PATHOLOGY OF MAFLD ROLE OF LIVER BIOPSY

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LIVER FIBROSIS IN THE ERA OF MAFLD EGYPT JULY 28 - 30,2022

- **Patterns of steatosis**
- Terminology
- NAFLD Pathology
- Liver biopsy in NAFLD in Children
- Scoring of NAFLD
- Staging of steatosis related fibrosis
- Morphometry and AI

Steatosis

- Liver mass is 5% lipid
- More than 5% lipid accumulation in the liver is steatosis
- Steatosis is the key-feature of MAFLD

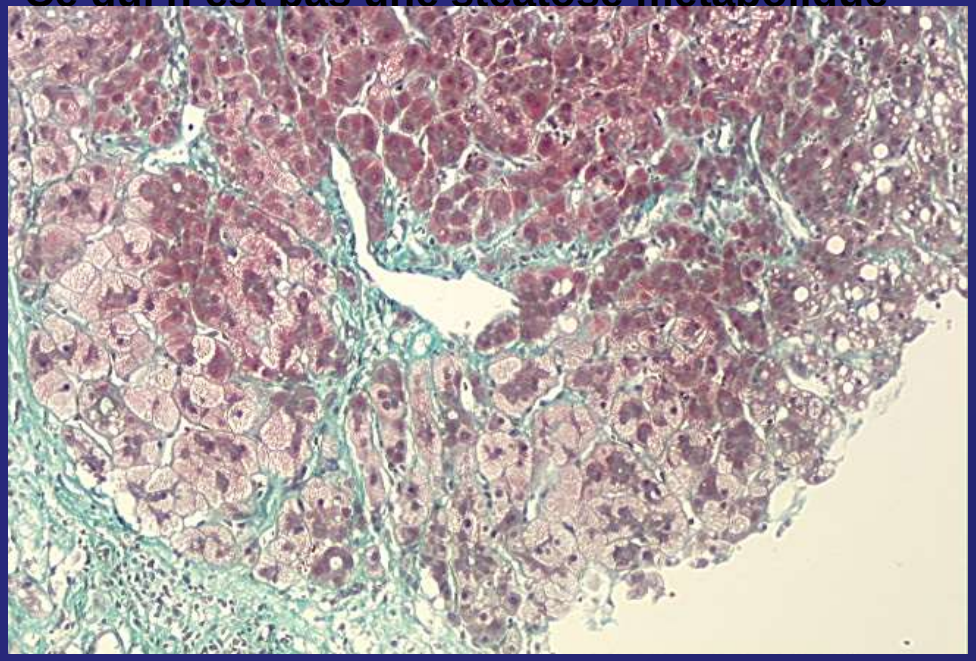
Prevalence of Steatosis

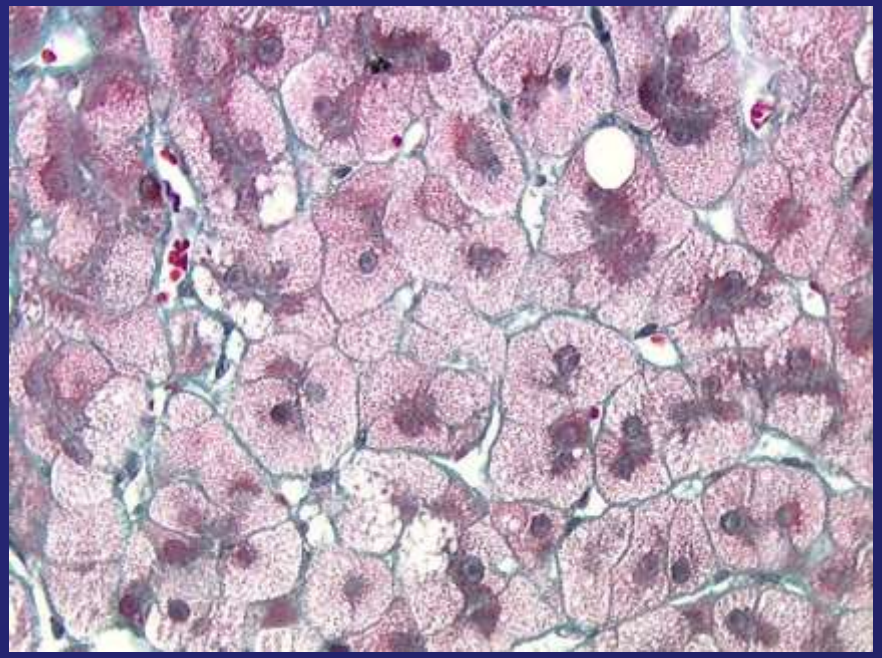
- 20-30% of general population have steatosis
- 50% in diabetics
- 75% in obese patients
- Strong relationship between steatosis and risk factors of cardiovascular diseases

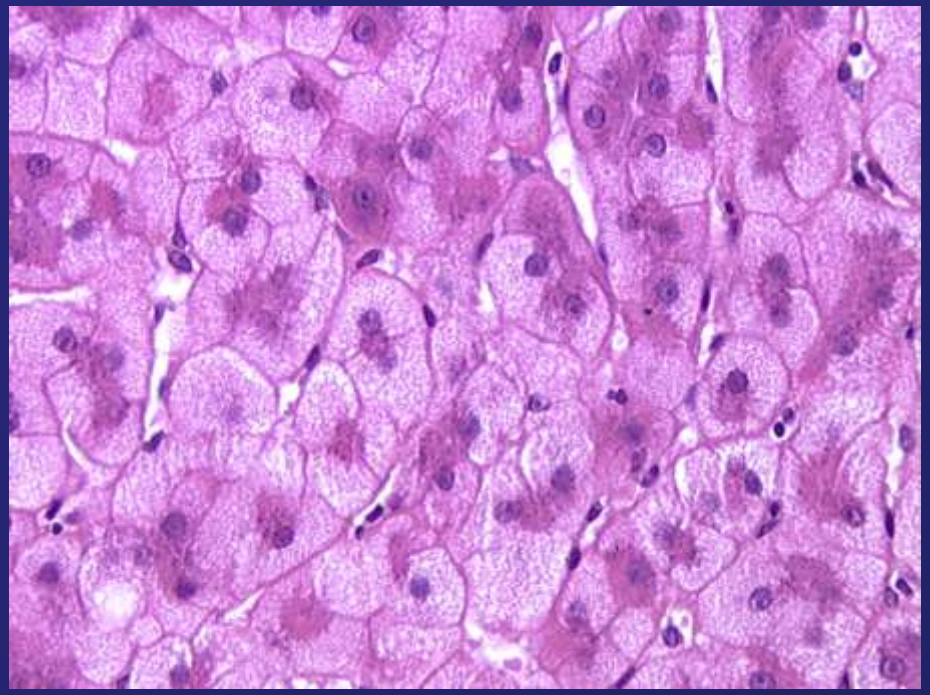
Steatosis, Microvesicular steatosis

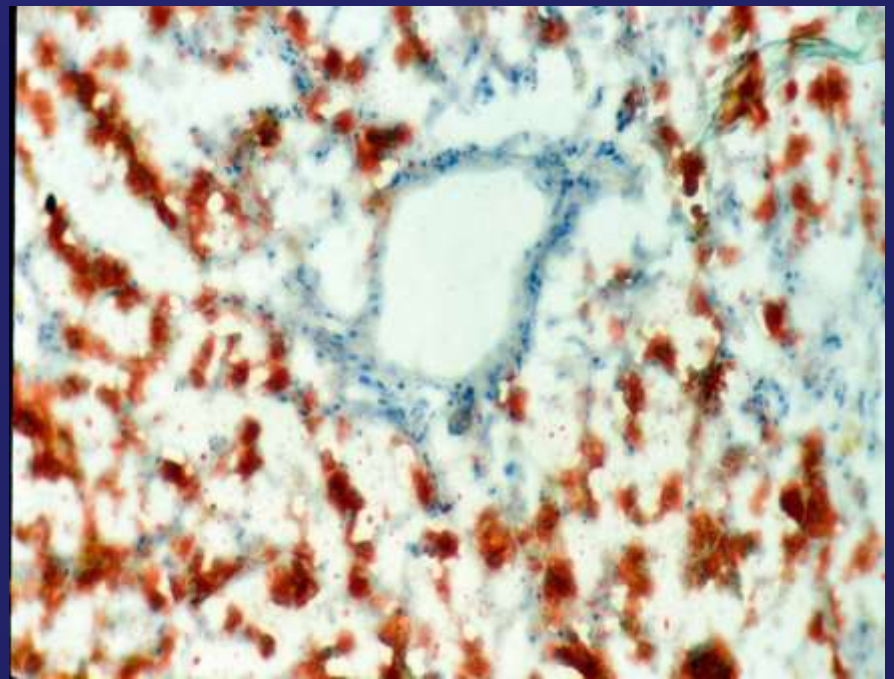
- Rare lesion
- Special staining (oil Red O)
- Acute and severe liver dysfunction
- Etiology :
  - Reye's syndrom
  - Fatty liver of pregnancy
  - Tetracyclin, valproic acid
  - Defect in mitochondrial β-oxydation of FA
  - Inborn errors of metabolism (e.g., LCAT defi ciency, cholesterol ester storage disease, Wolman disease)

Ce qui n’est pas une stéatose métabolique









Steatosis, Macrovesicular steatosis :

- Etiology:
  - NAFLD : a key-feature
  - Hepatitis C
  - Alcoholic liver diseases
  - Medications (e.g., amiodarone, methotrexate, tamoxifen, corticosteroids)
  - Lipodystrophy
  - Starvation
  - Parenteral nutrition
  - Abetalipoproteinemia
  - Genetic: wilson’s disease
  - Tumors : adenoma, FNH, HCC

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NASH : the landmark

- J. Ludwig, 1980 « Nonalcoholic steatohepatitis: Mayo clinic experience with a hitherto unnamed disease »
- Retrospective identification of patients with features of alcohol-like liver diseases who denied alcohol intake
- 20 patients:
  - 65% women
  - 90% obese
  - 25% diabetes Type II
  - 25% hyperlipidemia
  - 15% hypertension
- Alcohol-like liver lesions on biopsy

(Ludwig et al. Mayo Clin Proc, 1980;55:434)

NASH

- The complete spectrum :
  - Steatosis, moderate-severe, Zone 3 or diffuse (100%)
  - Ballooning
  - Lobular inflammation : lymphocytes and polymorphonuclear leukocytes (100%)
  - Mallory's hyaline (70%)
  - Perisinusoidal fibrosis (70%)
  - Cirrhosis (15%)
  - Others : fat cysts, lipogranuloma

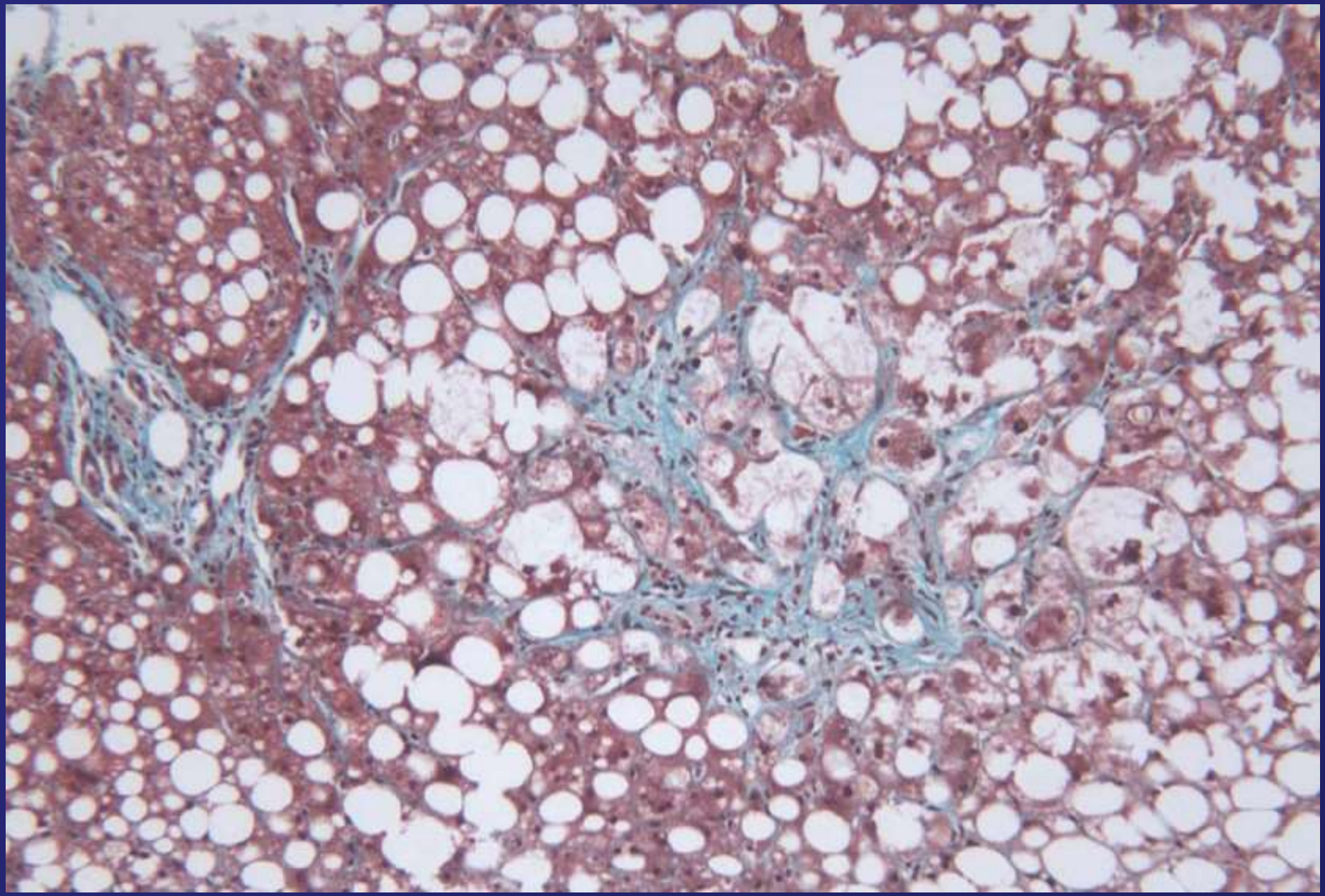
(Ludwig et al. Mayo Clin Proc, 1980;55:434)

Risk factors associated with NAFLD

Conditions with established Association (MAFLD)	Conditions with emerging association Not (MAFLD)
Obesity	Polycystic ovary syndrome
Type2 diabetes mellitus	Hypothyroidism
Dyslipidemia	Obstructive sleep apnea
Metabolic syndrome	Hypopituitarism
	Hypogonadism
	Pancreato-duodenal resection

Pathophysiology of NAFLD/MAFLD

- A “two-hit” theory was proposed in 1998 to describe the pathogenesis of NAFLD.
- It proposed that at the onset of disease, the “first hit” was represented by an increase In liver fat.
- Subsequently, the “second hit”, including inflammatory cytokines, adipokines, mitochondrial dysfunction, and oxidative stress, was needed for the progression to NASH and advanced fibrosis.



