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# Non-alcoholic fatty liver disease (NAFLD)

## Atlas of histological images

### Guidelines for diagnosis and scoring

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# The NAFLD Atlas

- Aim: To provide a set of histological images useful to diagnose and score NAFLD in a consistent manner
- The selected images show either typical features of NAFLD or borderline lesions
- Multiple choice questions for self-testing follow the Atlas
- The Atlas is an ongoing LITMUS initiative that can be complemented by additional relevant images

“The LITMUS project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No. 777377. This Joint Undertaking receives support from the European Union’s Horizon 2020 research and innovation programme and EFPIA”.

LITMUS aims to develop, robustly validate and advance towards regulatory qualification biomarkers that diagnose, risk stratify and/or monitor NAFLD/NASH progression and fibrosis stage.

The Liver Biopsy Report form used by the LITMUS Histopathology Group (LHG) members. It includes relevant items with sufficiently robust interobserver agreement.

Example of a reported liver biopsy with NAFLD in an Excel sheet used by LHG.

**LITMUS - LIVER BIOPSY REPORT**

PATHOLOGIST INITIALS

BIOPSY REFERENCE

**BIOPSY**

- Length (mm) \_\_\_\_\_
- Nbr of fragments \_\_\_\_\_
- Adequacy
  - Adequate
  - Marginal
  - Inadequate

If inadequate, it is because of:

- Size
- Fragmentation
- Technical

**STEATOSIS**

- Grade 0
- Grade 1
- Grade 2
- Grade 3

**BALLOONING**

NASH CRN

- 0
- 1
- 2

SAF

- 0
- 1
- 2

**LOBULAR INFLAMMATION**

•NASH CRN

- 0
- 1
- 2
- 3

•SAF

- 0
- 1
- 2

**ACTIVITY**

- NAS 1 2 3 4 5 6 7 8
- A (SAF) 1 2 3 4

**PORTAL INFLAMMATION**

- None or Minimal
- Mild
- More than mild

**MALLORY-DENK BODY**

- No
- Few
- Many

**OTHER FEATURE(S)**

\_\_\_\_\_

**FIBROSIS (NASH CRN)**

- 0
- 1a
- 1b
- 1c
- 2
- 3
- 4

**FIBROSIS (EPOS)**

- 0
- 1
- 2
- 3
- 4
- 5
- 6a
- 6b
- 6c

**DIAGNOSTIC CATEGORY**

- Normal
- NAFL (steatosis without definite NASH feature)
- Definite NASH
- Fibrosis / Cirrhosis **without steatosis**
  - But consistent with NAFLD
  - No feature of NAFLD
- Other etiology \_\_\_\_\_

Biopsy reference	EX123659875			
Biopsy_length (mm)	25			
Biopsy_nr_fragments	2			
Biopsy_Adequacy	Adequate			
If Inadequate, because				
Steatosis	Grade 2			
Microvesicular steatosis (patch)	Yes			
Ballooning CRN	1			
Ballooning SAF	1			
Lob_Inflammation CRN	1			
Lob_Inflammation SAF	2			
Activity_NAS	4			
Activity_A (SAF)	3			
Portal_Inflammation	None or Minimal			
Mallory-Denk bodies	No			
Other features				
Fibrosis_CRN	2			
Fibrosis_EPoS	2			
Diagnostic Category	Definite NASH			
If fibrosis/cirrhosis without steatosis				
Other aetiology				

# LIVER BIOPSY REPORT FORM



## 1. Quality of biopsy

**BIOPSY**

- Length (mm) \_\_\_\_\_
- Nbr of fragments \_\_\_\_\_
- Adequacy
  - Adequate
  - Marginal
  - Inadequate

If inadequate, it is because of :

- Size
- Fragmentation
- Technical

Note that the number of portal tracts is not reported here. The pattern of injury in NAFLD is centrilobular (acinar zone 3), therefore the number of portal tracts is not as relevant as in other liver diseases. Furthermore, in advanced fibrosis/cirrhosis portal tracts may be difficult to identify

# LIVER BIOPSY REPORT FORM

## 1. Quality of biopsy

- **Number of fragments:**

- Assessed on haematoxylin & eosin (H&E)
- Ignore small splits if the core is in the same orientation
- Ignore less than 1 mm fragment(s) (except if all are 1 mm fragments)
- Ignore blood clots or not liver tissue fragments

- **Serial sections on the glass slides**

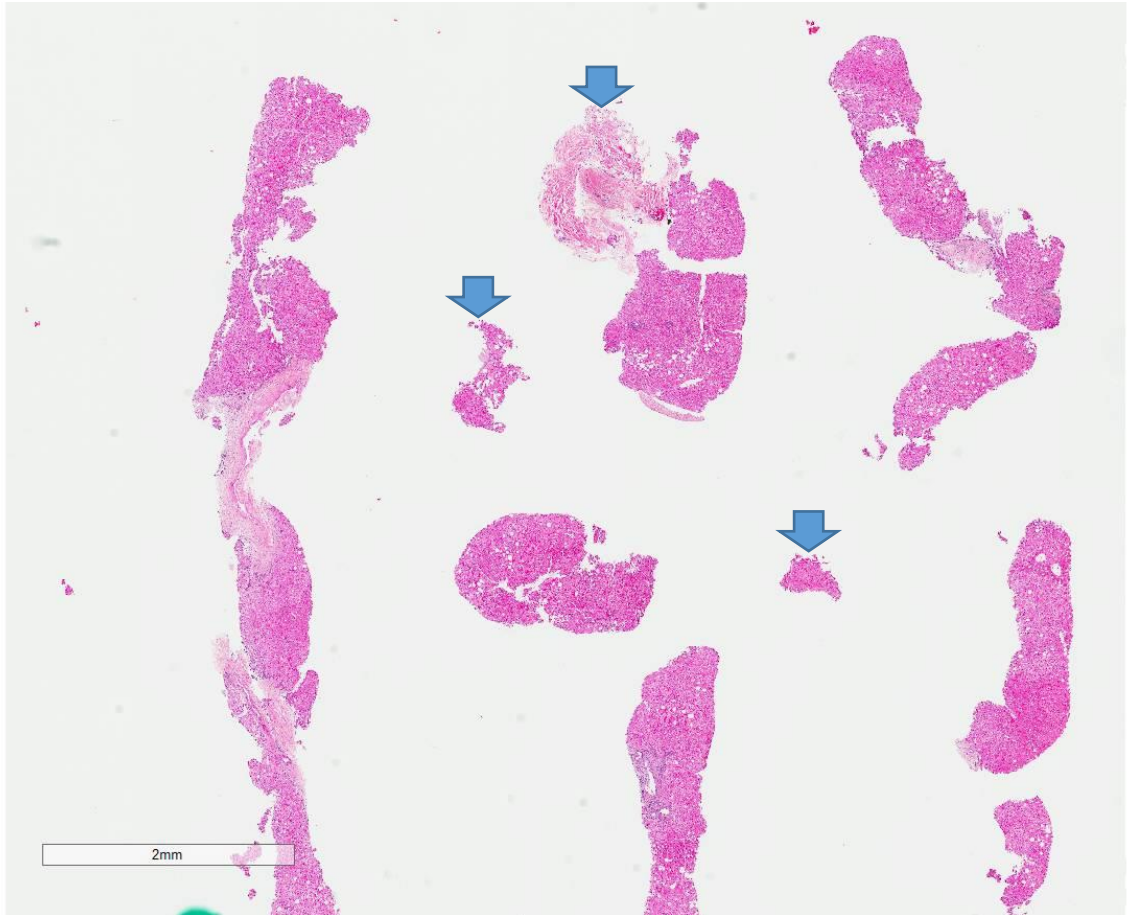
- Use the section with most tissue present

- **Adequacy:**

- Marginal quality means that some but not all features can be assessed or that the biopsy can be assessed but only with relative confidence because of technical issues
- If marginal, form can be filled in at least partially. Items that cannot be assessed are left blank
- If inadequate, the form cannot be filled at all

# Small splits but 1 single fragment





↓ fragments not counted

Number of fragments: 5

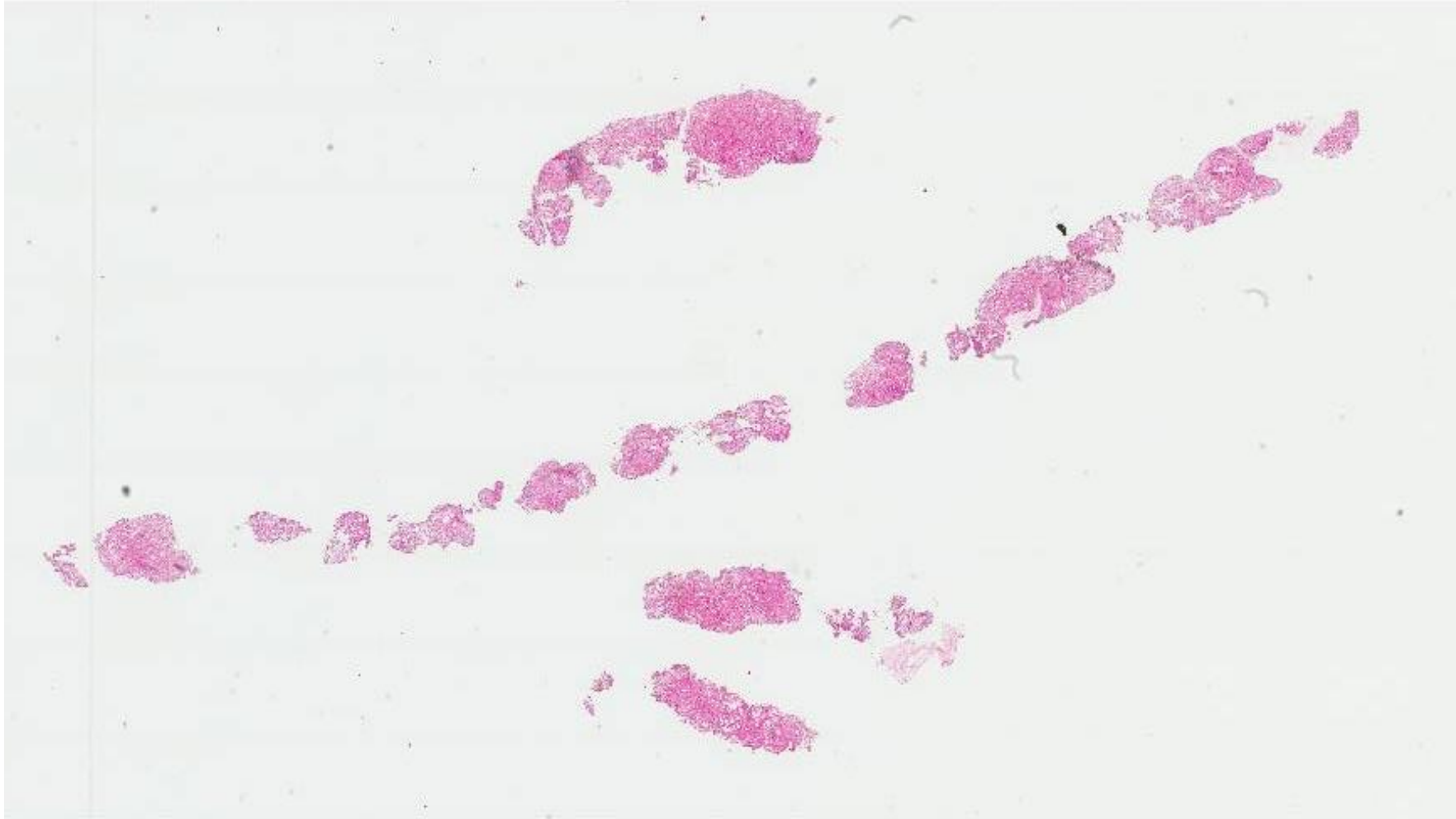




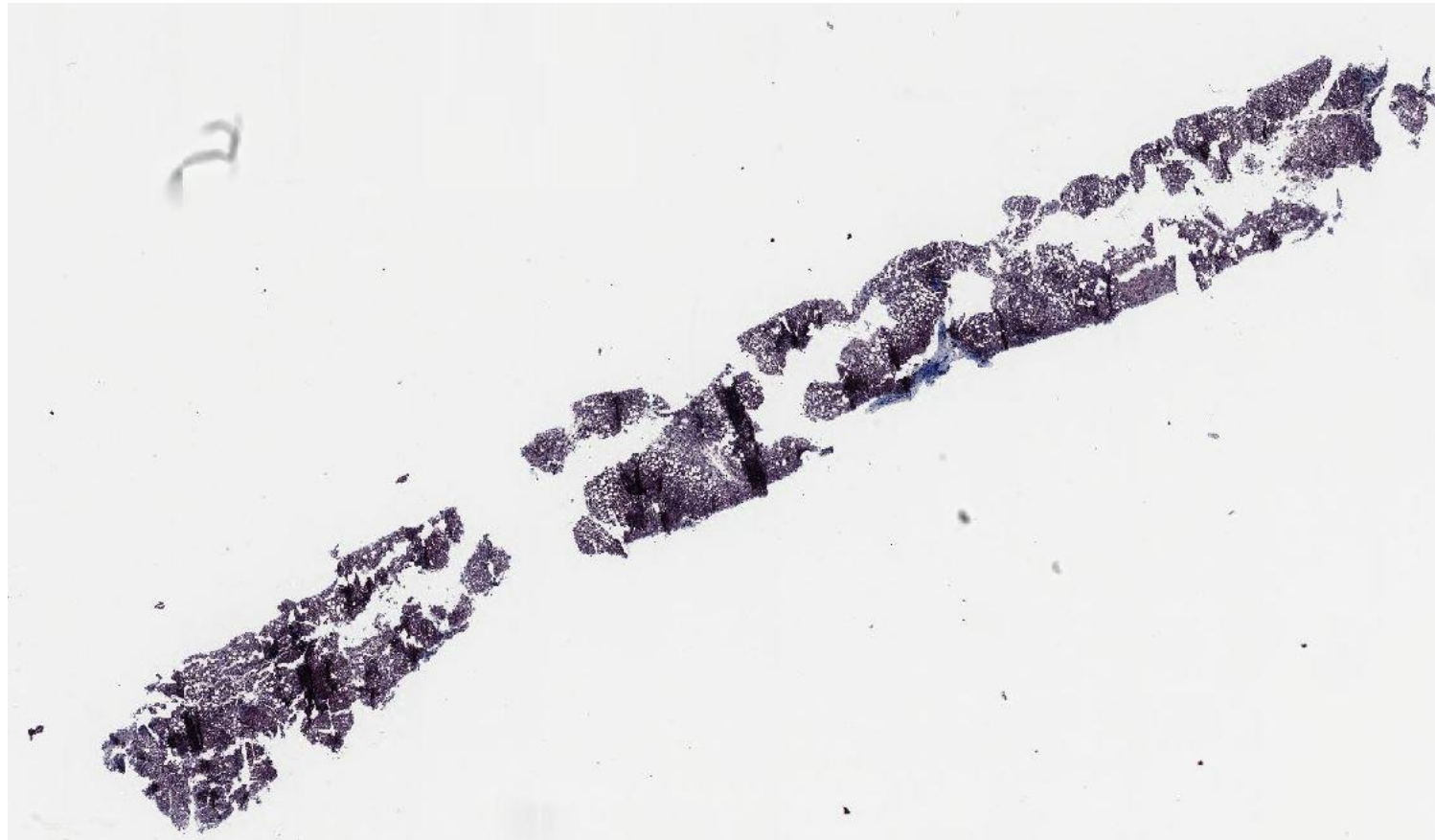
Number of fragments: 3



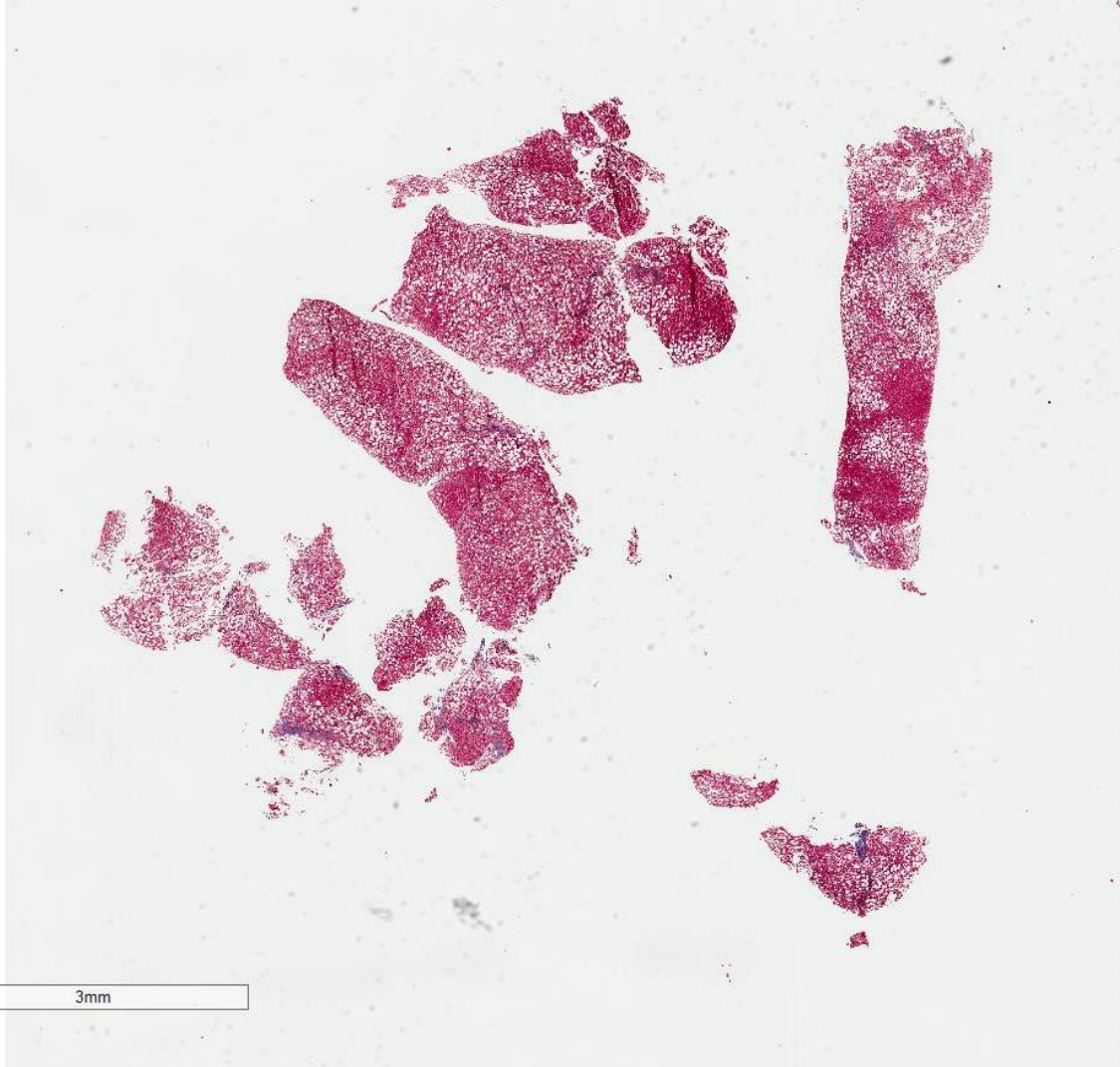
**Biopsy Inadequate:** fragmentation  
(Attrition of material in paraffin block after multiple recuts)



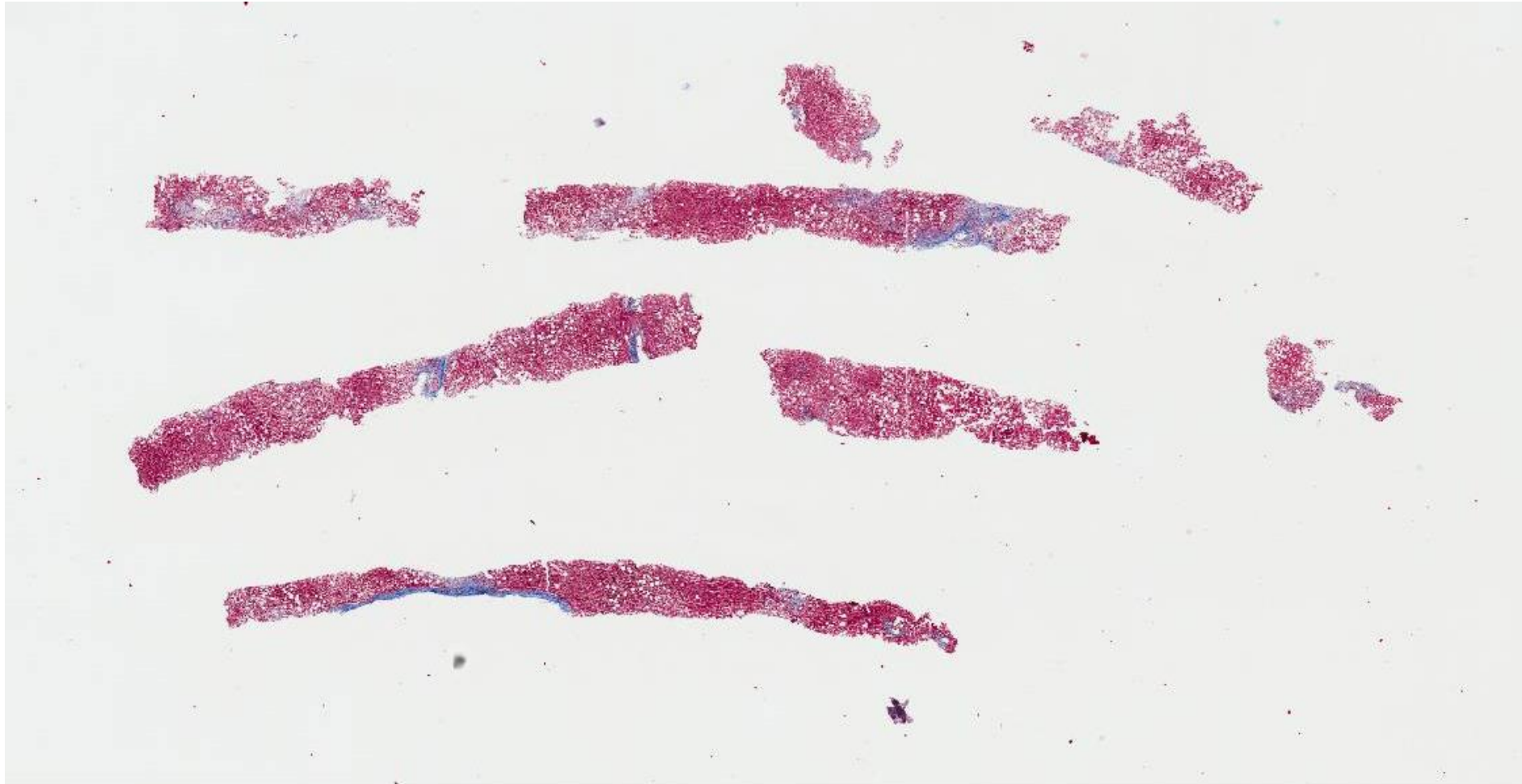
# Biopsy inadequate: technical issues



**Biopsy marginally adequate:** cannot stage fibrosis because of fragmentation but steatosis and other features can be assessed



**Biopsy marginally adequate:** fragmentation but fibrosis may be assessed with relative confidence



## STEATOSIS

Grade 0

Grade 1

Grade 2

Grade 3

## MICROVESICULAR STEATOSIS (PATCH) \*

NO

YES

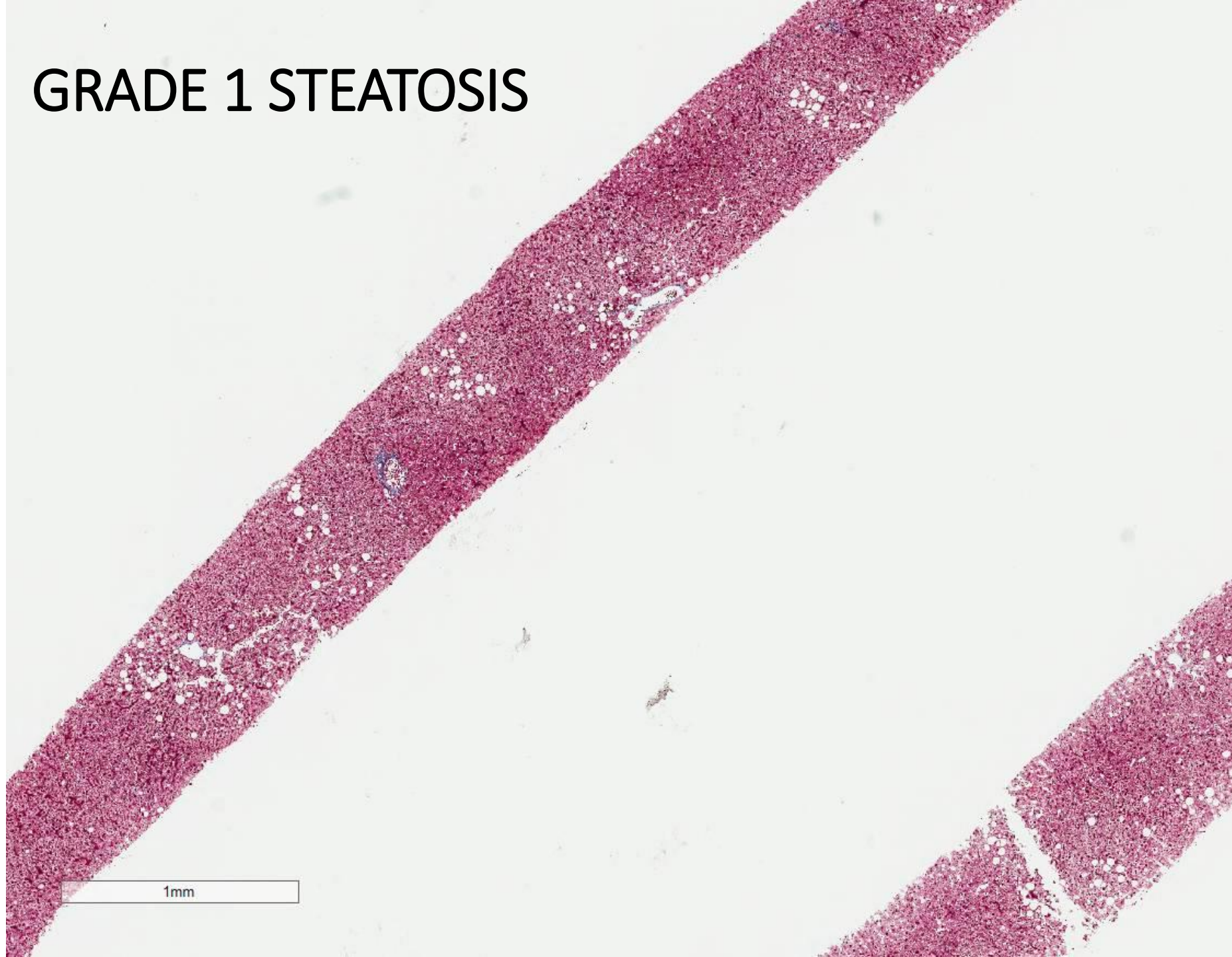
\* Patch is defined as a group of at least 10 hepatocytes

# Steatosis

- Grading according to NASH-CRN\* (<5%, 5-33%, >33-66%, >66% of hepatocytes involved by steatosis)
- % of hepatocytes with either large (macrovesicular) or small (mediovvesicular) fat droplets (not minute droplets/foamy cytoplasm)
- Assessed on H&E stained slide
- Assessed at low magnification (x4)
- When the extent of steatosis is variable along the biopsy core(s), take into account the whole section area

\*NASH CRN: Nonalcoholic Steatohepatitis Clinical Research Network, National Institutes of Health, USA  
Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41:1313-1321.

