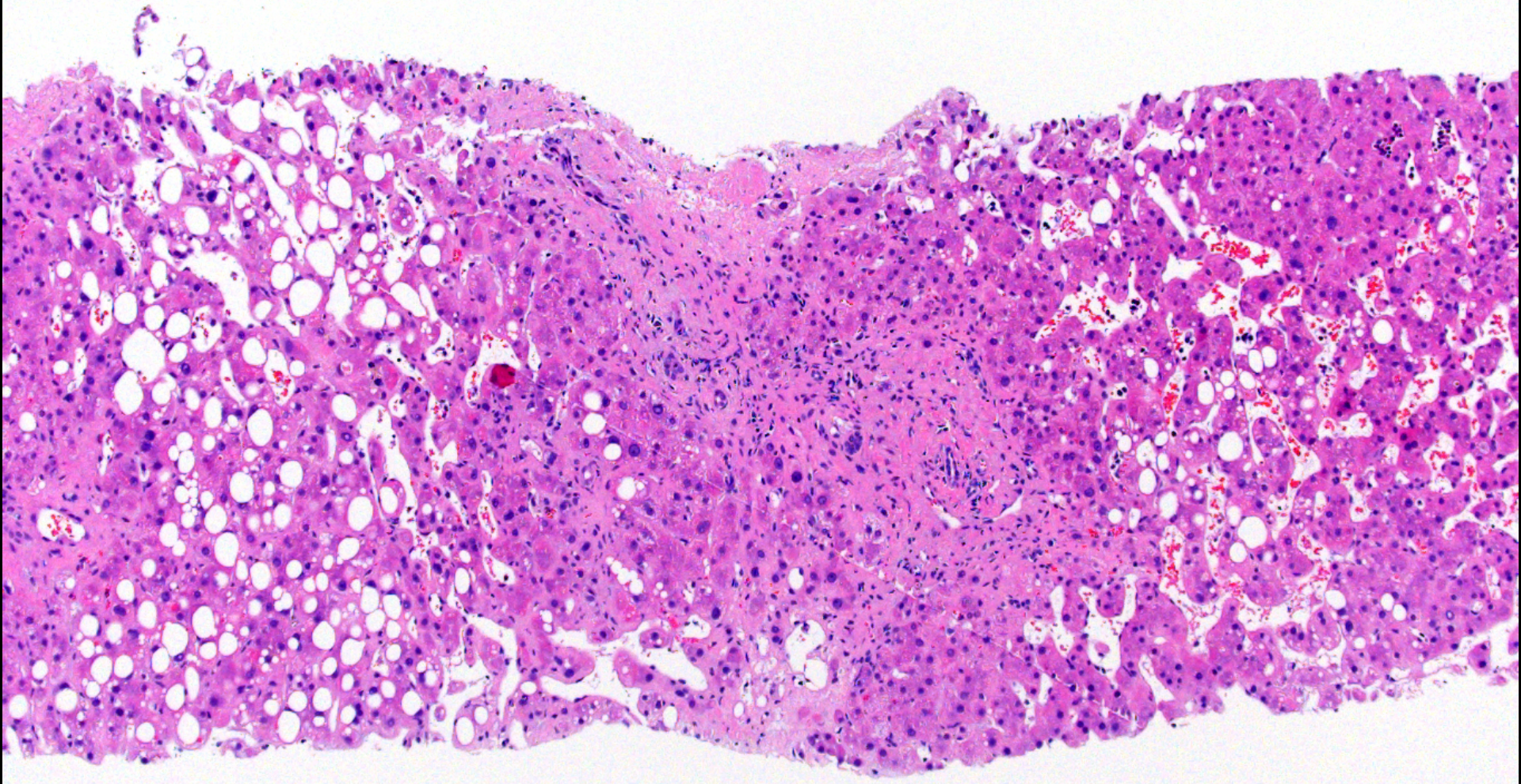
(*Am J Surg Pathol* 2014;38:205–214)

Gregor Krings, MD, PhD, Bilge Can, MD,† and Linda Ferrell, MD**

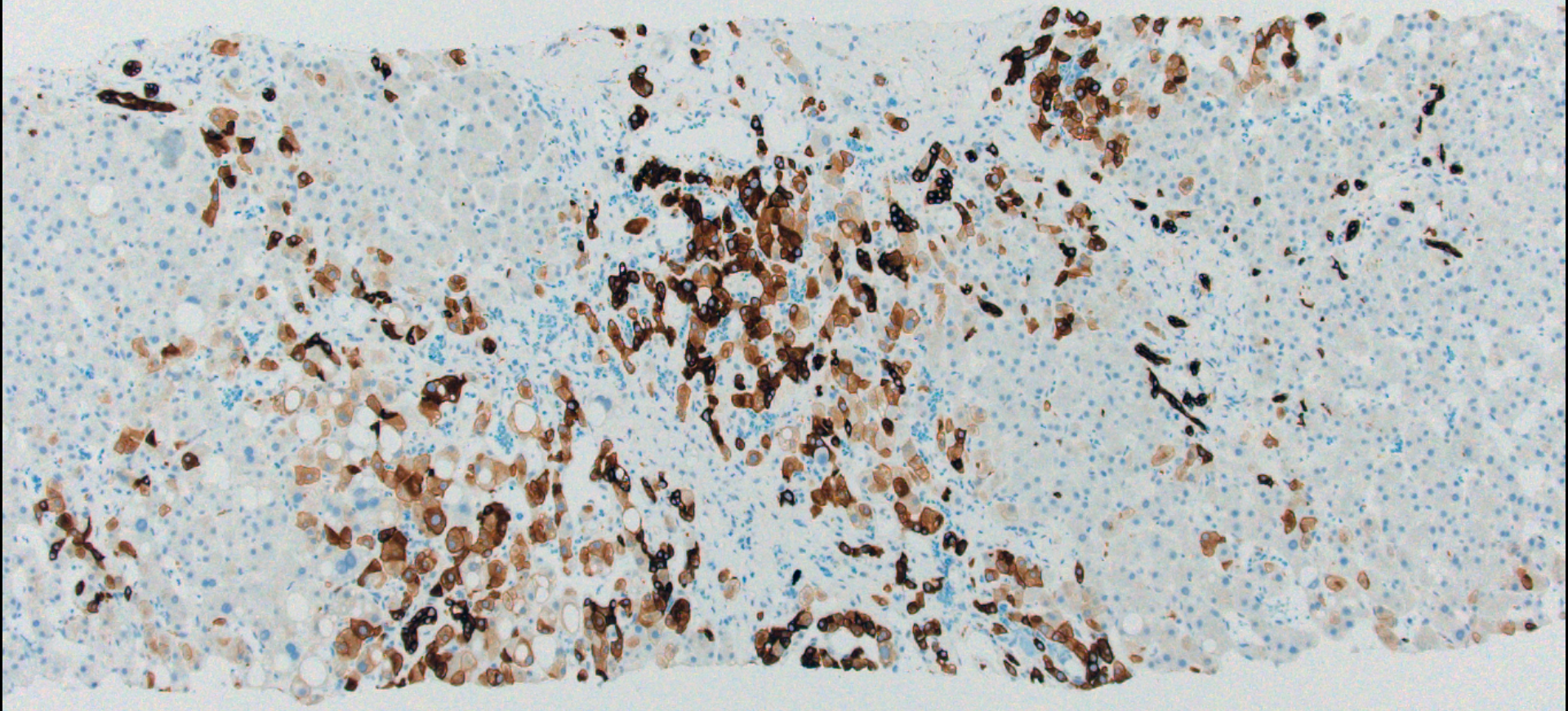
TABLE 6. Comparison of Fibrosis Stage With Prevalence of Ductular Metaplasia in Chronic Venous Outflow Obstruction[1]

Fibrosis Stage	Grade 0	Grade 1	Grade 2	Grade 3	Total (n [%])
0/1a	4	0	0	0	0/4 (0)
1b	7	10	1	0	11/18 (61.1)
2	4	9	2	0	11/15 (73.3)
3	2	9	3	6	18/20 (90.0)
4	0	1	1	2	4/4 (100)
Total	17	29	7	8	44/61 (72.1)

Semiquantitative scoring of ductular metaplasia: 0, none; 1, mild; 2, moderate; and 3, marked.