NAFLD To MAFLD

- Recently, a consensus of international experts has proposed the disease name being changed from NAFLD to metabolic associated fatty liver disease (MAFLD).
- The criteria are based on the evidence of hepatic steatosis, plus any of the following three conditions:
  - overweight/obesity,
  - presence of type 2 diabetes mellitus (T2DM), or
  - evidence of metabolic dysregulation.

Pathophysiology of NAFLD/MAFLD

A “multiple-hit” hypothesis, which incorporates various processes, such as insulin resistance, lipotoxicity, inflammation, imbalance of cytokines, activation of innate immunity, and microbiota, in the context of environmental and genetic factors, offers a more comprehensive delineation of the pathogenesis of NAFLD/MAFLD.

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MAFLD Pathology

- The nature of inflammation in MAFLD might be chronic, relapsing or intermittent as in many other chronic inflammatory and liver disorders, Like Crohn's disease, etc.
- It could simply be missed by liver biopsy (Snap Shot).
- Classification of patients as with or without NASH has not proven useful and needs to be reconsidered.
- From now on let us call it MAFLD (on their words).

NAFLD Pathology

- Liver biopsy is the gold standard or the Best Standard FOR;
  - Confirmation or exclusion
  - Distinguish NASH from NAFL
  - Establish severity of fibrosis, steatosis and activity
  - Searching for Co-morbedity

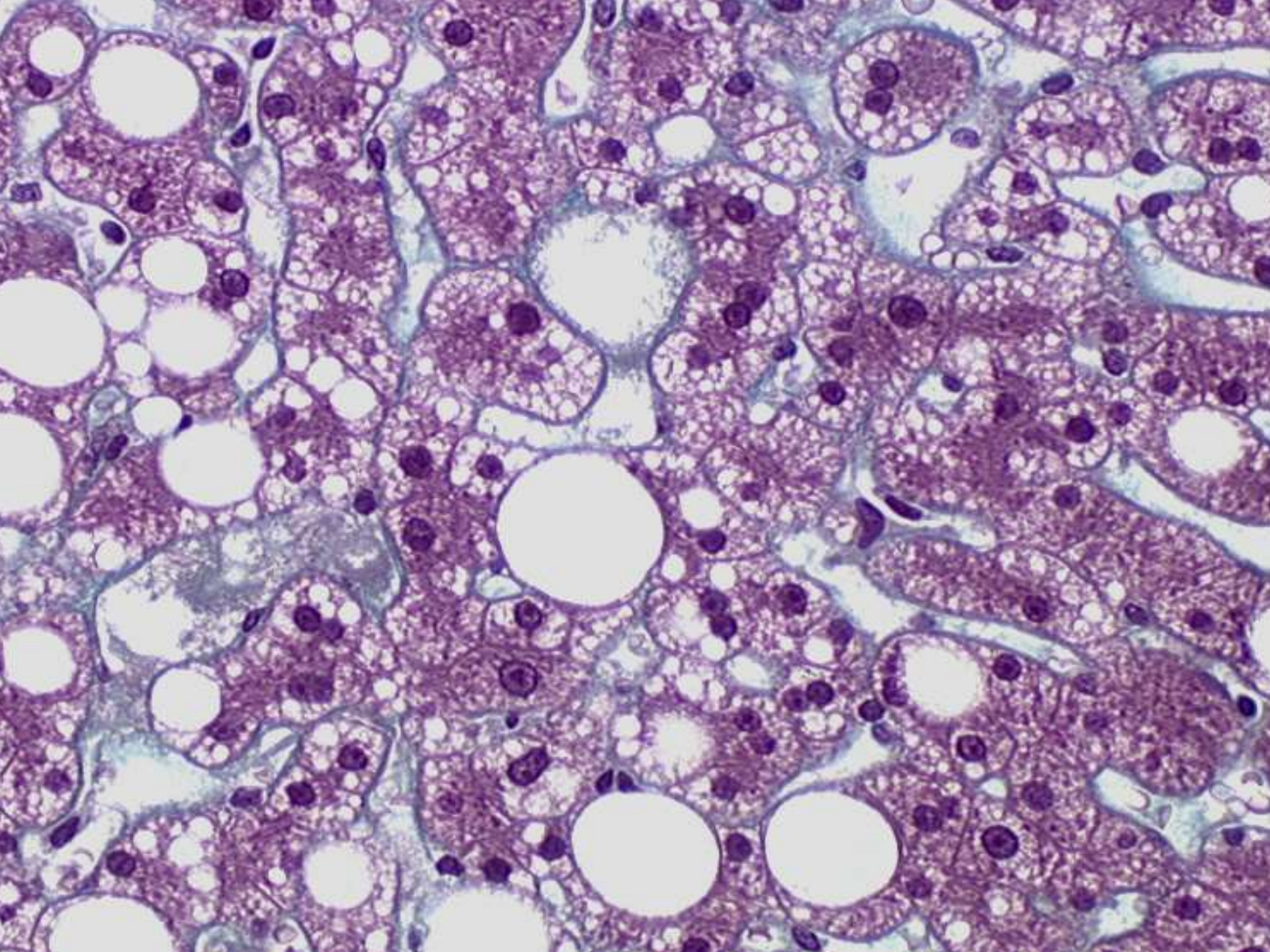
– Gunn NT, Shiffman ML 2018.

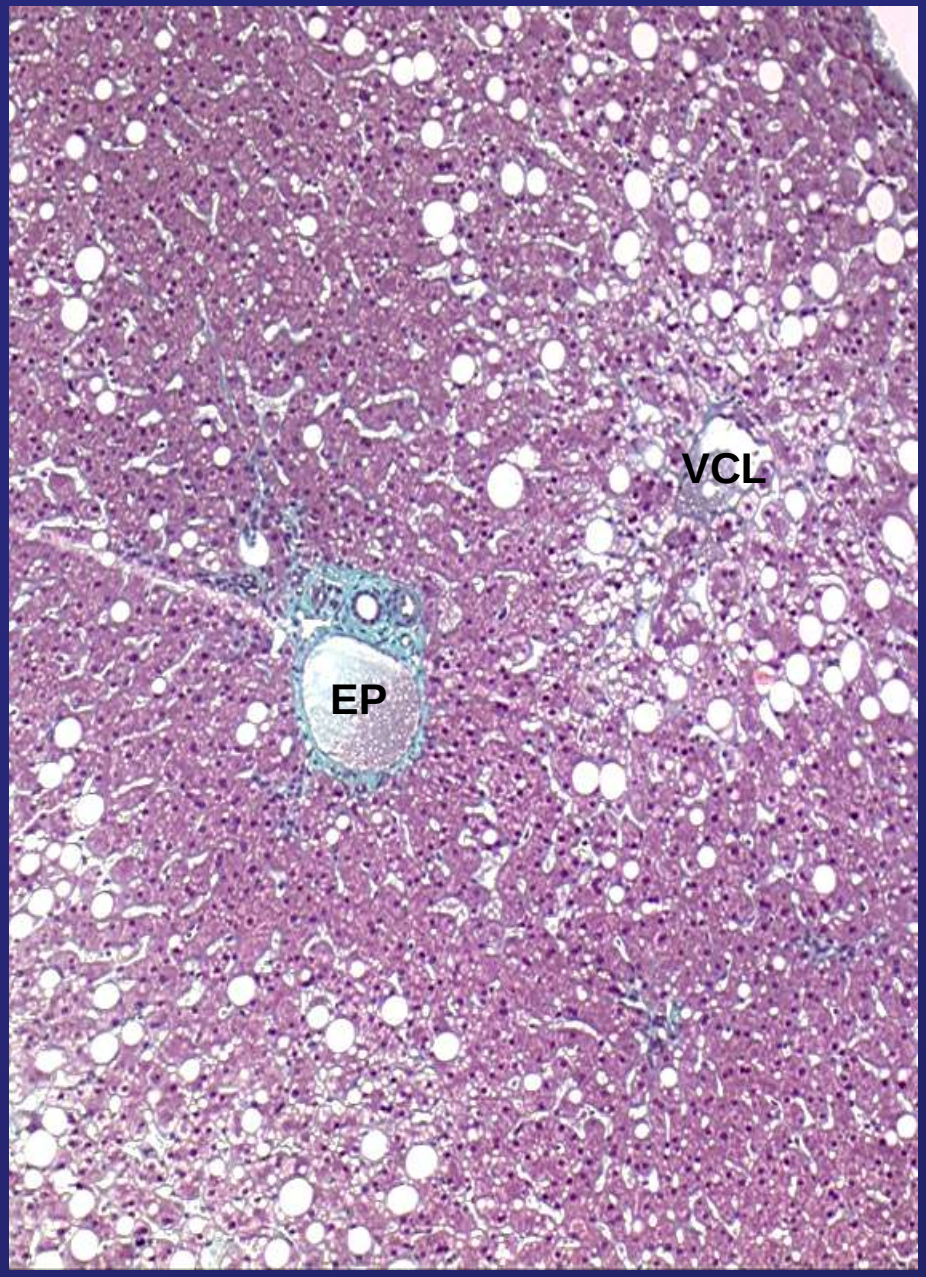
NAFLD : pathology

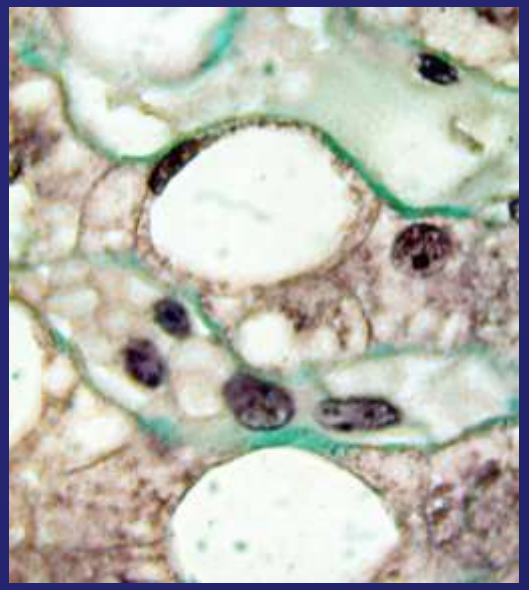
- The components of the pathologic spectrum :
  1. Steatosis
  2. Liver cell damages
     - Ballooning
     - Inflammation
     - Mallory’s hyaline
  3. Fibrosis

Steatosis in NAFLD

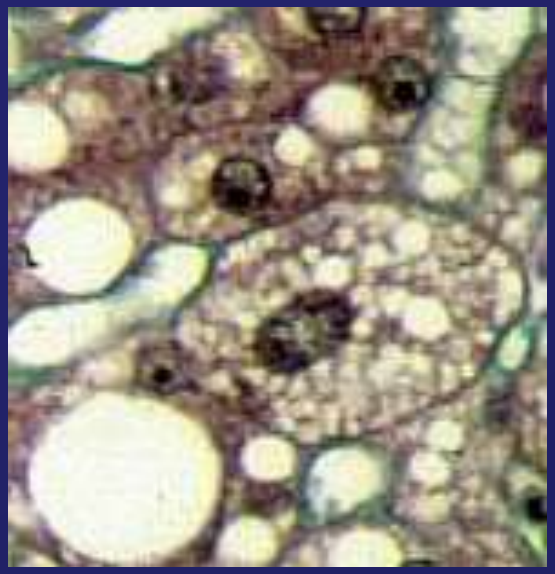
- Necessary feature for Dx
- Type : Macro / Mediovesicular
- Location : Zone 3 ++ or diffuse
  - »In Children Zone 1 periportal.
- Amount :
  - <5%
  - 5-30%
  - 30-60 %
  - > 60%
- Fat cysts, lipogranulomas