# GRADE 1 STEATOSIS



1mm



# GRADE 2 STEATOSIS



900um

# GRADE 2 STEATOSIS

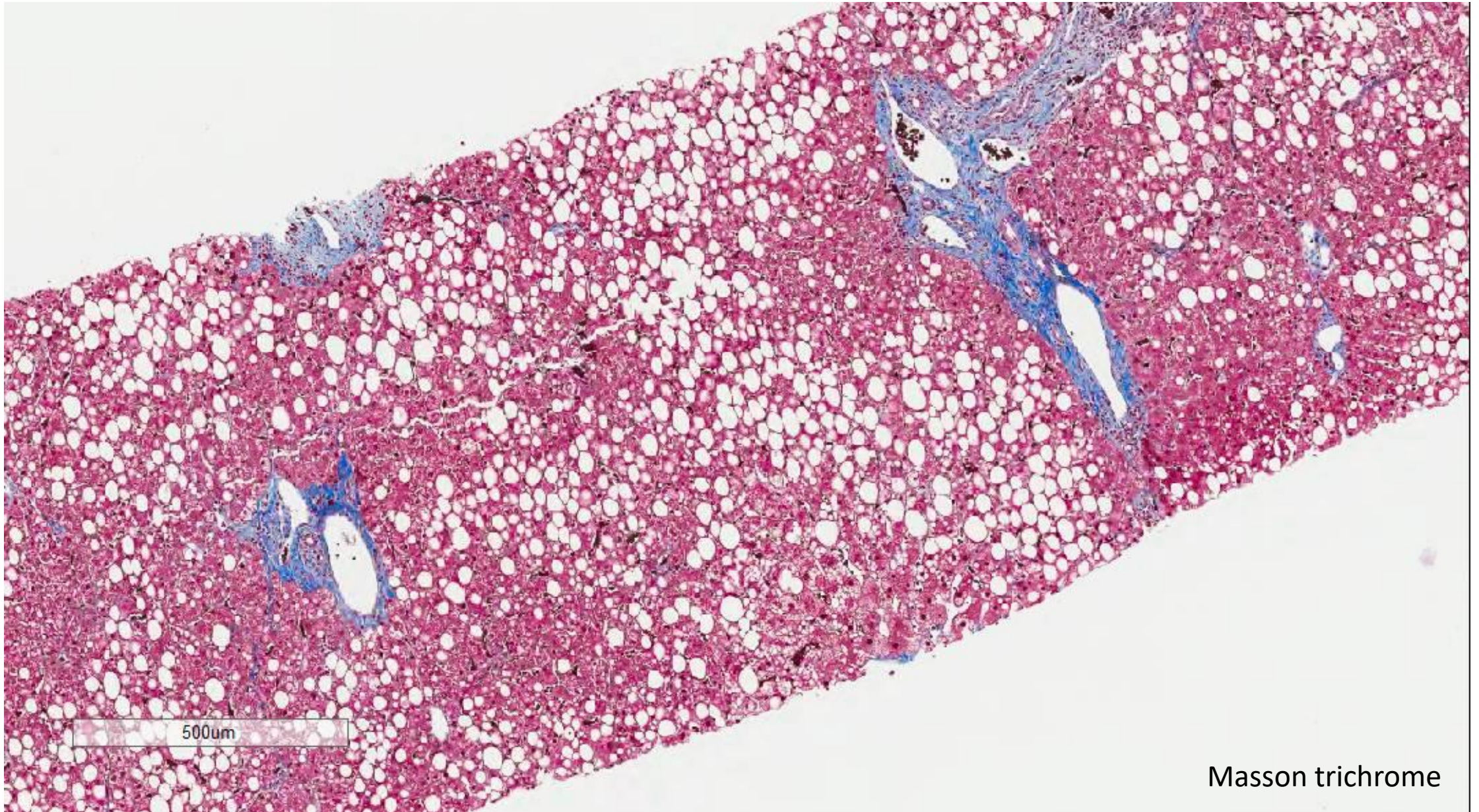


# GRADE 3 STEATOSIS



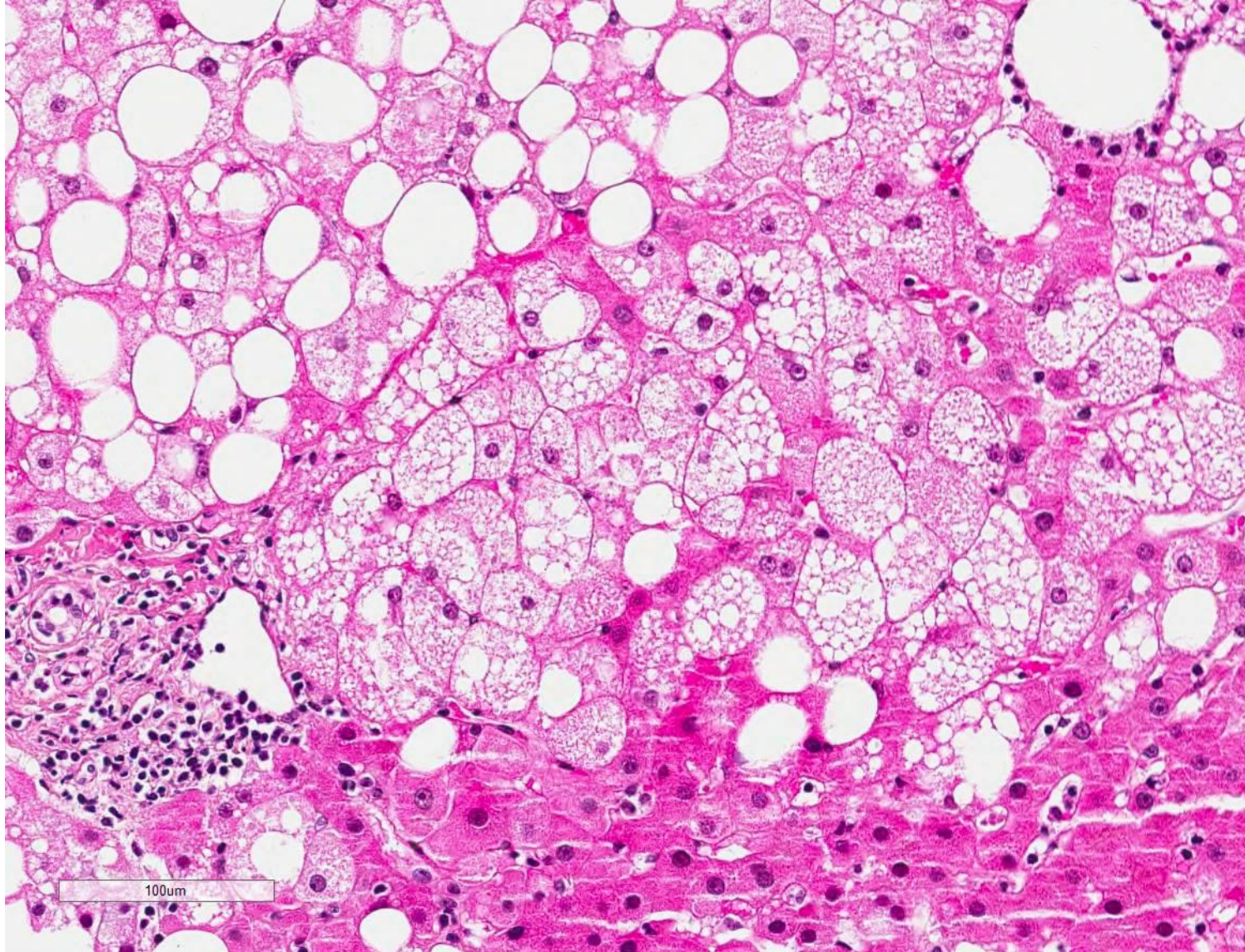
900um

# GRADE 3 STEATOSIS

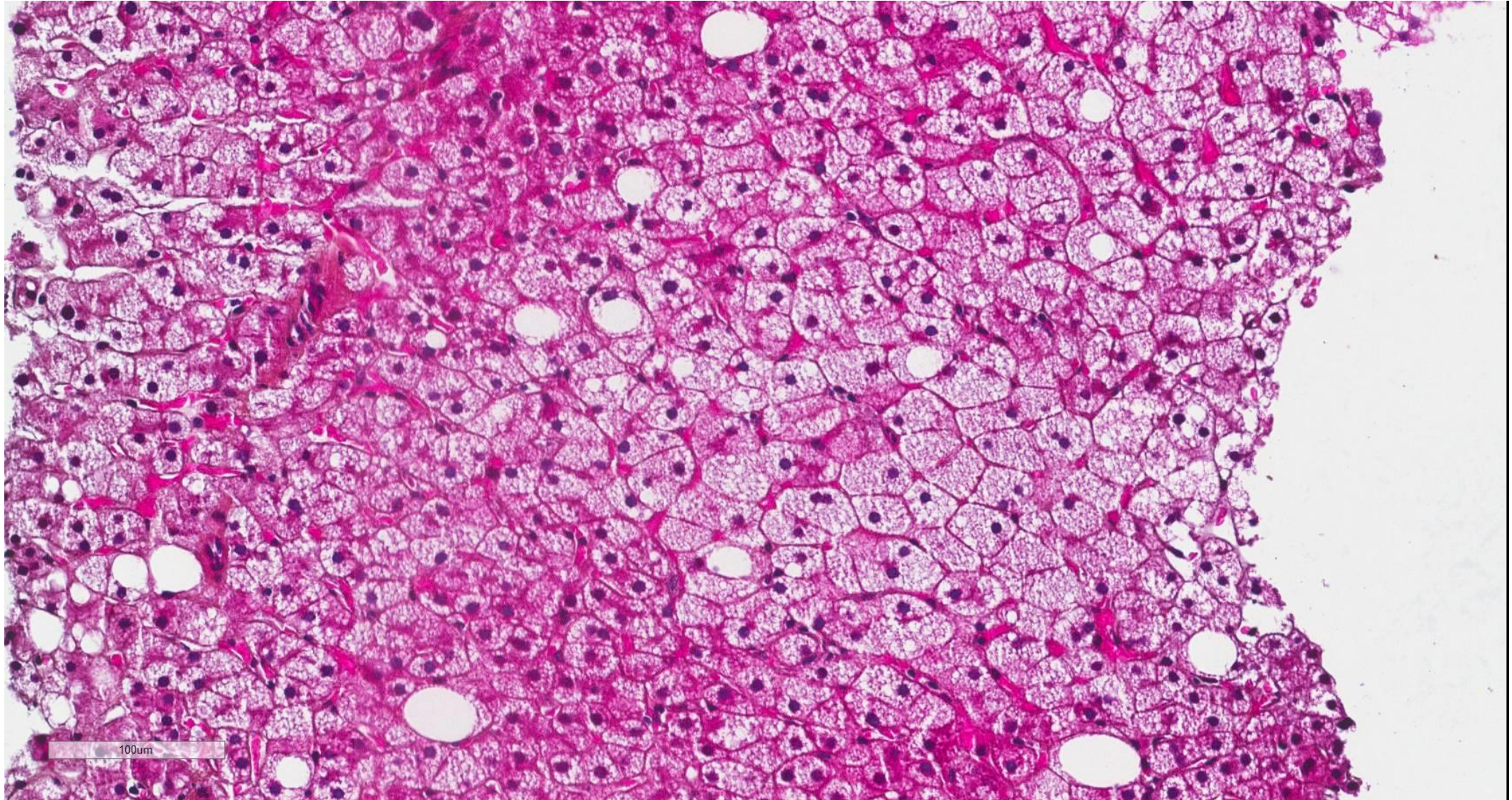


# Microvesicular steatosis

(patch: a group of at least 10 hepatocytes with foamy cytoplasm)



# Microvesicular steatosis (patch)



## BALLOONING

NASH CRN

0

1

2

SAF

0

1

2

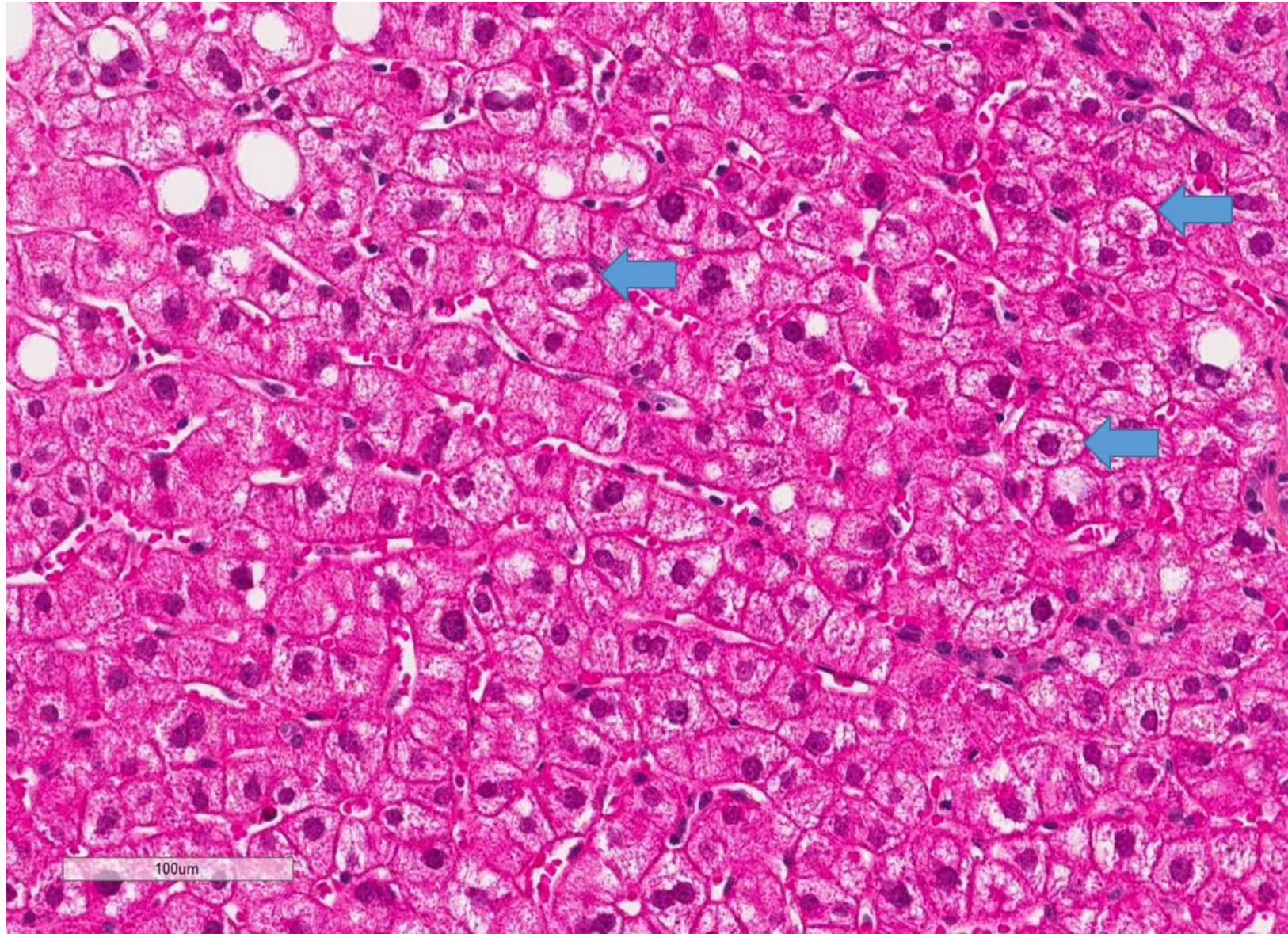
## • HEPATOCYTE BALLOONING

- Round shape **AND** clear, pale or reticulated cytoplasm
- Diagnosis on H&E and/or Masson trichrome (not with immunostaining)
- Scoring of ballooning:
  - According to NAS (0=no, 1=few, 2=many, prominent) (Kleiner et al. Hepatology 2005)
  - According to SAF (0,1,2)\*
    - 0:** normal hepatocytes with cuboidal shape, sharp angles and pink eosinophilic cytoplasm or rounded hepatocytes without cytoplasmic clearing
    - 1:** presence of clusters of hepatocytes with round shape and pale cytoplasm, usually reticulated. Although the cell shape is different, the size is similar to that of normal hepatocytes
    - 2:** as for score 1, but where there is also at least one enlarged ballooned hepatocyte (at least twice the size [2x] of a normal hepatocyte, within a cluster of hepatocytes with score 1 ballooning)

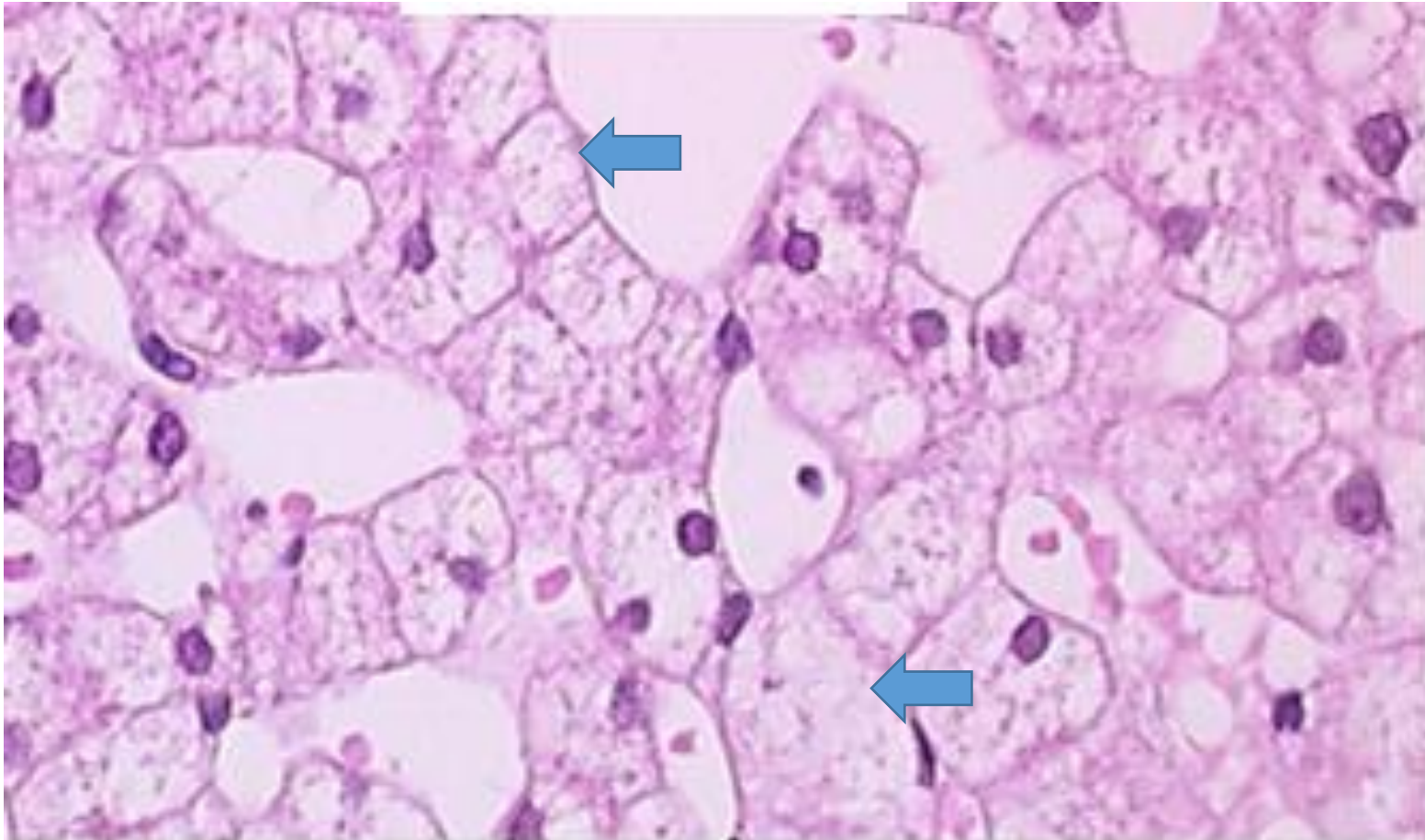
\*Bedossa P, FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of non-alcoholic fatty liver disease. Hepatology 2014; 60:565-575.



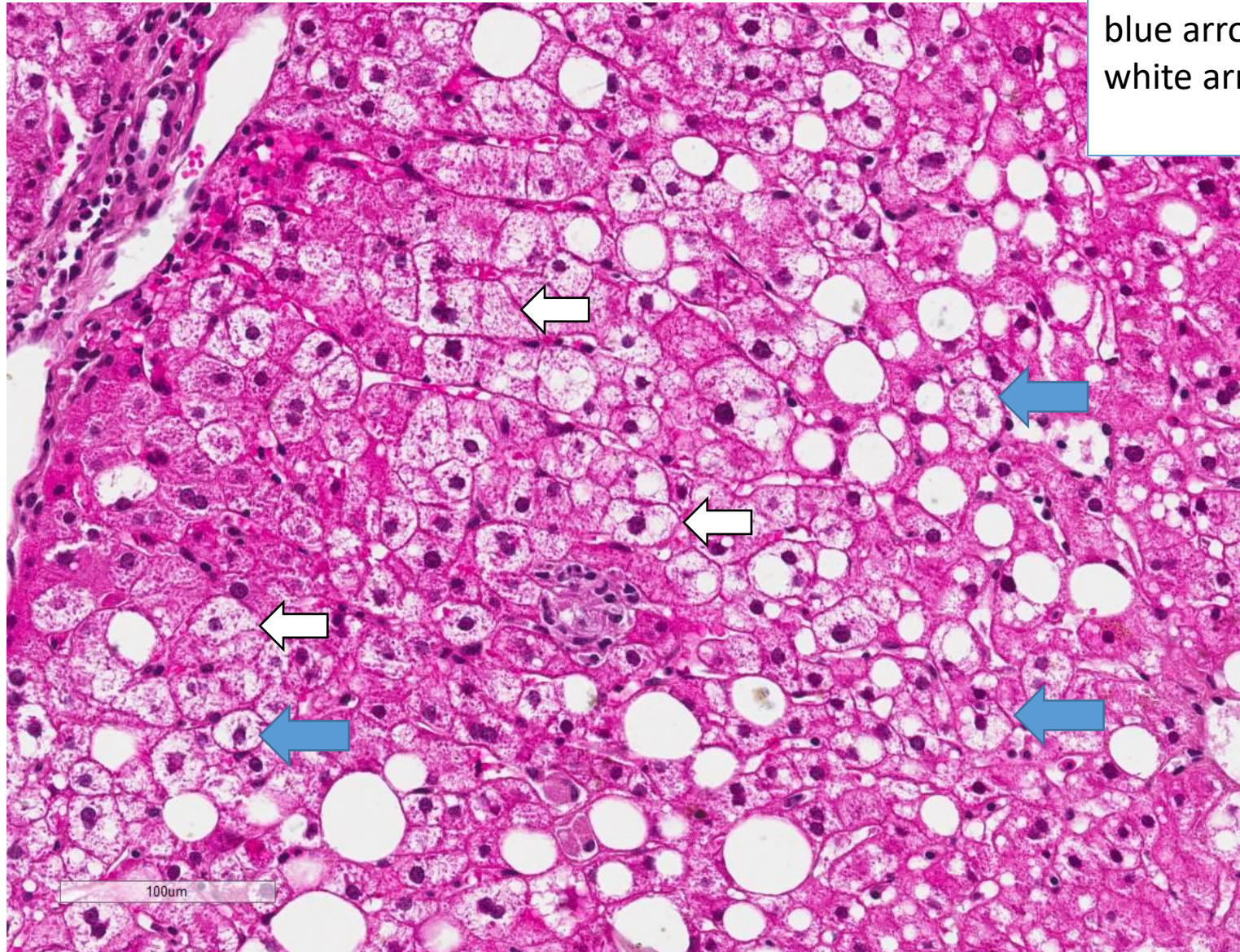
# Ballooning 0: few ballooned hepatocytes without cytoplasmic clearing



**Ballooning 0:** clear hepatocytes without round shape

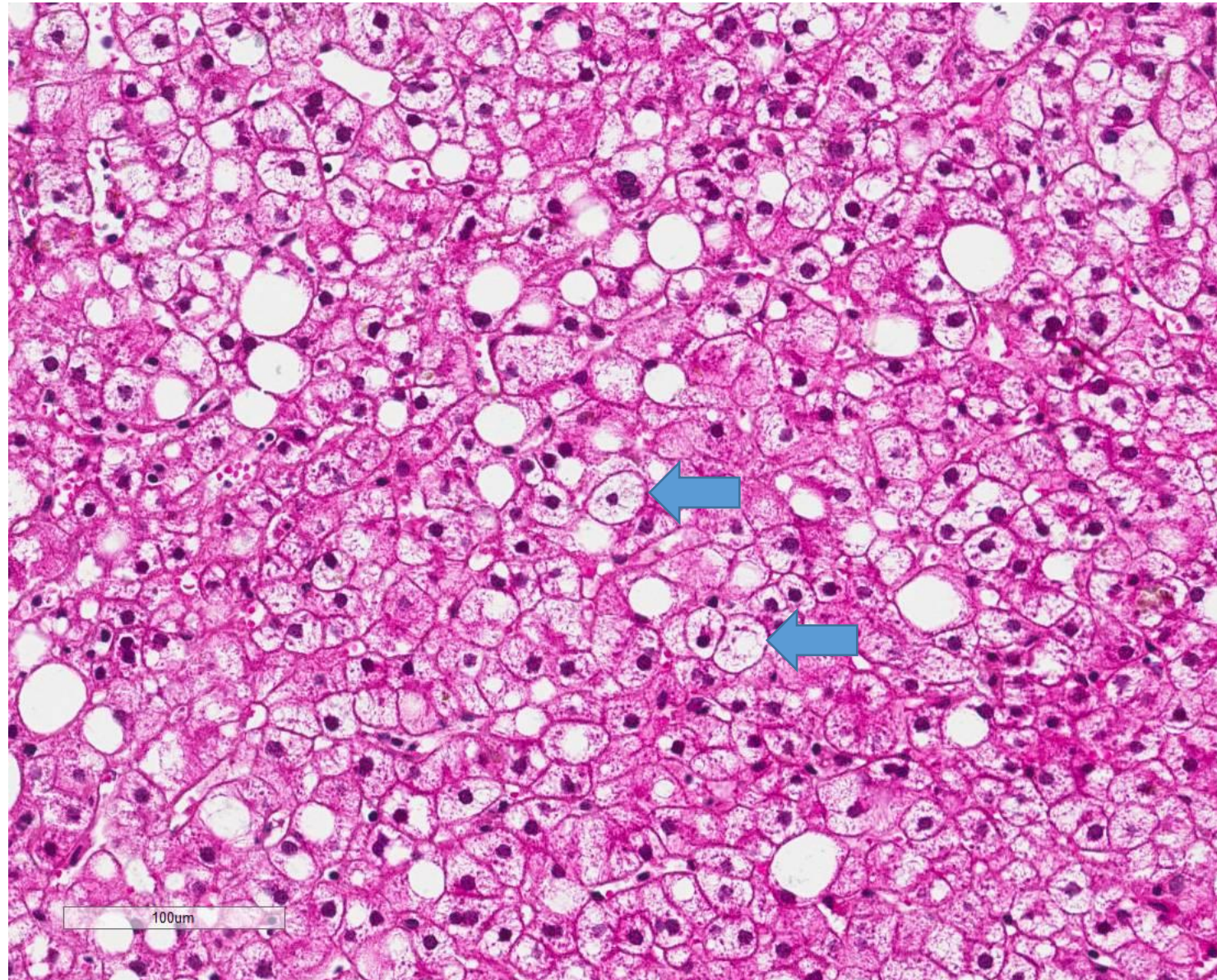


# Ballooning 1 (SAF & NASH CRN)

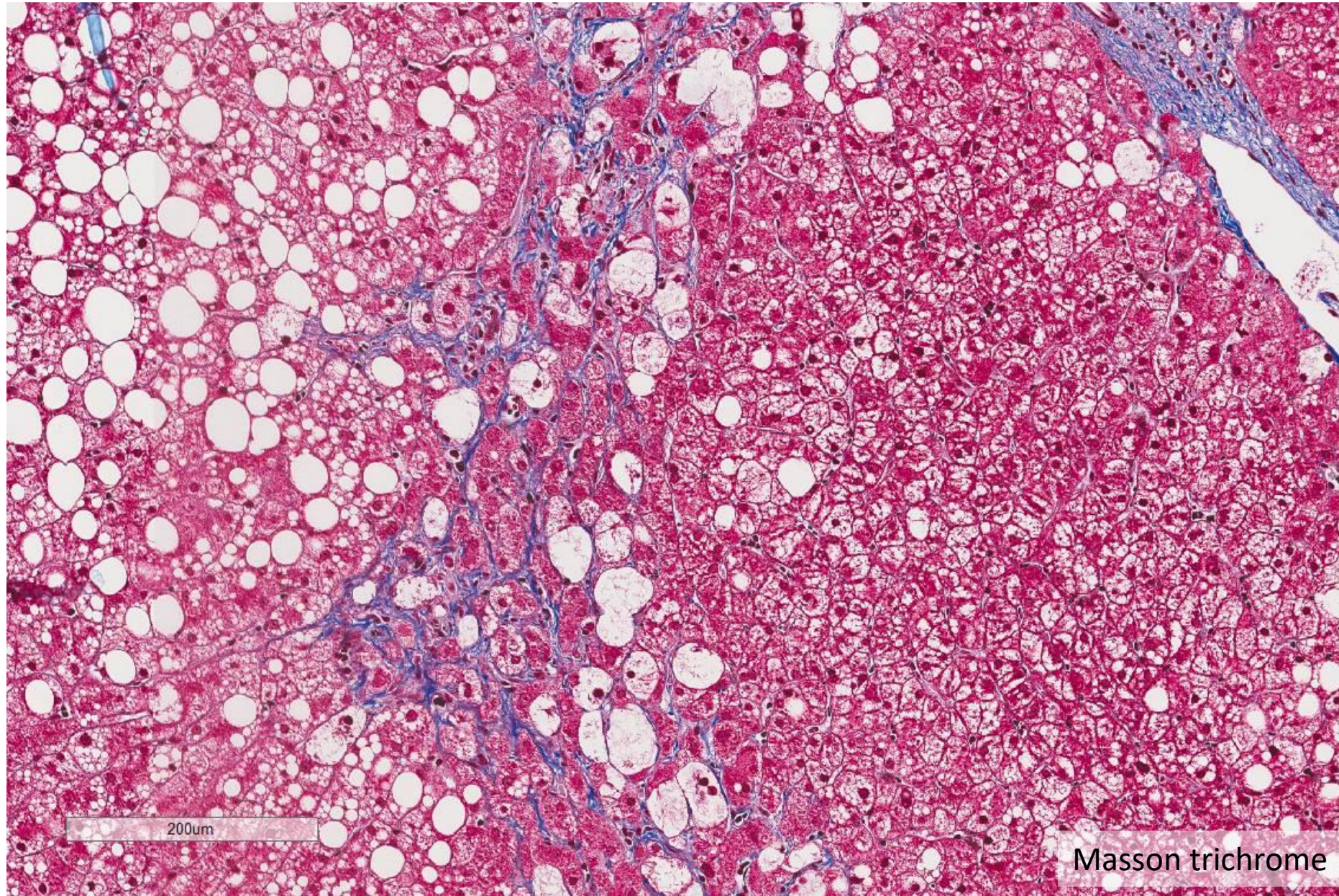


blue arrow: ballooned hepatocytes  
white arrow: clear but not ballooned hepatocytes

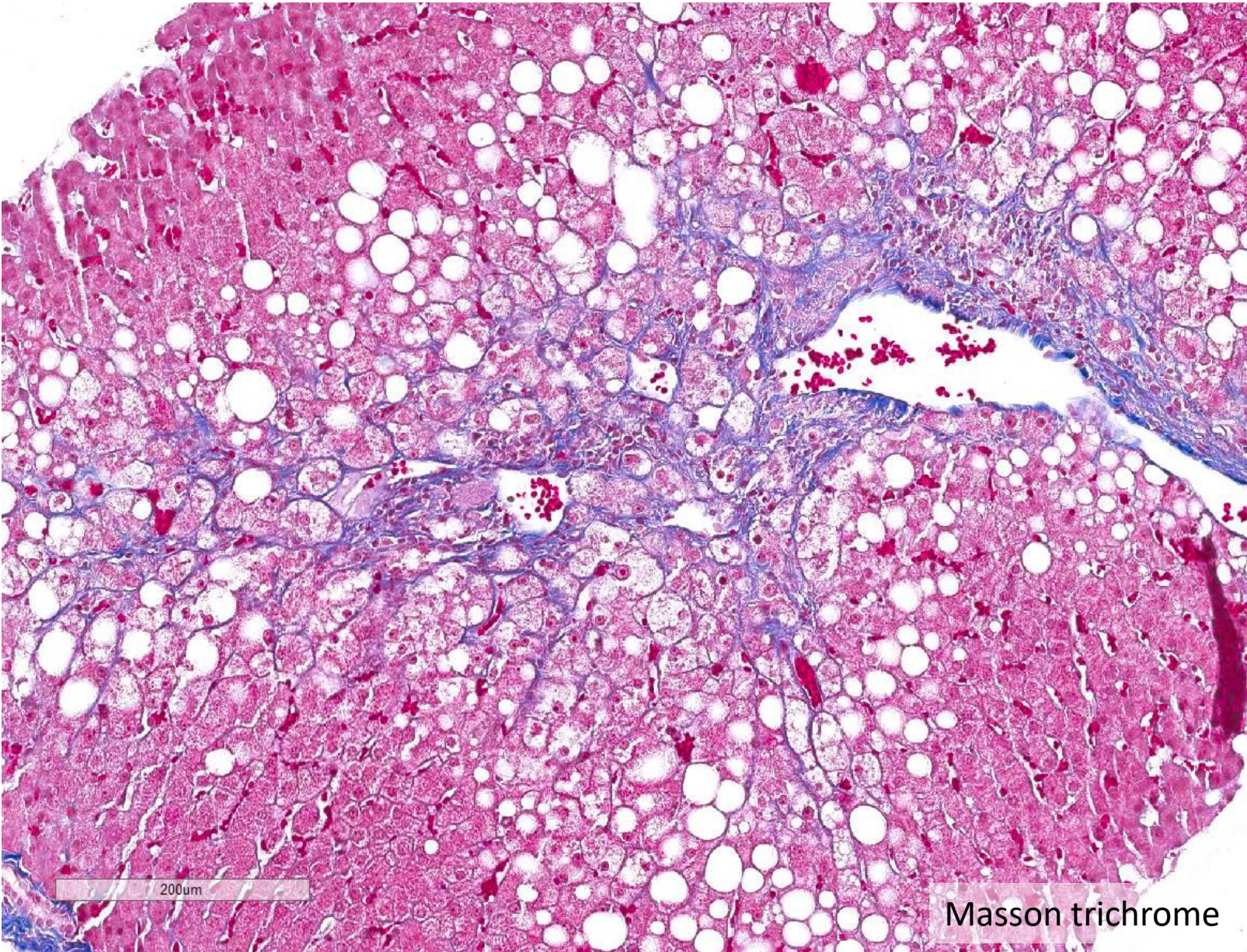
# Ballooning 1 (NASH CRN and SAF)