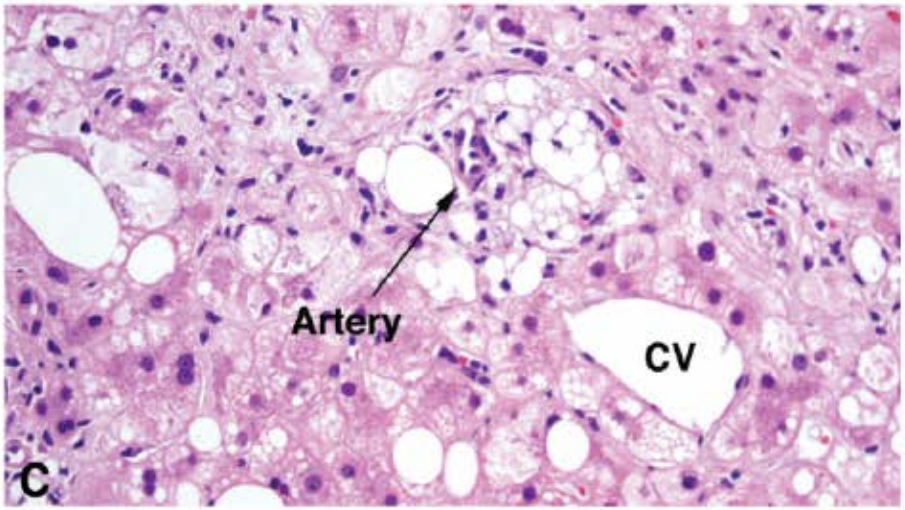
hepatic venous outflow obstruction due to severe chronic heart failure

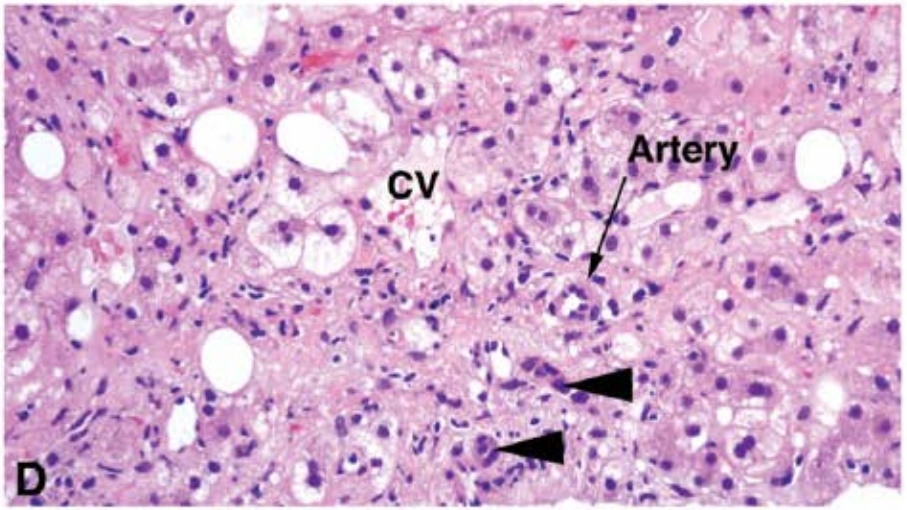

CK7

Centrizonal Arteries and Microvessels in Non-Alcoholic Steatohepatitis

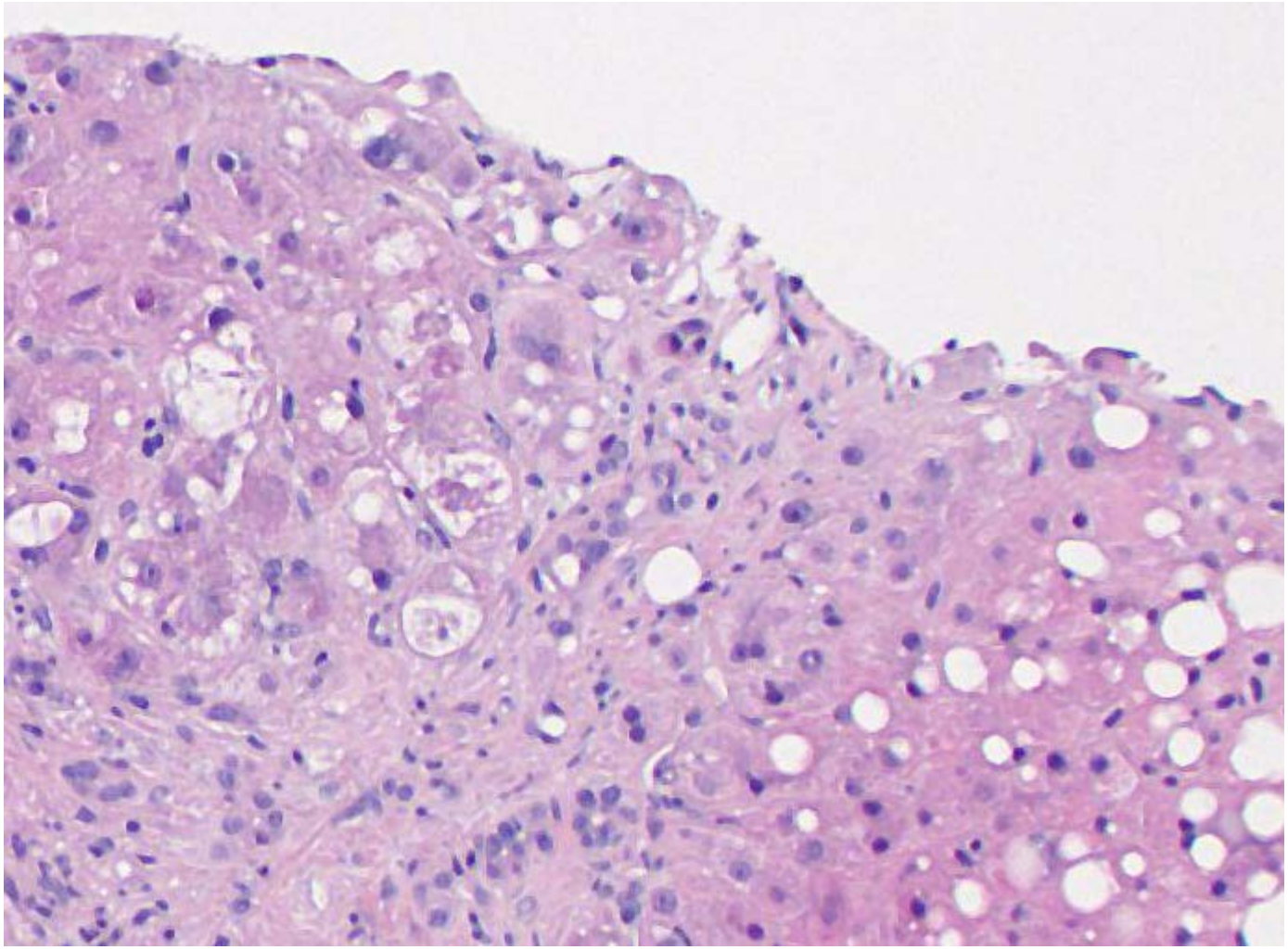
Am J Surg Pathol. 2011 September ; 35(9): 1400–1404.

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





Does centrilobular ductular reaction correlate with fibrosis in NASH?