Macrovacuolar steatosis



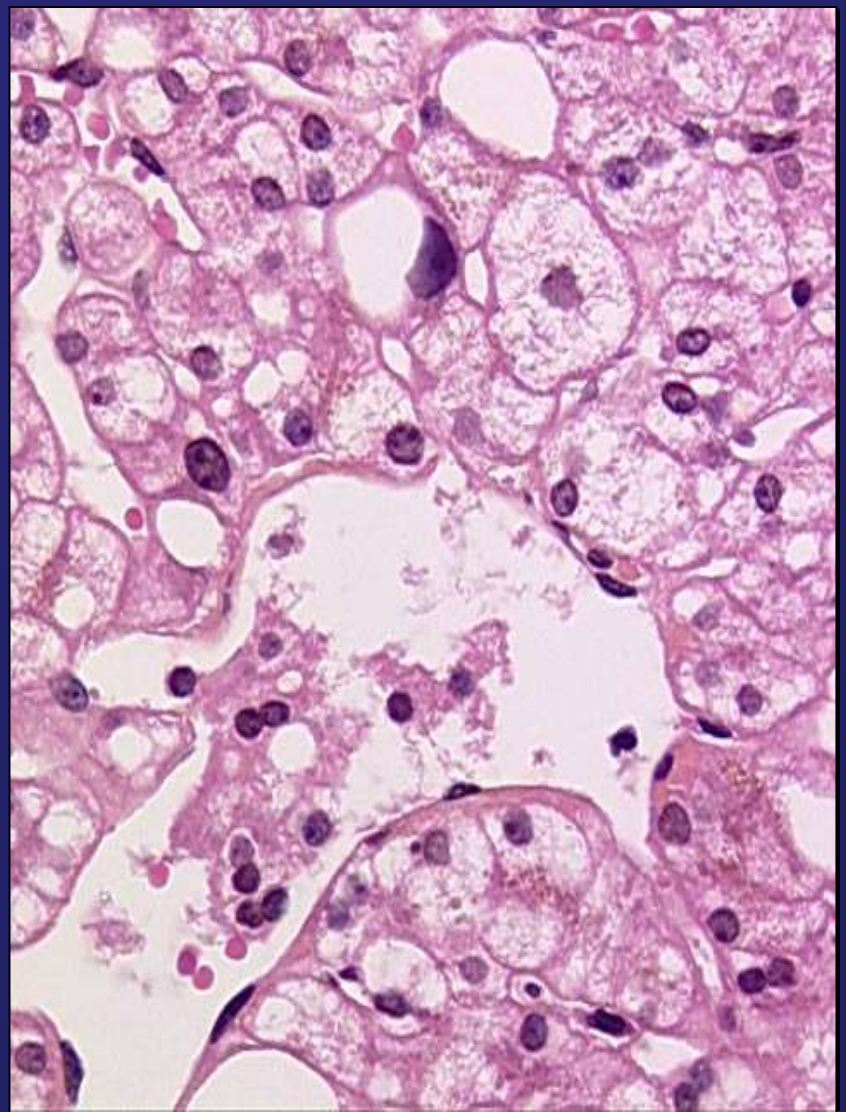
Mediovacuolar steatosis

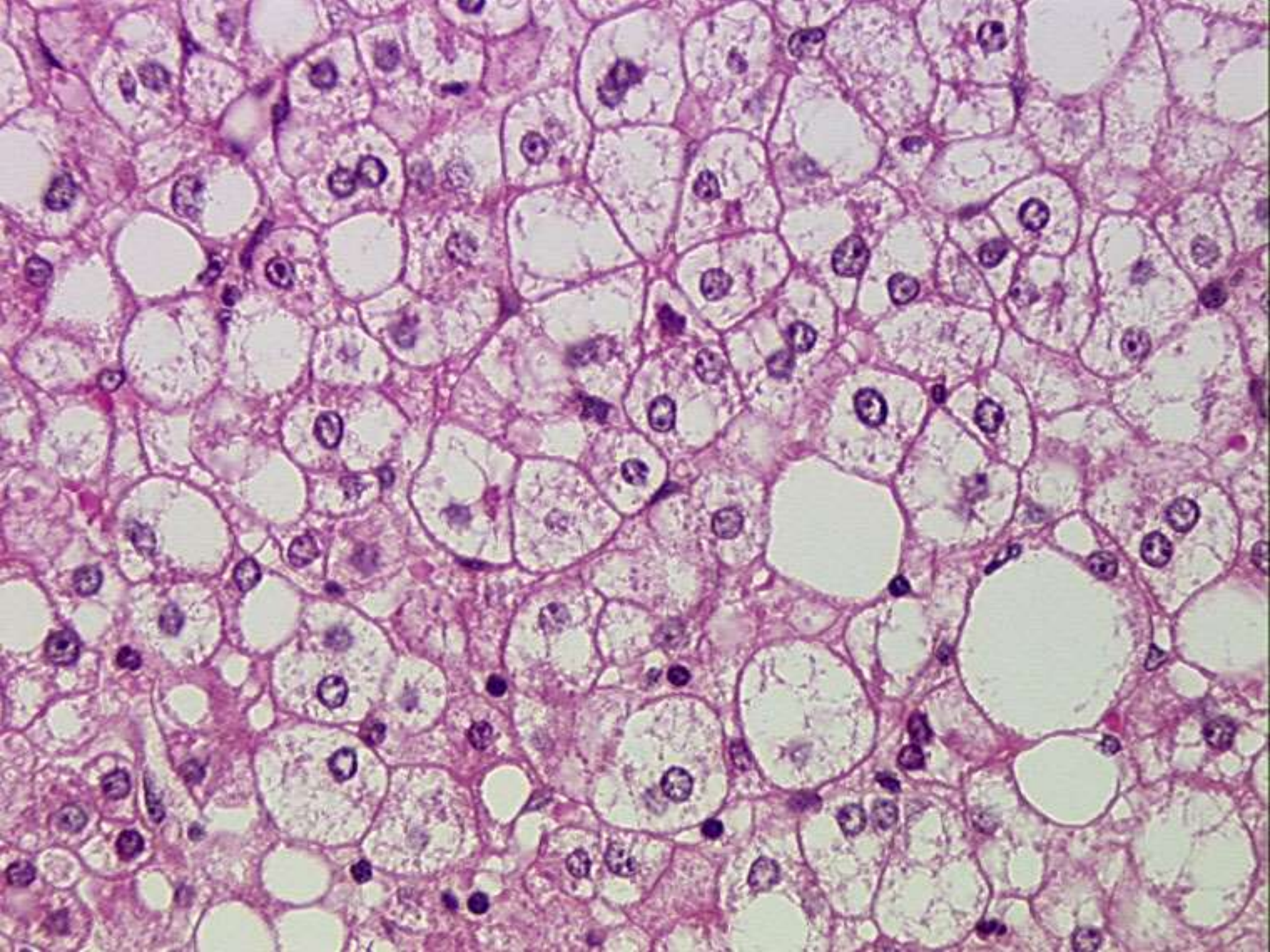
NAFLD

- The components of the pathologic spectrum :
  - Steatosis
  - Liver cell damages
    - Ballooning
    - Inflammation
    - Mallory's hyaline
  - Fibrosis

NAFLD : Pathologic spectrum

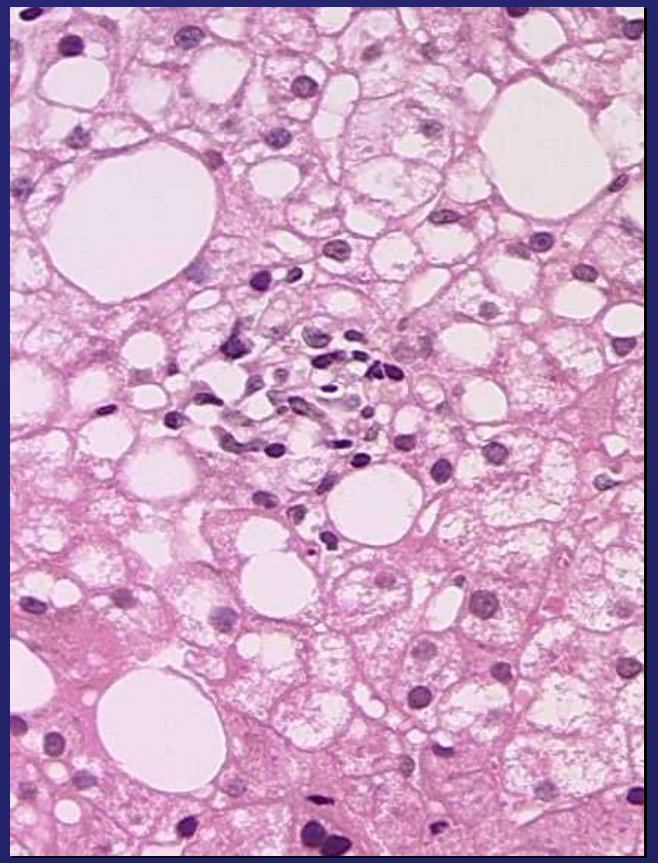
- Ballooning, swelling
  - Very common
  - Criteria variable
  - Poor reproducibility between observers
  - Pathophysiology unknown
  - No inflammation

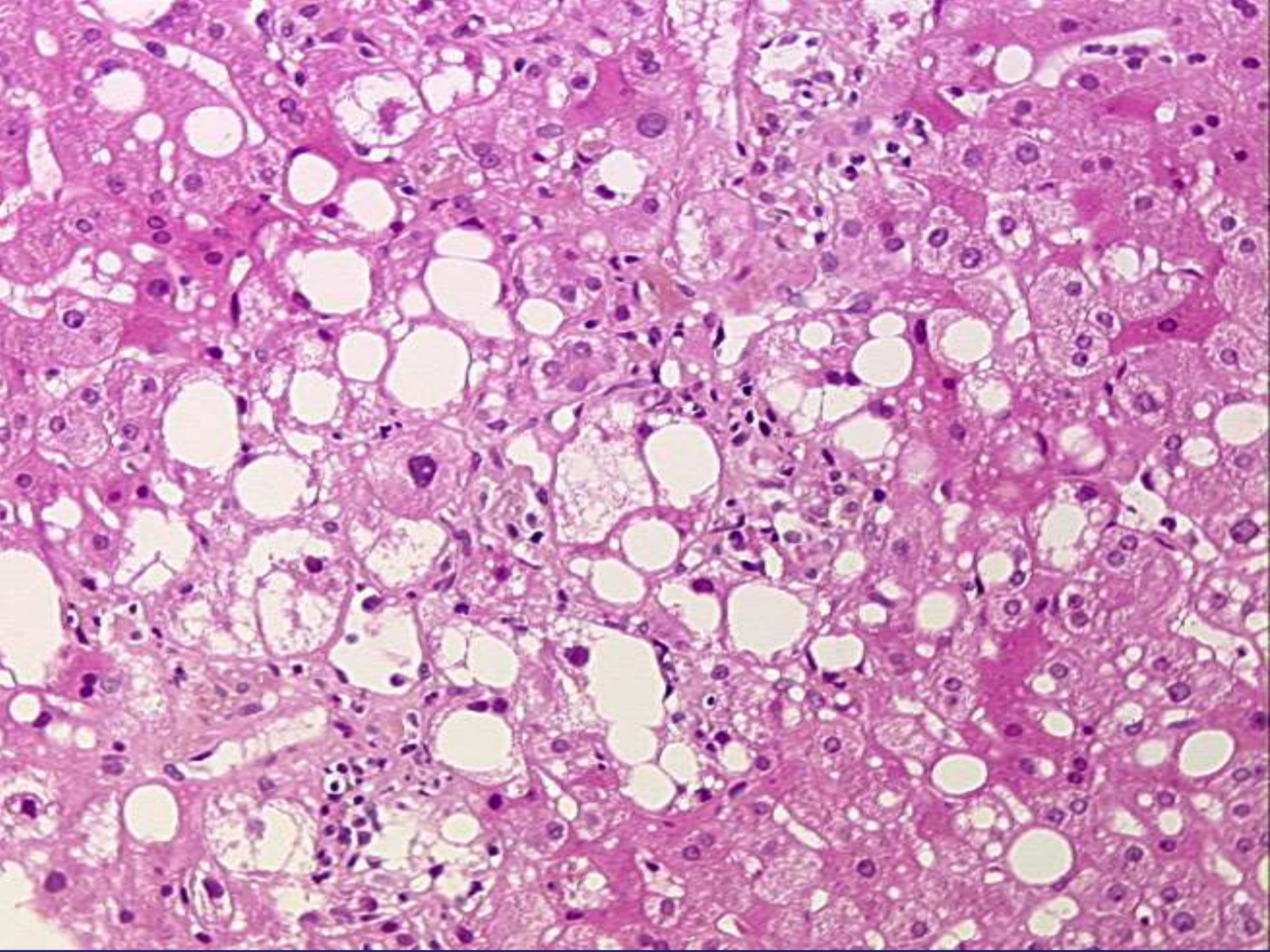




NAFLD : Pathologic spectrum

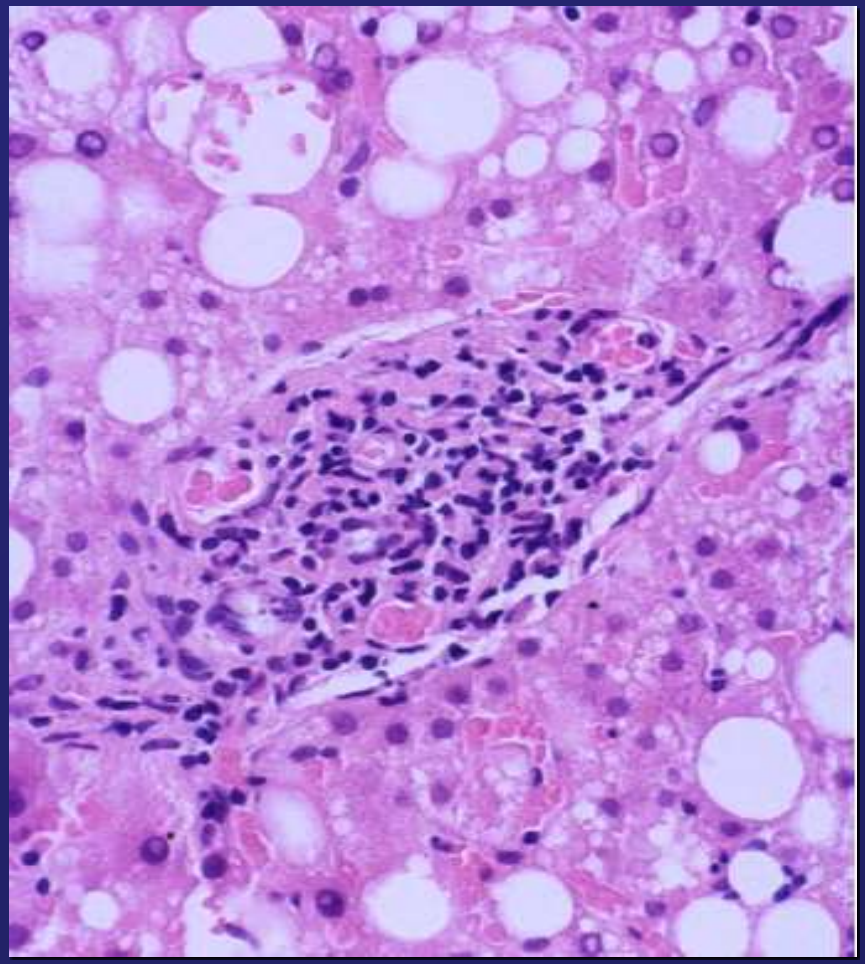
- Lobular inflammation :
  - Very mild
  - Mononuclear cells
  - Rare polymorphonuclear leukocytes
  - Few apoptotic bodies