# Ballooning 2 (NASH CRN and SAF)

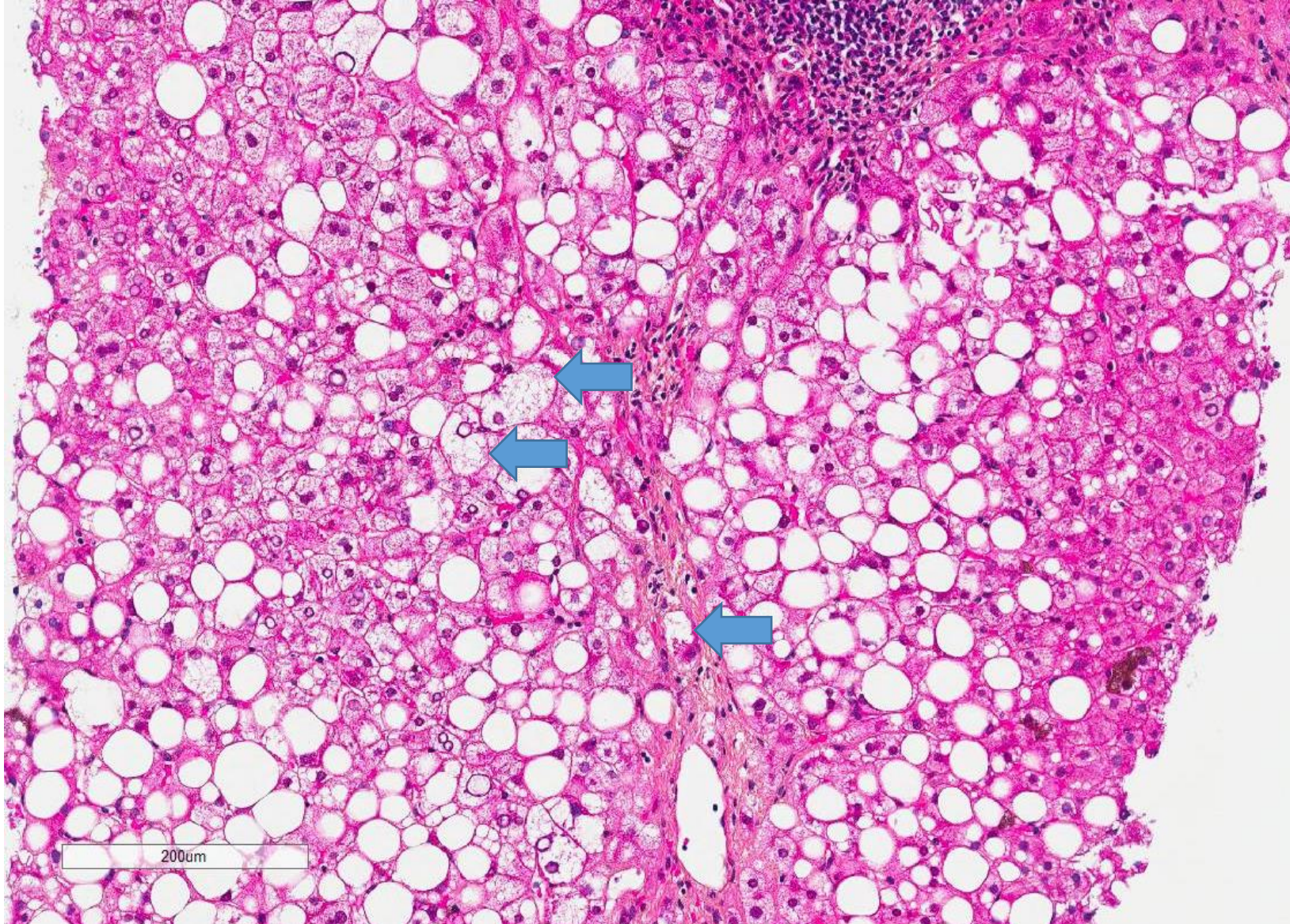


# Ballooning 2 (NASH CRN and SAF)

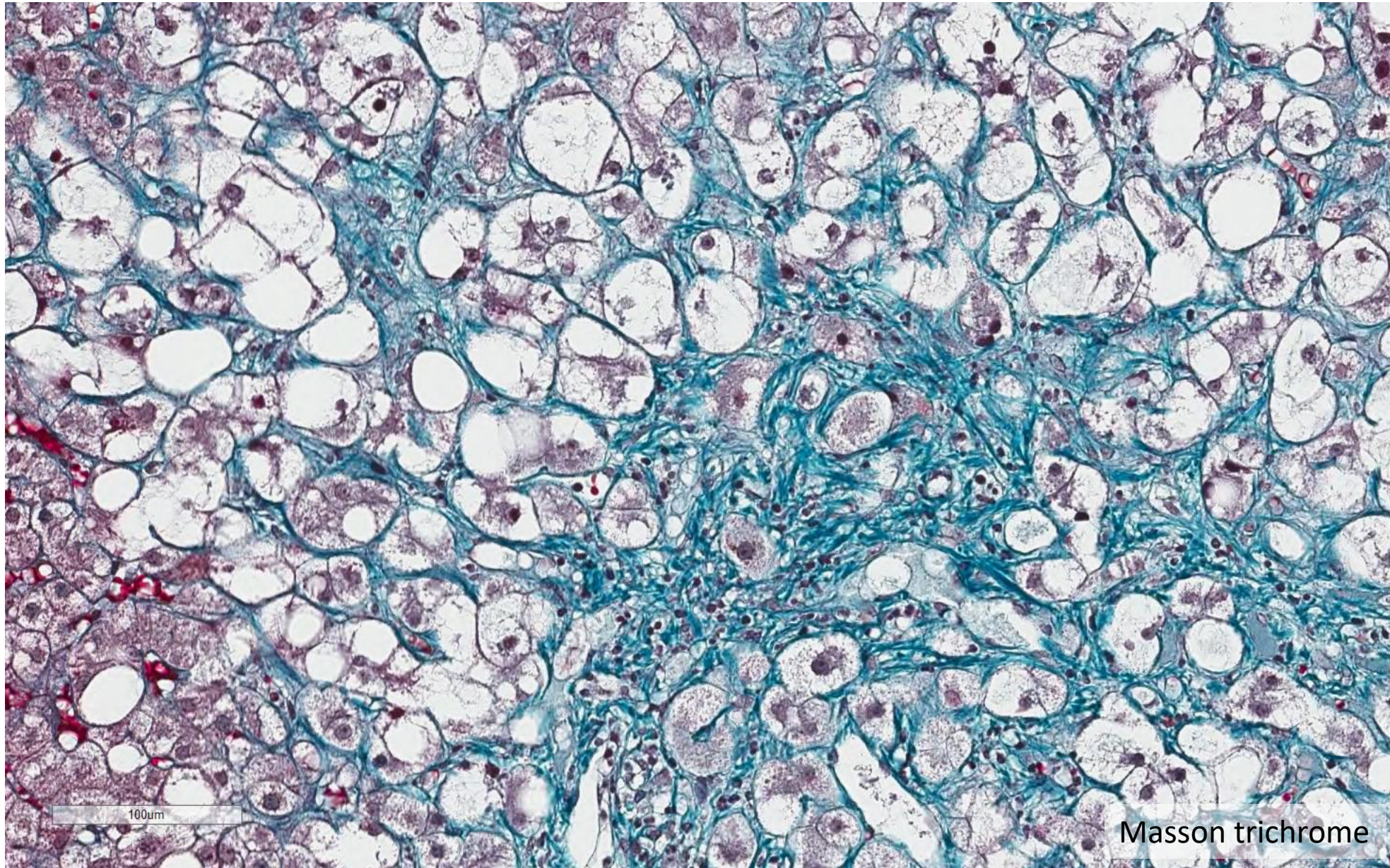


# Ballooning 1 (NASH CRN, few ballooned hepatocytes)

## Ballooning 2 (SAF, enlarged ballooned hepatocytes)



# Ballooning 2 (NASH CRN & SAF, many ballooned and enlarged hepatocytes)





## Lobular inflammation

- Based on global assessment (not hot spots)
- Consider foci (clusters), not linear pattern of intrasinusoidal inflammatory cells
- Foci with more than 2 inflammatory cells
- x20 magnification
- Scoring :
  - According to NAS (1 to 3)  
NASH CRN : **0** None; **1** < 2 foci; **2** 2-4 foci; **3** > 4 foci
  - According to SAF (1-2)  
SAF : **0** none; **1**  $\leq 2$  foci; **2** >2 foci

### LOBULAR INFLAMMATION

- NASH CRN

0

1

2

3

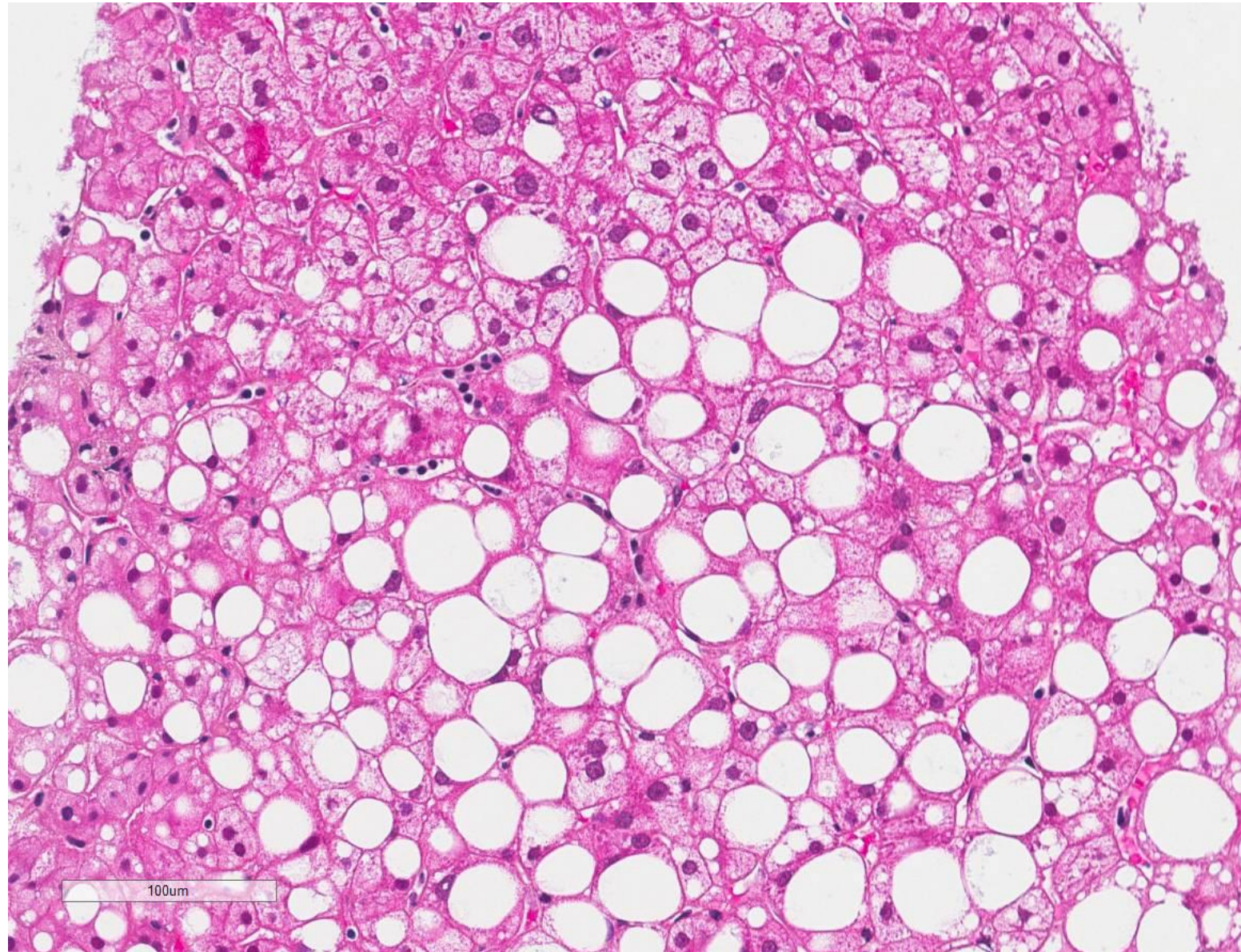
- SAF

0

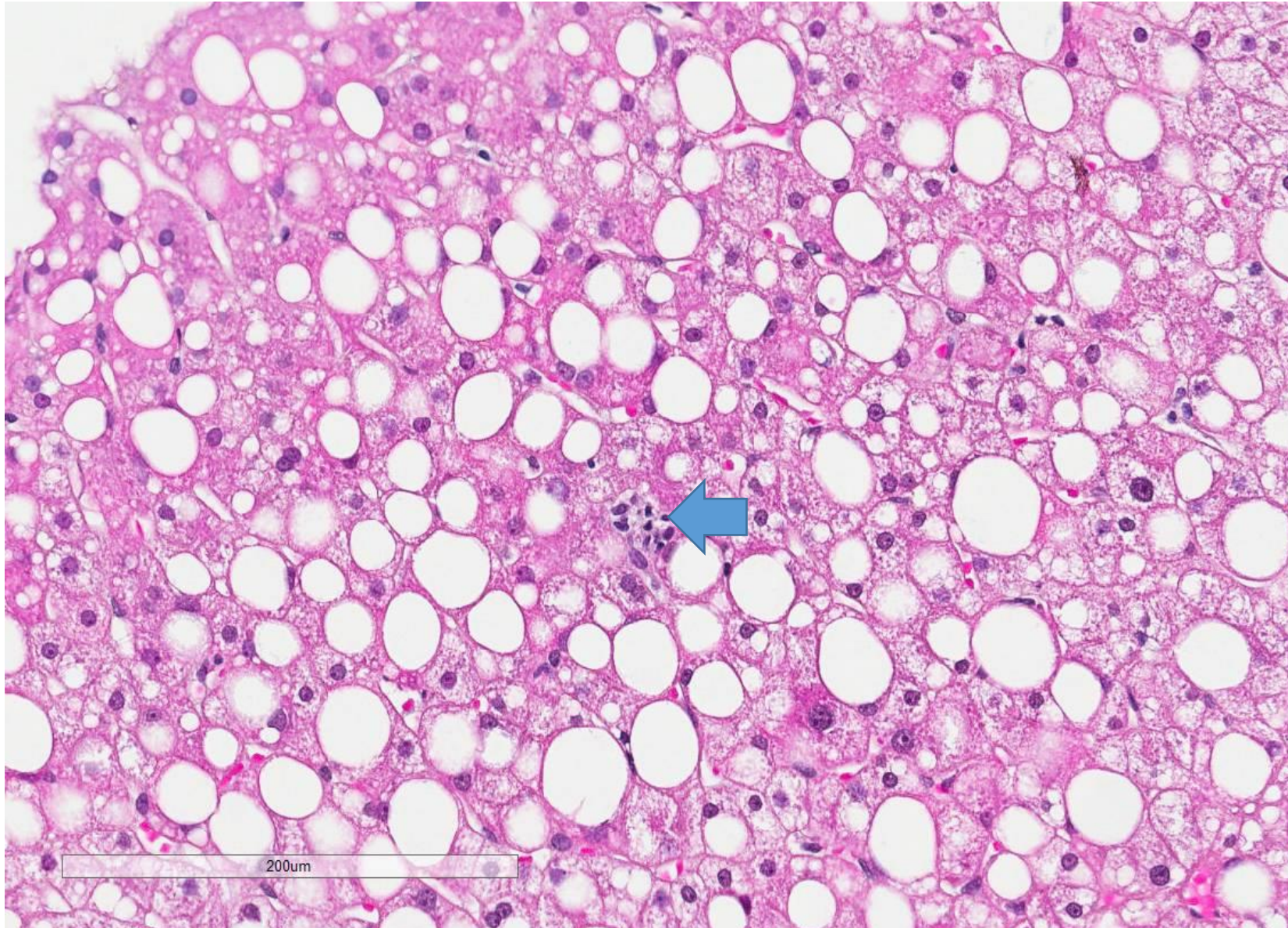
1

2

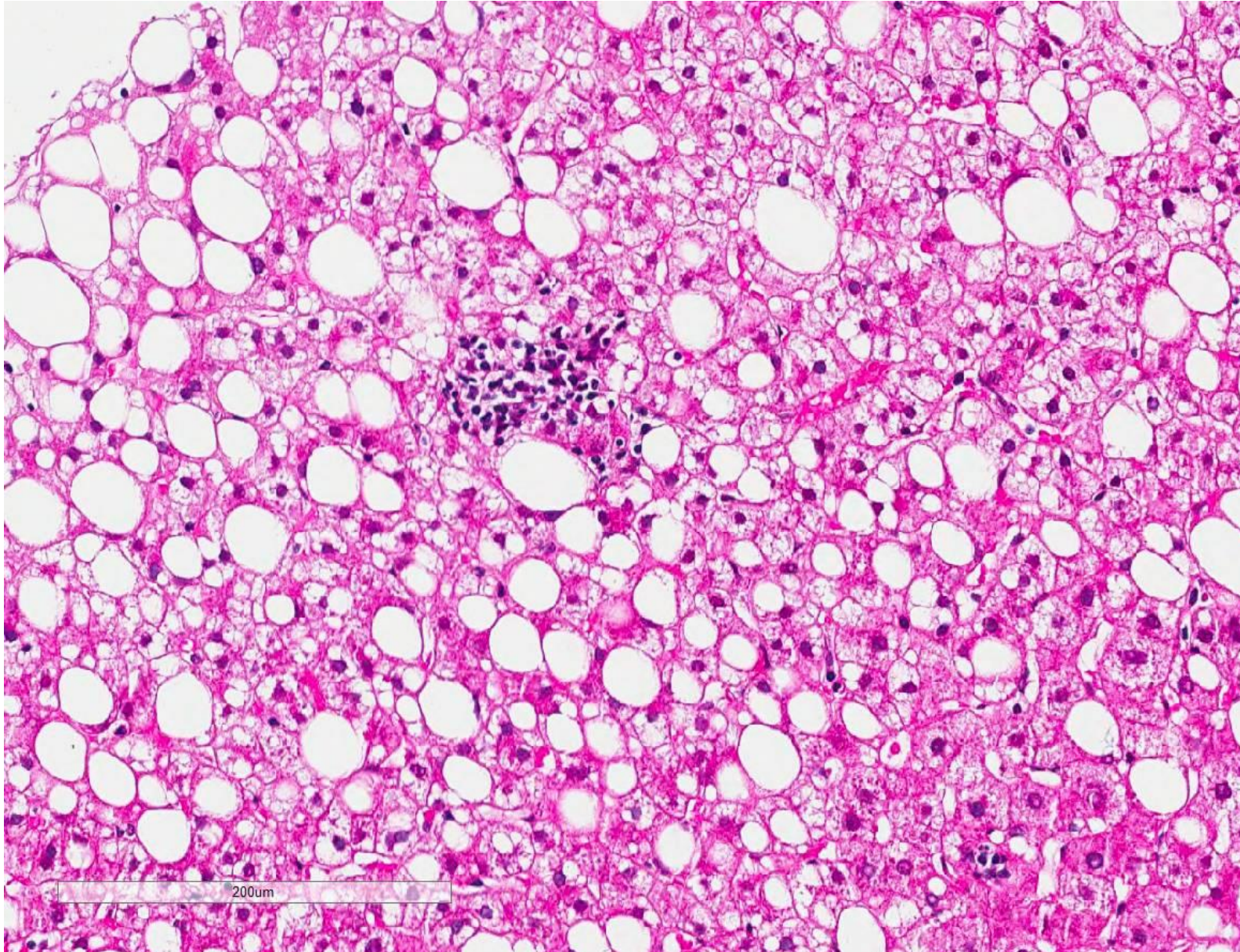
# Lobular inflammation 0



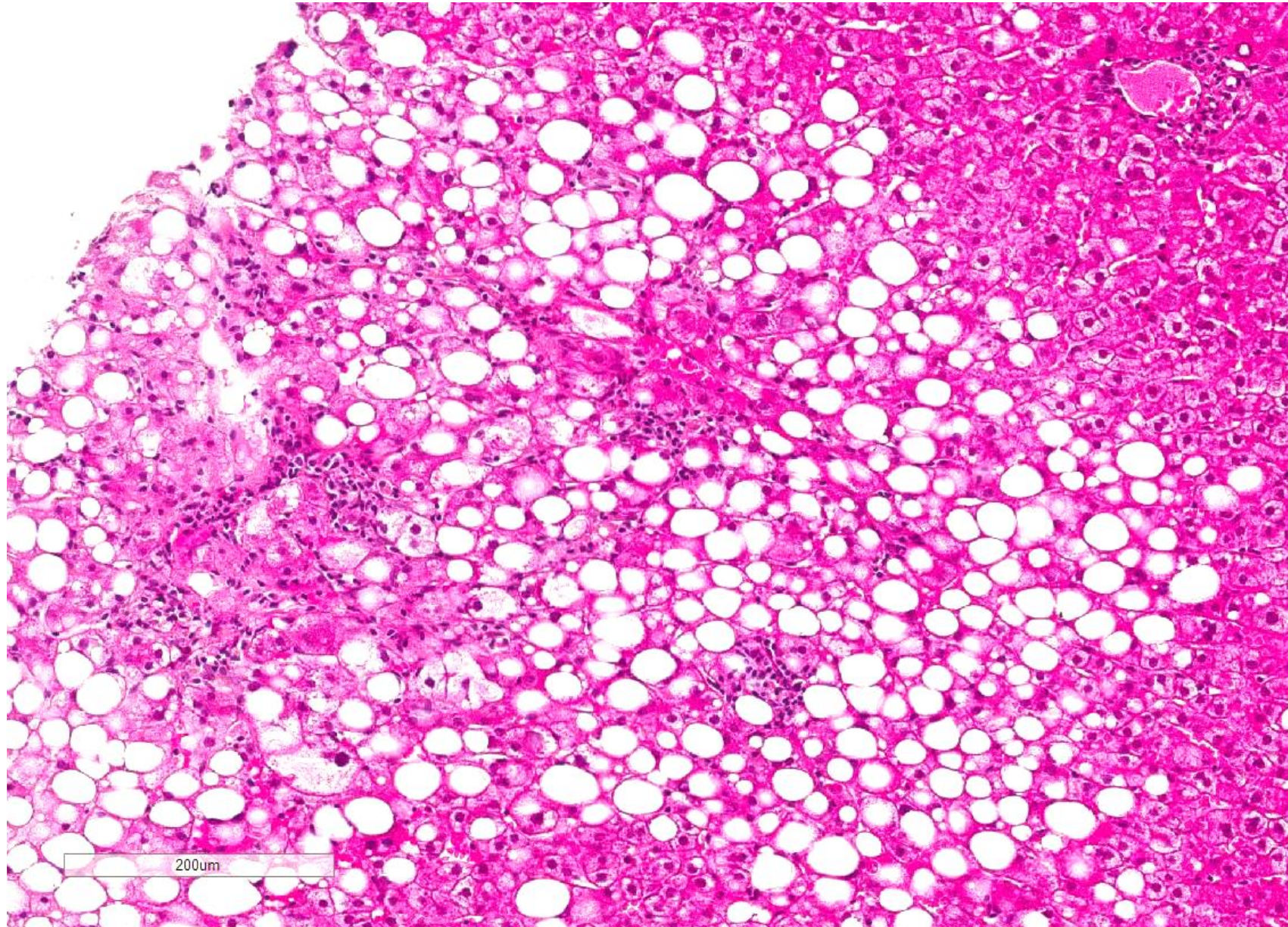
# Lobular inflammation 1 (NASH CRN & SAF)



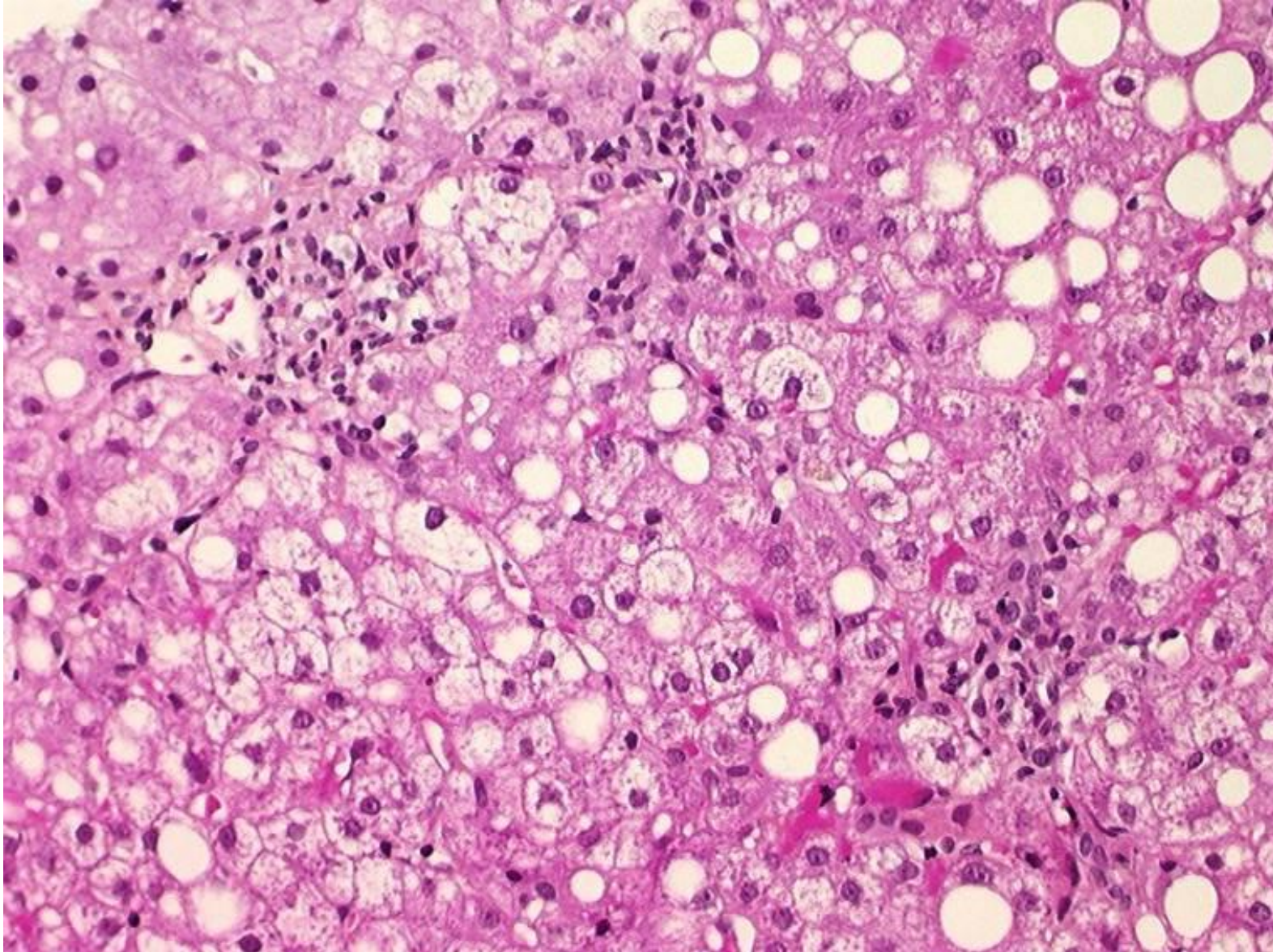
# Lobular inflammation 1 (NASH CRN & SAF)



# Lobular inflammation 2 (NASH CRN & SAF)

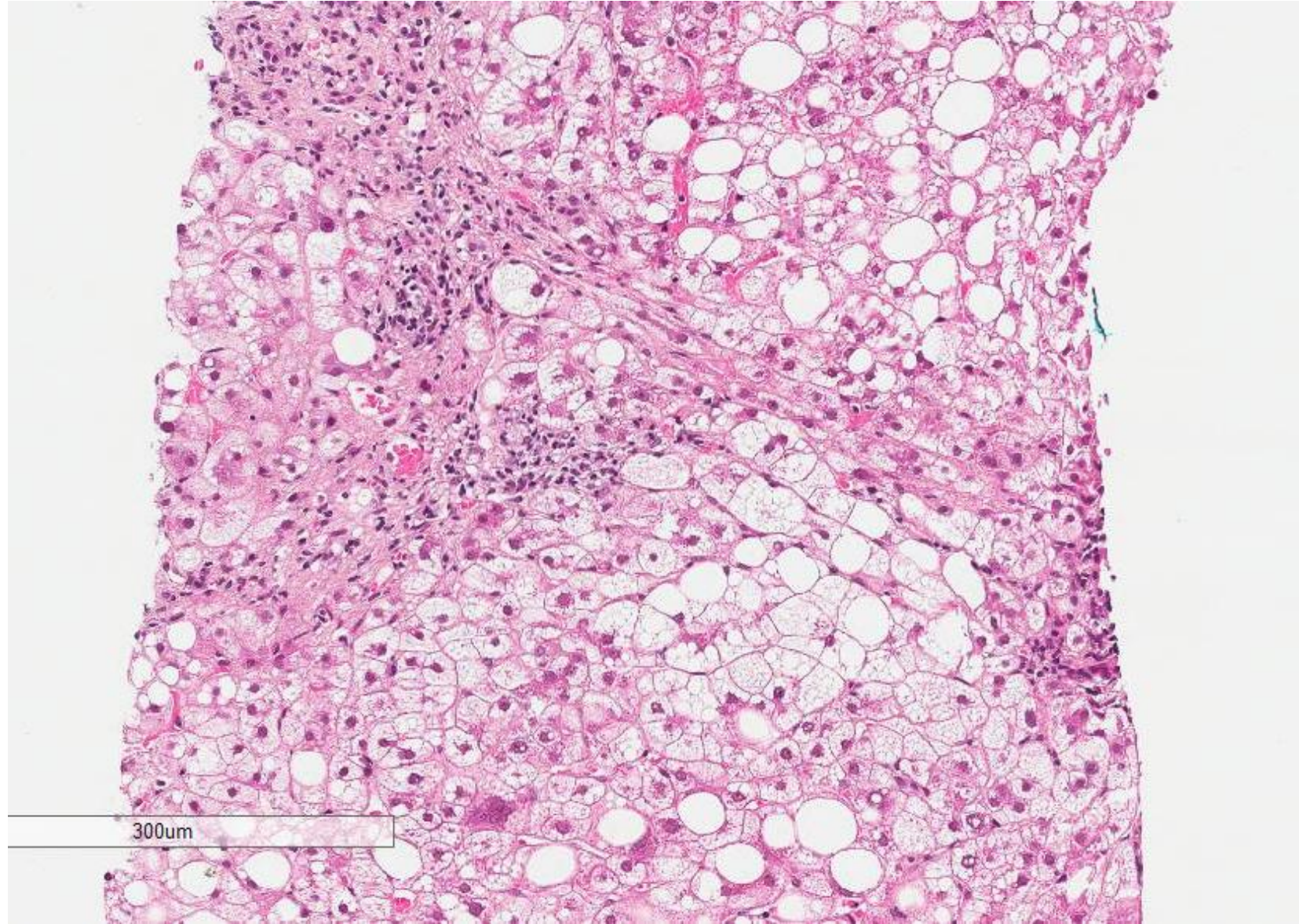


# Lobular inflammation 3 (NASH CRN) Lobular inflammation 2 (SAF)



# Lobular inflammation 3 (NASH CRN)

## Lobular inflammation 2 (SAF)



- **Portal inflammation**

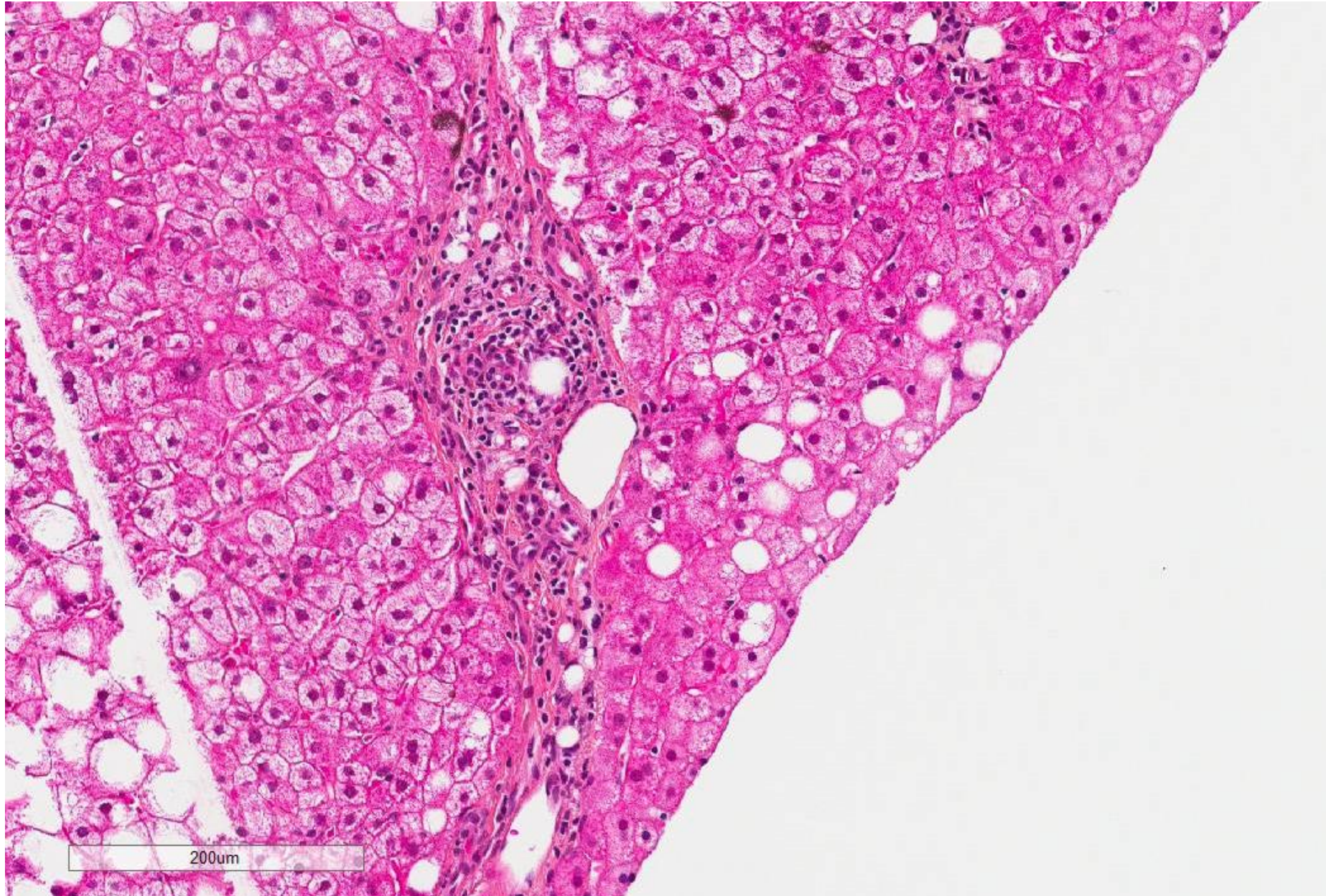
- Assess portal and/or septal inflammation
- Lymphocytes, macrophages, plasma cells...
- Assessed on hot-spots