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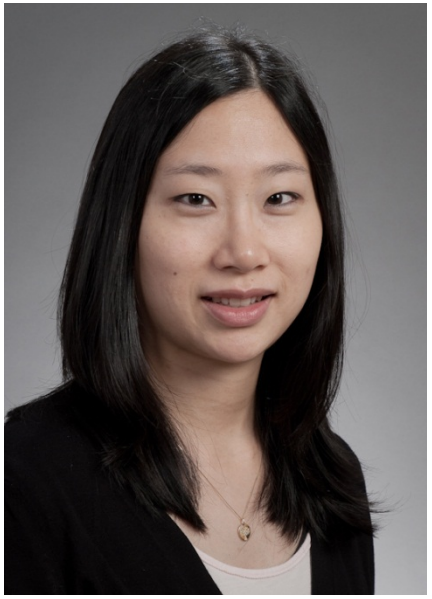

Maria Westerhoff, M.D
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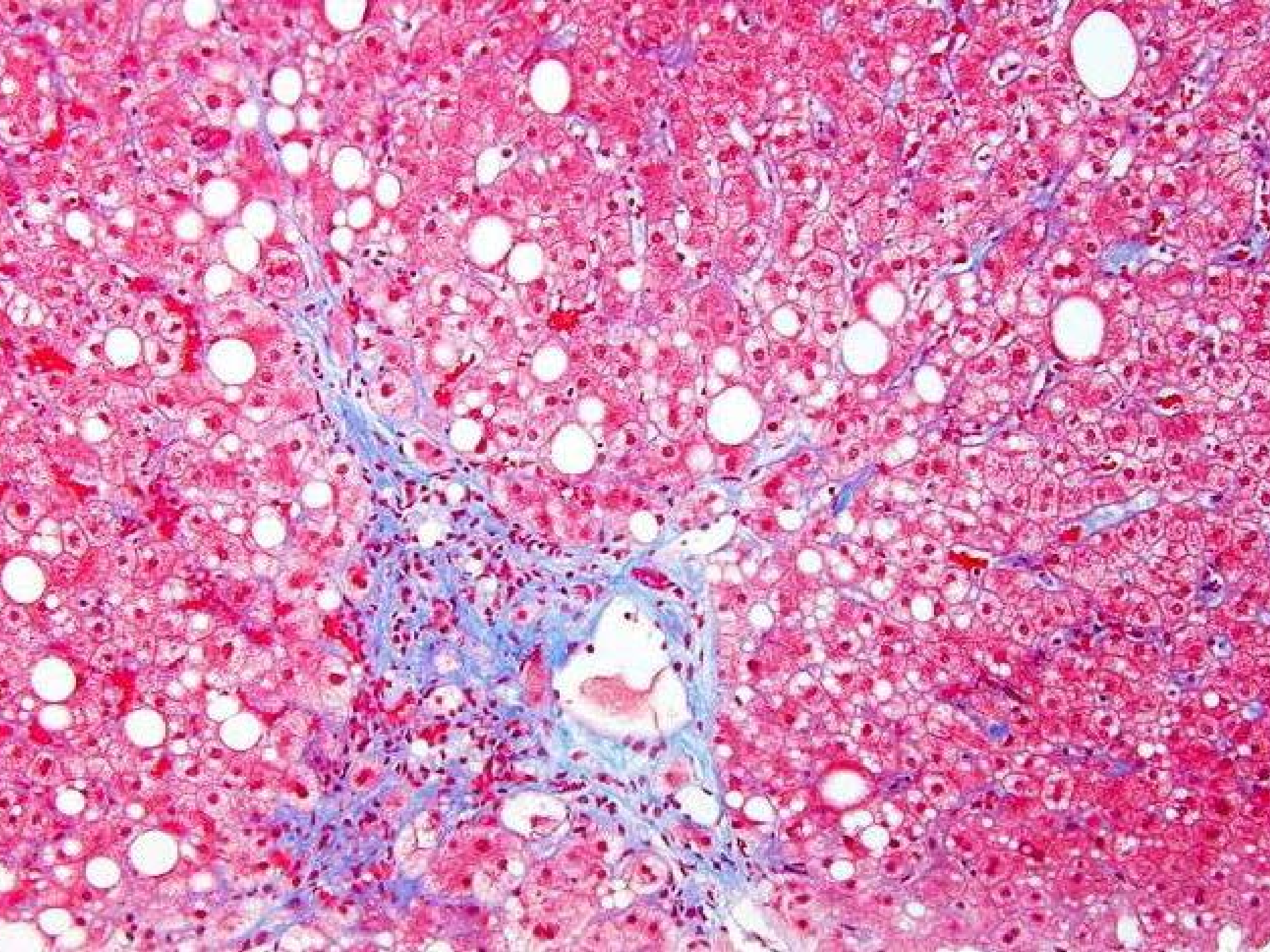
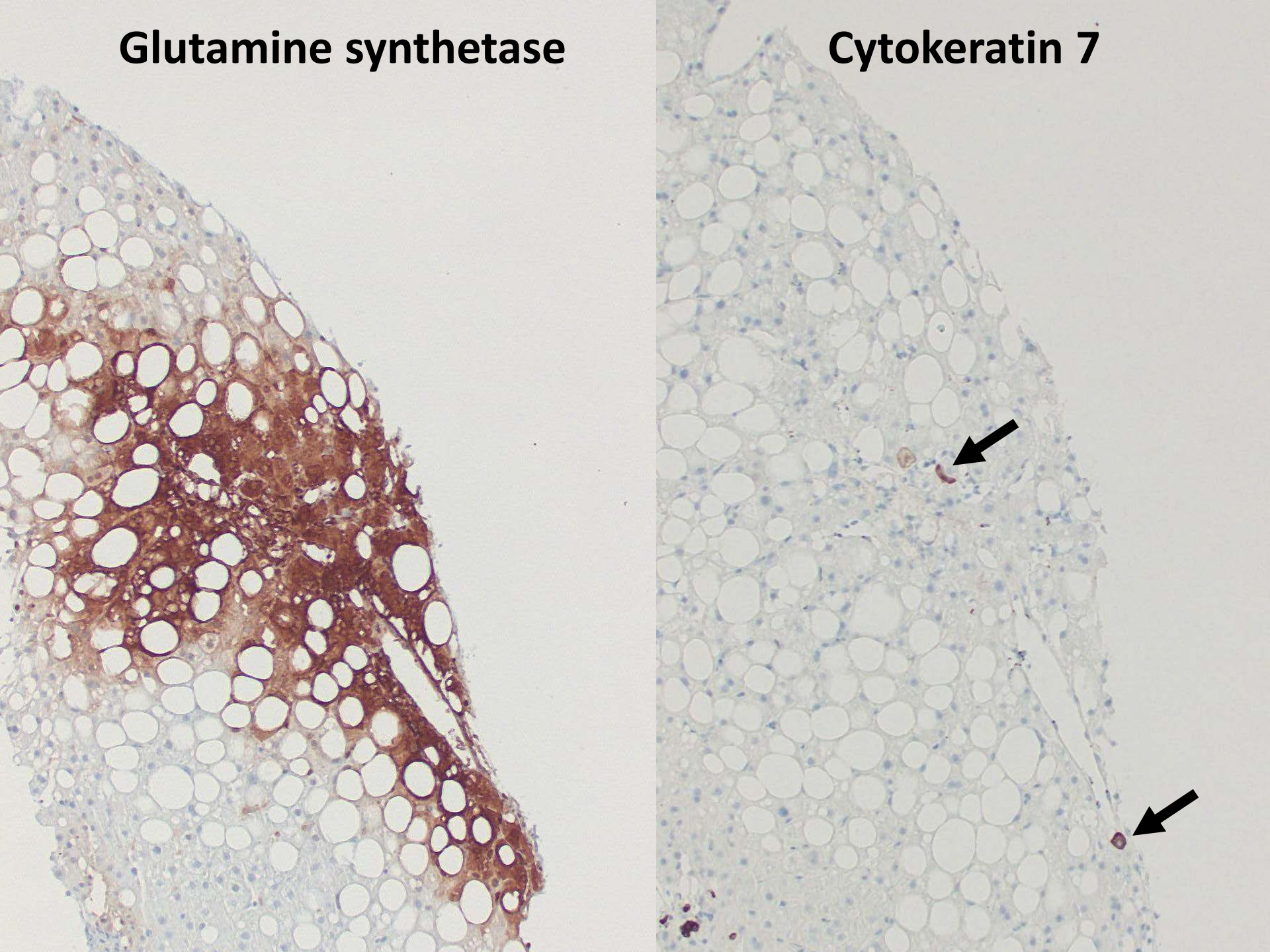
Study Design

Number of patients		**52**
Age (yrs)		**33-78, median 54**
Sex (M/F)		**1:1.36**
NASH stage*		
	0	**7 (13.5%)**
	1	**6 (11.5%)**
	2	**22 (42.3%)**
	3	**17 (32.7%)**
NASH grade*		
	1	**17 (32.7%)**
	2	**30 (57.7%)**
	3	**5 (10.0%)**