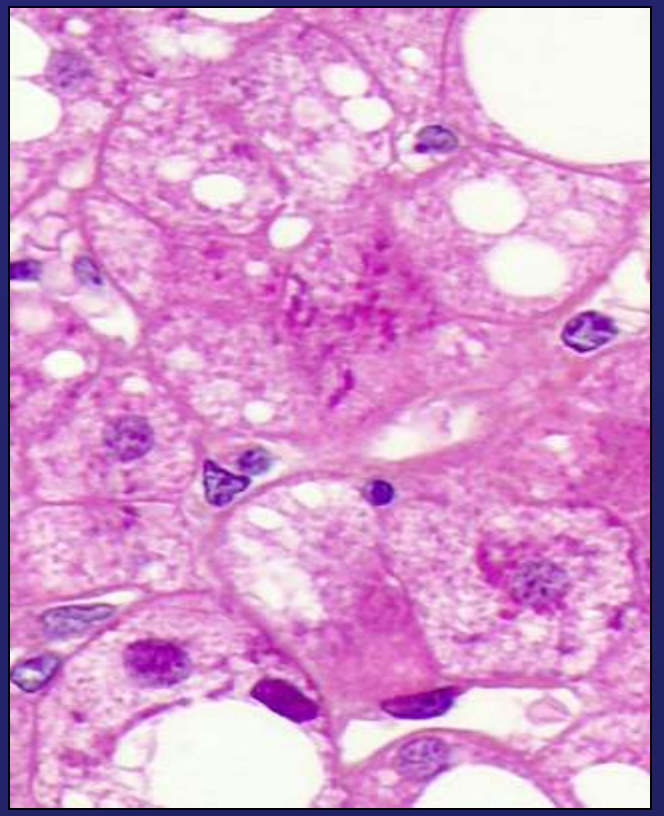
NAFLD : Pathologic spectrum

- Portal inflammation :
  - Usualy mild
  - Mainly mononuclear cells
  - More common in children
  - If significant, coexistent liver disease



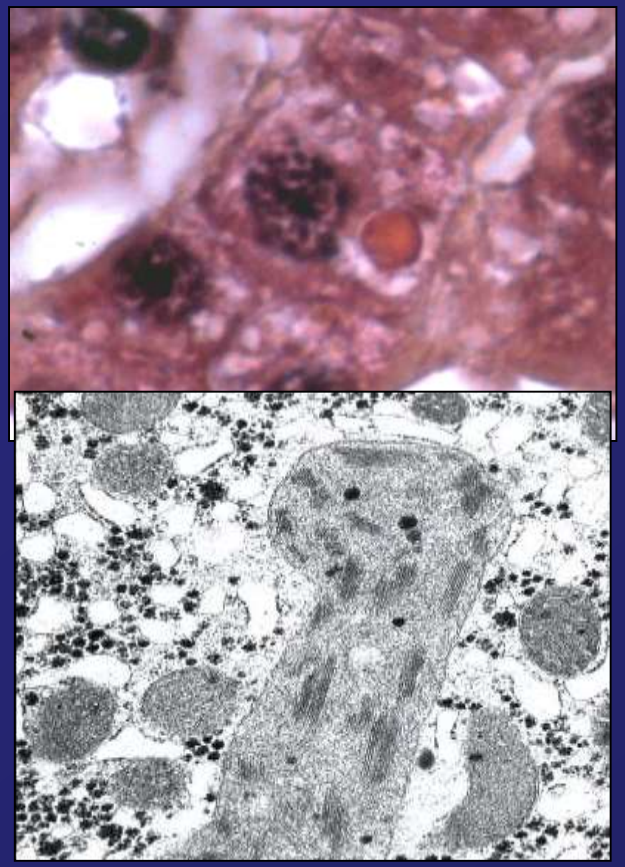
NAFLD : Pathologic spectrum

- Mallory's Hyaline
  - Rare
  - Not well-formed
  - Association with clarification (Wilson, PBC, etc)
  - Immunohistochemistry (ubiquitin, p62)

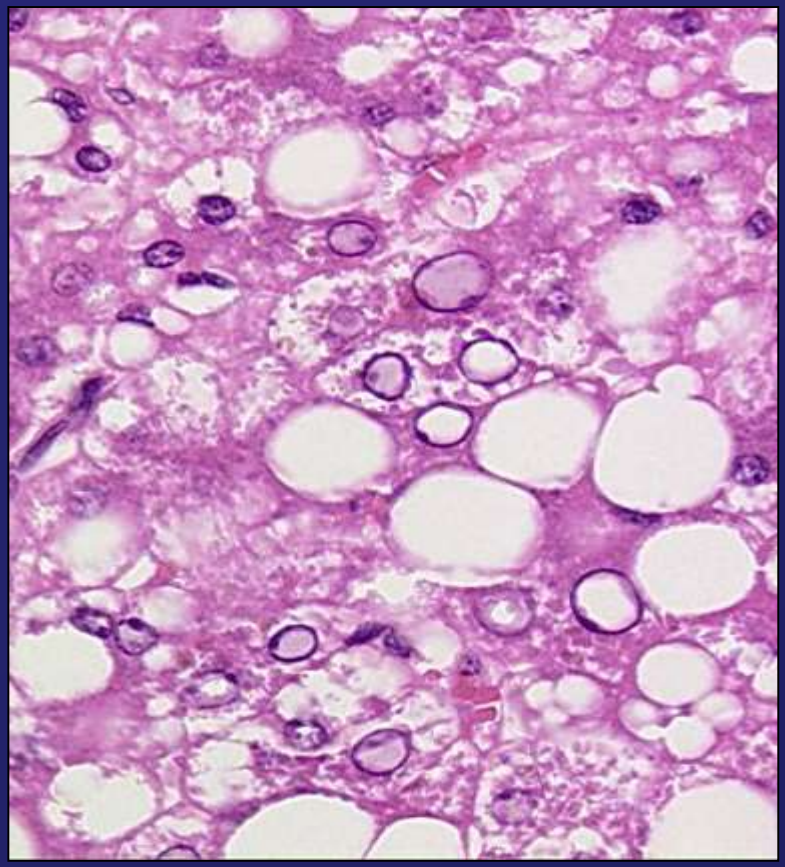


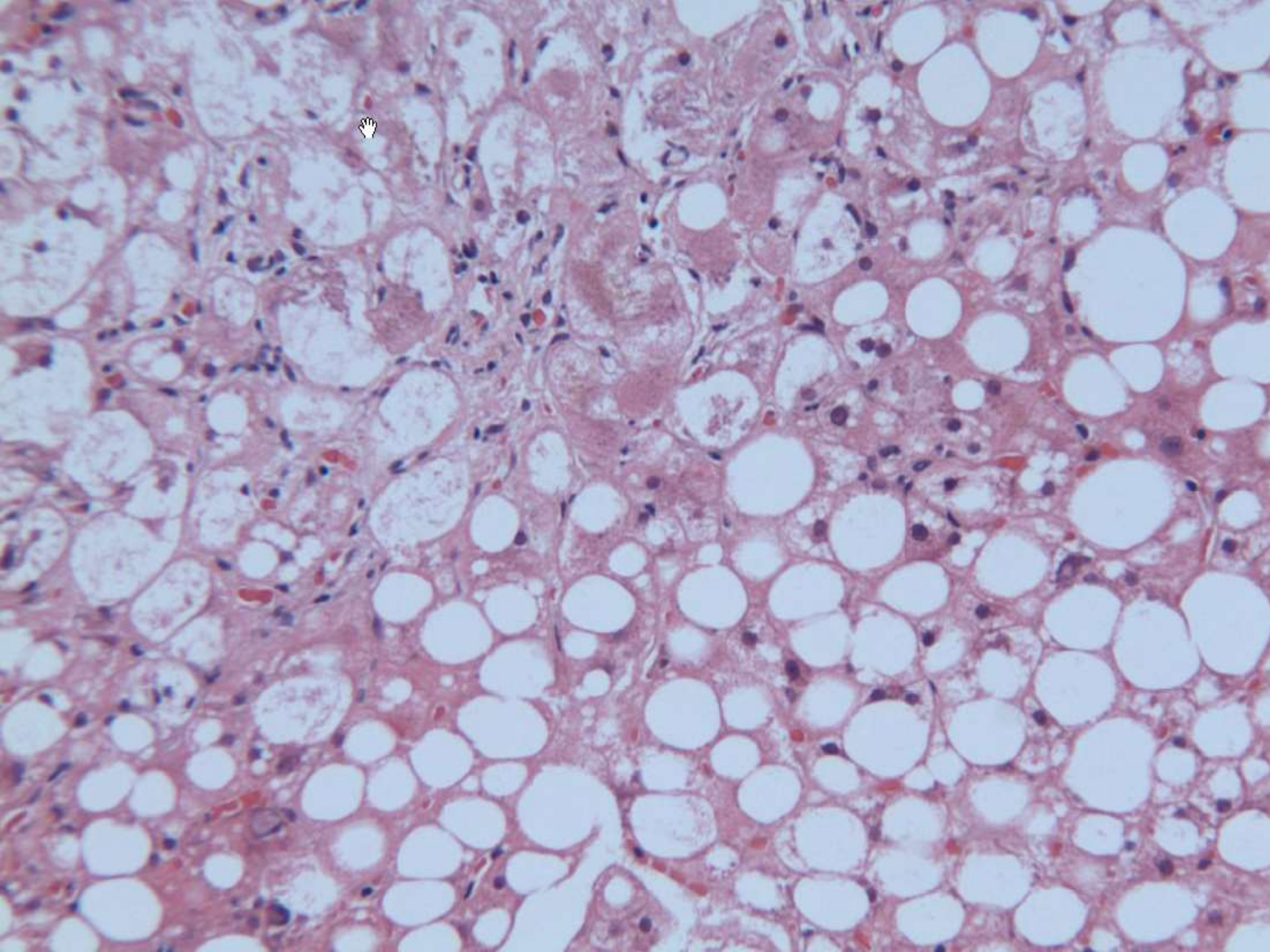
NAFLD : Pathologic spectrum

- Megamitochondria



- Glycogenated nuclei





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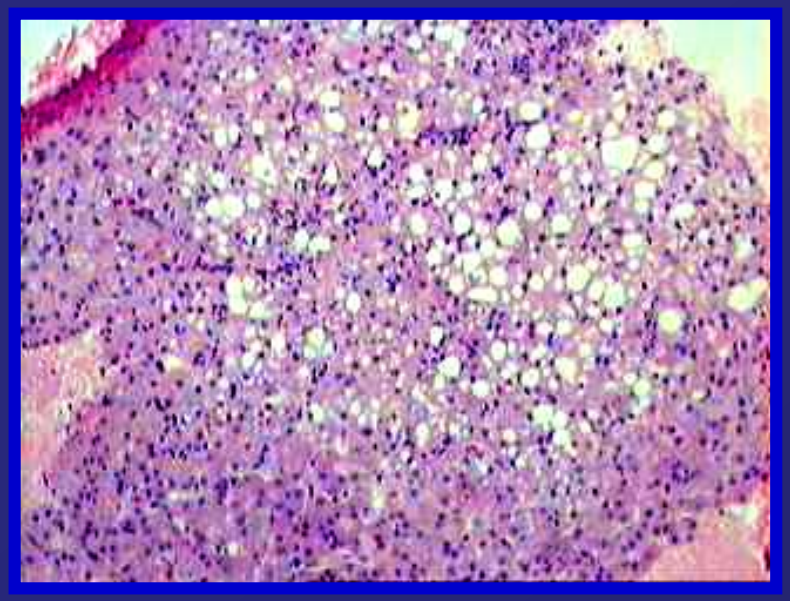
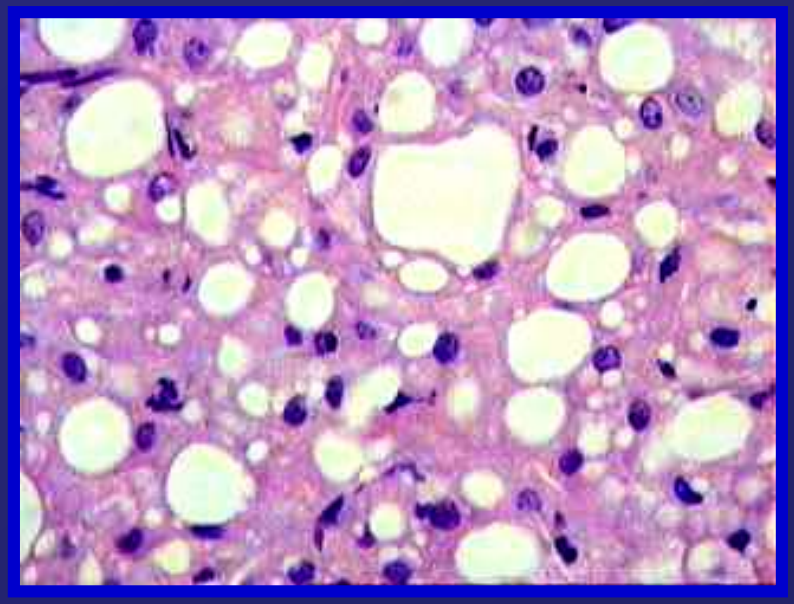
Recommendations about liver biopsy in NAFLD in Children by AASLD .