**PORTAL INFLAMMATION**

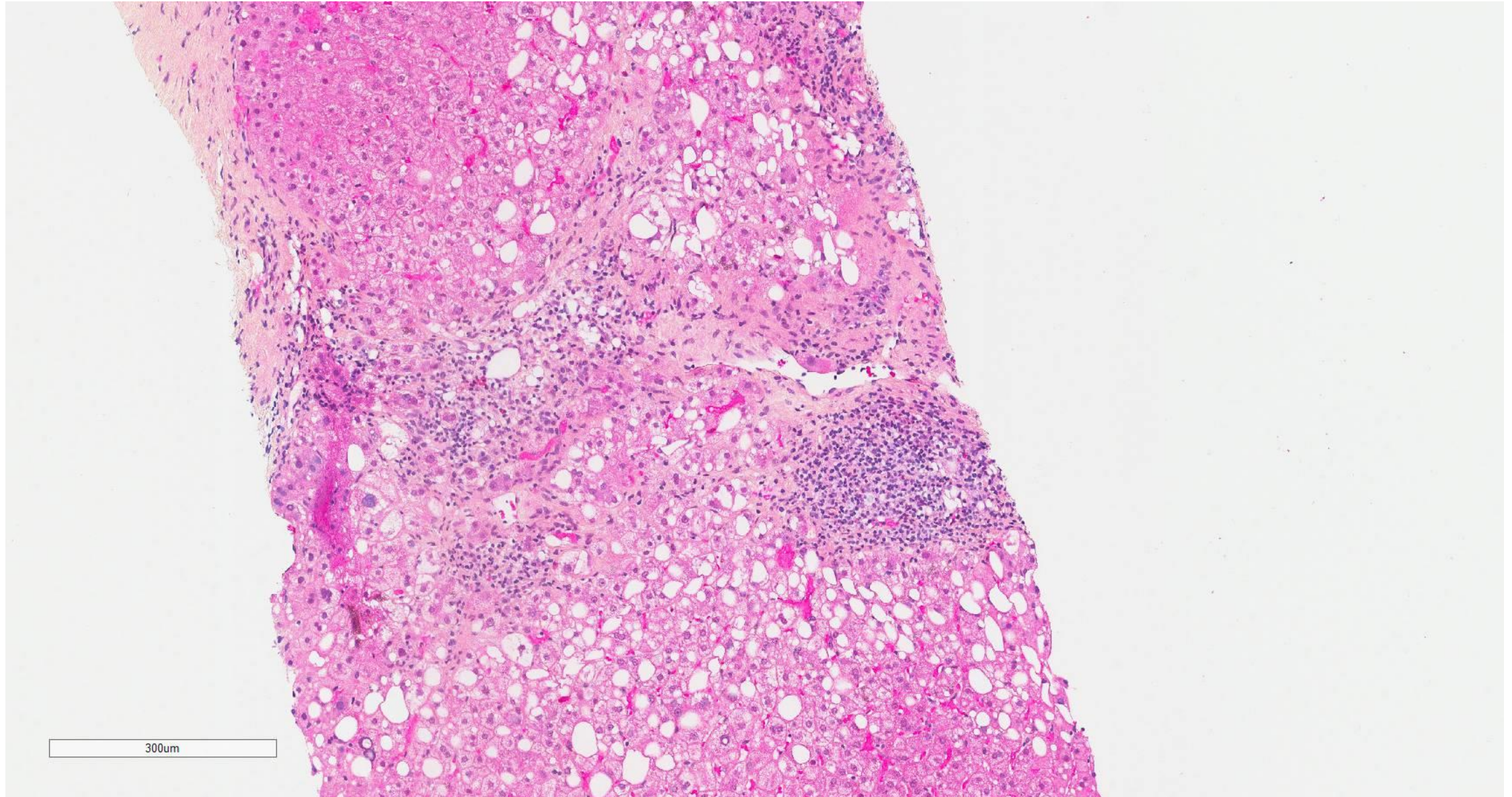
- None or Minimal
- Mild
- More than mild



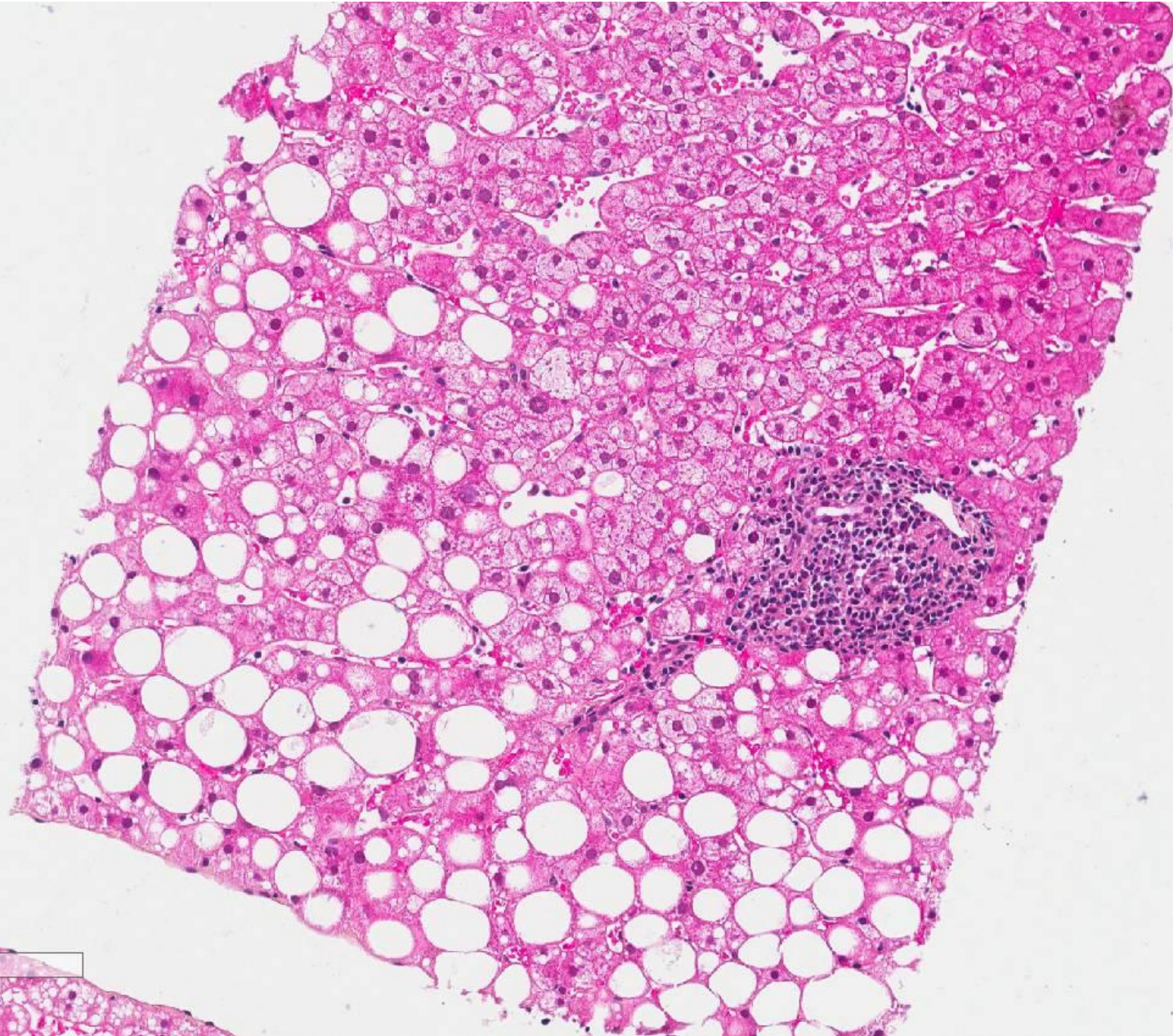
# Portal inflammation 1 (mild)



# Portal inflammation 2 (more than mild)

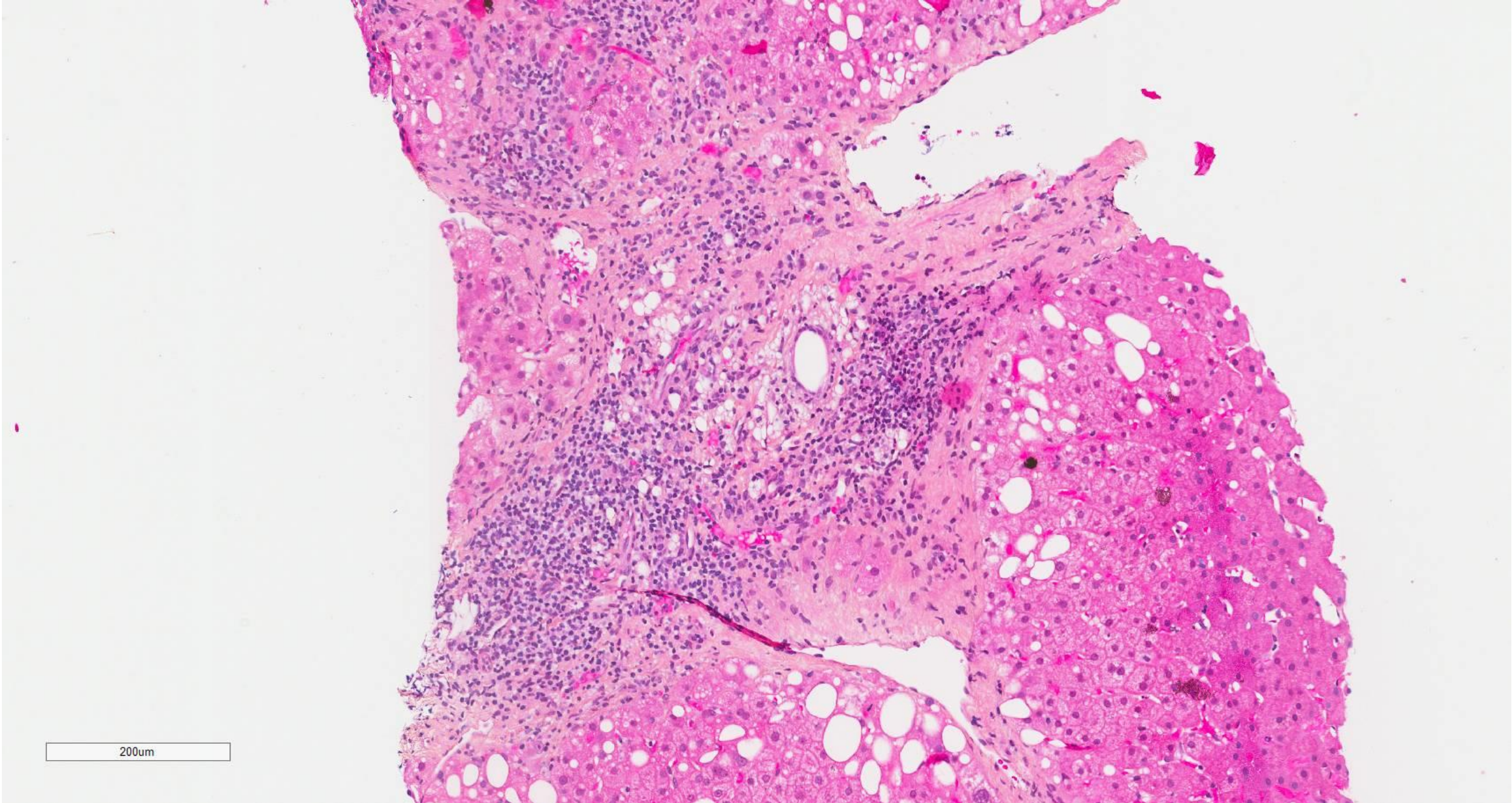


# Portal inflammation 2 (more than mild)



200um

# Portal inflammation 2 (more than mild)



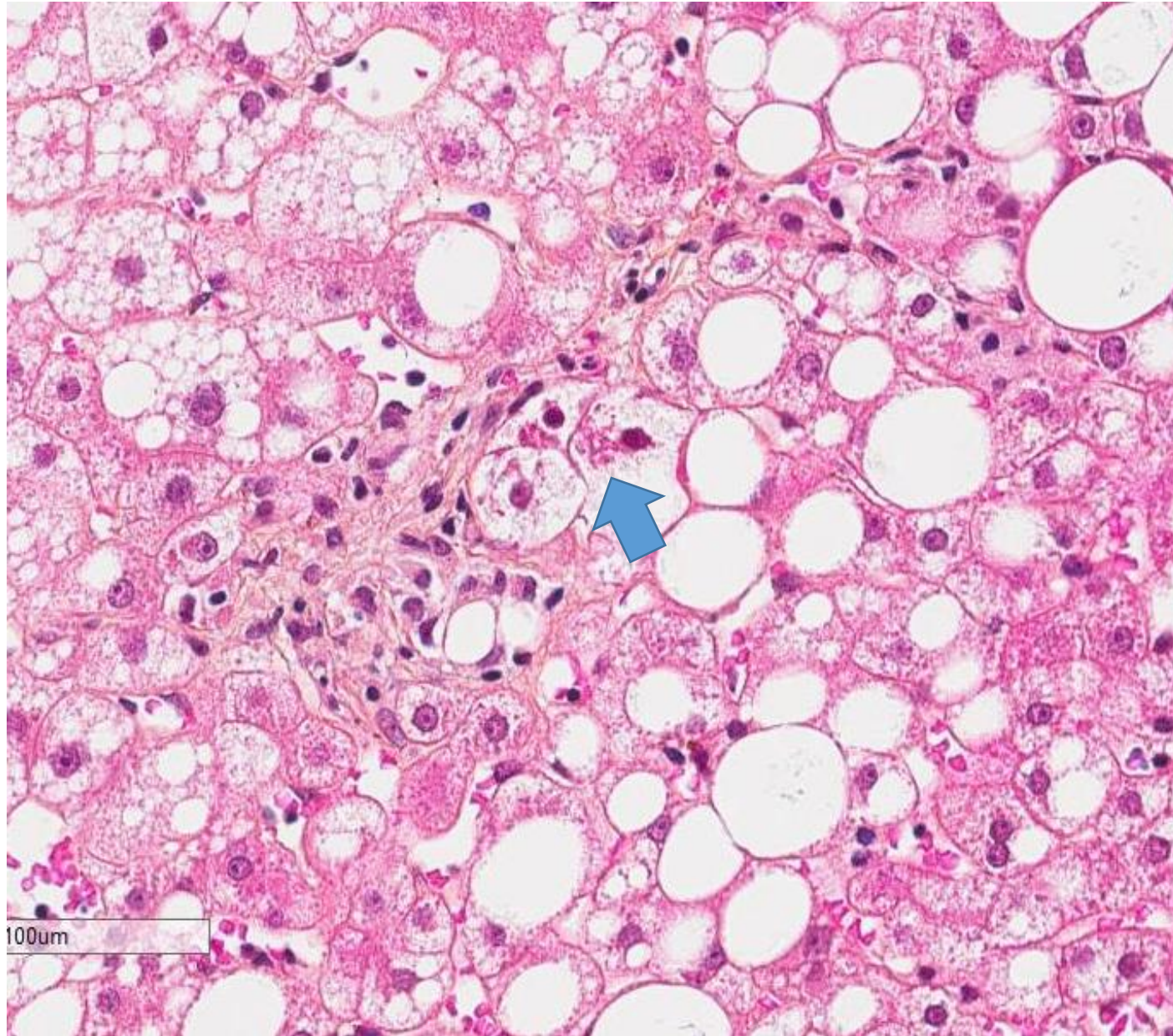
- **Mallory- Denk Bodies (MDB)**

- Few = at least 1, often not easy to recognise because they are rare or incomplete
- Many = numerous and easy to recognise (might suggest associated ALD)
- On H&E (not with immunostains)

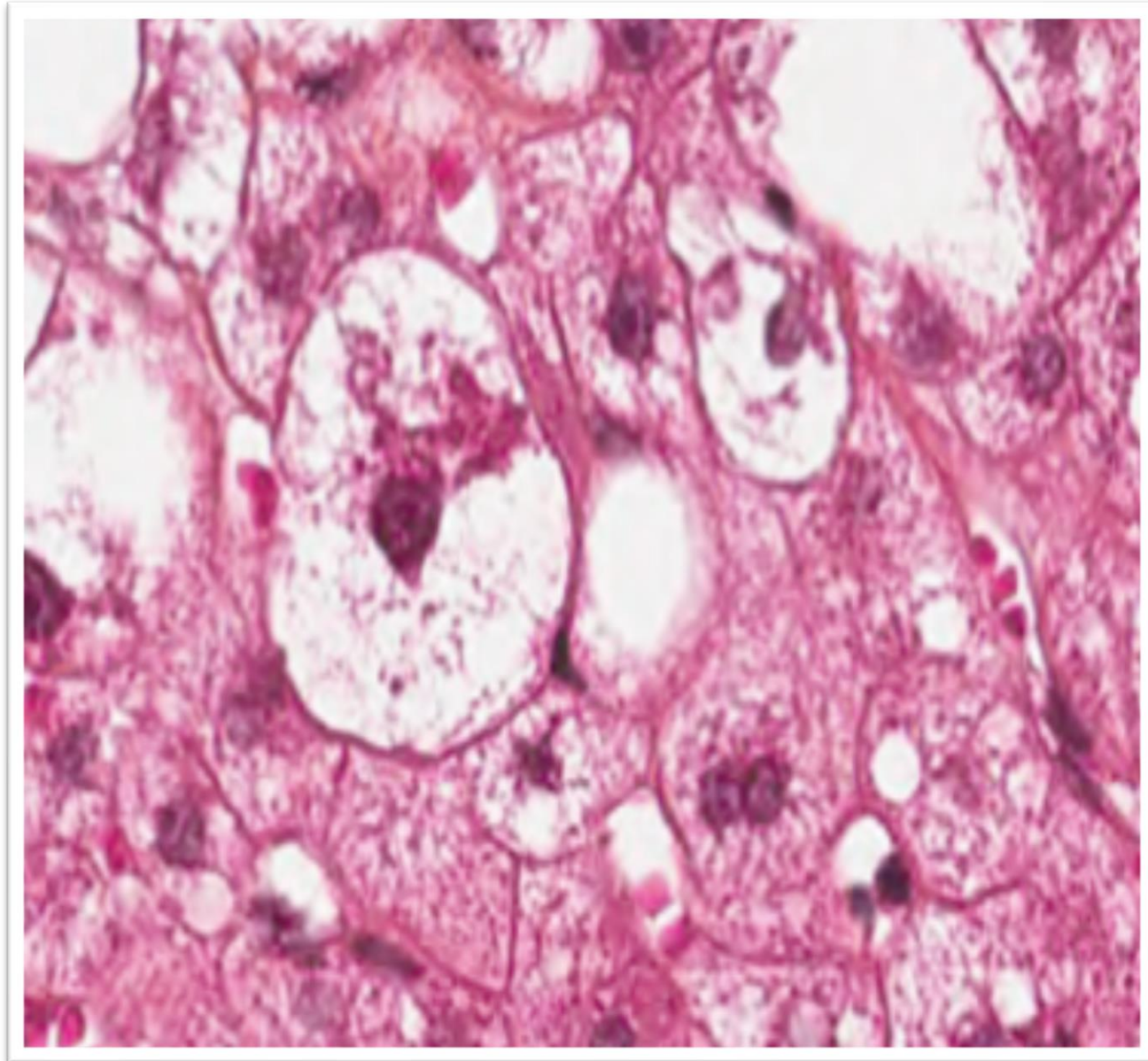
**MALLORY-DENK**

- No
- Few
- Many

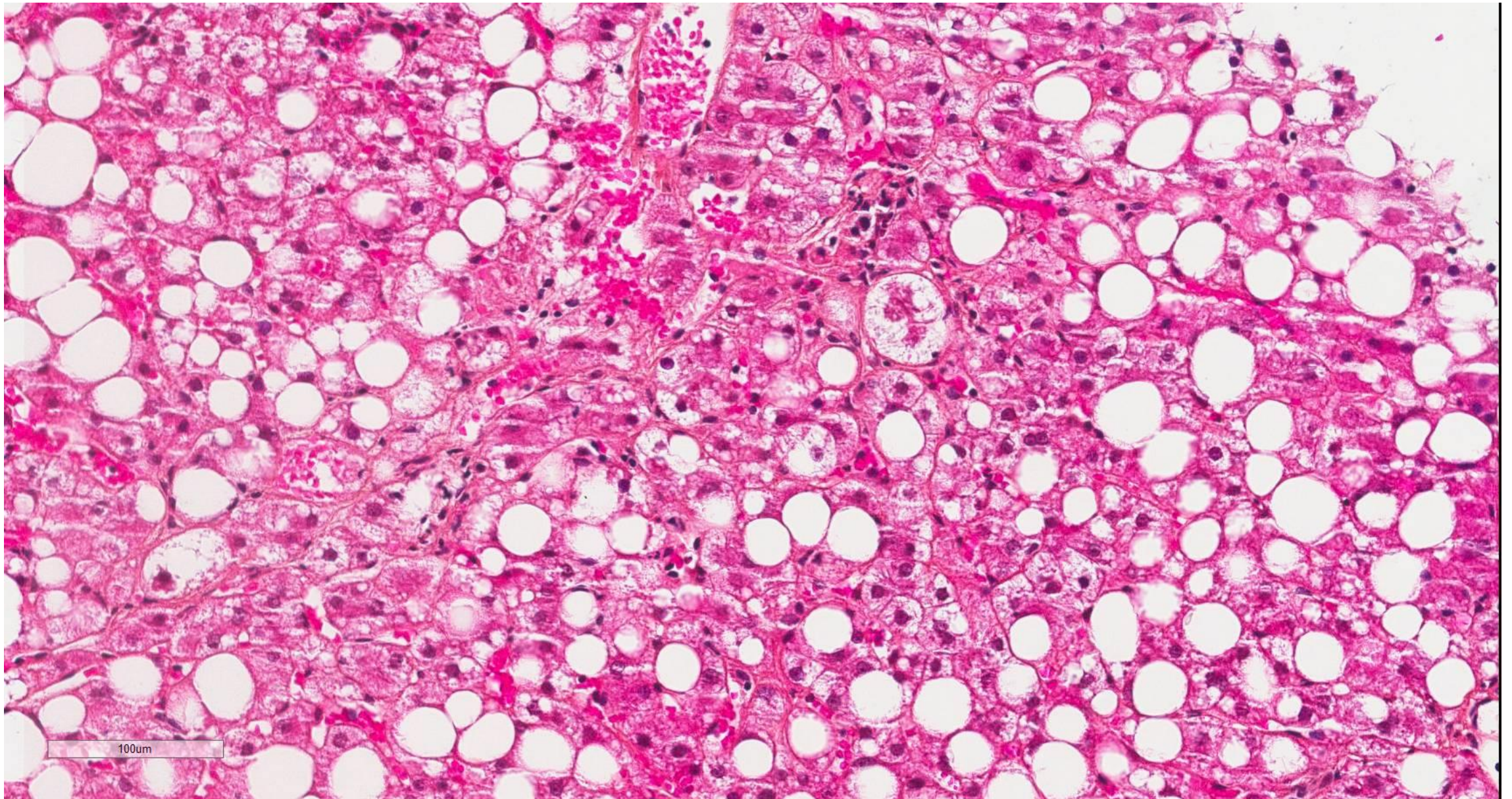
# Incomplete/immature MDB



# Fully developed MDB

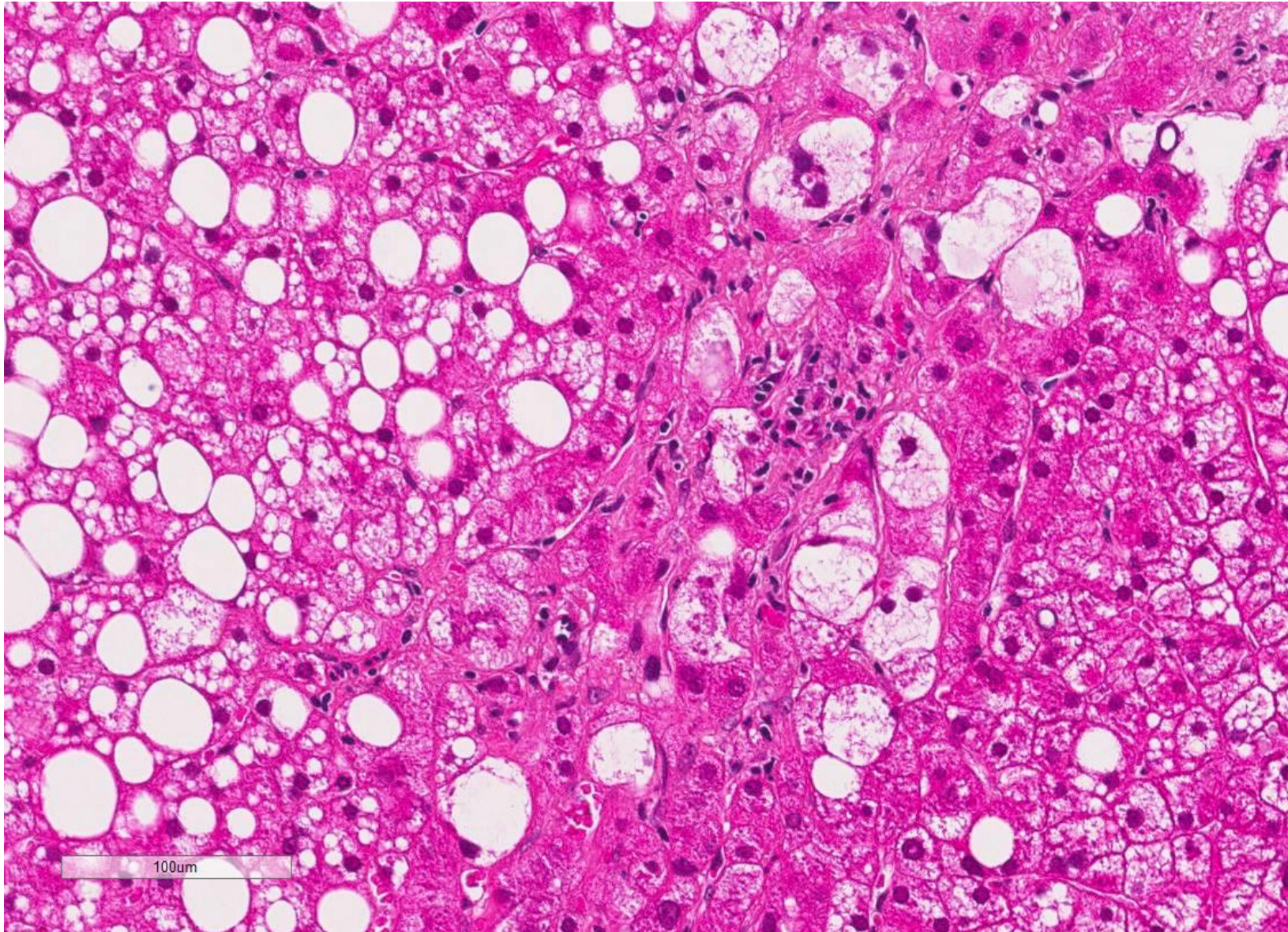


# MDB 1 (few)

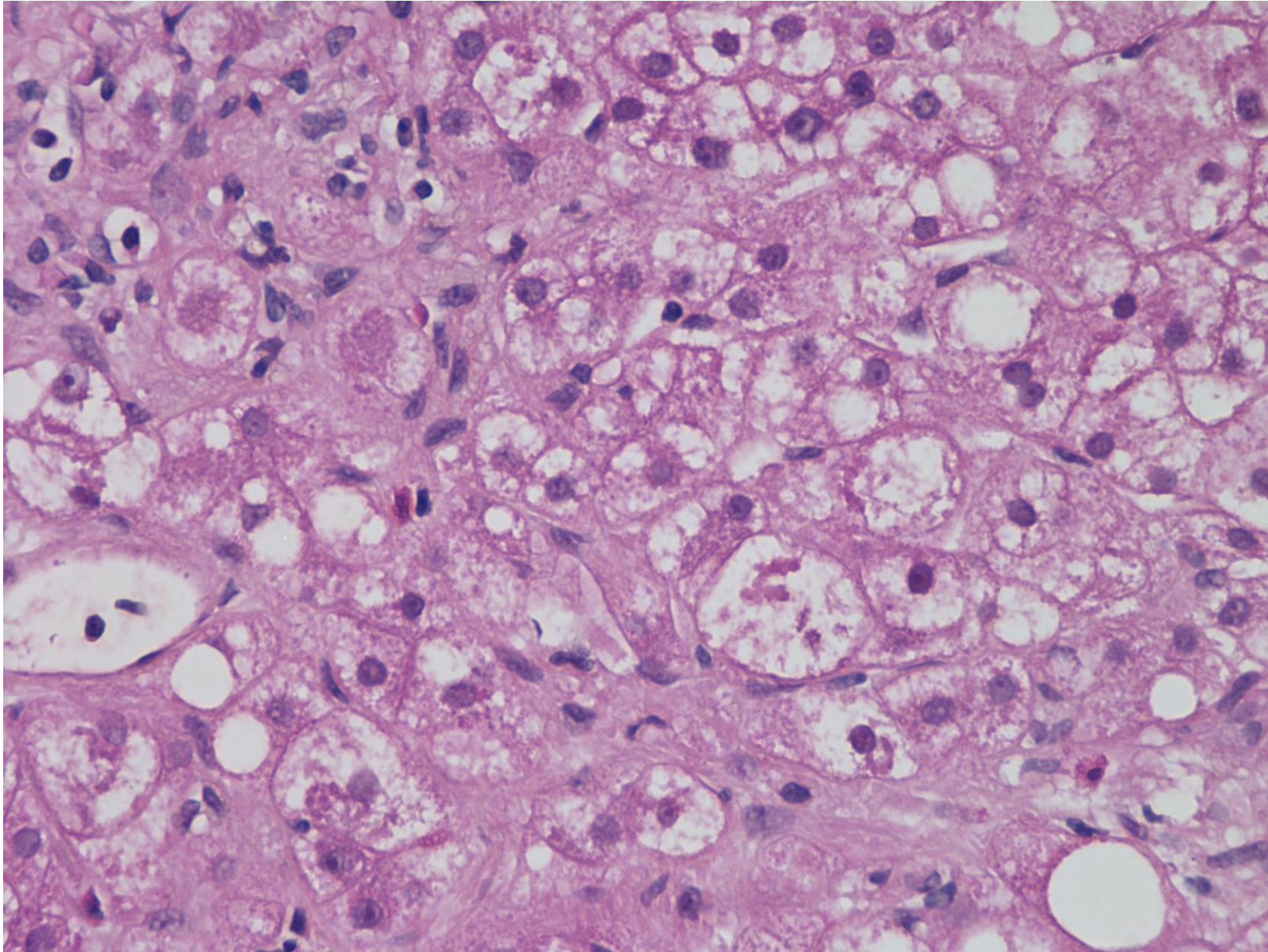




# MDB 2 (many)



# **MDB 2 (many)**



## Stage of fibrosis

- Stage according NASH-CRN (stage 0 to 4) and according to EPOS (stage 0 to 6)\*
- Ignore subcapsular area (evaluate at a distance > 5mm from the capsule)
- Fibrous septa: from one vascular structure to another or a continuous transversal fibrous bridge that crosses the whole width of the biopsy
- Fibrous septa: thin or thick. Ignore bridging collapse with residual hepatocytes between individual collagen fibres

\*Bedossa P, Arola J, Davies S, et al. The EPoS staging system is a reproducible 7-tier fibrosis score for NAFLD adapted both to glass slides and digitized image (e-slides). J Hepatol 2018; 68 (suppl 1):S553 (abstract).