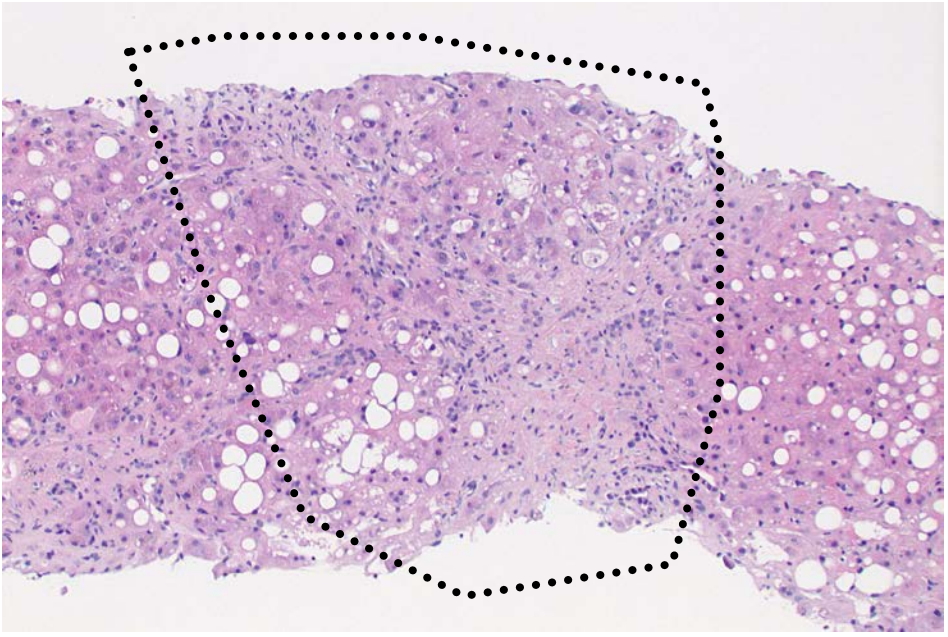
*** modified Brunt system**

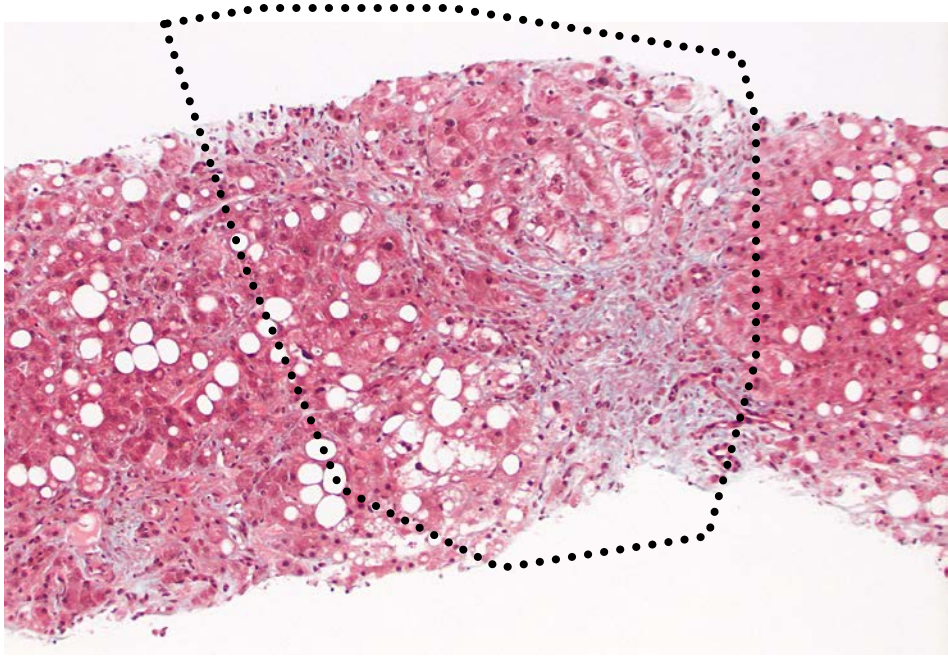


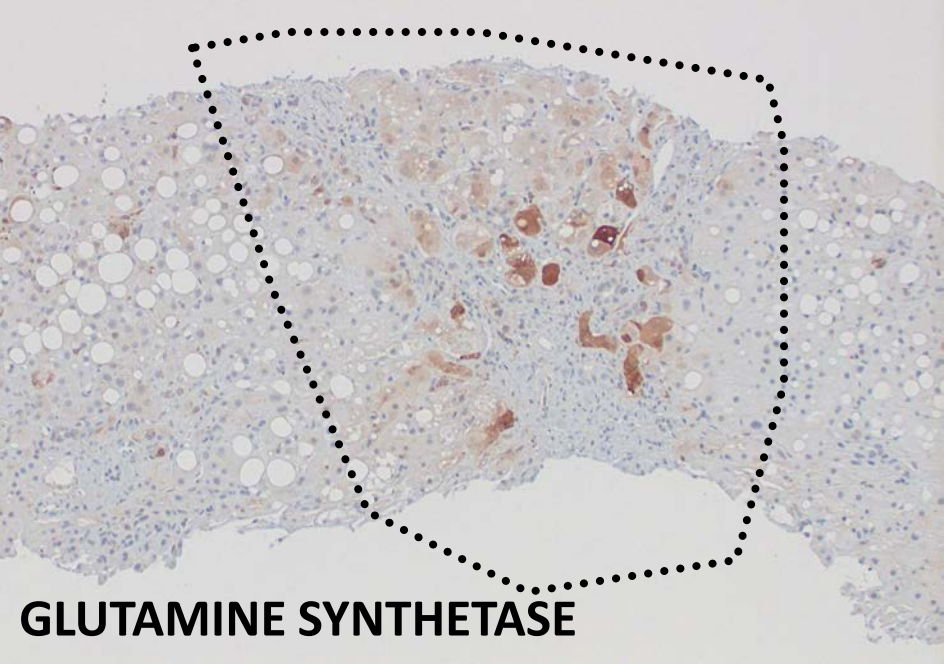
GS and CK7 immunostains on consecutive sections of each biopsy

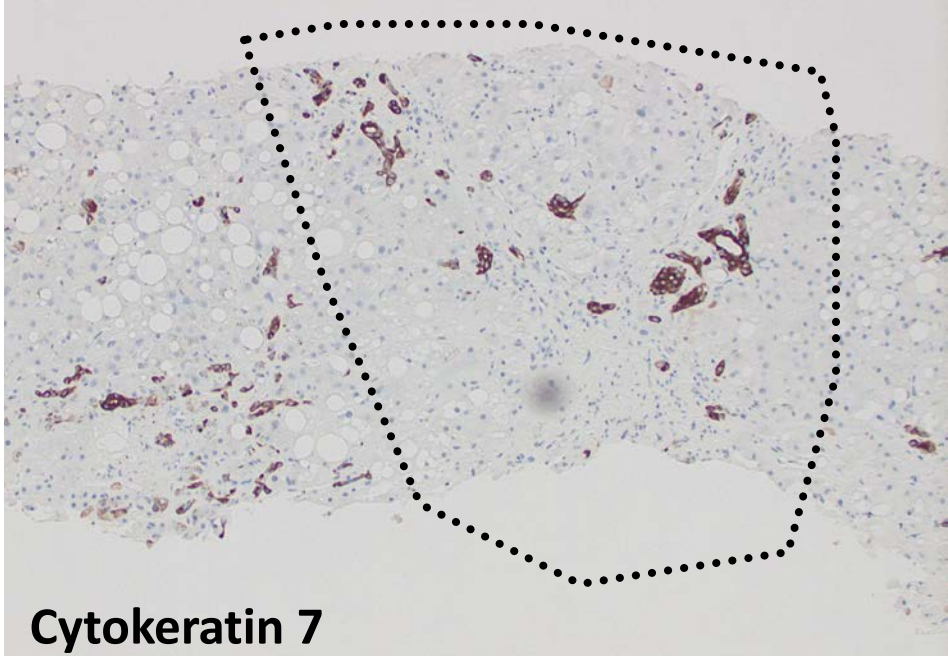

Glutamine synthetase
Cytokeratin 7

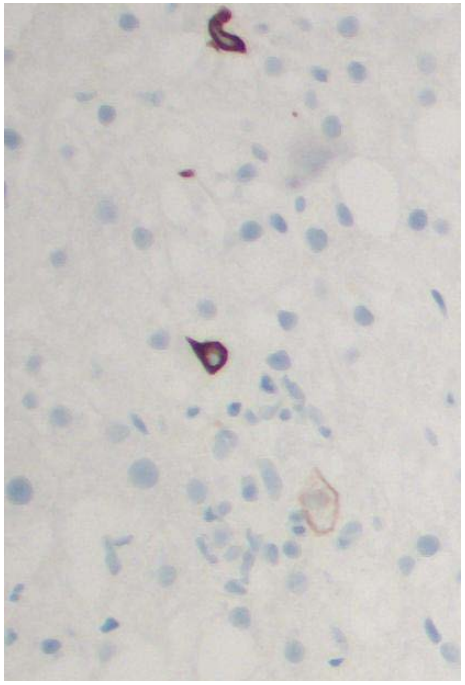
Case 45 S07-23911 (grade 2 / stage 2)