- *Children with fatty liver who are very young or not overweight should be tested for monogenic causes of chronic liver disease such as fatty acid oxidation defects, lysosomal storage diseases, and peroxisomal disorders, in addition to those causes considered for adults.*



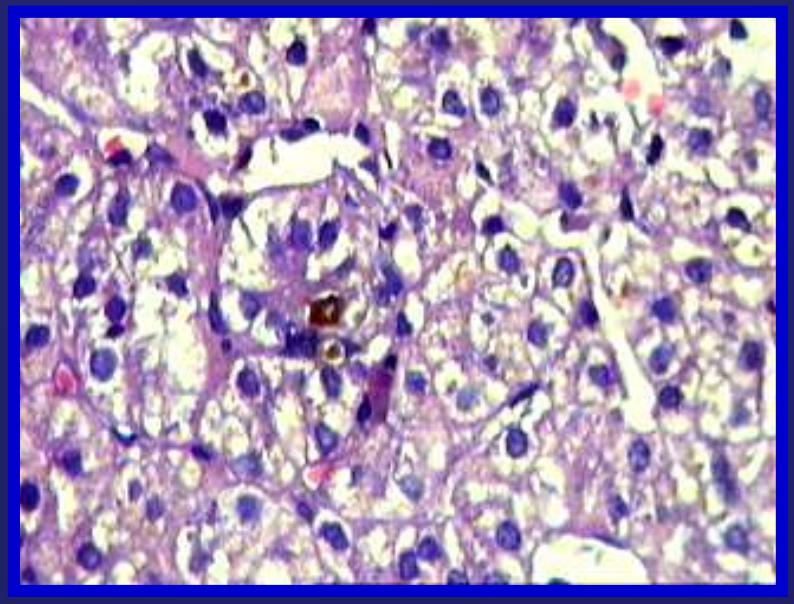


Fatty change with cholestasis:

? Galactosemia,

? Tyrosinemia,

? Hereditary fructose intolerance.



NAFLD histology in children

- Histopathology of children with NAFLD can differ from that found in adults.
- As in adults, children can present with pronounced features of hepatocellular injury, lobular inflammation, and peri-sinusoidal fibrosis, but there is a unique pattern of unclear significance recognized in children.
- This pattern is typified by marked macrovesicular steatosis, portal inflammation, and portal fibrosis in the absence of ballooning

Recommendations, Cont. Children.

- *Low serum titers of autoantibodies are often present in children with NAFLD, but higher titers, particularly in association with higher serum aminotransferases and high globulin should prompt a liver biopsy to evaluate for possible autoimmune hepatitis.*
- *Liver biopsy in children with suspected NAFLD should be performed in those where the diagnosis is unclear, where there is possibility of multiple diagnoses, or before starting therapy*

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NAFLD activity score (NAS)

Steatosis (S), Activity (A) and Fibrosis (F). (SAF) **score**

NAFLD activity score (NAS)

- « the primary purpose of the NAFLD activity score (NAS) is to assess overall histological changes; it is not intended that numeric values replace the pathologist's diagnostic determination of steatohepatitis » *(Kleiner et al. Hepatology 2005)*

- The frontier between NAFL and NASH remains unclear

Steatohepatitis score, NAFLD activity score (NAS)

- Extent Score Steatosis
  - < 5% 0
  - 5-33% 1
  - 33-66% 2
  - >66% 3
- Lobular inflammation
  - No foci 0
  - 2 foci/200 1
  - 2–4 foci/200 2
  - 4 foci/200 3
- Hepatocyte ballooning
  - Few balloon cells 1
  - Many cells 2
- Total scoreL 0-8
- >5 Diagnostic of NASH
- 3-4 Probable NASH
- 1-2 Not NASH

(Kleiner et al. Hepatology 2005)

- In children there is a modification with adding of portal inflammation to steatosis, Ballooning and inflammation)
- with score 0-2. to give total score 0-10 instead of 0-8 in adults

Algorithm for categorizing liver lesions in NAFLD patients

- An algorithm for segregating lesions into normal liver, NAFLD or NASH was built based on semi-quantitative evaluation of steatosis, hepatocellular ballooning and lobular inflammation.
- For each case, the SAF score was created including the semi-quantitative scoring of steatosis (S), activity (A) and fibrosis (F).

Bedossa et al, Hepatology 2014

SAF score

- The steatosis score (**S**) from 0 to 3 (S0: <5%; S1: 5-33%, mild; S2: 34- 66%, moderate; S3: >67%, marked).
- Activity grade (**A**, from 0-4) was the unweight addition of hepatocyte ballooning (0-2) and lobular inflammation (0-2) .
- Cases with A**0** (A=0) had no activity, A**1** (A=1), mild activity, A**2** (A=2), moderate activity, A**3** (A>3) severe activity.
- Stage of fibrosis (**F0-F4**)
- SAF score **S3A2F1** (marked steatosis, moderate activity and mild fibrosis)
- Another SAF score **S1A3F4** (cirrhosis with severe activity and mild steatosis).

- Finally, no scoring system is meant to replace the analytical description of the biopsy which enables introducing refinements, but both the NAS algorithm and the SAF score might provide practical tools for pathologists that would simplify comprehension of liver lesions by hepatologists

Steatosis related sinusoidal wall fibrosis

- Early fibrotic changes are concentrated in the centrilobular vein area:
- (i) around the sinusoids (i.e., perisinusoidal fibrosis) with capillarisation of the sinusoids
- (ii) around groups of hepatocytes, with chicken-wire pattern
- (iii) around the central vein, with perivenular fibrosis

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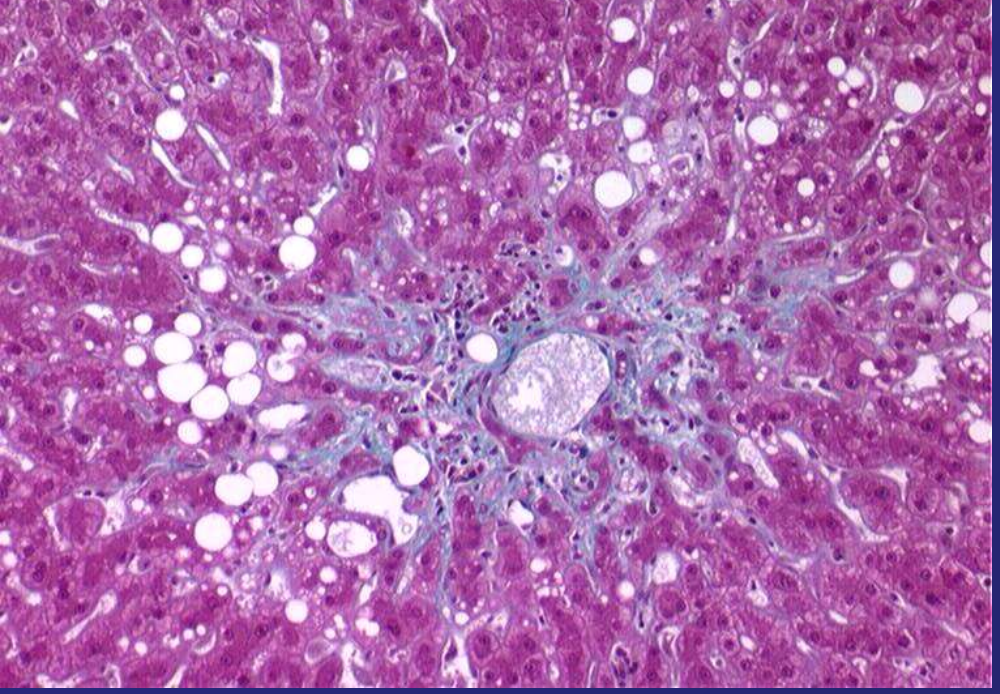
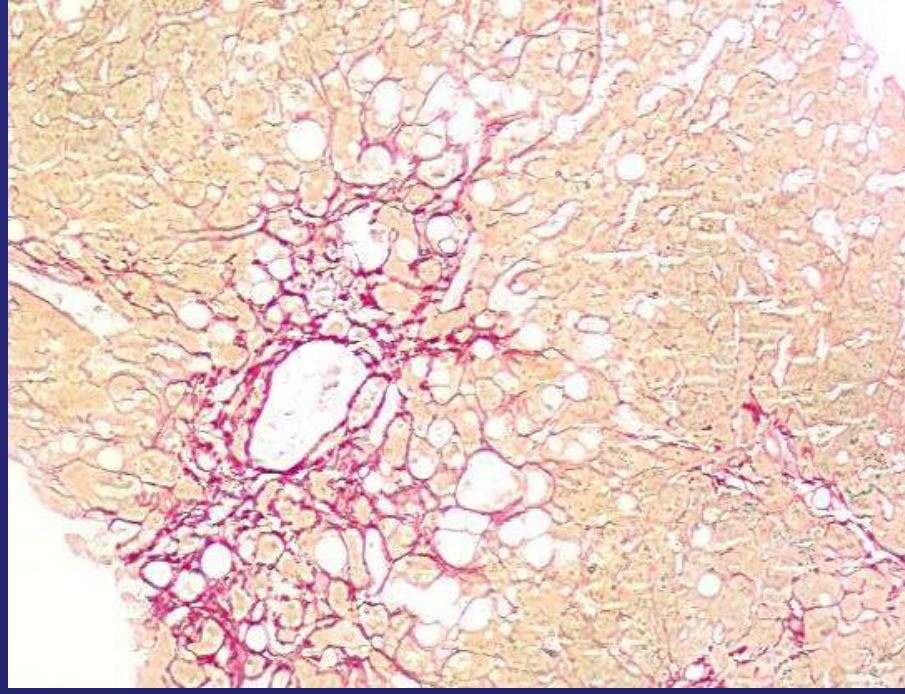
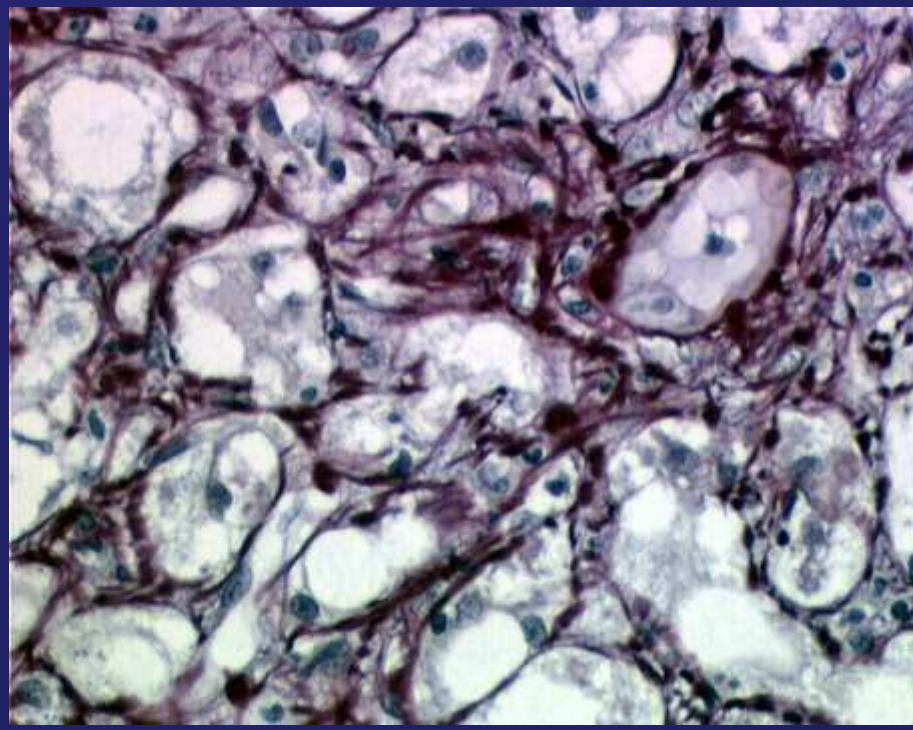
Staging of fibrosis in NAFLD