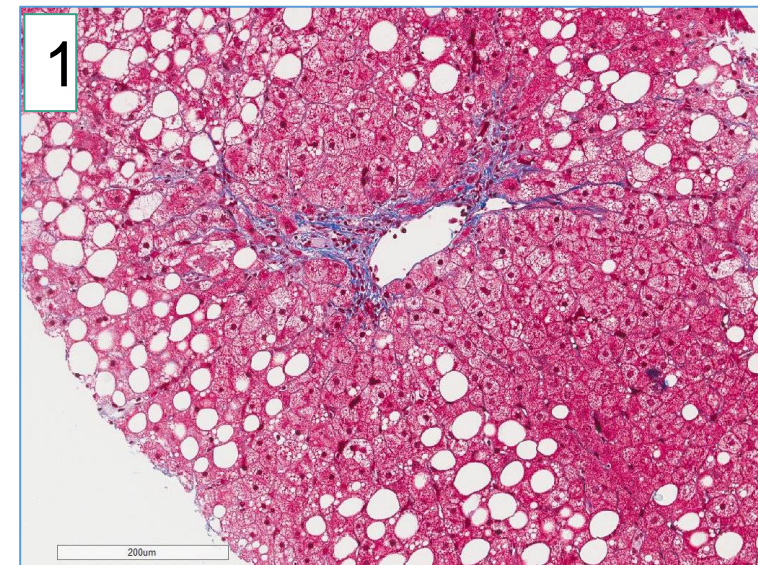
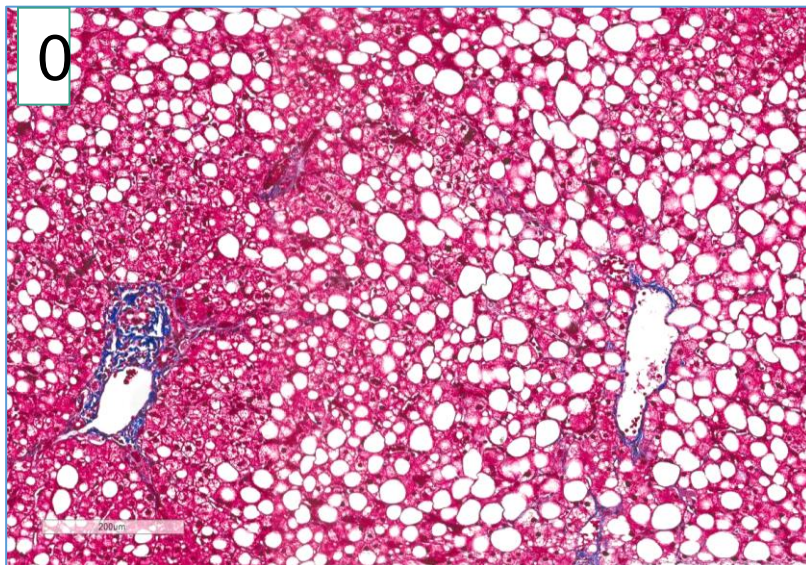
## Stage of fibrosis (NASH CRN)

- None = 0
- Perisinusoidal or periportal = 1
  - Mild, zone 3, perisinusoidal = 1A
  - Moderate, zone 3, perisinusoidal = 1B
  - Portal/periportal = 1C
- Perisinusoidal and portal/periportal = 2
- Bridging fibrosis = 3
- Cirrhosis = 4

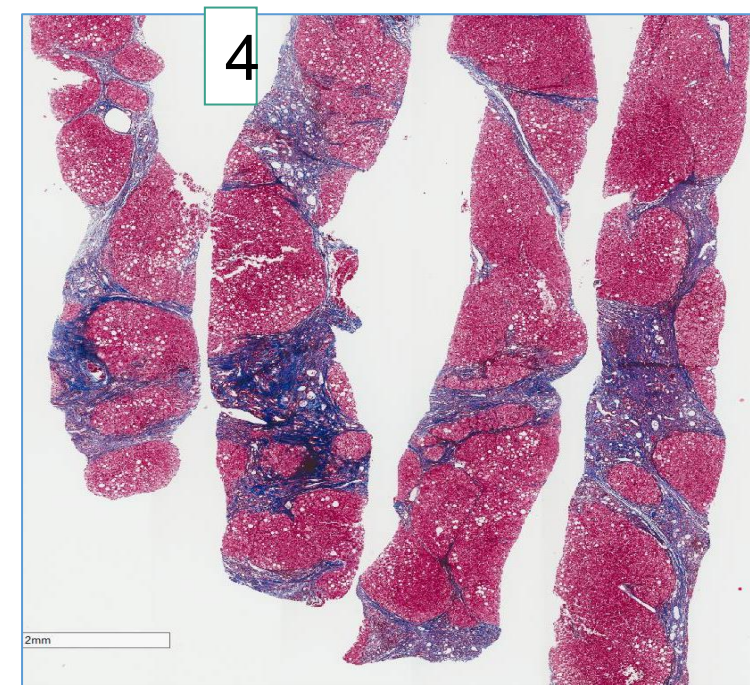
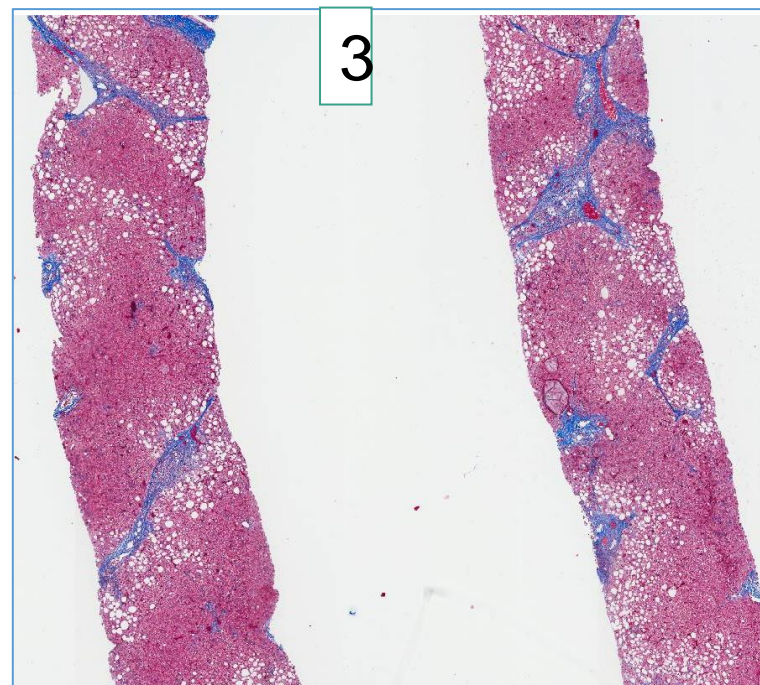
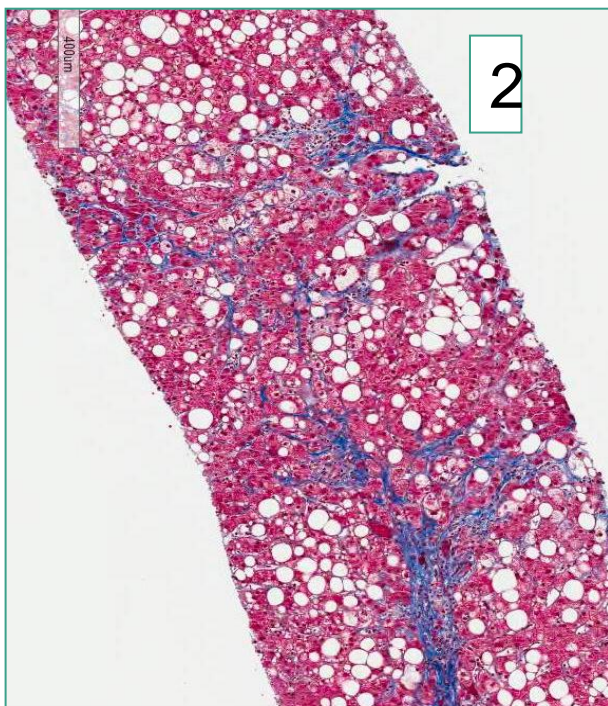
### FIBROSIS (NASH CRN)

0	<input type="checkbox"/>
1a	<input type="checkbox"/>
1b	<input type="checkbox"/>
1c	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input type="checkbox"/>
4	<input type="checkbox"/>

# Stage of fibrosis (NASH CRN)


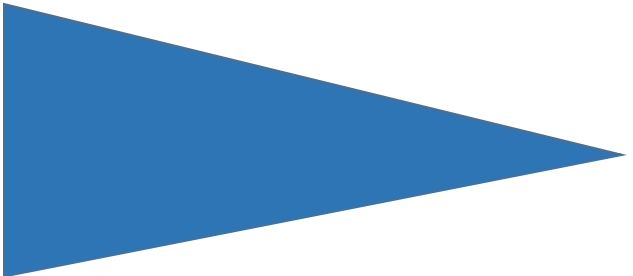

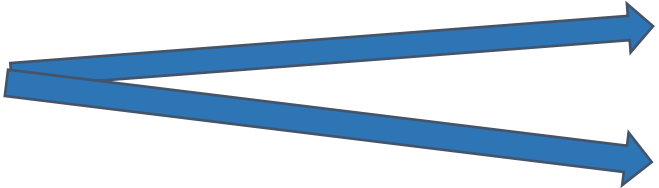
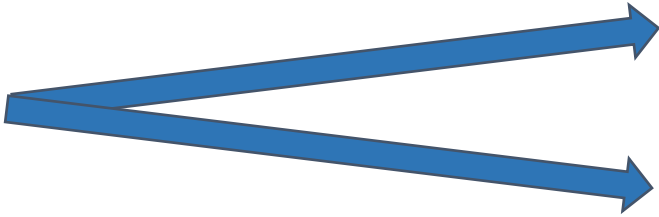


Masson trichrome



# EPOS staging of fibrosis (0-6)

FIBROSIS (EPOS)	
0	<input type="checkbox"/>
1	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input type="checkbox"/>
4	<input type="checkbox"/>
5	<input type="checkbox"/>
6a	<input type="checkbox"/>
6b	<input type="checkbox"/>
6c	<input type="checkbox"/>

NASH CRN		EPOS	Comments
1a 1b 1c		1	<b>Lumping together stages 1a, 1b, 1c</b>
2		2	<b>Changing definition :</b> Fibrosis in at least 2 areas within the lobule/acinus (centrilobular /zone 3, periportal/zone 1, intralobular/zone 2 fibrosis) without fibrous septa
3		3	<b>Increase granularity:</b> Few septa (no more than 2/10 mm length of biopsy)
		4	Many septa (> 2/10 mm) without nodule formation
4		5	<b>Increase granularity:</b> Many septa with rare nodules (incomplete cirrhosis)
		6	Cirrhosis with many nodules

# EPOS staging of fibrosis (0-6)

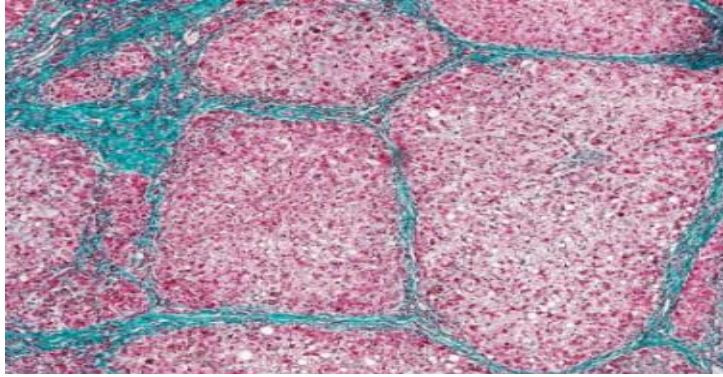


- **Stage 0: none or scant fibrosis** (perivenular and/or portal)  
Mild portal fibrosis without periportal expansion OR scant perivenular fibrosis around up to 2 terminal hepatic venules with only delicate sinusoidal fibrosis but without pericellular fibrosis
- **Stage 1: Mild fibrosis.** Perivenular and pericellular fibrosis (up to 5 hepatocytes, fibrosis all around hepatocyte) and/or sinusoidal (linear) fibrosis involving more than 2 terminal hepatic venules OR Periportal fibrosis (collagen fibers extending to the periportal parenchyma in at least 1 portal tract)
- **Stage 2: Moderate fibrosis.** Sinusoidal and/or pericellular fibrosis extending from the portal tract and/or perivenular zone to the midzone without complete septa
- **Stage 3: Occasional bridging/septal fibrosis.** At least 1 complete fibrous septum and no more than 2 septa /10mm biopsy length (a septum is a fibrous band that transverses the biopsy or links 2 vascular structures).  
Avoid subcapsular area (evaluate at a distance > 0.5 cm from the capsule)
- **Stage 4: Advanced bridging/septal fibrosis.** More than 2 fibrous septa /10mm, no nodule
- **Stage 5 : Incomplete cirrhosis.** Septal fibrosis with isolated nodule/s (completely circumscribed by fibrosis)  
Avoid subcapsular area (evaluate at a distance > 0.5 cm from the capsule)
- **Stage 6 : cirrhosis** (6a – 6b – 6c). According to the Laennec staging system (see next slide)

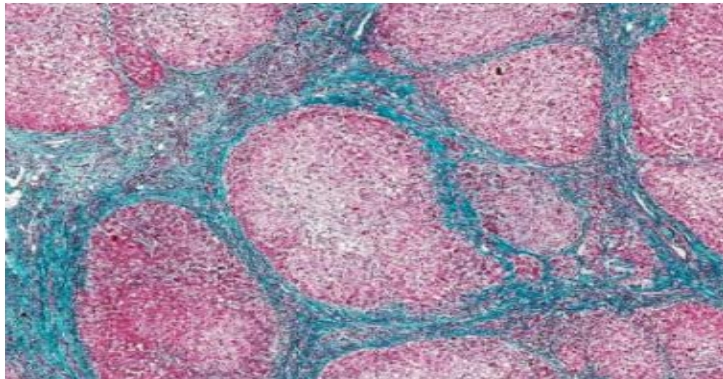


# Substaging of cirrhosis (4 a, b, c / Laennec staging system)\*

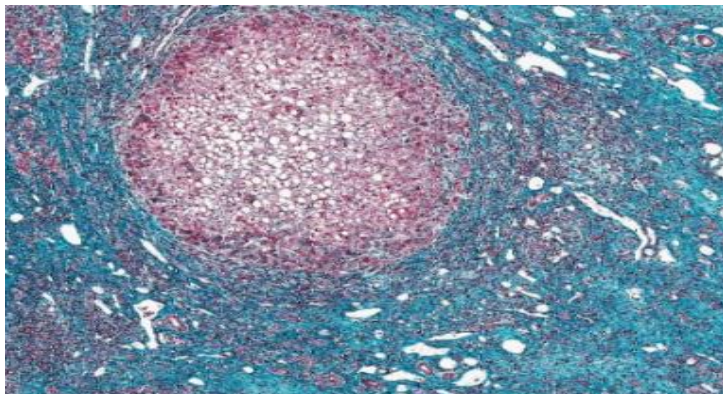
Based on the thickness of fibrous septa and width of hepatocyte nodules



- 4a** Marked septation with rounded contours or visible nodules  
Most septa are thin (one broad septum allowed)



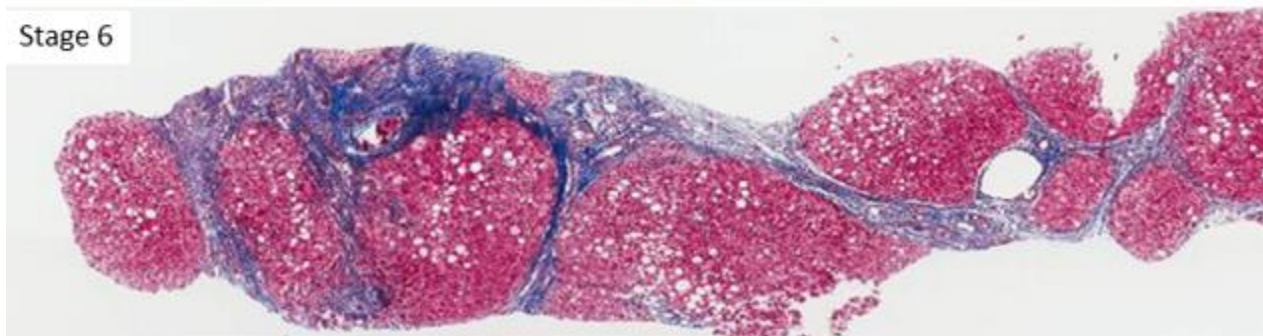
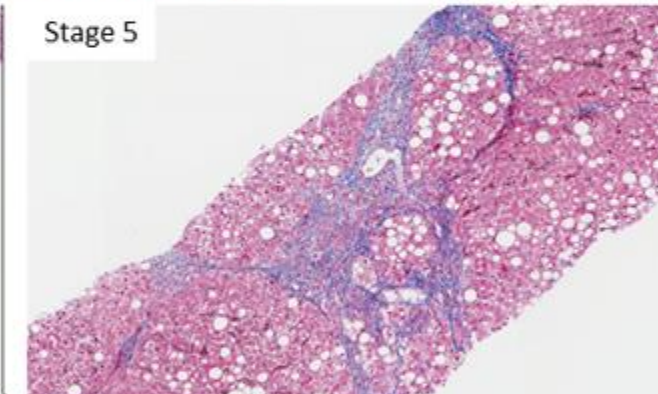
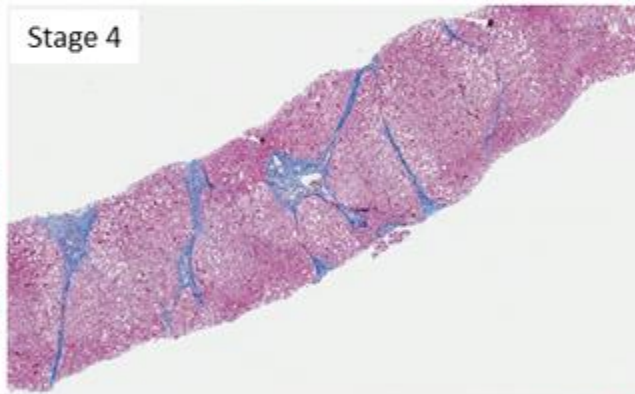
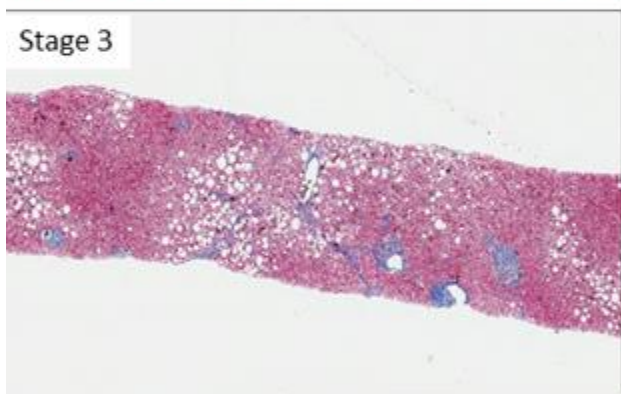
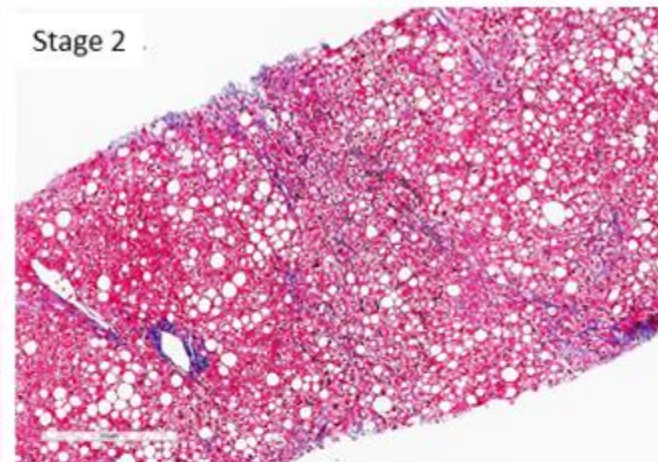
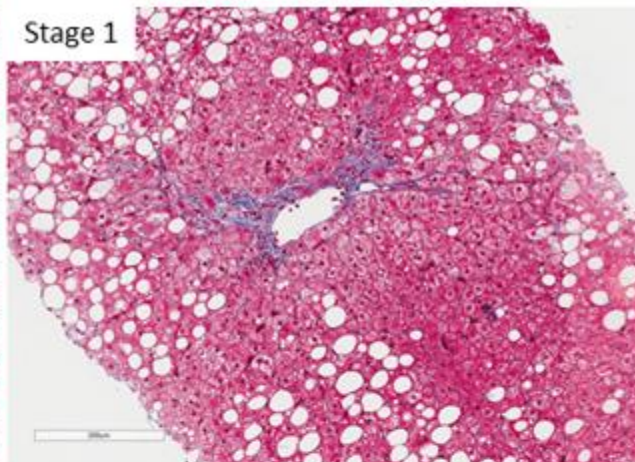
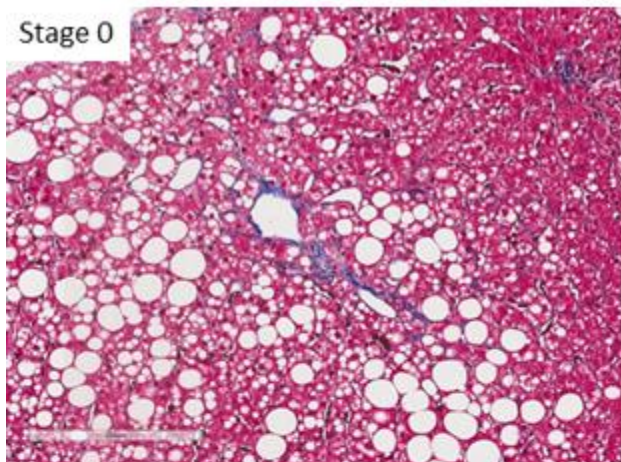
- 4b** At least two broad septa, but no very broad septa and  
less than half of biopsy length composed of minute nodules



- 4c** At least one very broad septum or more than half of biopsy length  
composed of minute nodules (micronodular cirrhosis)

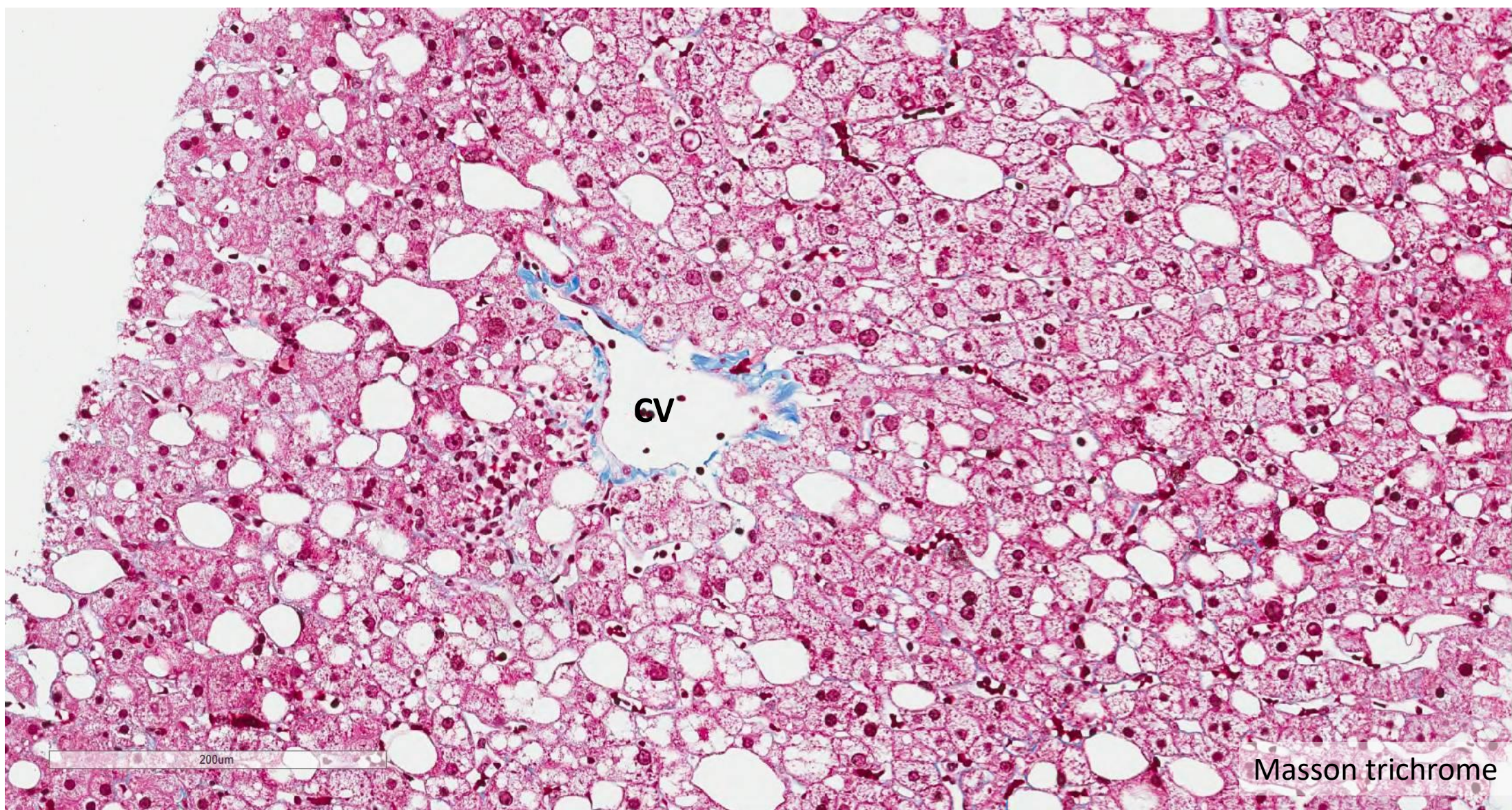
\*Wanless IR, Sweeney G, Dhillon AP, Guido M, Piga A, Galanello R, et al. Lack of progressive hepatic fibrosis during long-term therapy with deferiprone in subjects with transfusion-dependent beta-thalassemia. *Blood* 2002;100:1566–1569.

# EPOS staging of fibrosis (0-6)

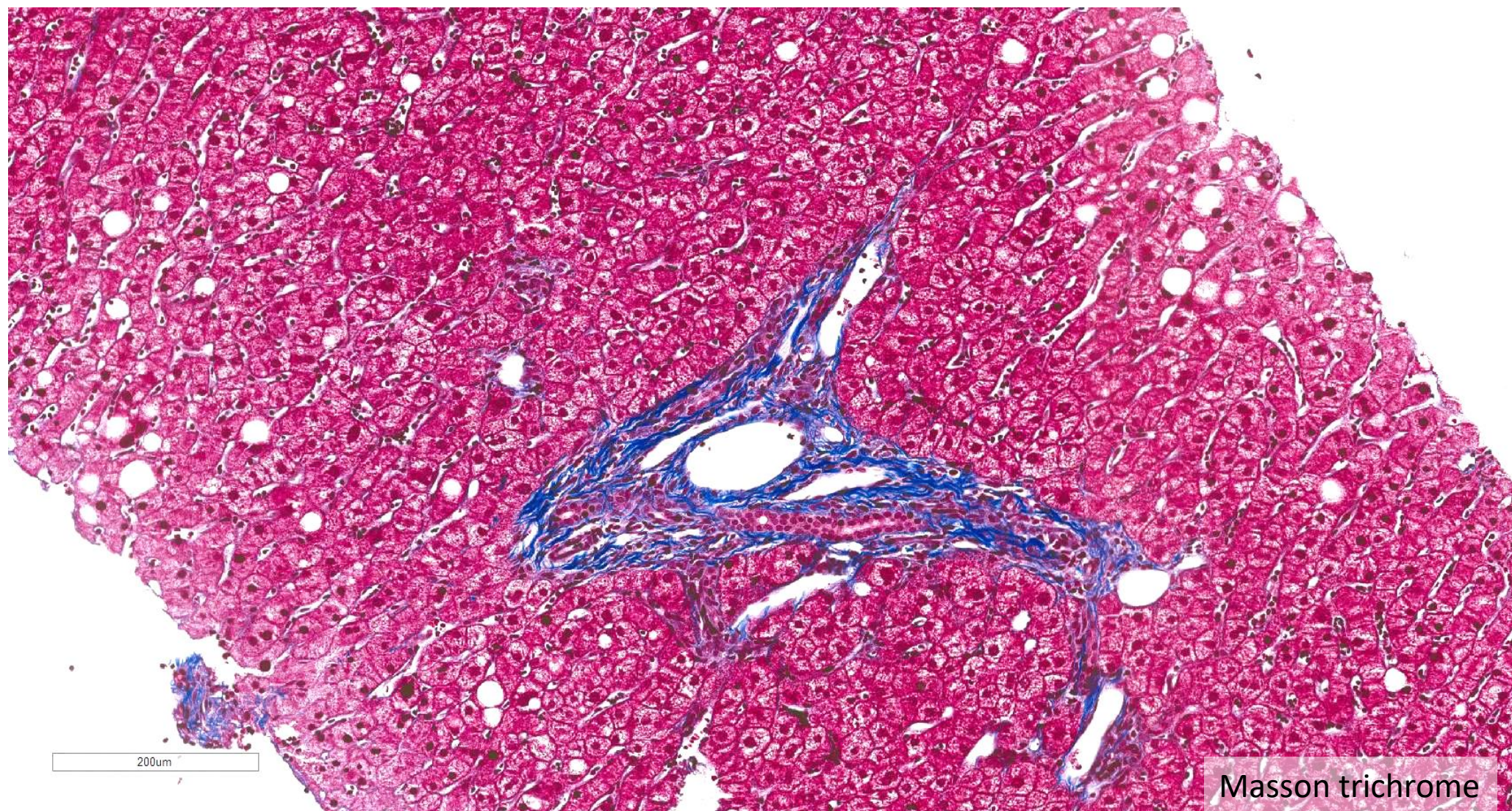


Masson trichrome

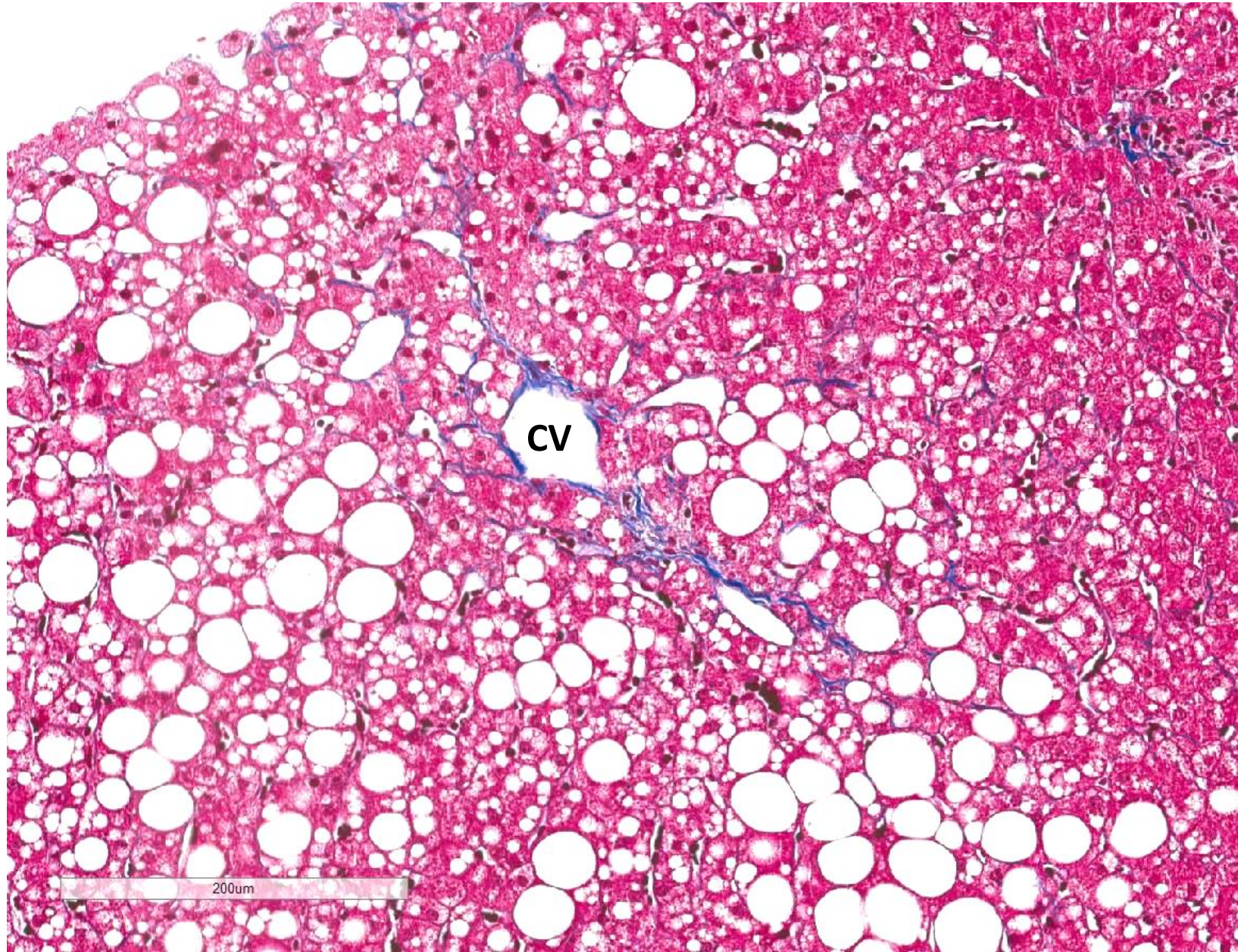
# Stage 0 (NASH CRN and EPOS)