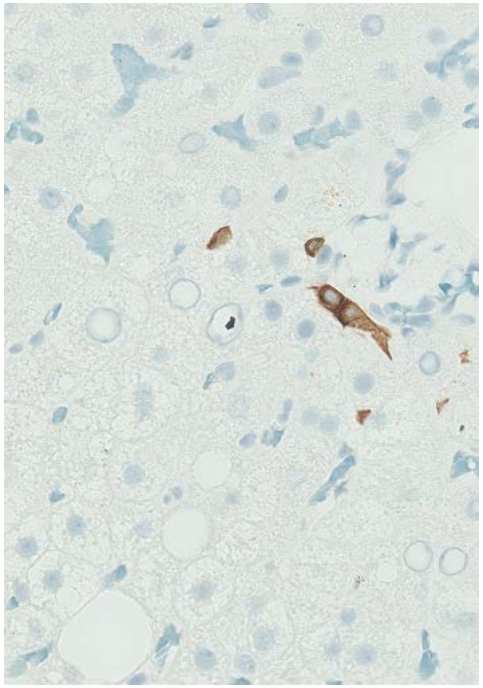


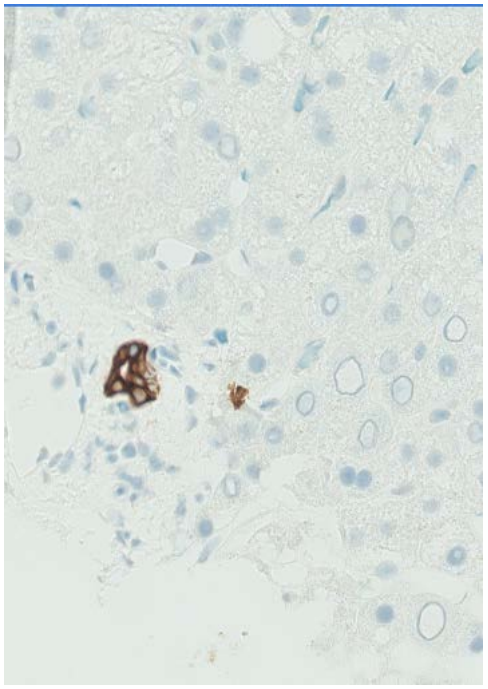








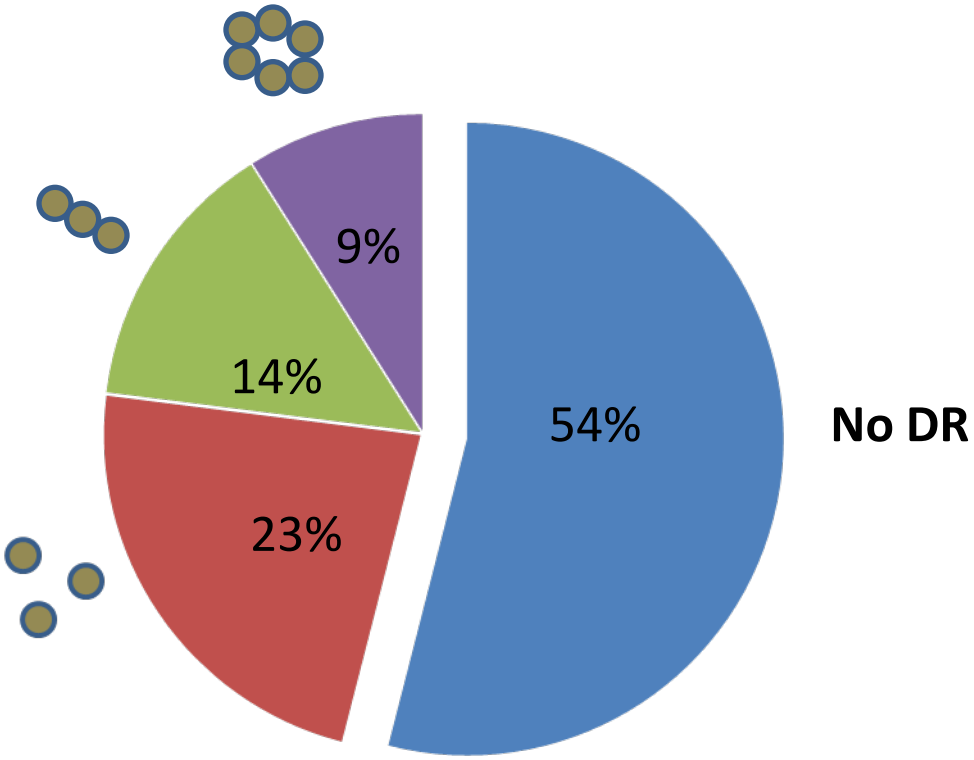




- **The presence of CK7+ cells within each GS+ centrilobular zone (CLZ) of every biopsy was recorded as either: no CK7+ cells, isolated single CK7+ cells, CK7+ cells in strings, or CK7+ ductular structures.**

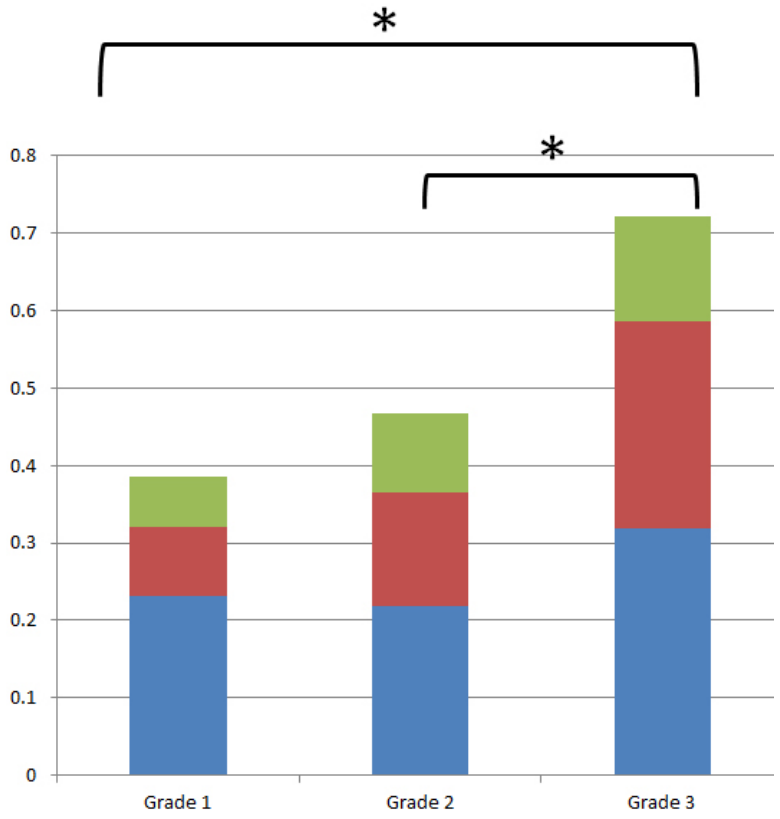
- **In addition every portal tract (PT) in the CK7 stained slides was graded as either: no ductular reaction, mild ductular reaction, or florid ductular reaction.**