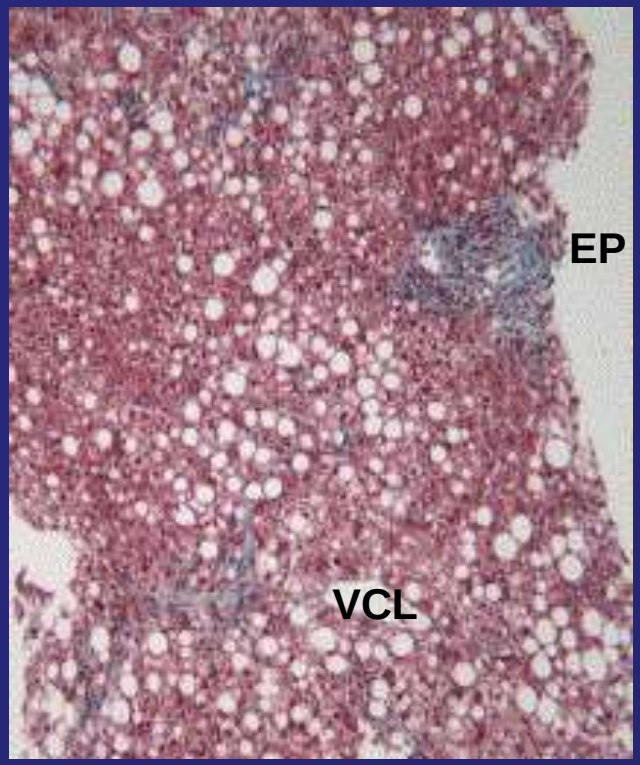
- According to Brunt, 1999 and Kleiner 2005
  - Early lesion (Stage 1)
- subclassifications of stage 1 to account for delicate (1a) or dense (1b) perisinusoidal fibrosis and stage 1c to include 'portal only' fibrosis when noted.
    - Centrolobular Fibrosis delicate (1a)
    - Centrolobular Fibrosis dense (1b)
    - Portal Fibrosis only (1c)
  - Centrolobular Fibrosis + Portal Fibrosis (Stage 2)
  - Septal (Bridging) Fibrosis (Stage 3)
  - Cirrhosis (Stage 4)