# Stage 0 (tangential section of portal tract)



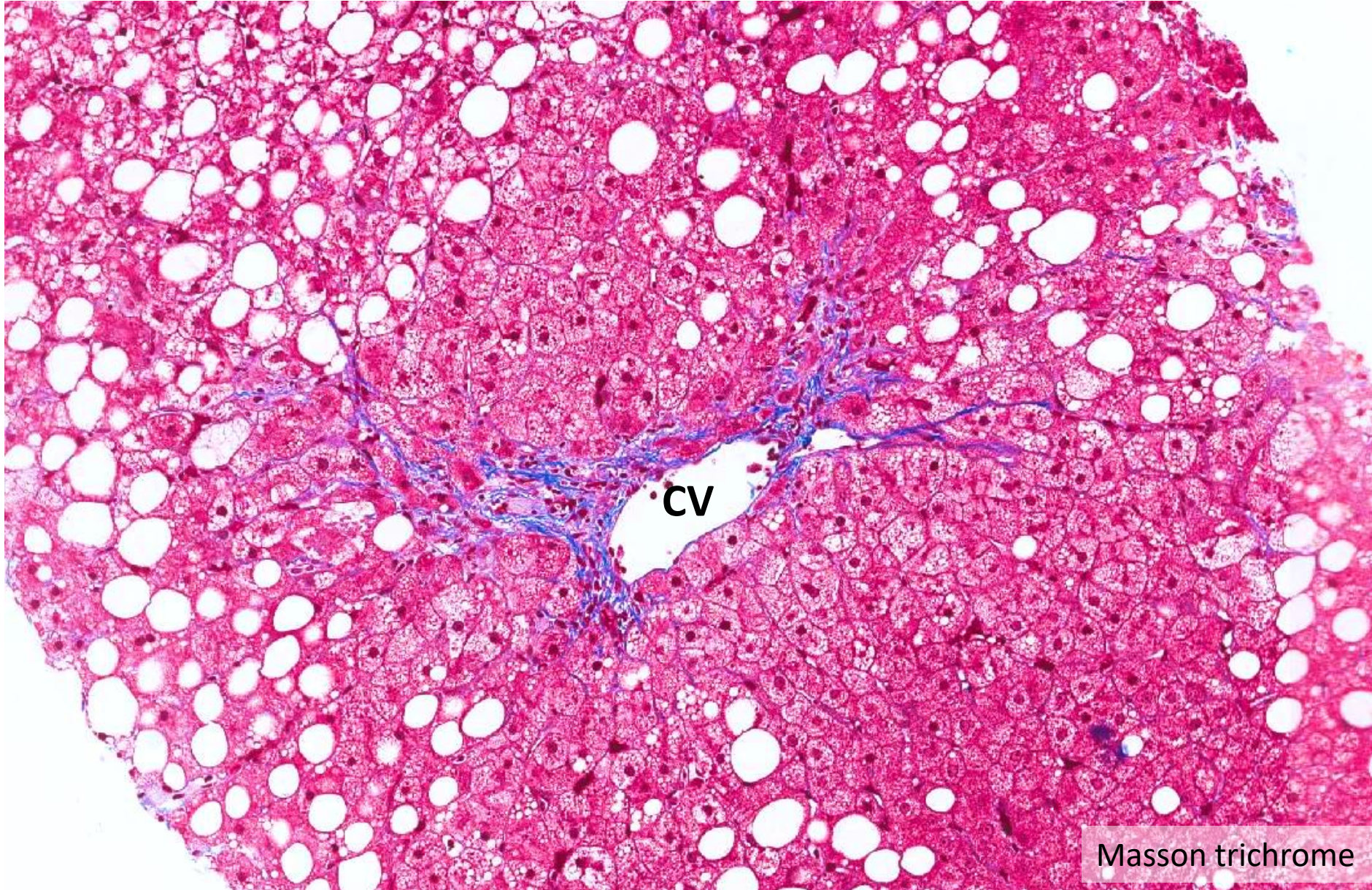
# Stage 1a (NASH CRN), Stage 0 (EPOS)



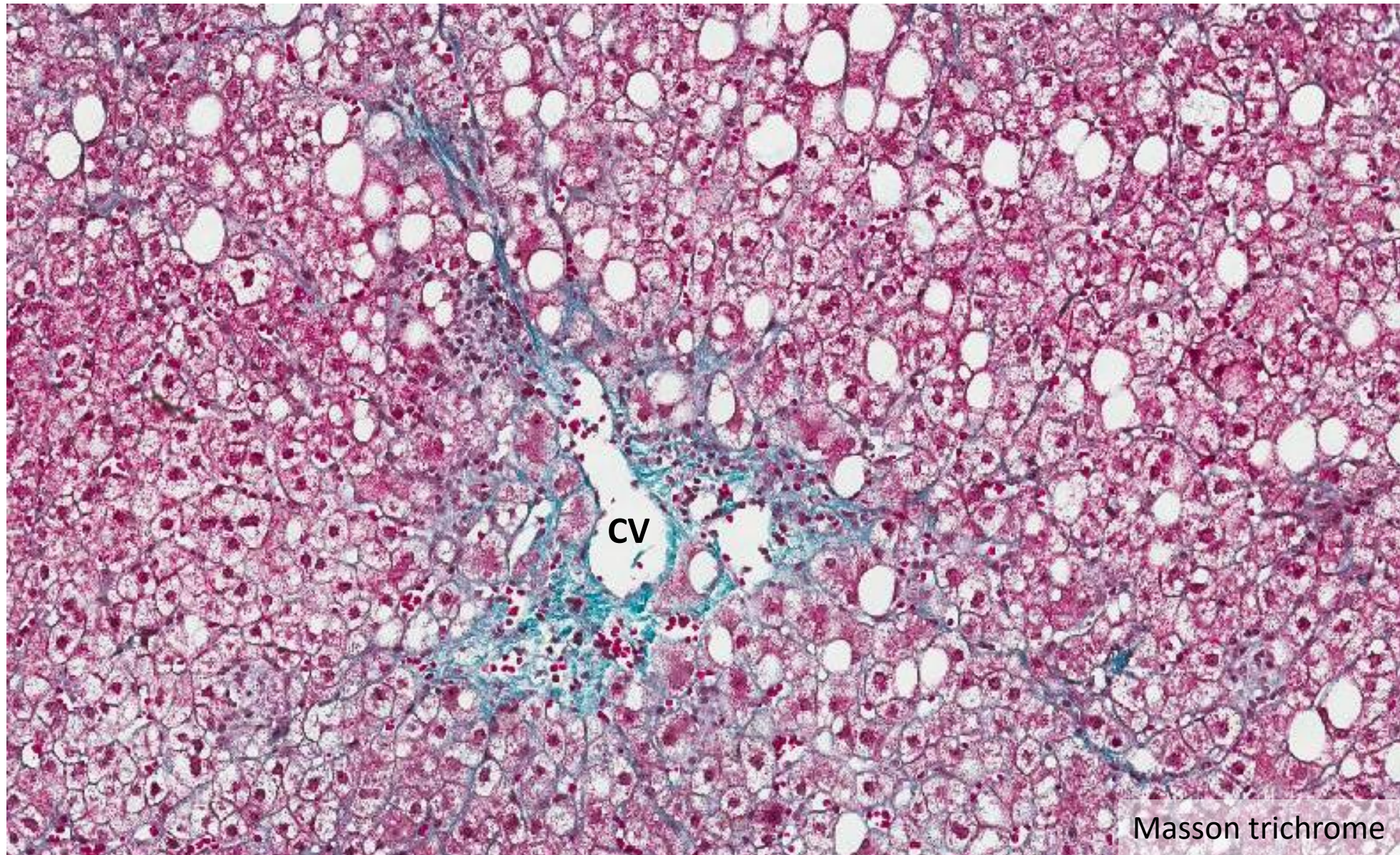
CV: central venule

Masson trichrome

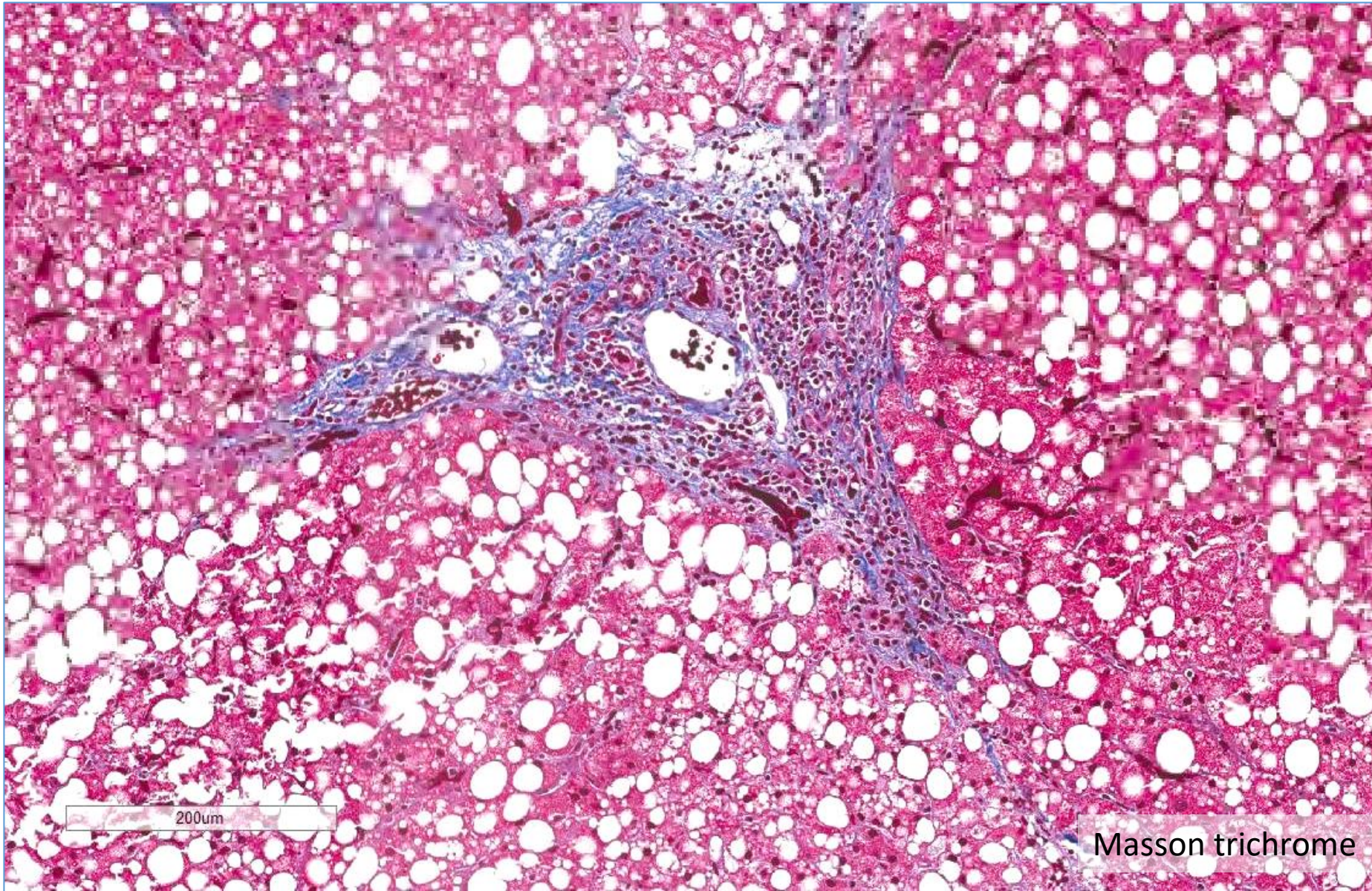
# Stage 1b (NASH CRN), Stage 1 (EPOS)



# Stage 1b (NASH CRN), Stage 1 (EPOS)

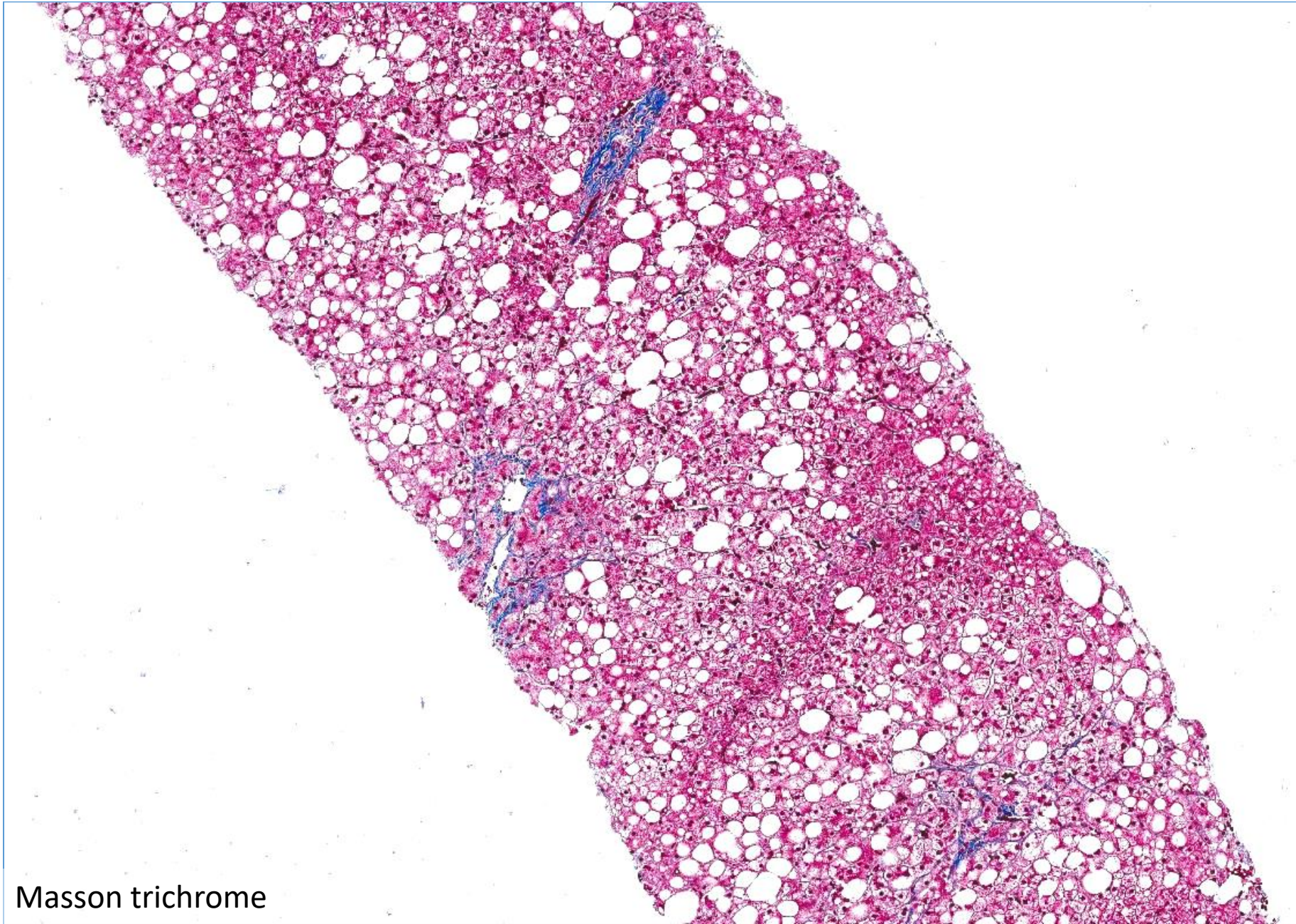


# Stage 1c (NASH CRN), Stage 1 (EPOS)



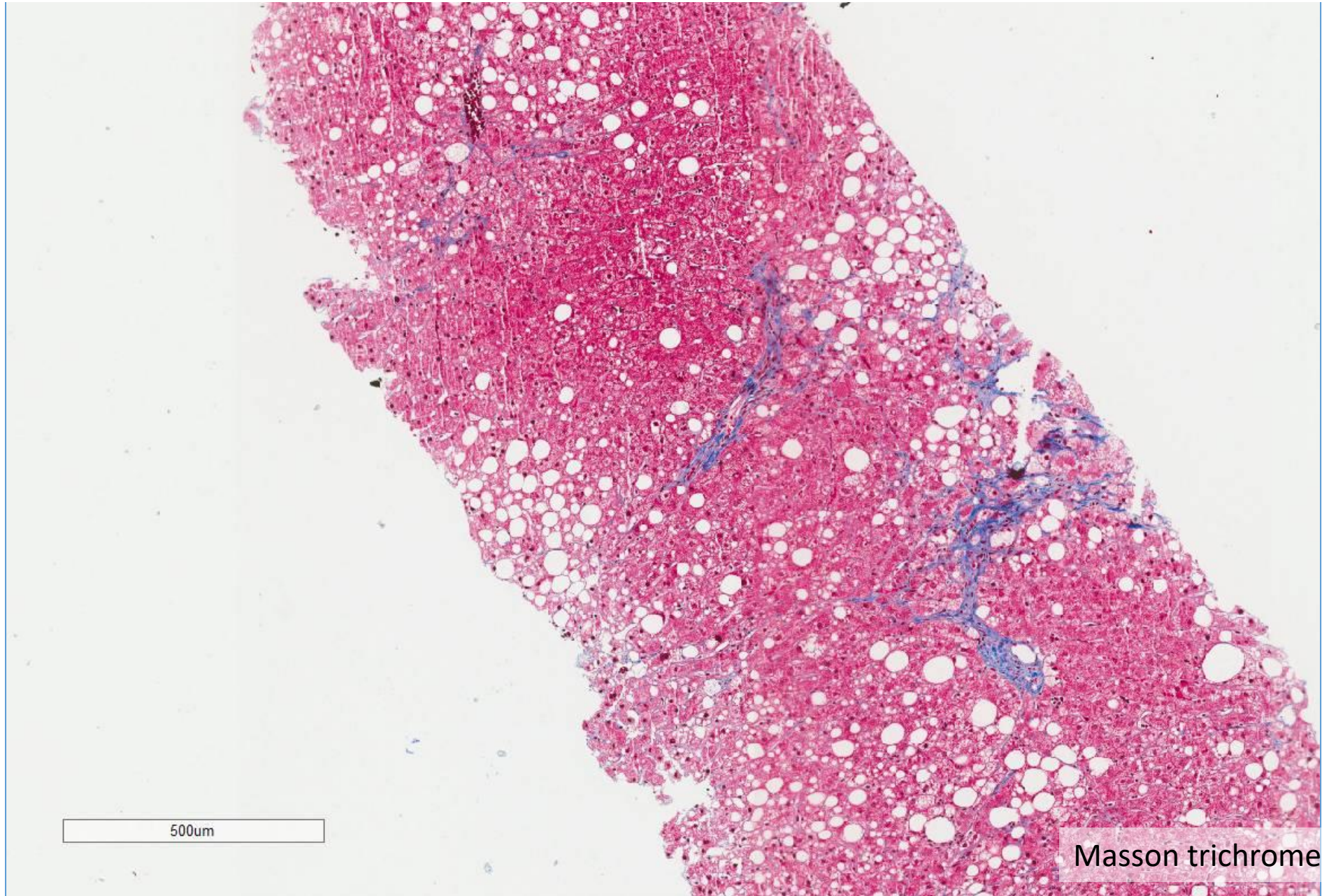


# Stage 2 (NASH CRN and EPOS)



Masson trichrome

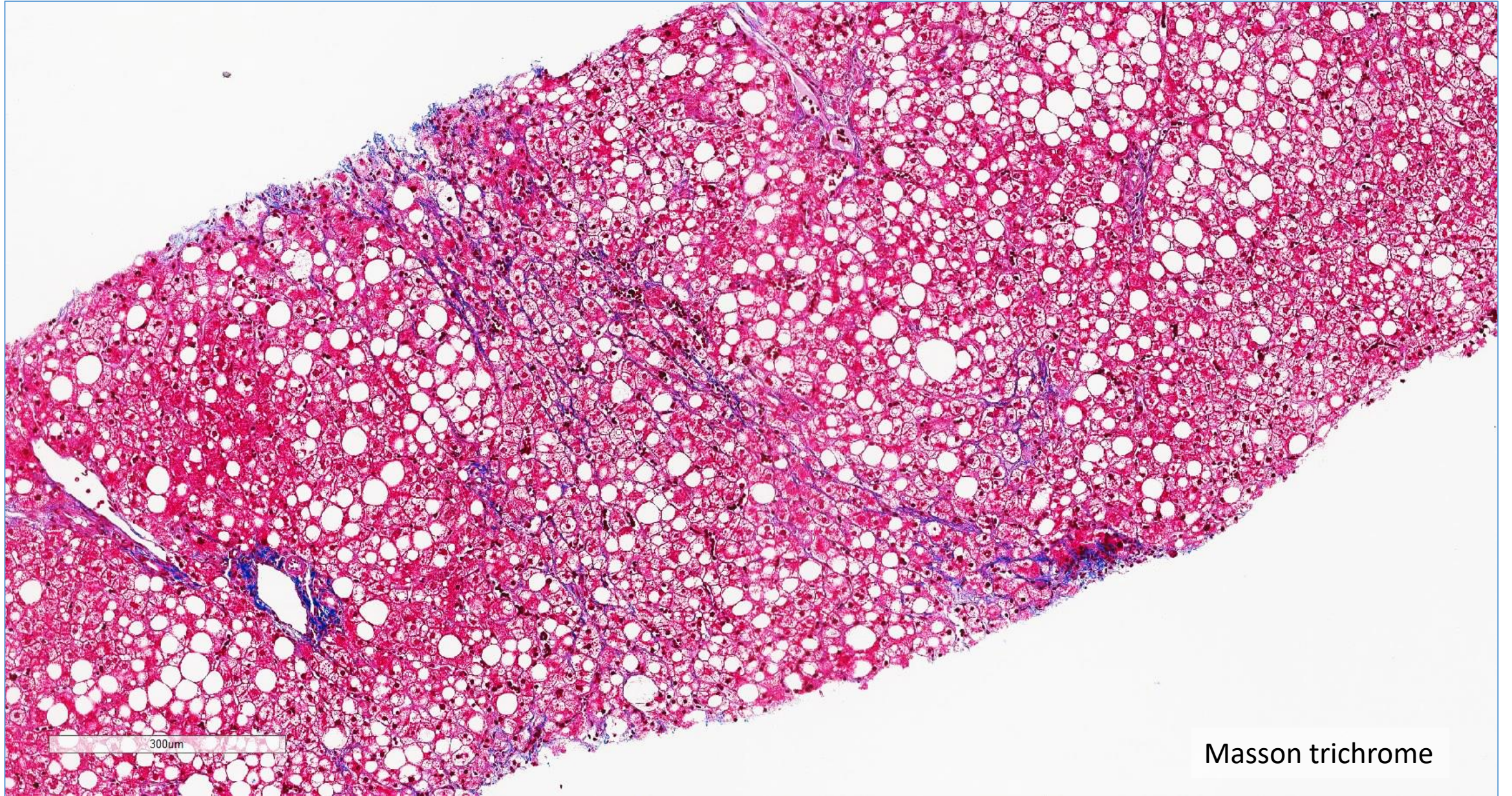
# Stage 2 (NASH CRN and EPOS)



500µm

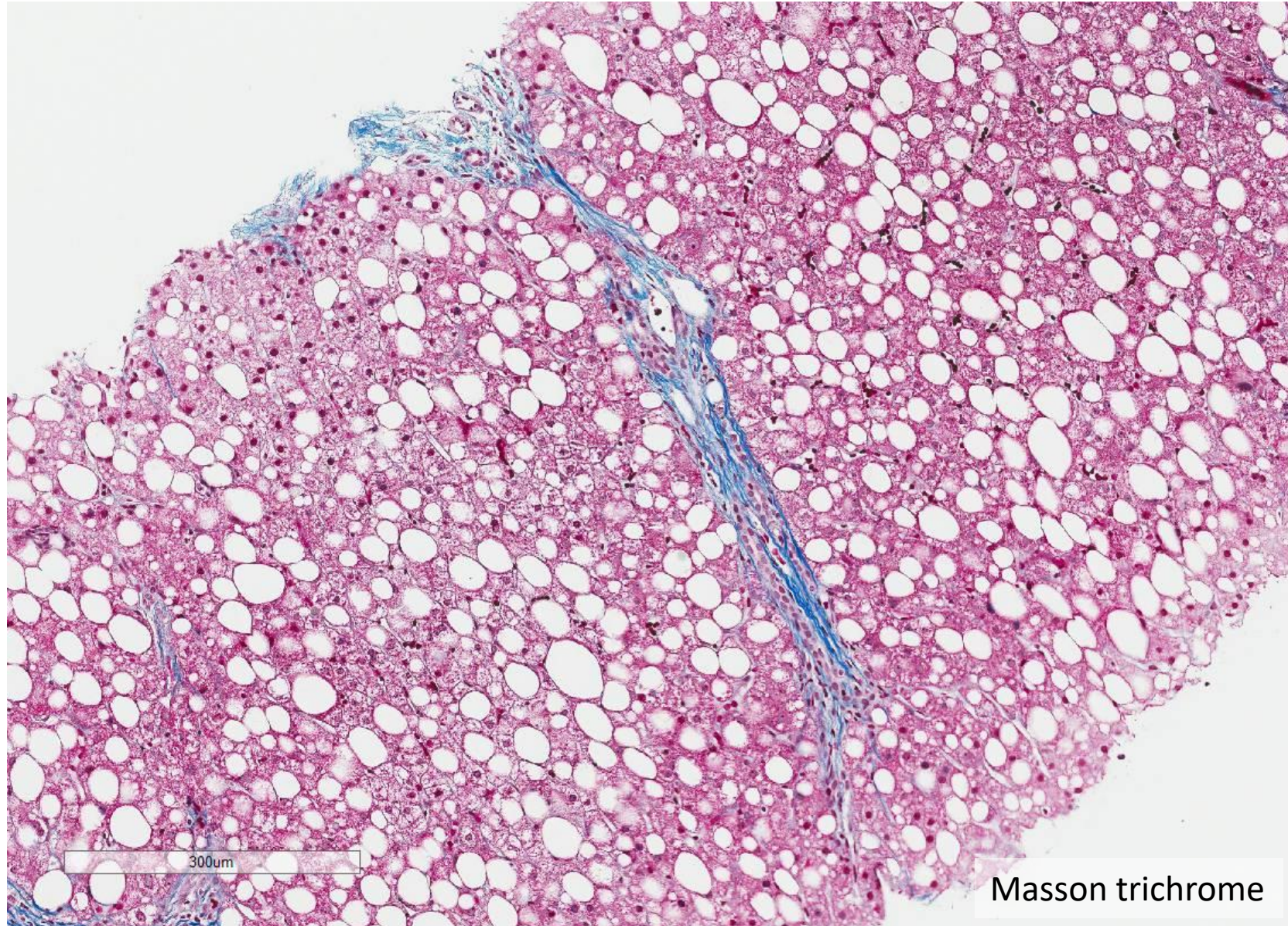
Masson trichrome

# Stage 2 (NASH CRN and EPOS)

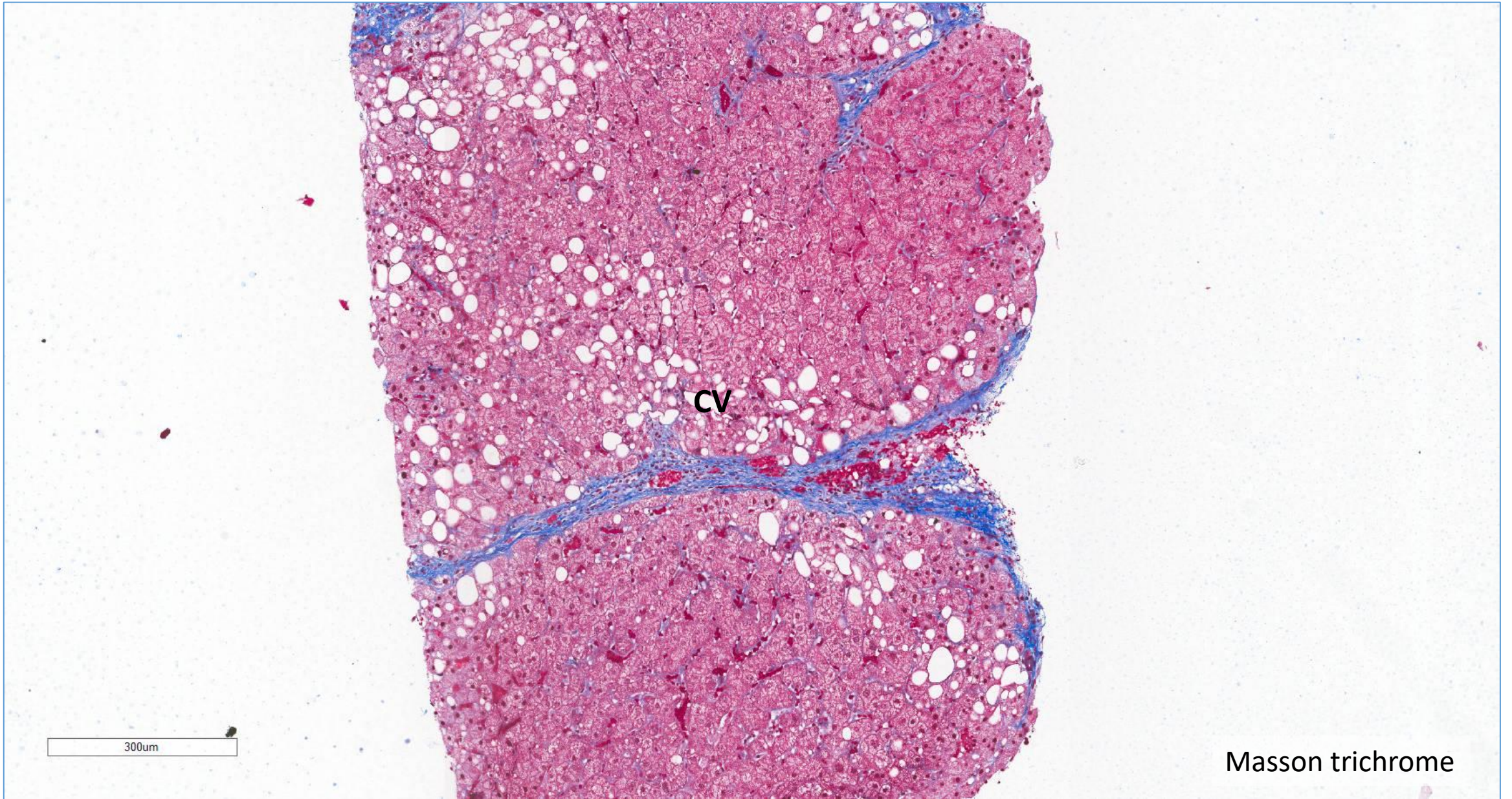


Masson trichrome

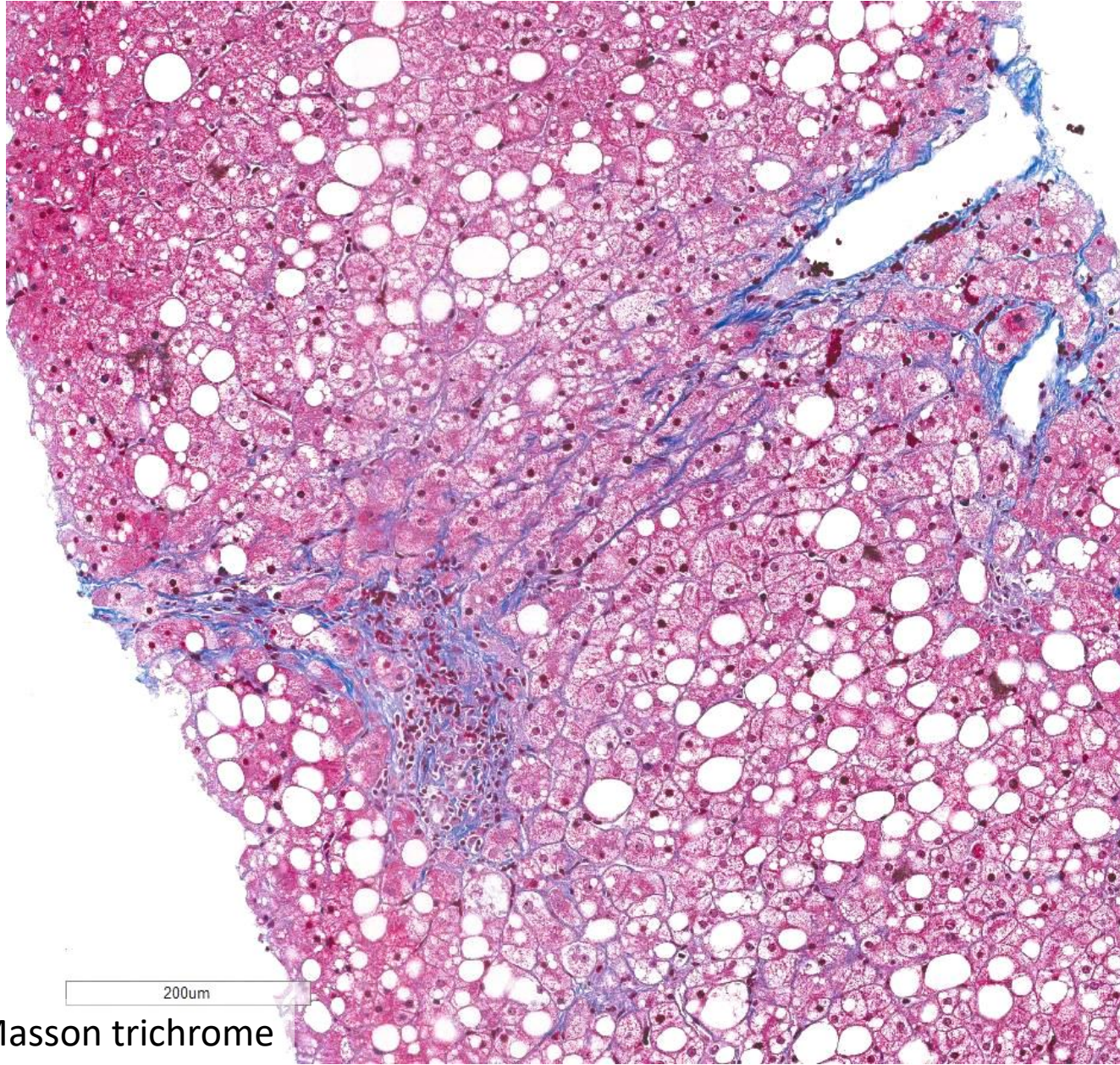
# Stage 3 (NASH CRN and EPOS, real fibrous septum crossing biopsy)