Centrilobular DR is common in NASH



a total of 1250 GS positive centrilobular zones were scored

Centrilobular DR increases as grade increases



* P<0.05

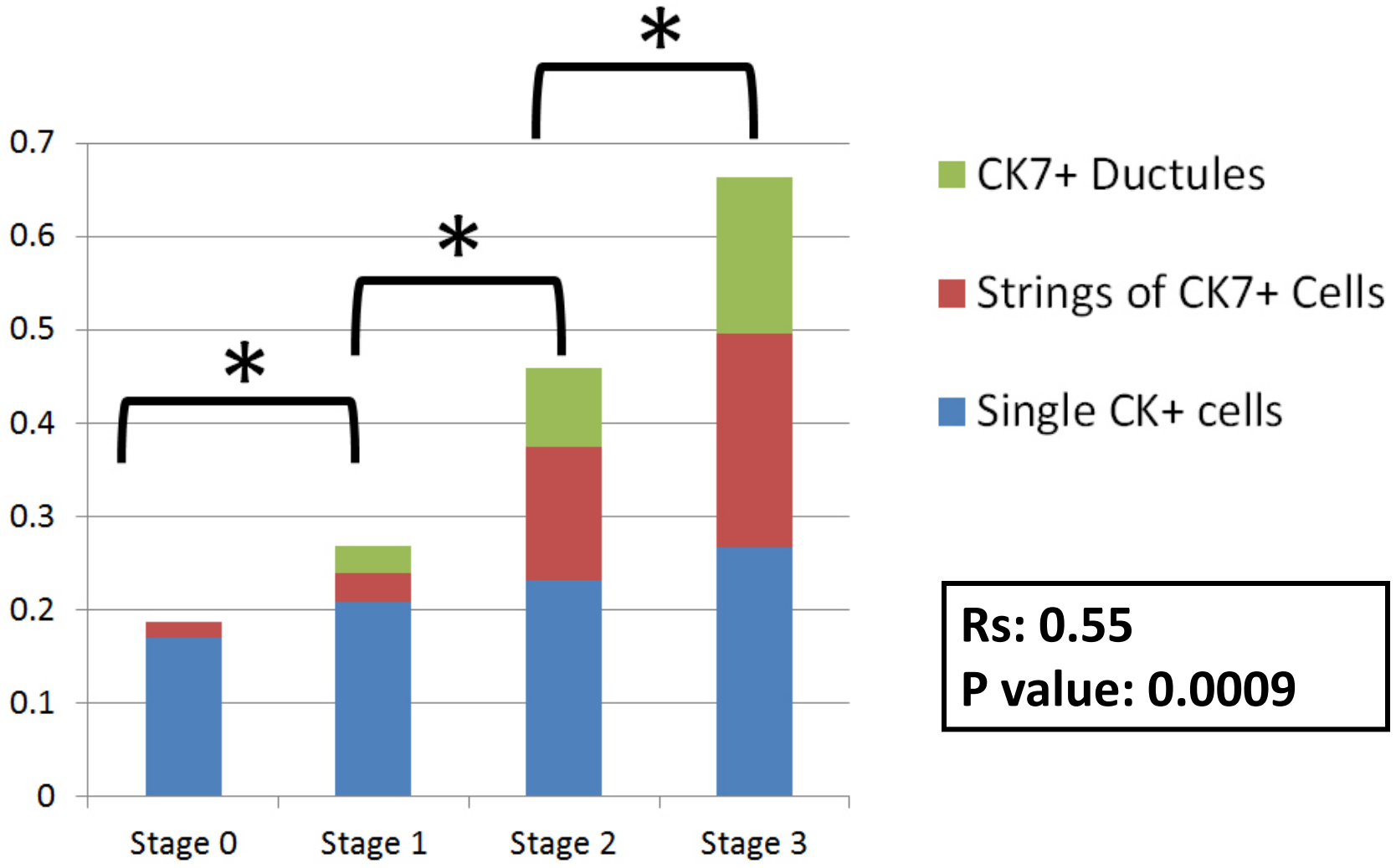
	Grade 1	Grade 2	Grade 3
Single CK+ cell	23%	22%	32%
String of CK+ cells	9%	15%	27%
CK7+ Ductules	6%	10%	13%

Rs: 0.5
P value : 0.015

Factors associated with centrilobular DR

	Centrilobular Ductular Structures (single cells, strings & complete ductules)	
	Rs	***p value***
→ **Ballooning score**	0.54	0.007
→ **Presence of Mallory-Denk bodies**	0.44	0.04
→ **Lobular inflammation score**	0.5	0.016
Extent of Steatosis	-0.14	0.5
Location of Steatosis	0.03	0.22
Portal inflammation	-0.14	0.72