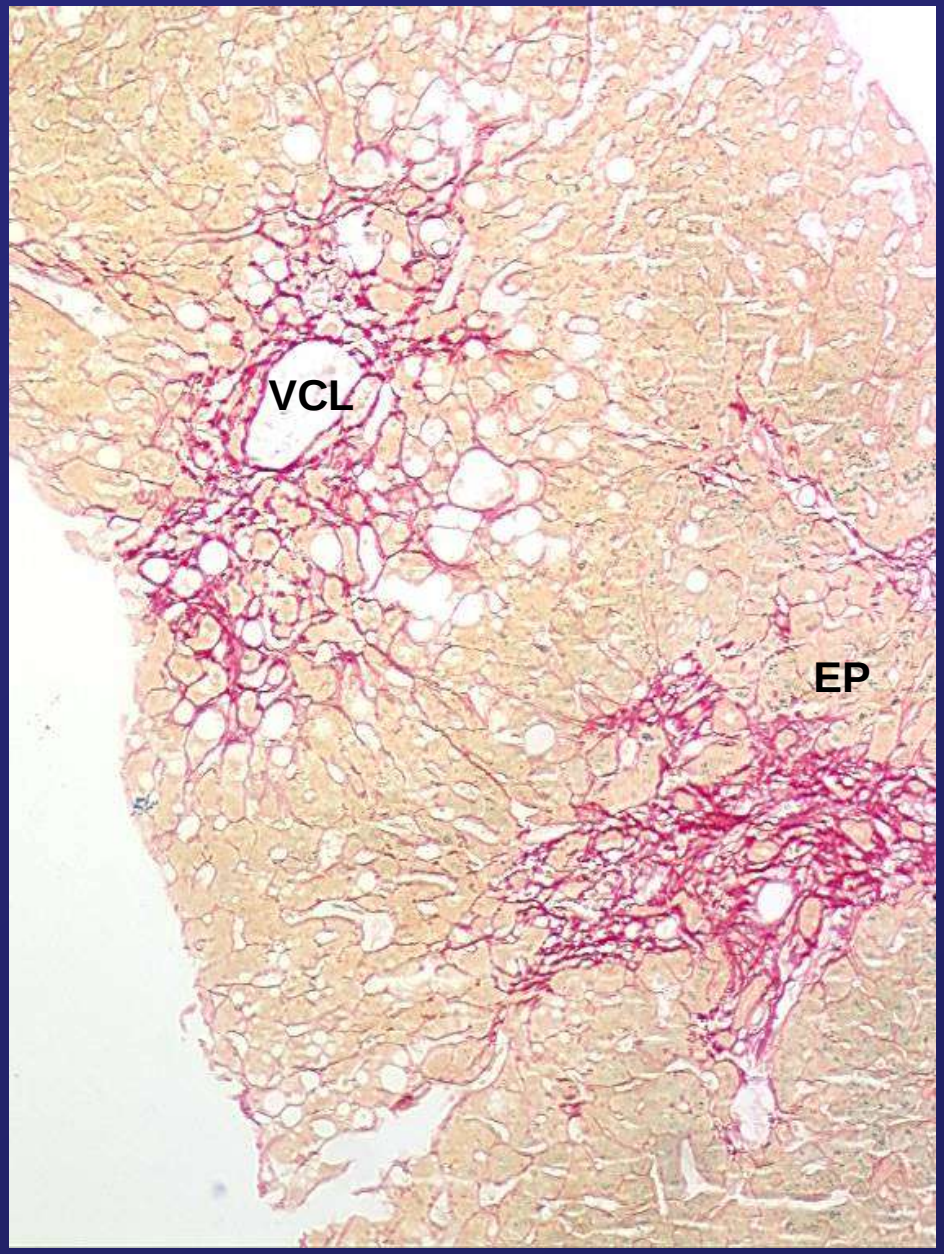


Stage 1 (a and b)
Zone 3 perisinusoidal fibrosis

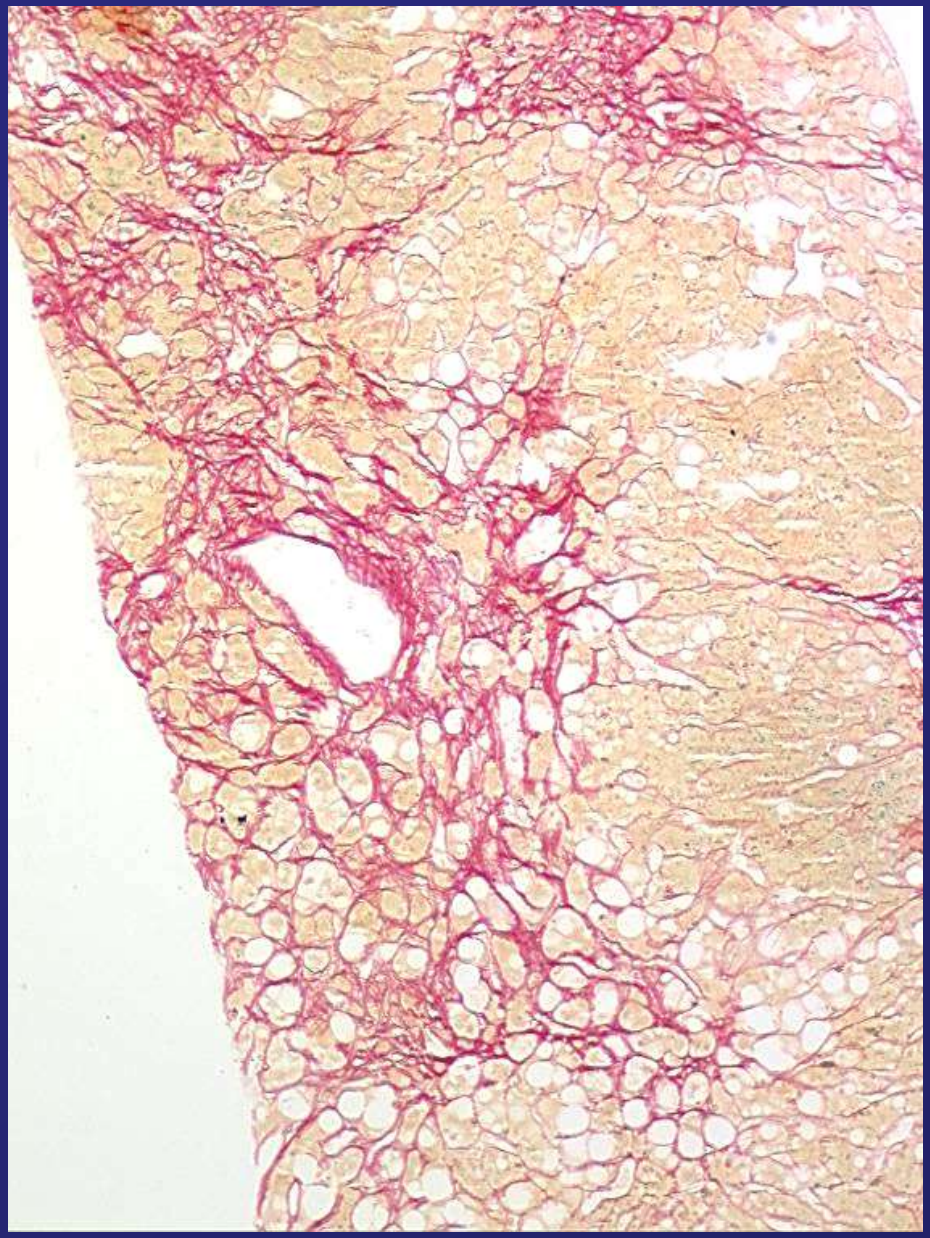


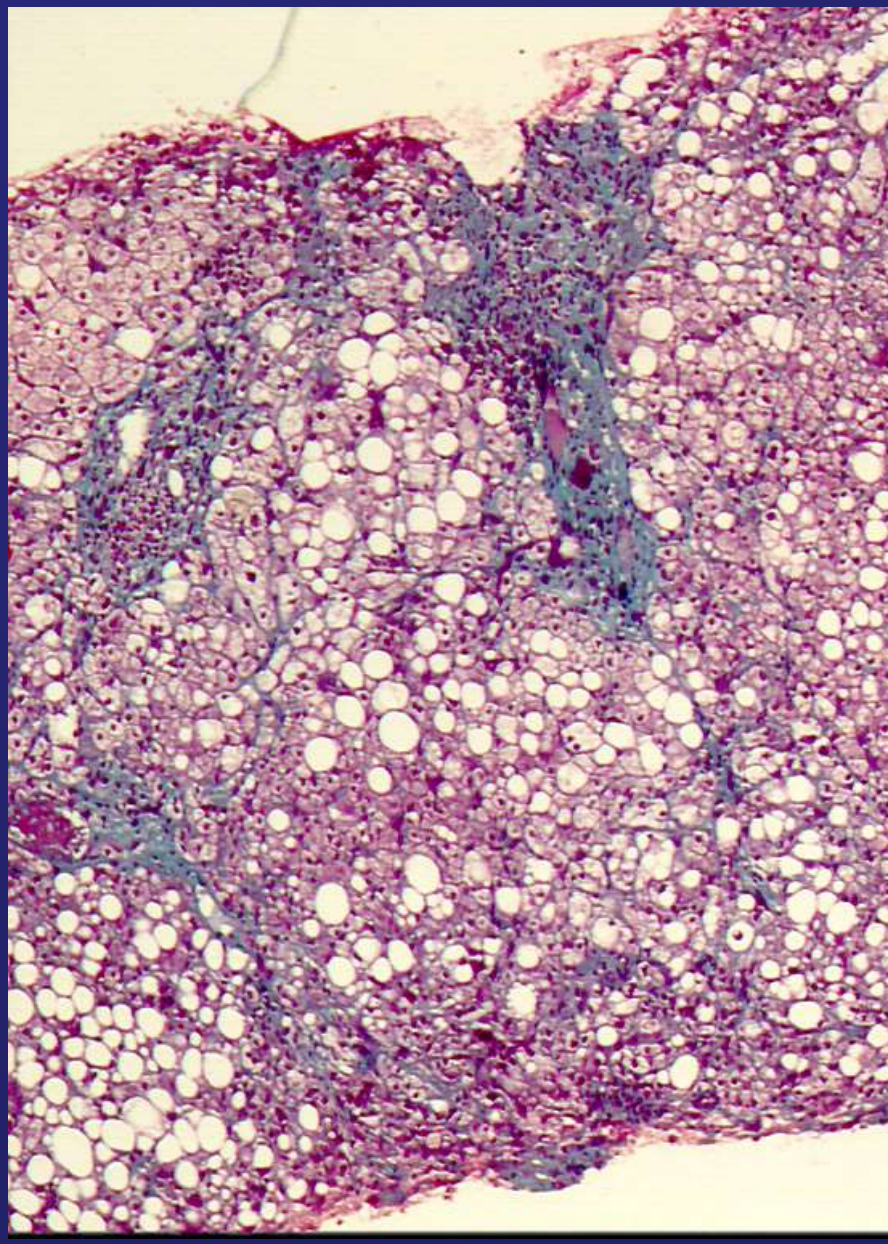


Stage 1c : Periportal fibrosis

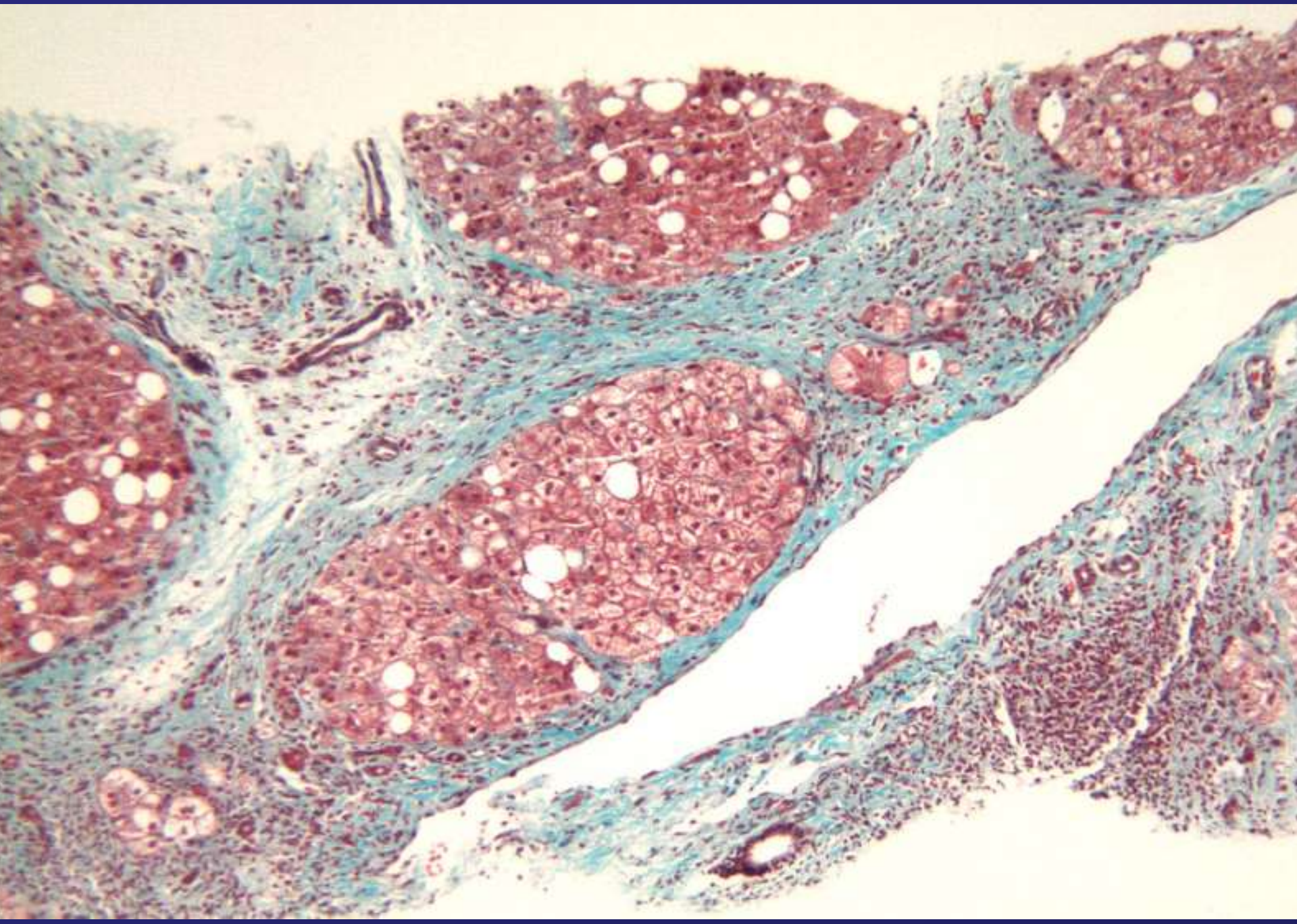


Stage 2 : pericentral and periportal fibrosis





Stage 3 : Septal Fibrosis



Cirrhosis in NAFLD

- May loose or retain active features of NASH
- NAFLD may be the primary cause of cryptogenic cirrhosis :
  - Caldwell *(Hepatology 1999)* : CC with 70% females, 73% obese/diabetes, older but otherwise similar to NASH
  - Poonawala, *(Hepatology, 2000) :* obesity and diabetes more common in CC than other cirrhosis

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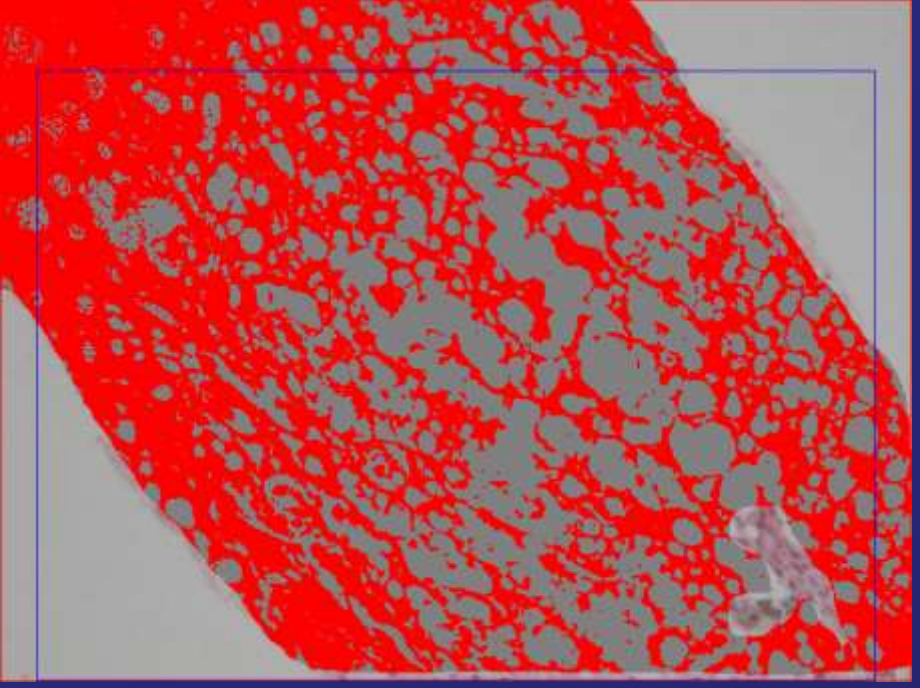
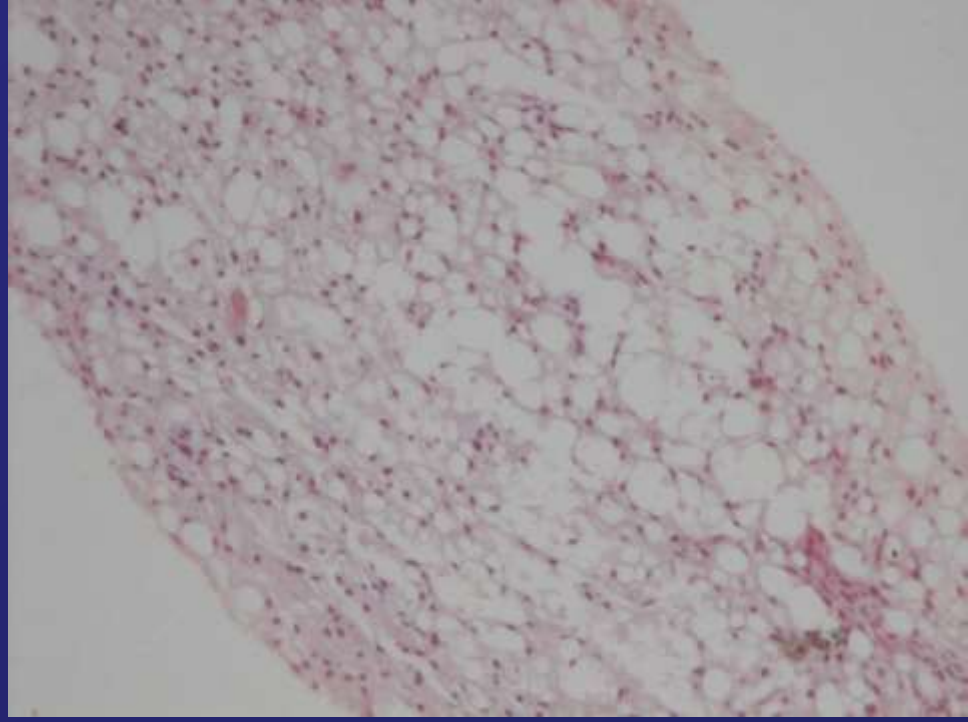
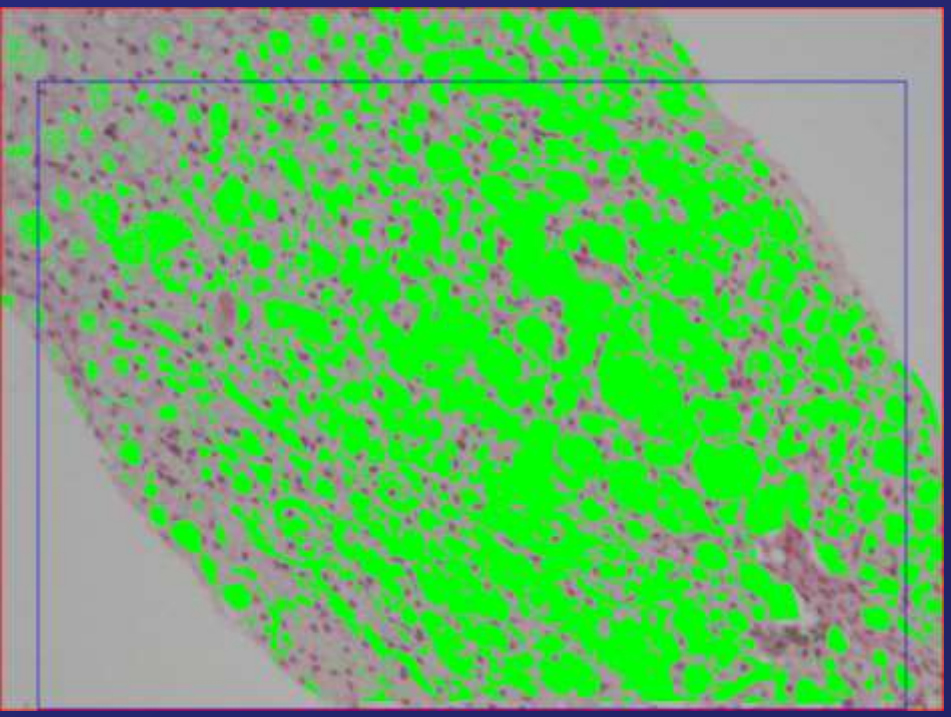
Does semiquantitative assessment of steatosis is accurate and reproducible ????

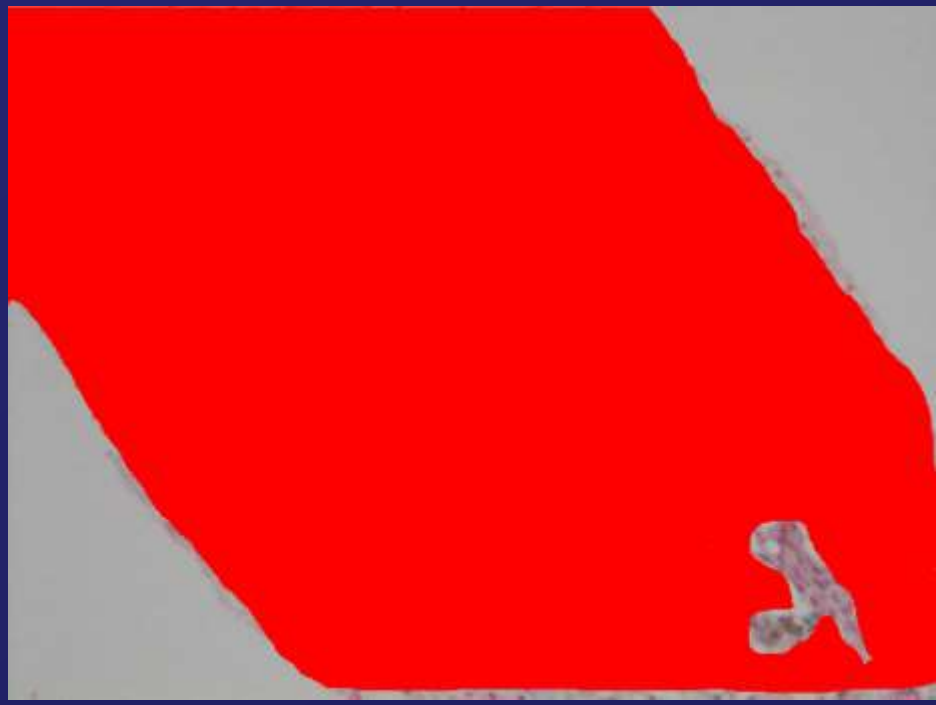
Morphometric assessment of steatosis as gold standard

- Morphometry to assess the area and diameter of fat globules in H&E slides of paraffin biopsies with steatosis
- Most trials apply on fresh frozen tissue with oil red O stain