# Stage 3 (NASH CRN and EPOS, fibrous septum)

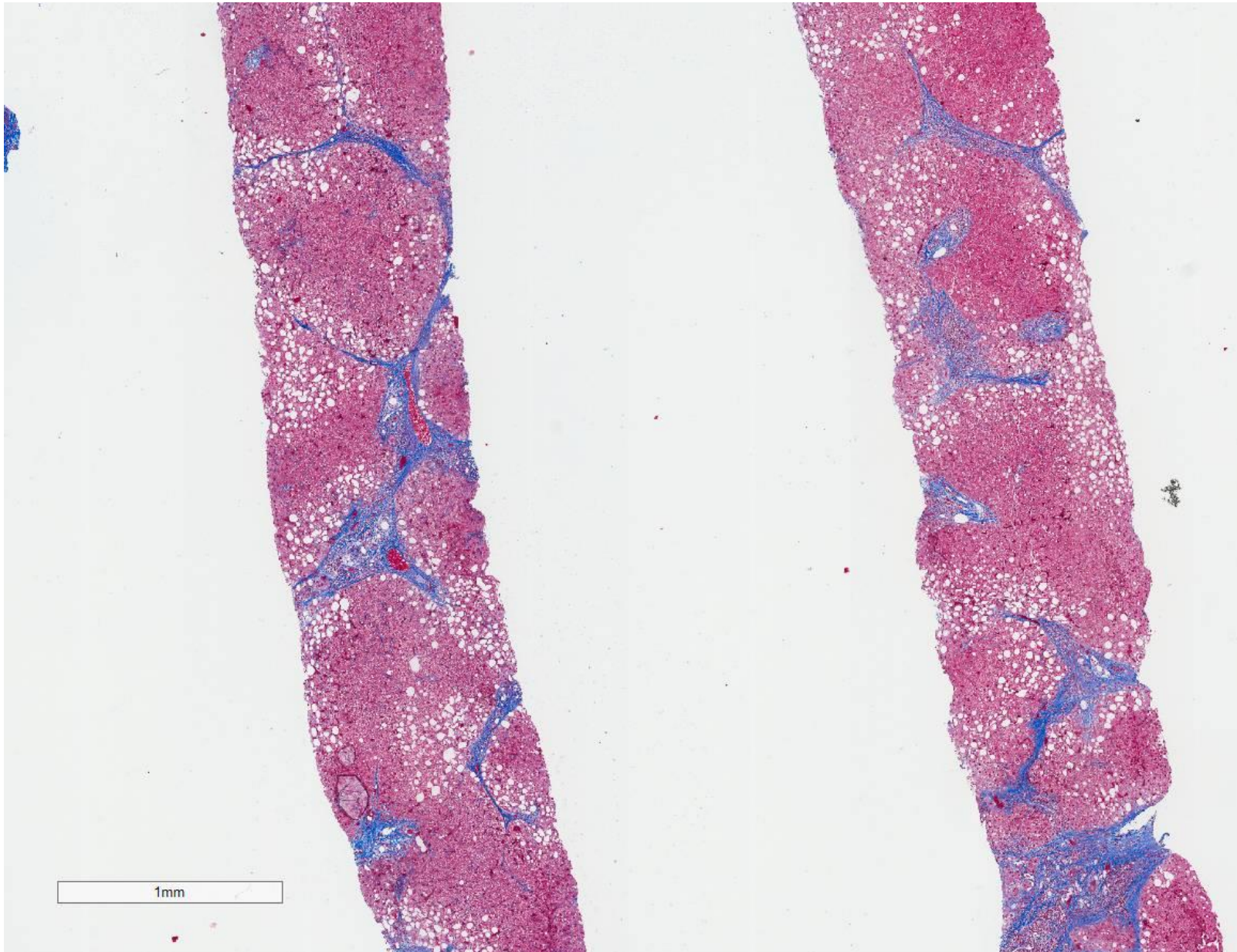


# Stage 2 (NASH CRN and EPOS)



- This is perisinusoidal fibrosis between hepatocyte trabeculae linking 2 vascular structures. Compared to previous slides, this is not a fully structured septum (septum in progress).

# Stage 3 (NASH CRN), Stage 4 (EPOS)



- Many fibrous septa
- No nodular morphology

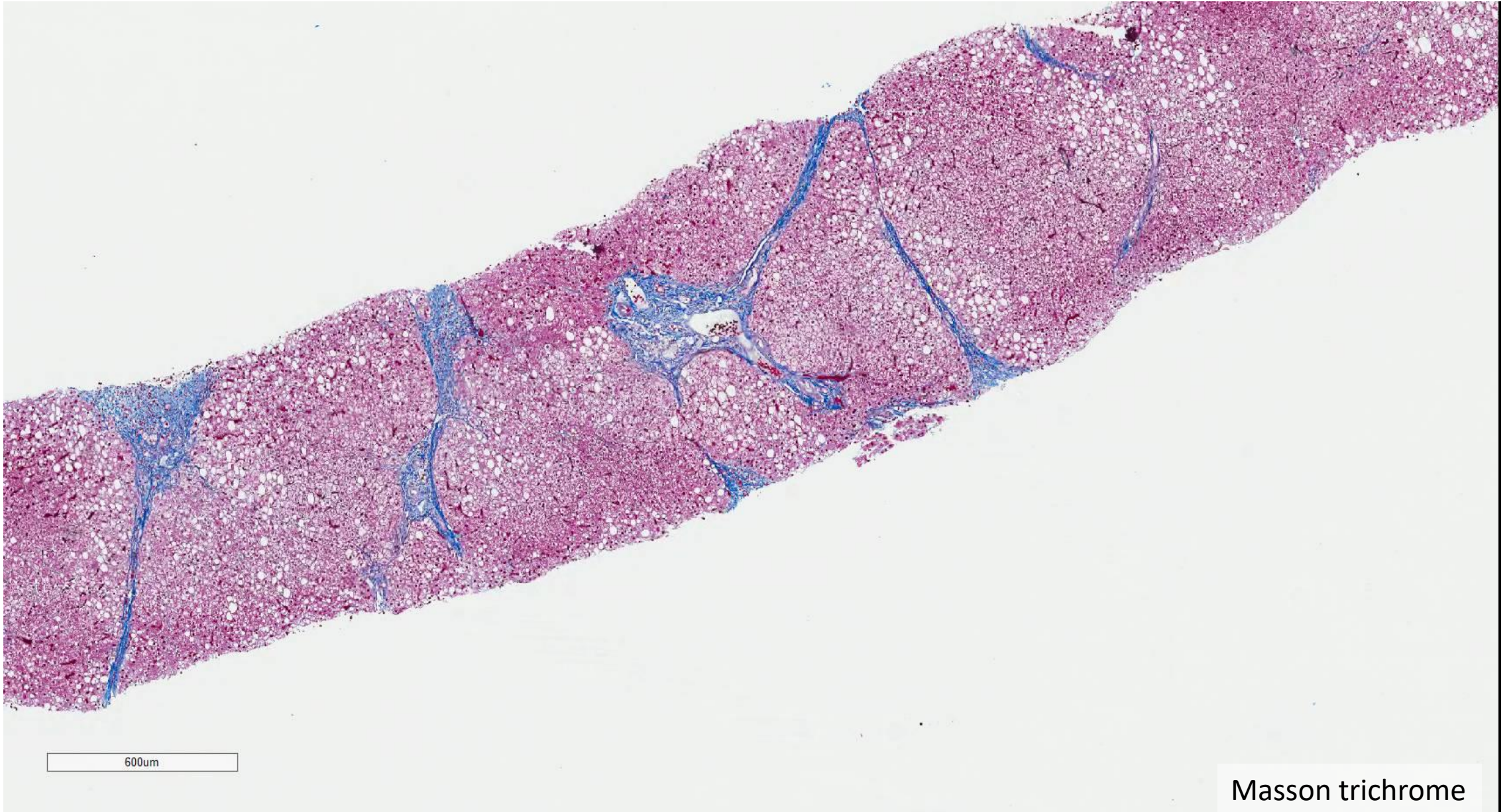
Masson trichrome

# Stage 3 (NASH CRN), Stage 4 (EPOS)

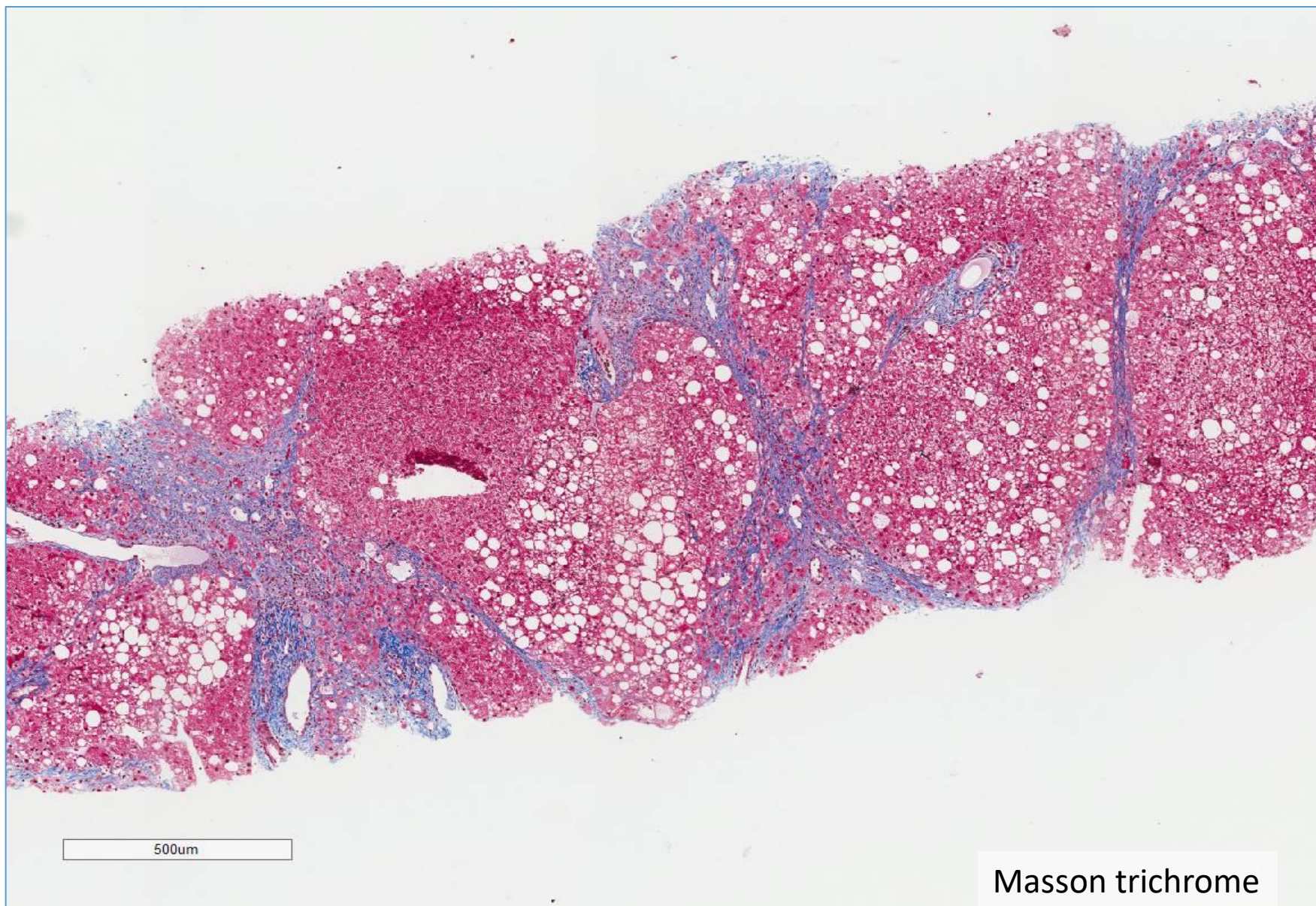




# Stage 3 (NASH CRN), Stage 4 (EPOS)

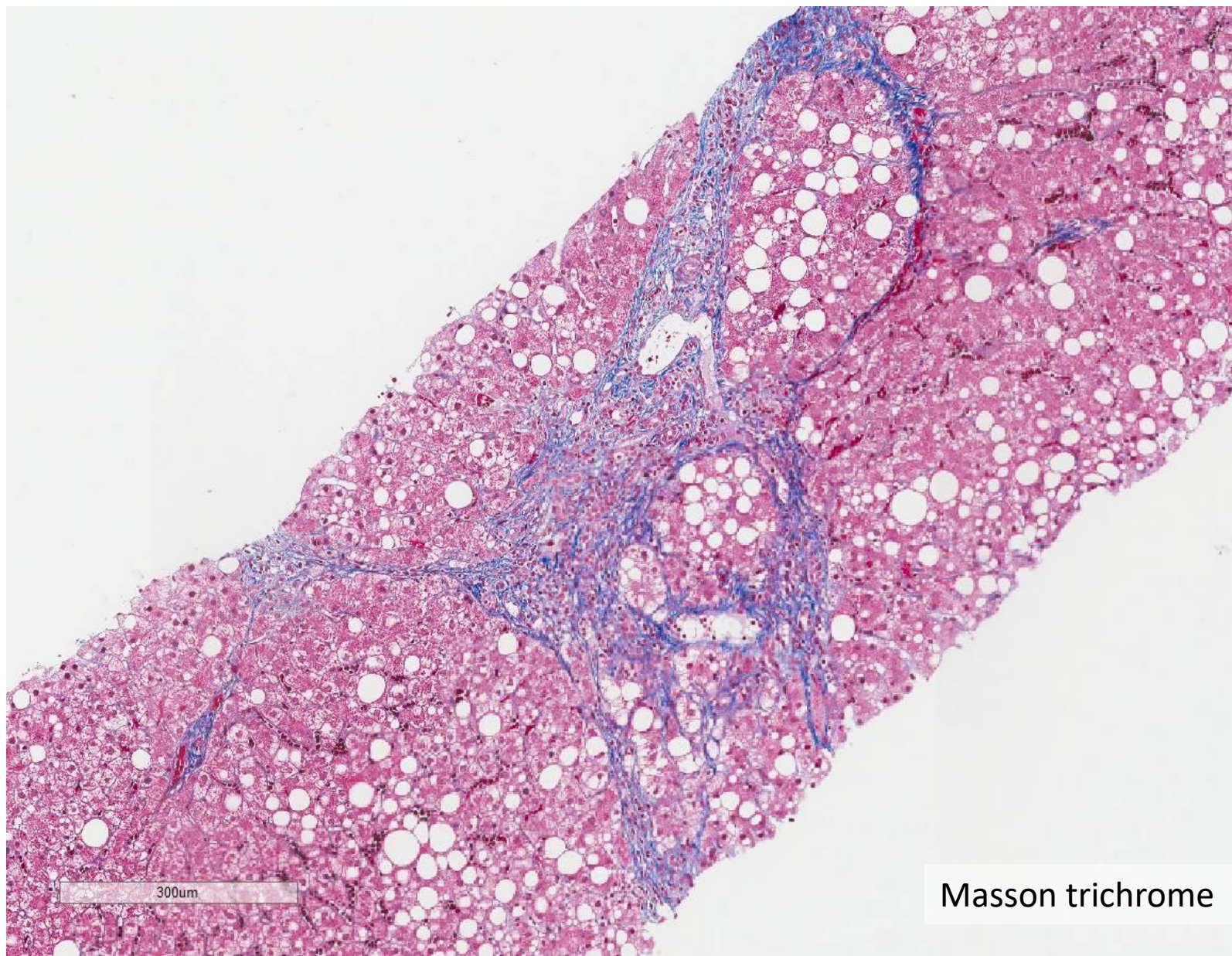


## Stage 3 (NASH CRN), Stage 5 (EPOS)



- Many fibrous septa
- Focal but not complete nodular morphology
- Incomplete cirrhosis

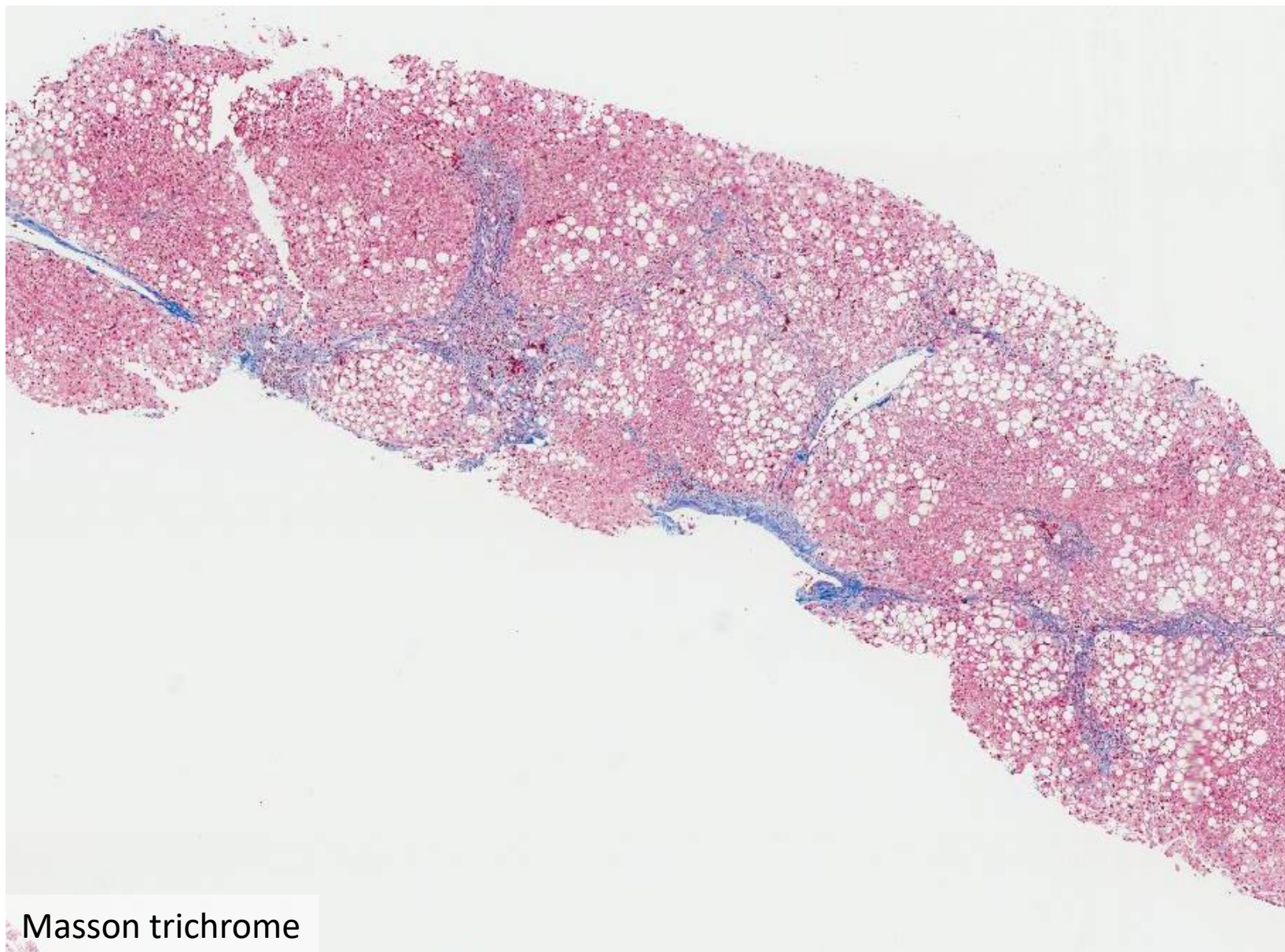
# Stage 3 (NASH CRN), Stage 5 (EPOS)



300um

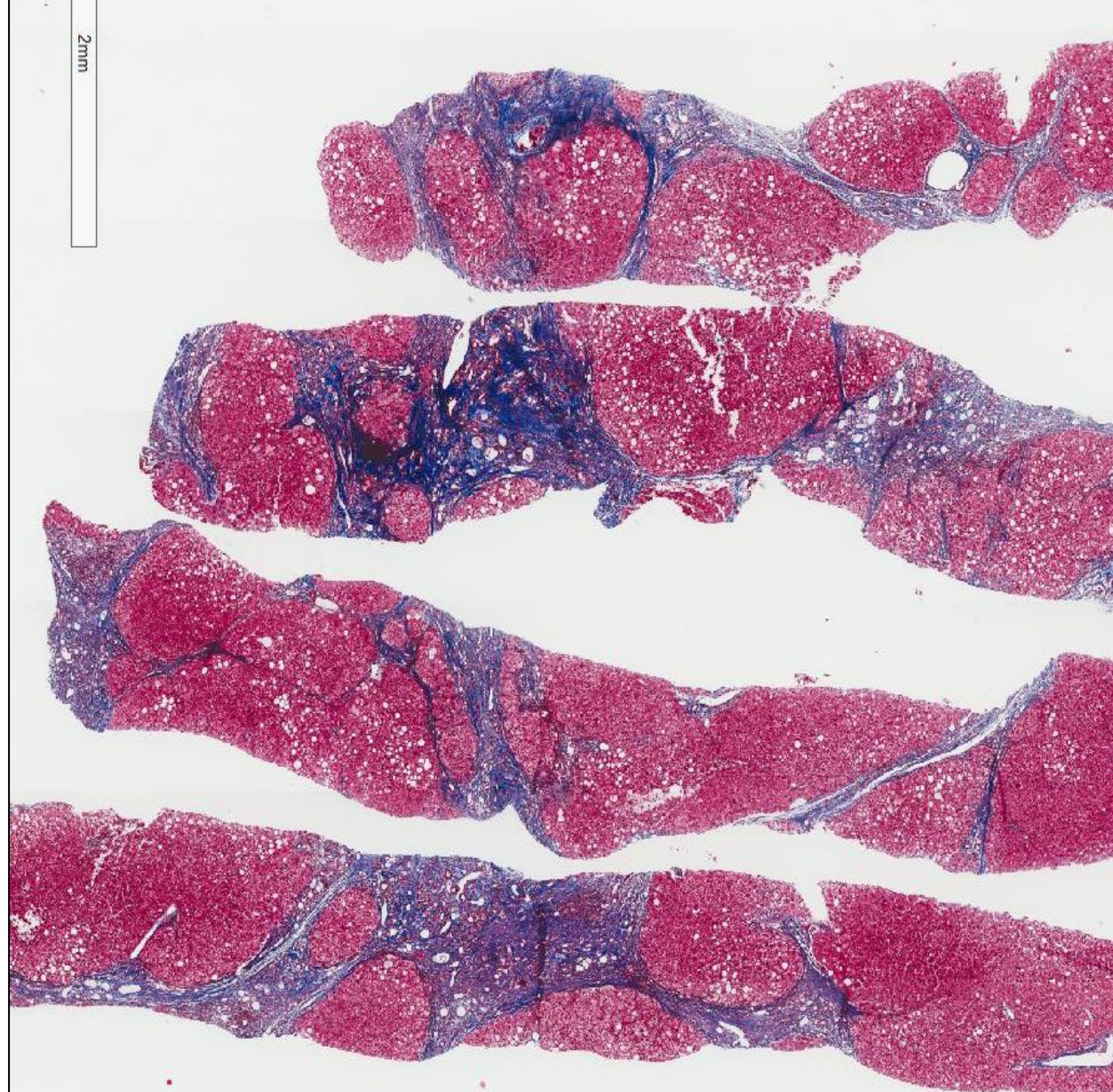
Masson trichrome

# Stage 3 (NASH CRN), Stage 5 (EPOS)



Masson trichrome

# Stage 4 (NASH CRN), Stage 6 (EPOS)



Masson trichrome

### DIAGNOSTIC CATEGORY

- Normal
- NAFL (steatosis without definite NASH)
- Definite NASH
- Fibrosis / Cirrhosis without steatosis 
  - Consistent with NAFLD
  - No NAFLD
- Other etiology \_\_\_\_\_

### ADDITIONAL COMMENTS

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## CRITERIA FOR DEFINITION OF CATEGORIES

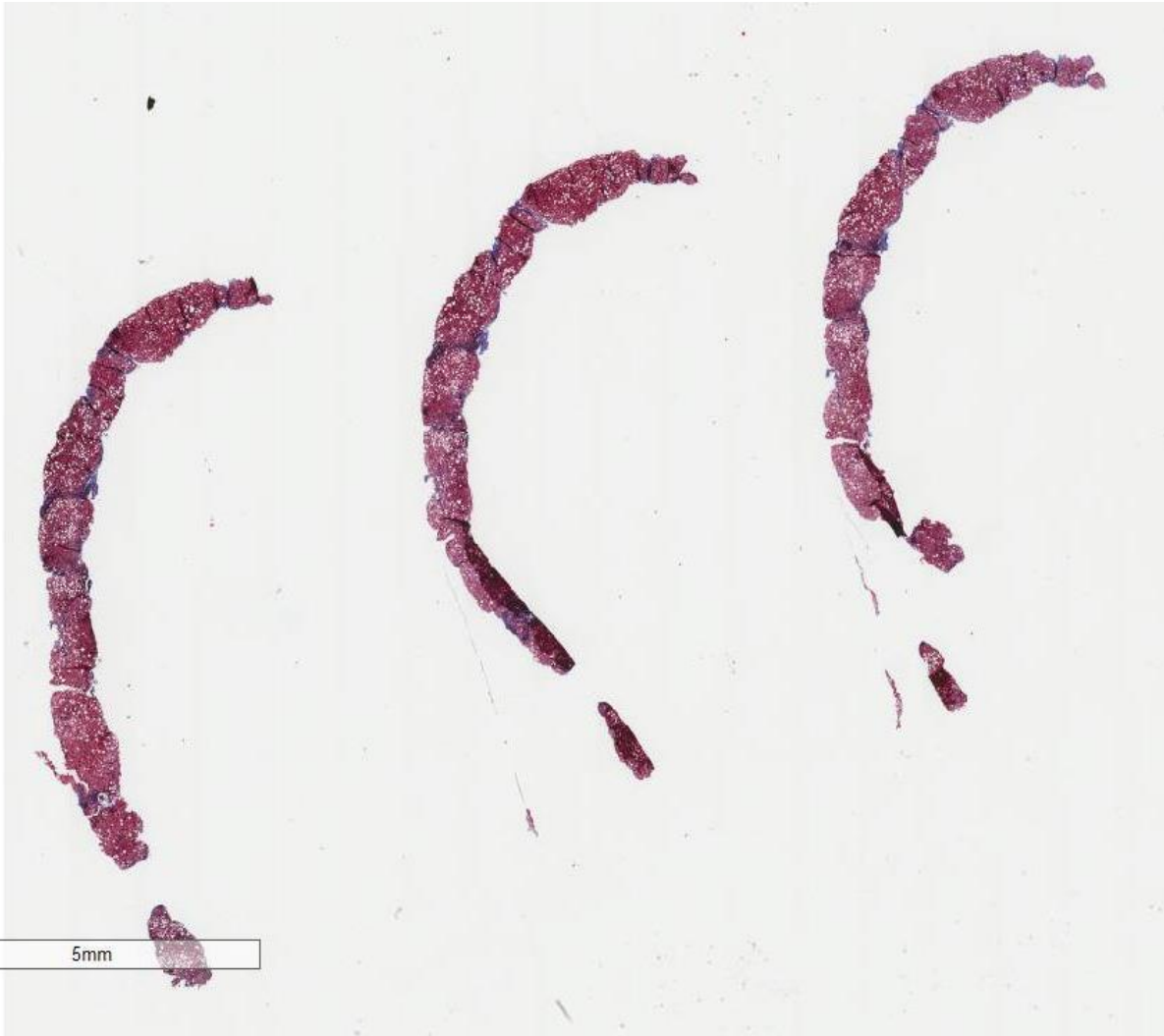
- **NORMAL** : fat in less than 5% of hepatocytes
- **NAFL** (steatosis without definite NASH features )
  - Fat only or
  - Fat and inflammation but no ballooning or ballooning but not inflammation
  - Fat and fibrosis or fat and cirrhosis
- **NASH**
  - Fat and
  - Ballooning and
  - Lobular Inflammation
- **NASH cirrhosis:**
  - Cirrhosis with steatosis+ballooning+inflammation
- **Fibrosis / Cirrhosis without steatosis :**
  - Consistent with NAFLD (presence of other NASH features/s i.e. ballooning, MDB, perisinusoidal fibrosis....)
  - No features of NAFLD («burned-out cirrhosis »)
- **Other** : possible other liver disease isolated or associated with NAFLD (granulomas, autoimmune hepatitis, .....)