Centrilobular DR increases as fibrosis increases



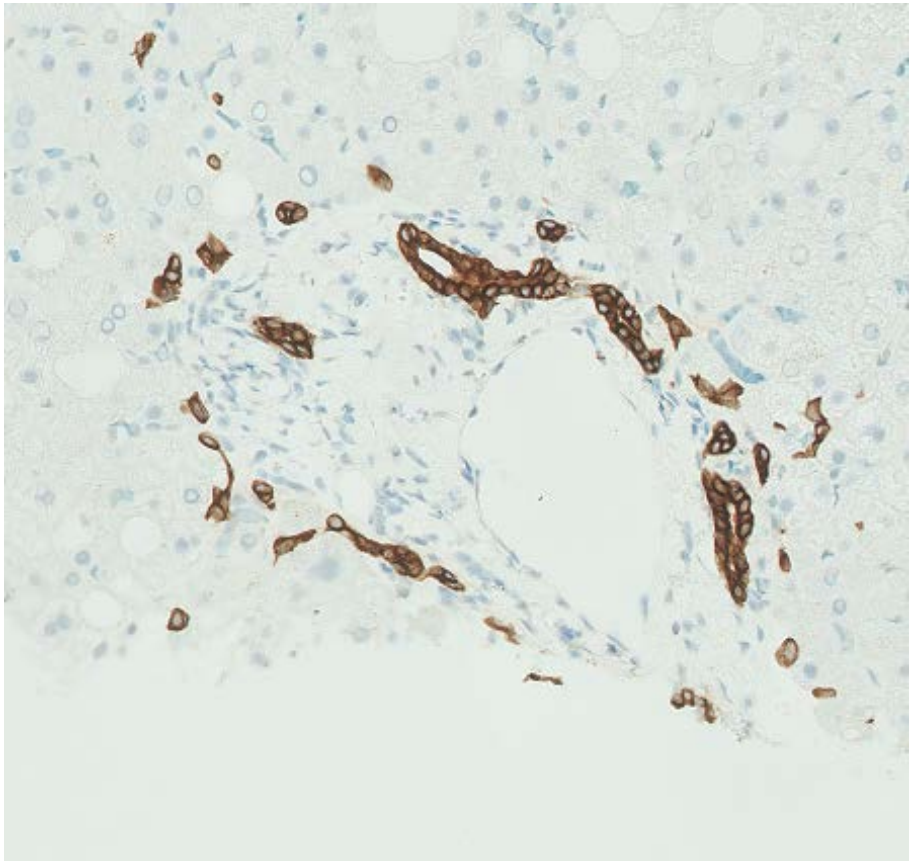
Rs: 0.55
P value: 0.0009

*** P<0.05**

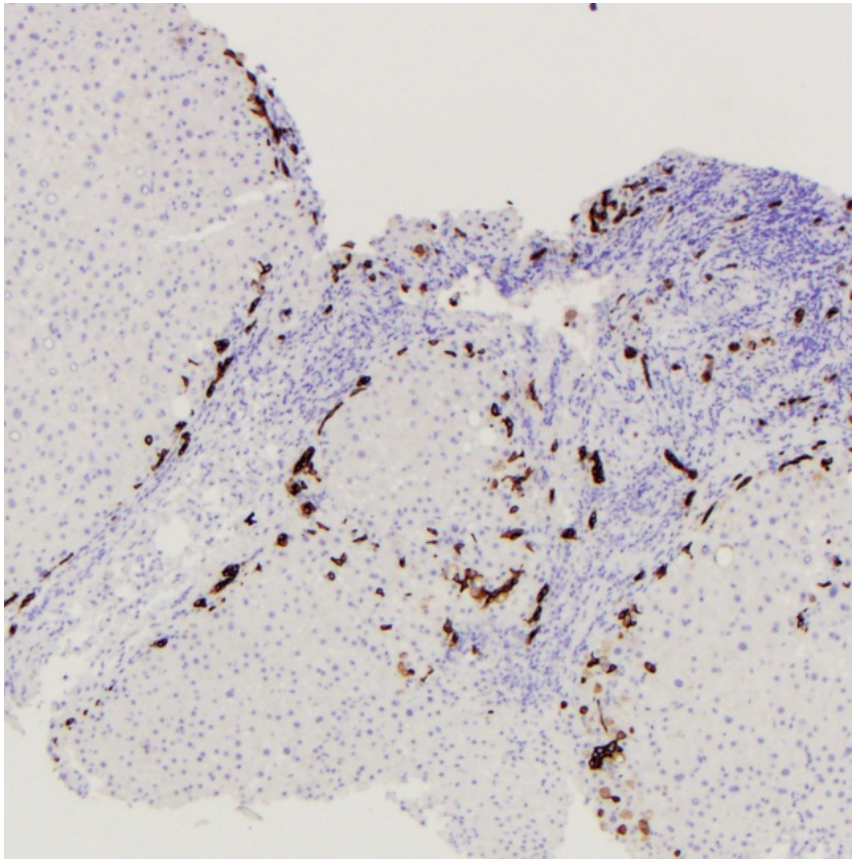
Portal DR is common in NASH

Total portal area scored = 897

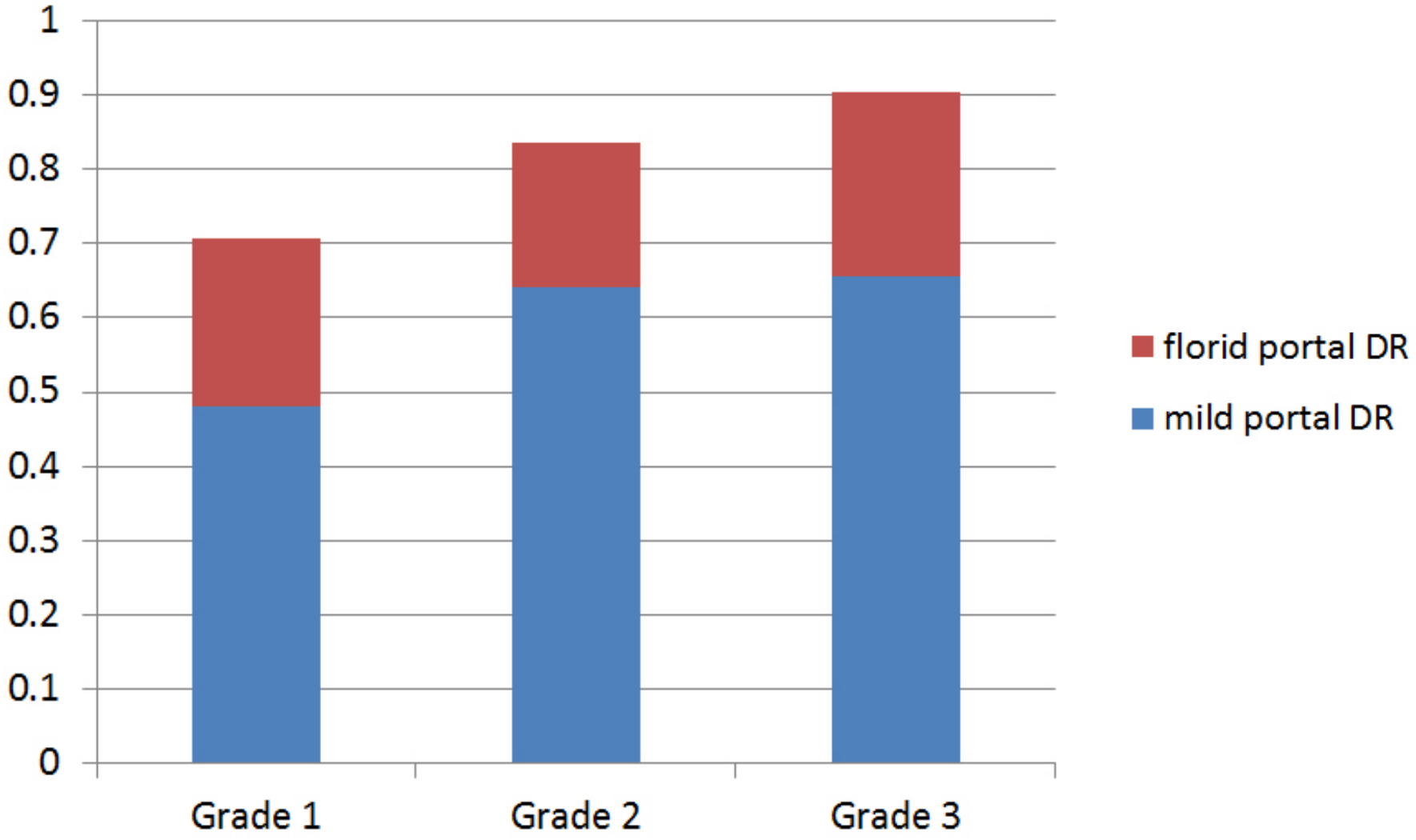
mild portal DR = 511 (57%)



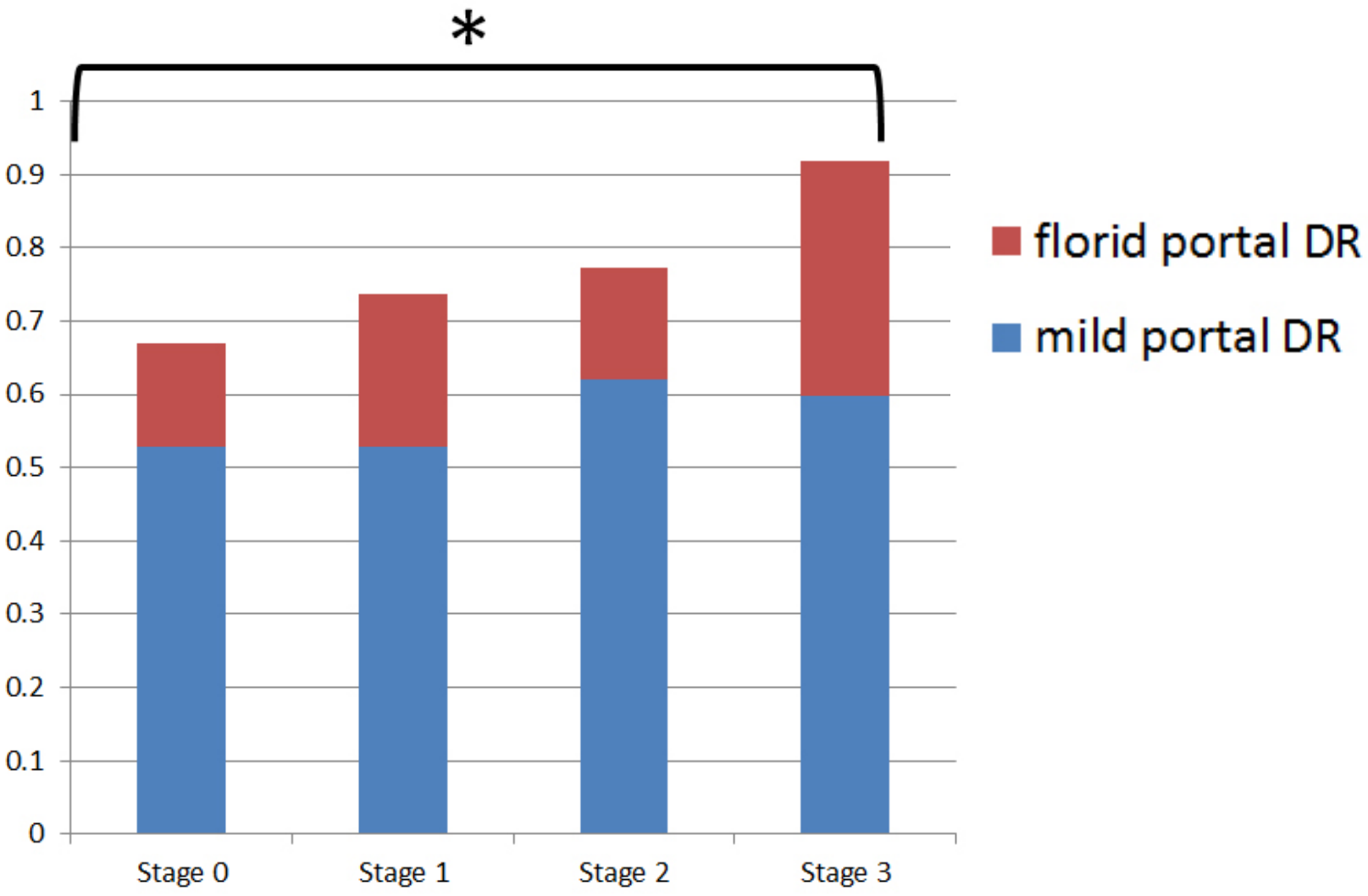
florid portal DR = 188 (21%)