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2	102373.98	0.40	0.66	39.80

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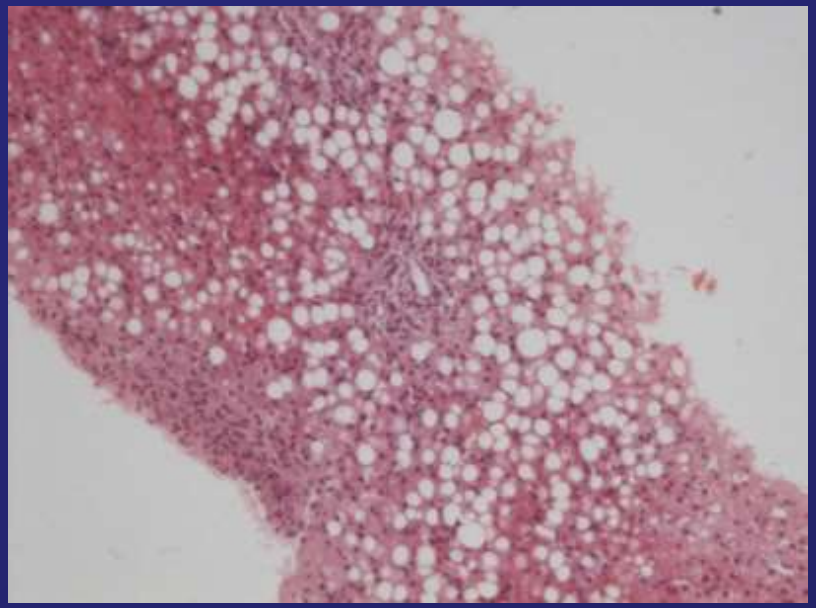
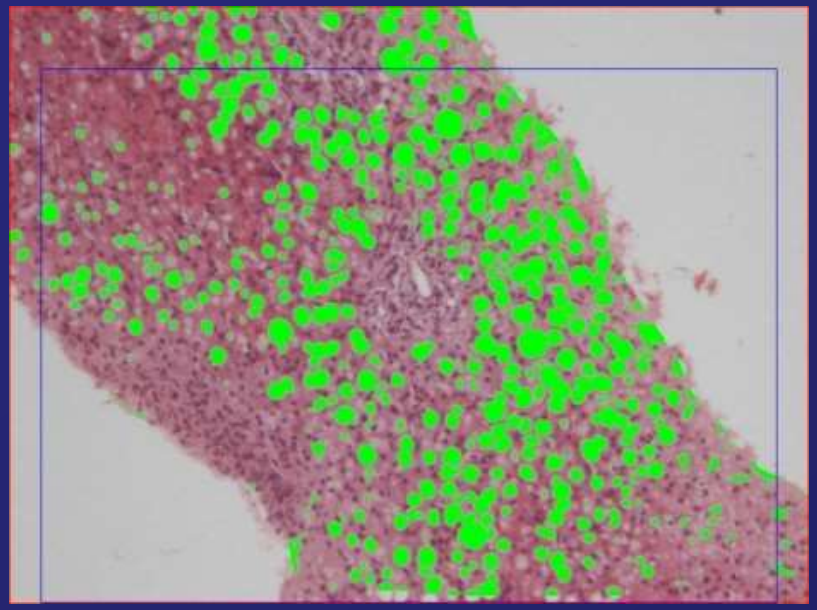
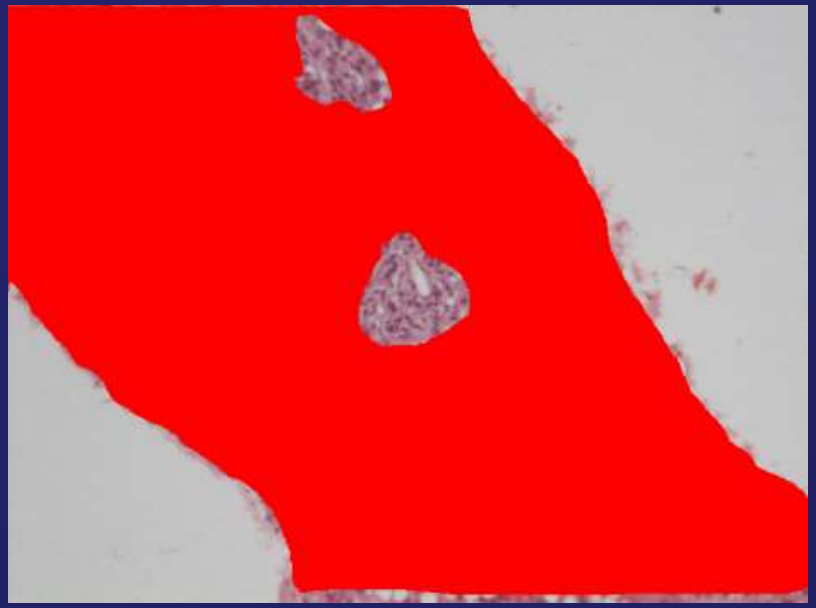
2	102373.98			

- **Semiquantitative measurment of steatosis:**
- **Wagdi: 80%**
- **Khaled: 70%**



- % of steatosis ????





Field #	Area	Area Fract	Area Fill	Area%
1	179122.95	0.70	2.29	69.64
2	34649.60	0.13	0.16	13.47

Field #	Area	Area Fract	Area Fill	Area%
1	179122.95	0.19	0.24	19.34%
2	34649.60			

- Semiquantitative measurment of steatosis:
- Wagdi: 40%
- Khaled: 30%

Advantage

- Performed on H&E stained slide
- No need for special stains for fat
- No need for frozen section
- Performed on Paraffin sections, so retrospective studies could be applied
- Not time consuming, one case takes less than 5 minutes.

Regression of fibrosis in paediatric autoimmune hepatitis: morphometric assessment of fibrosis versus semiquantiatative methods

Fibrogenesis & Tissue Repair

BioMed Central

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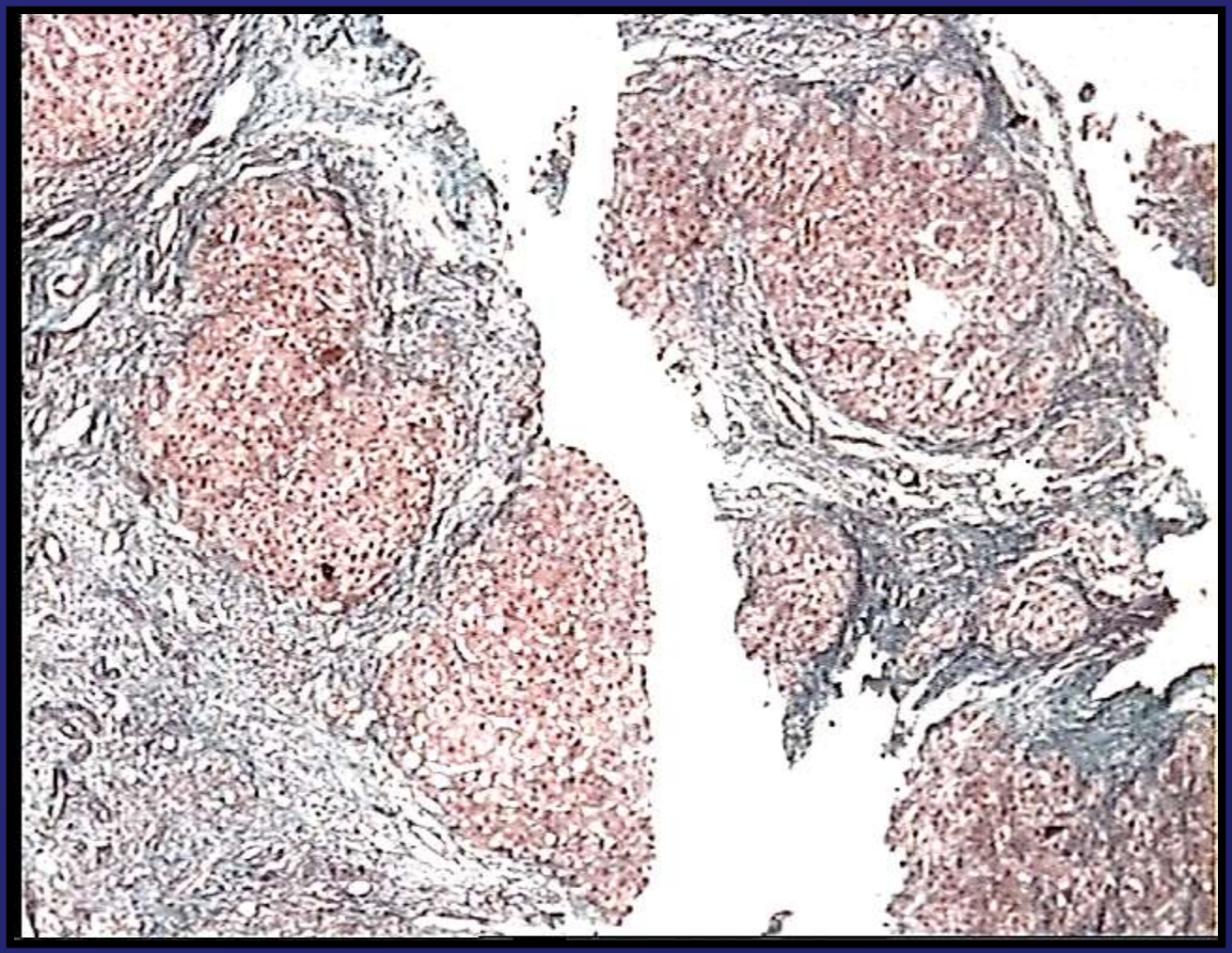
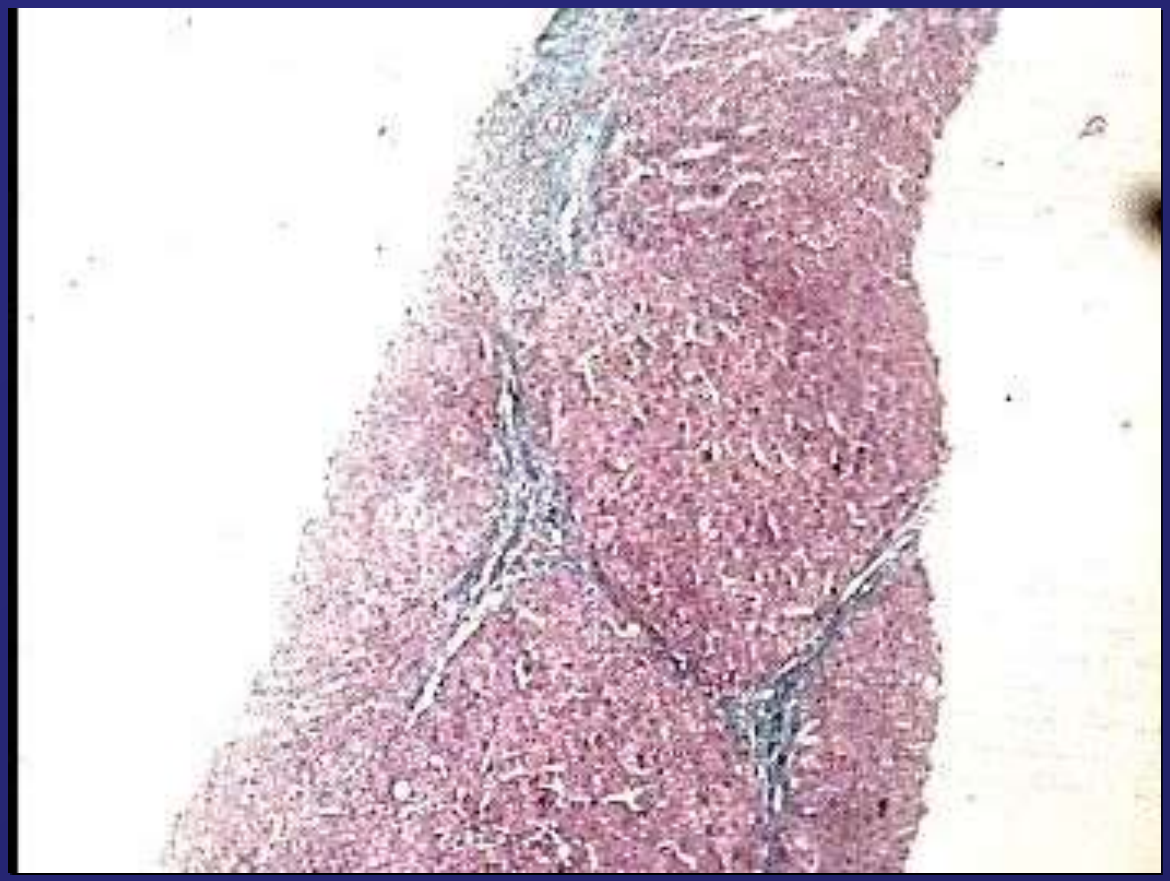


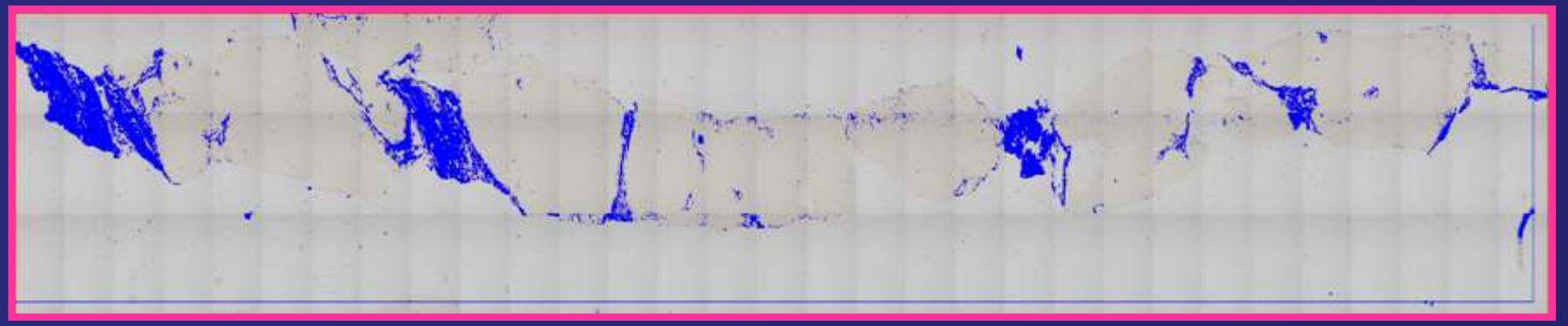
Pretreatment, Liver cirrhosis, thick fibrosis



Post-treatment pathology photo

Post-treatment, mosaic picture

Delineation of the whole liver core



Computer marking of the fibrosis in binary image

Combined marking of fibrosis and the liver parenchyma

The mean of fibrosis before and after therapy of 13 children with AIH


EGYPTIAN LIVER
TRANSPLANT
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Connecting
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ELTS 2022
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November 17th - 19th
Cairo, Egypt


THANKYOU