# Multiple Choice Questions

(correct answers follow at the end of the document)

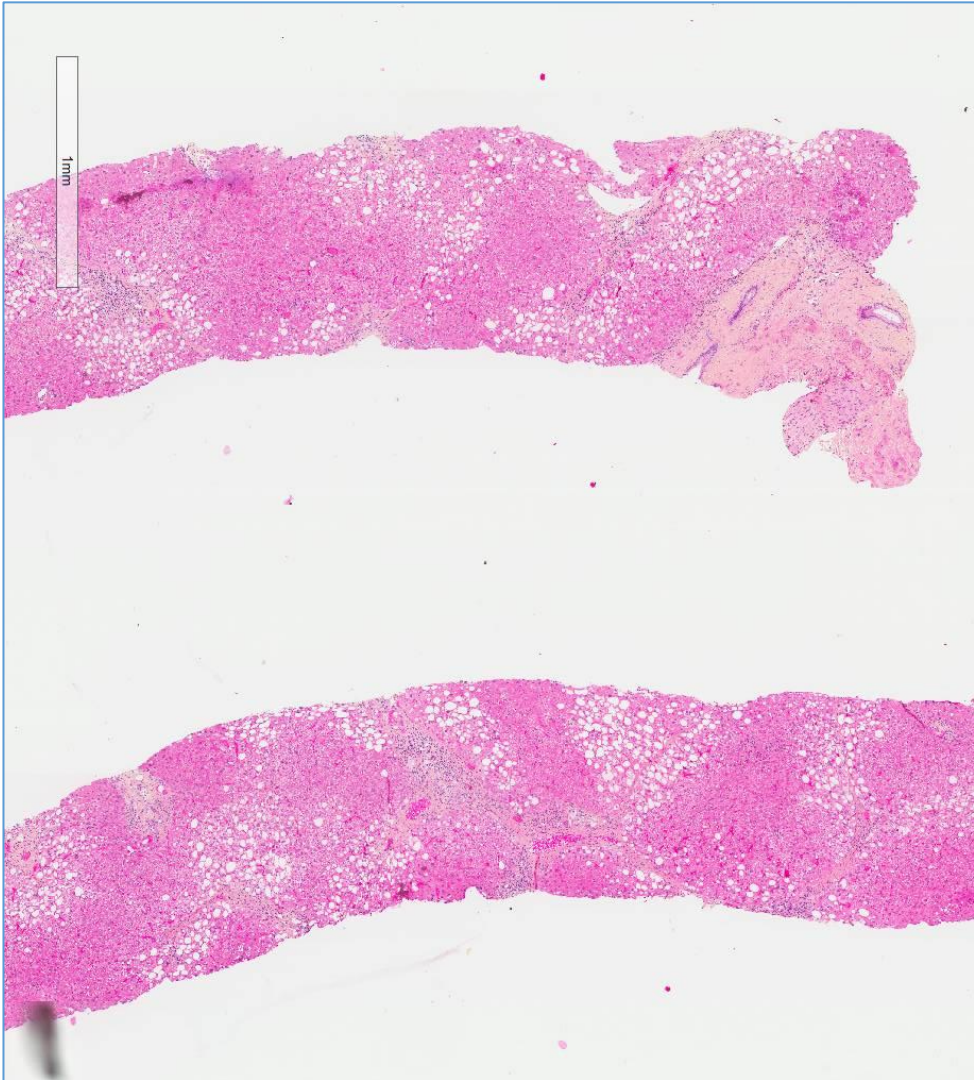


# Multiple Choice Question 1



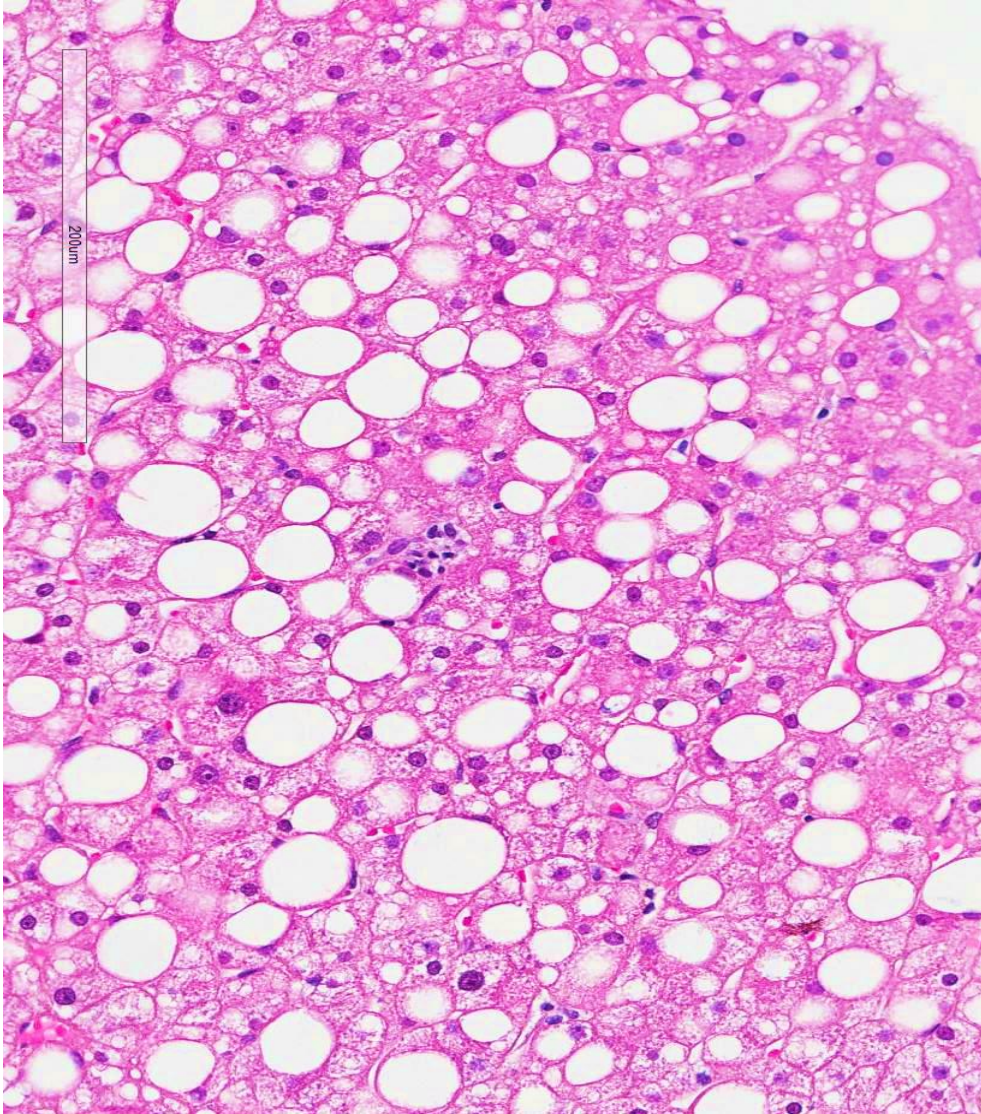
- How many fragments would you count in this biopsy ?
  - 1 = 3
  - 2 = 6
  - 3 = 2
  - 4 = 1

# Multiple Choice Question 2



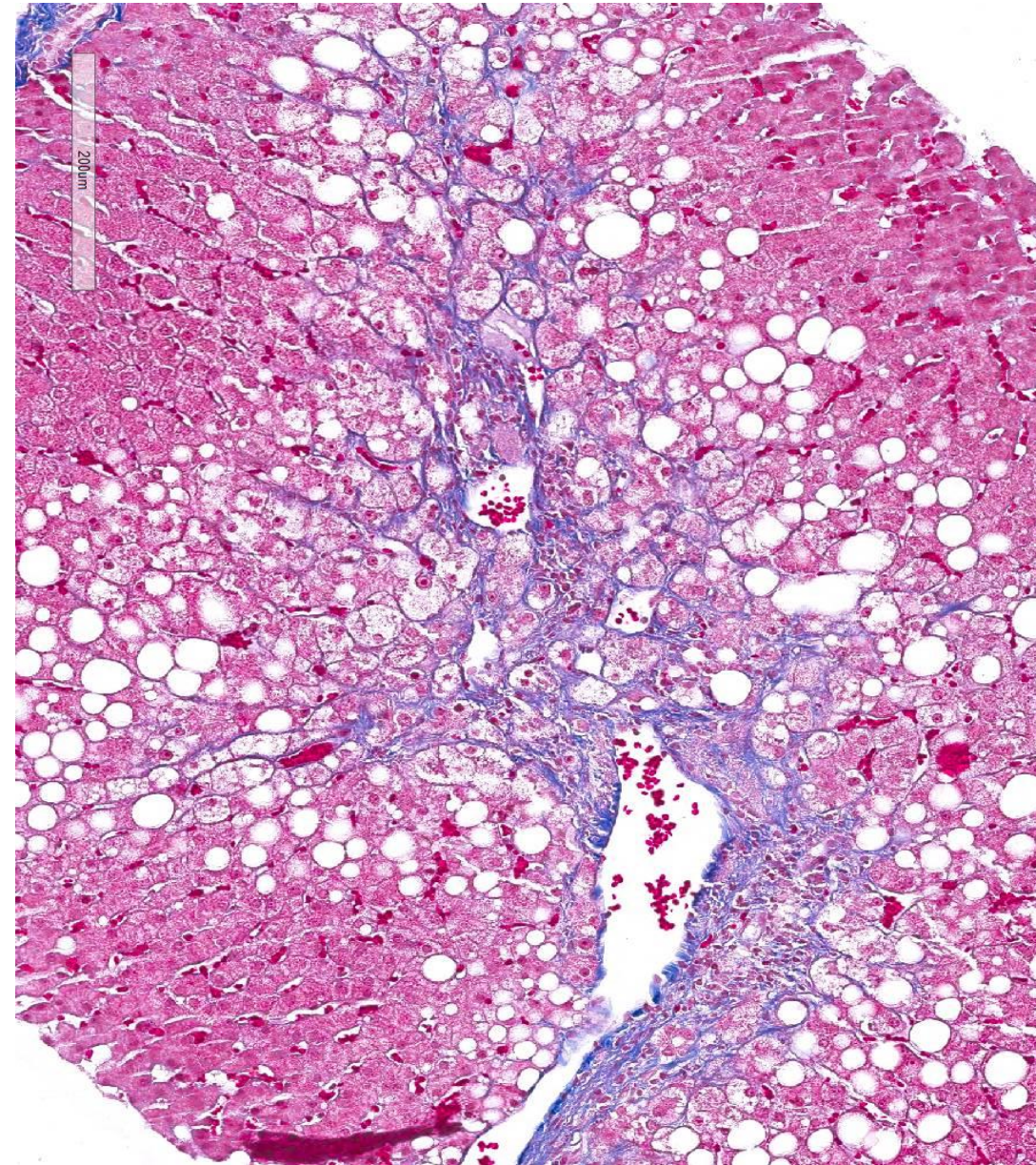
- What is the grade of steatosis ?
  1. Grade 0
  2. Grade 1
  3. Grade 2
  4. Grade 3

# Multiple Choice Question 3



- Does this picture fulfill the criteria of:
  1. NAFLD
  2. NAFL
  3. NASH
  4. I don't know

# Multiple Choice Question 4

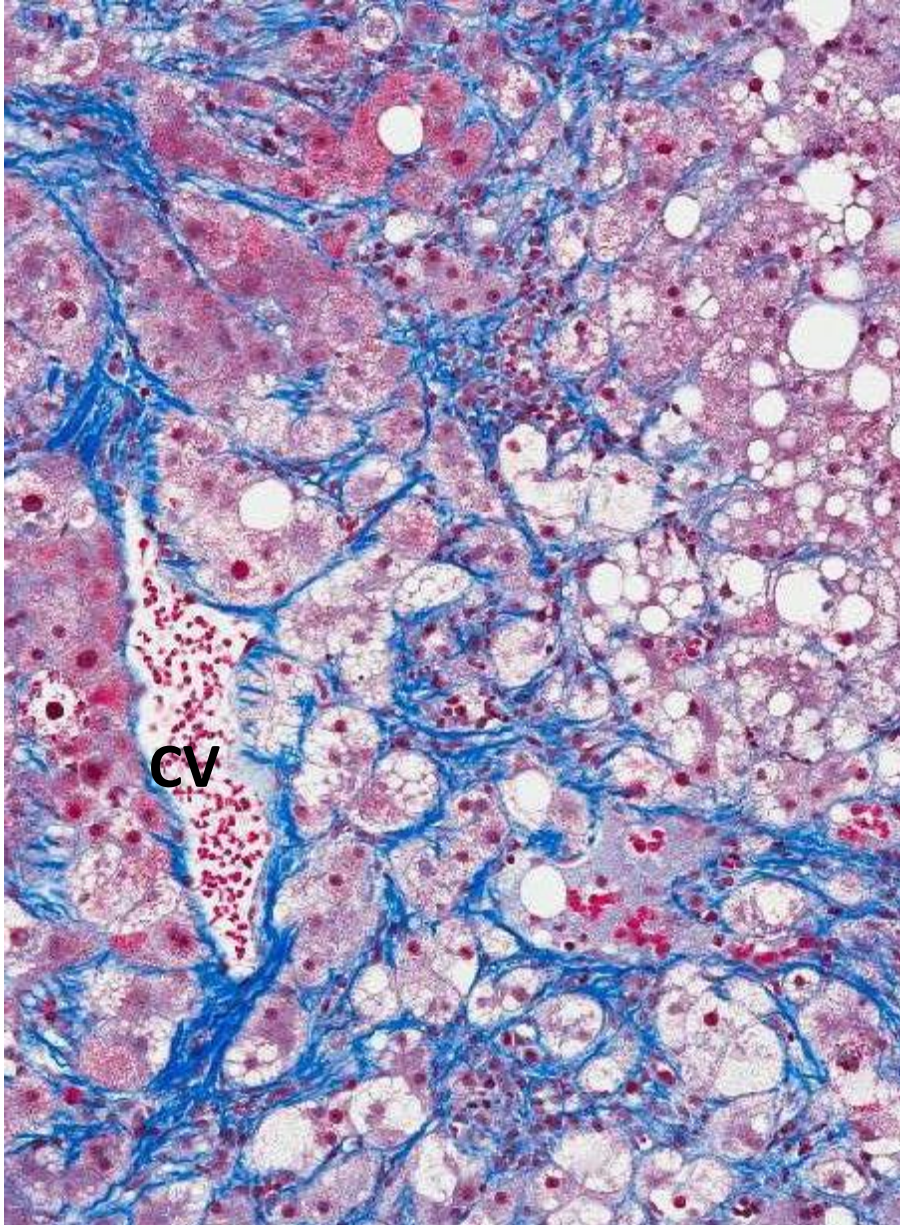


- Does this picture fulfill the criteria of:
  1. NAFLD
  2. NAFL
  3. NASH
  4. I don't know

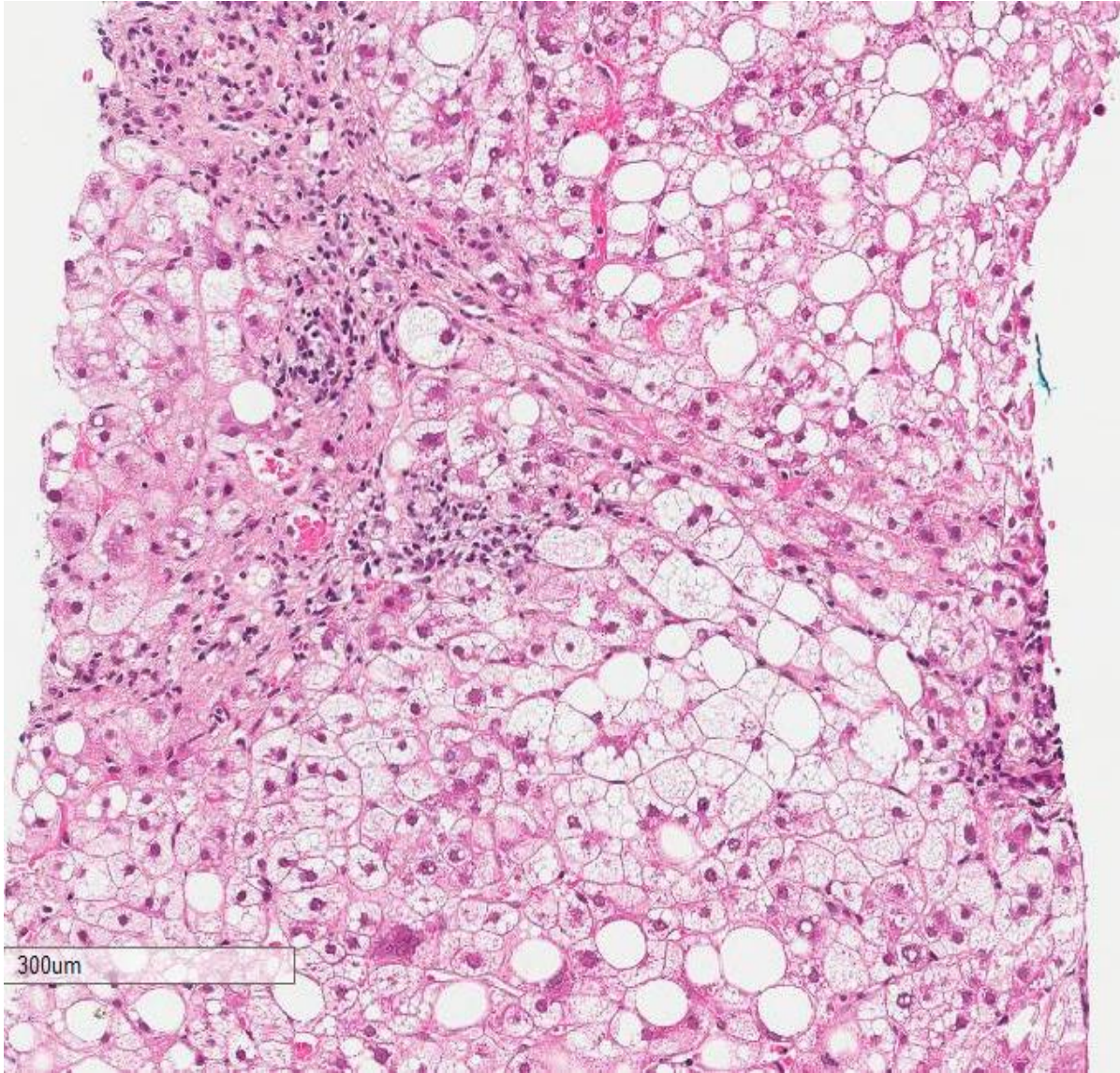
# Multiple Choice Question 5

- In this picture what are the features that are mandatory for the diagnosis of NASH ?

1. Steatosis
2. Ballooning/clarification
3. Lobular Inflammation
4. Perisinusoidal fibrosis
5. Predominant in Zone 3



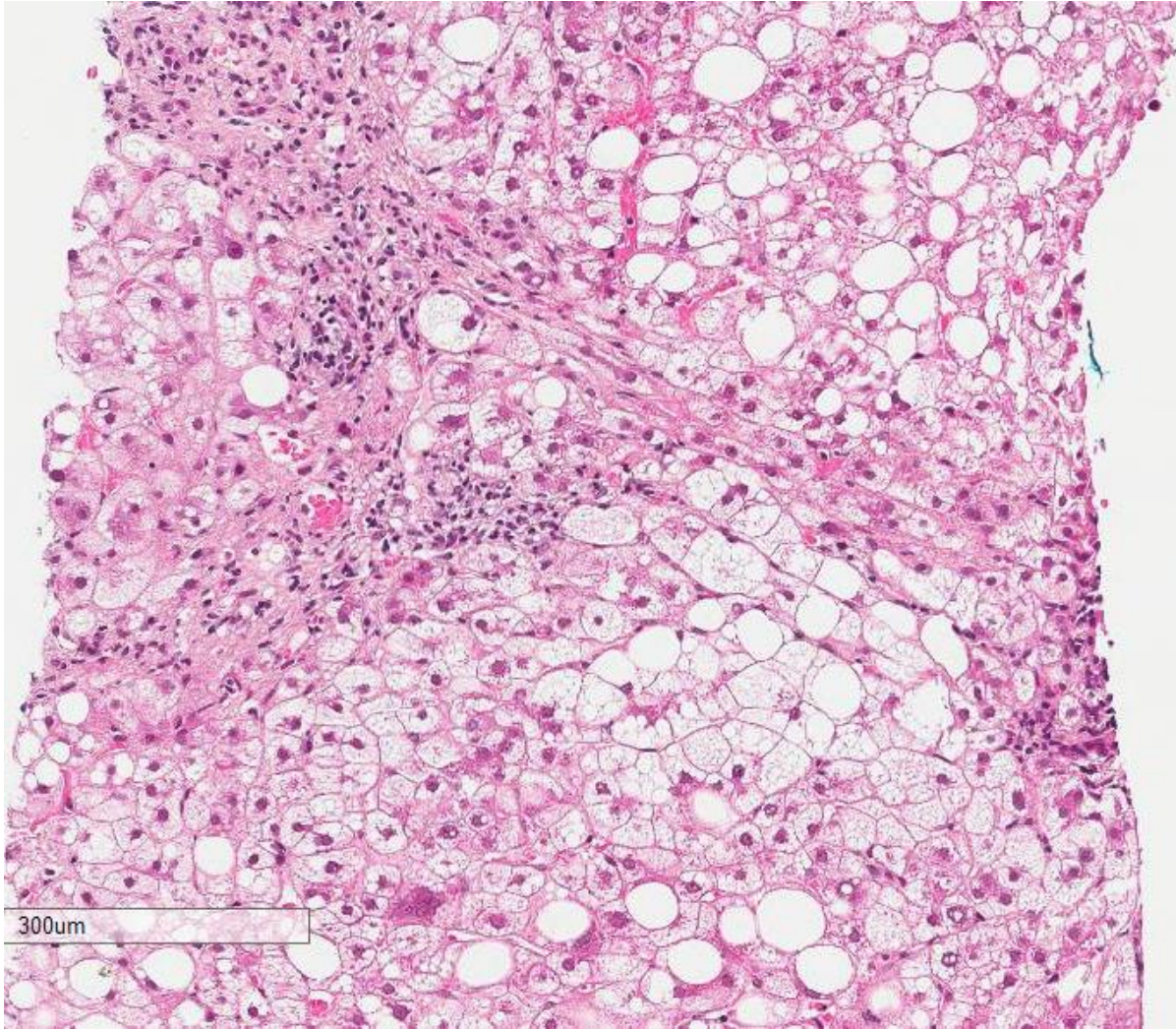
# Multiple Choice Question 6



- How would you score this NASH case according to NAS ?

1. <4
2. 5
3. 6
4. >6

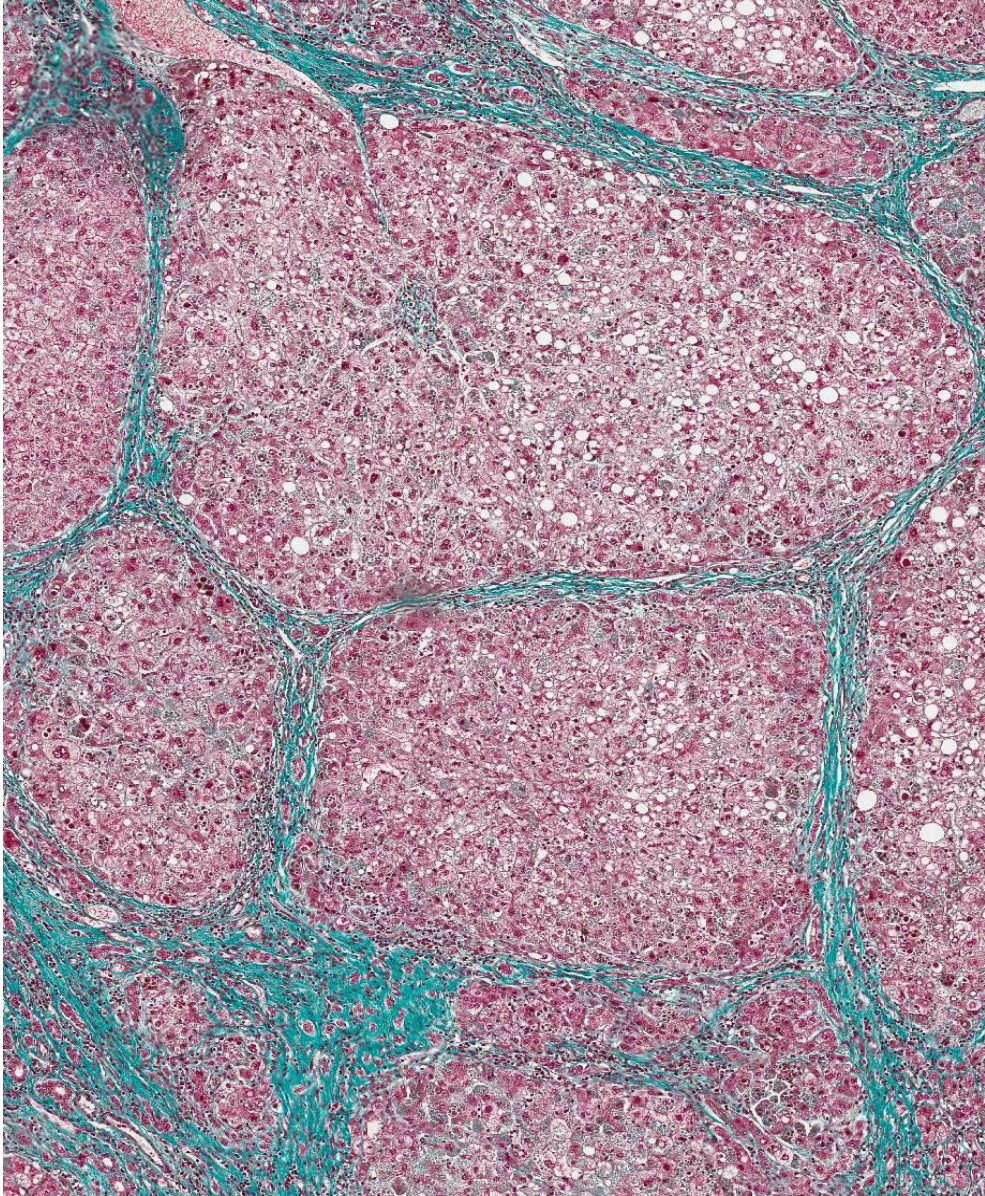
# Multiple Choice Question 7



- How would you grade Activity of this NASH case according to SAF ?

1. A=1
2. A=2
3. A=3
4. A=4

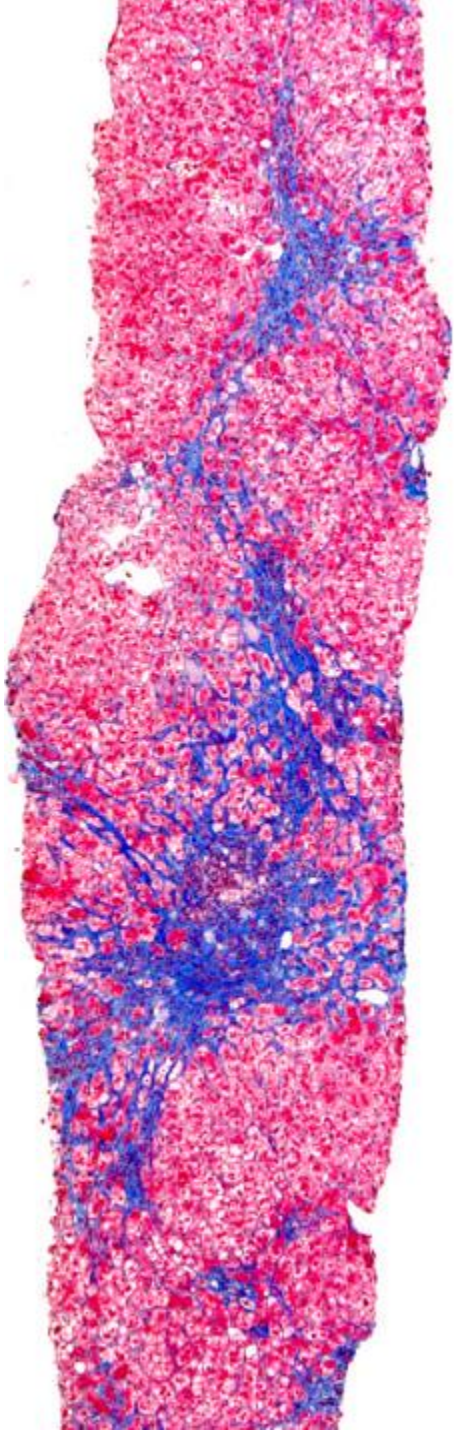
# Multiple Choice Question 8



- How would you substage this cirrhosis case according to the Laennec staging system ?
  - 1= 4a
  - 2= 4b
  - 3= 4c
  - 4= 4d

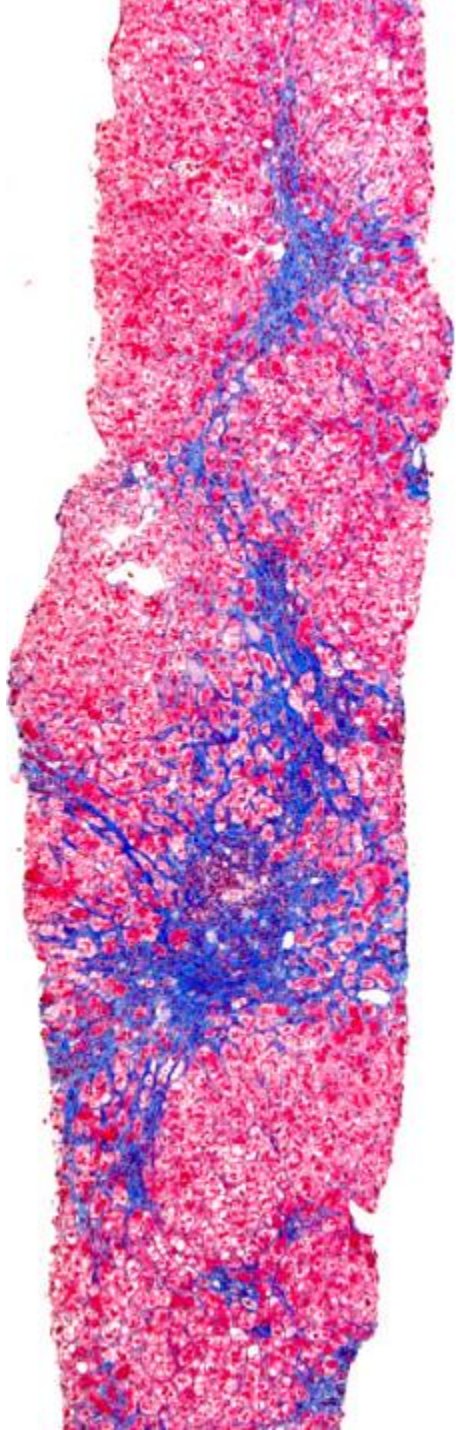


# Multiple Choice Question 9



- How would you stage fibrosis in this biopsy according to NASH CRN ?
  - 1 = 2
  - 2 = 3
  - 3 = 4
  - 4 = 5

# Multiple Choice Question 10



- How would you stage fibrosis in this biopsy according to EPOS ?
  - 1 =3
  - 2 =4
  - 3 =5
  - 4 =6

# Answers to MCQ

- MCQ 1: 1
- MCQ 2: 3
- MCQ 3: 1, 2
- MCQ 4: 1, 3
- MCQ 5: 1, 2, 3
- MCQ 6: 4
- MCQ 7: 4
- MCQ 8: 1
- MCQ 9: 2
- MCQ 10: 3