Portal DR and degree of necroinflammatory activity



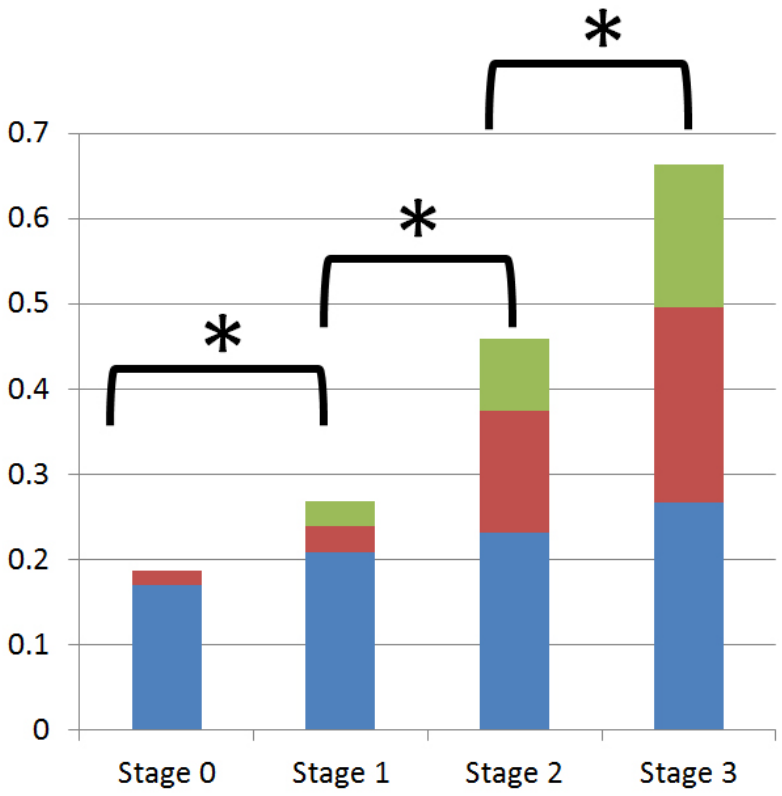
Portal DR and fibrosis

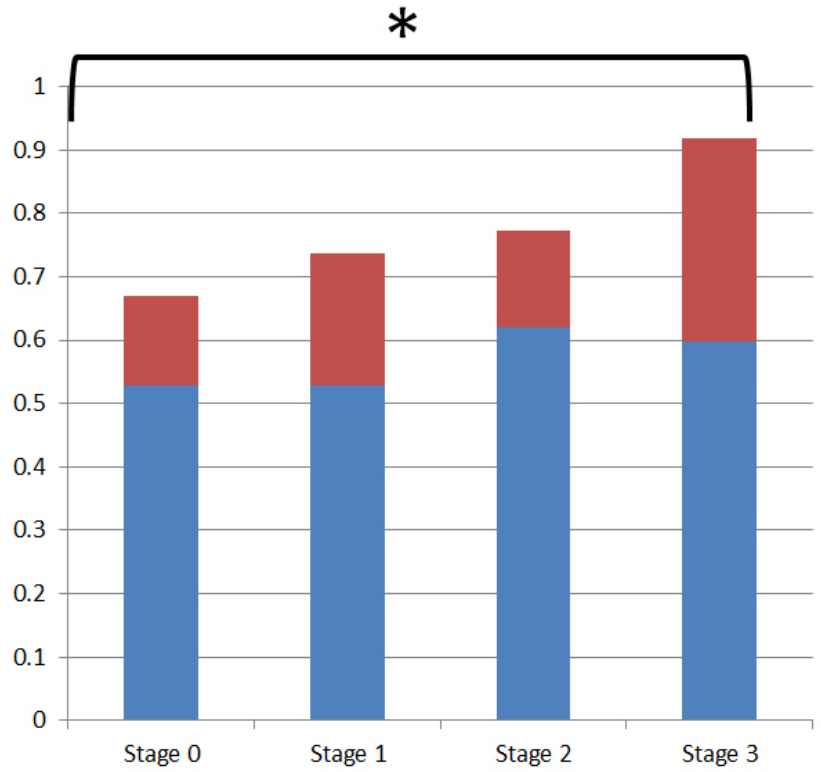


*** P<0.05**

Correlation with fibrosis

centrilobular DR vs. portal DR





Multivariate analysis:

Rs: 0.56

p Values: Centrilobular DR **0.03**

Portal DR **NS**

Conclusions

- CL CK7+ ductular elements may cause confusion in distinguishing portal tracts from CL zones, and GS immunostains may be helpful in this regard
- CK7+ CL ductular elements are common in NASH
- The development of CK7+ CL ductular elements correlates with increasing necroinflammatory activity and fibrosis.
- It is possible that the development of CK7+ CL ductular elements contributes to the development of fibrosis in NASH.

Histopathologic Features Related to Progression of Fibrosis in Sequential Liver Biopsies in Non-Alcoholic Steatohepatitis

- 51 NASH patients who underwent 2 liver biopsies at least 1 year apart were studied.
- Hepatocyte ballooning, Mallory-Denk bodies, lobular & portal inflammation, lobular neutrophils, steatosis & fibrosis stage were scored in initial biopsies.
- Centrilobular CK7+ elements were quantitated by form and degree in initial biopsies (CK7 & GS immunostains).
- Portal ductular reaction was scored as mild or florid.
- Fibrosis stage (NIDDK) was scored for follow-up biopsies

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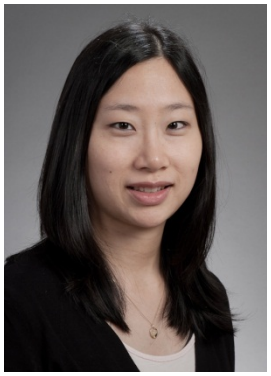

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Cleveland Clinic

Zu-Hao Gao, M.D., PhD
McGill Univ

Results

- Mean interval between biopsies was 2.5 years (range 1.0-7.5)
- Fibrosis stage progressed in 51%, was stable in 33%, and regressed in 16%.

Histologic Features in Initial Biopsy	Fibrosis Stage in Initial Biopsy		Progression of Fibrosis	
	Rs	P value	Rs	P value
Steatosis	0.26	0.03	-0.14	0.16
Lobular inflammation	0.36	0.004	0.11	0.23
Neutrophils	0.31	0.01	0.01	0.46
Ballooning	0.44	0.0006	0.004	0.49
Mallory bodies	0.44	0.0007	-0.05	0.36
Portal inflammation	0.31	0.01	0.03	0.42

Results

CL CK7+ elements & portal DR in initial biopsy	Fibrosis stage in initial biopsy		Progression of fibrosis	
	Rs	P value	Rs	P value
CLZ single cells	0.13	0.19	-0.11	0.22
CLZ strings	0.54	0.00002	0.01	0.46
CLZ ductules	0.54	0.00003	0.17	0.11
CLZ mild CK7	0.28	0.02	-0.14	0.16
CLZ florid CK7	0.52	0.00004	0.21	0.06
PDR mild	-0.06	0.32	-0.18	0.1
PDR florid	0.31	0.01	-0.08	0.28

Summary

- NAFLD is the most common liver disease in the U.S. and the prevalence is increasing yearly
- Current treatment options for NASH are suboptimal
- Predictors of future fibrosis/prognosis require refinement
- Histologic grading of NASH is currently inadequate in terms of prediction of future fibrosis