Highlights from Gnomes Meeting
Ravello, Italy
May 28th – 31st 2014

Stefan Hübscher,
School of Cancer Sciences, University of Birmingham
Dept of Cellular Pathology, Queen Elizabeth Hospital, Birmingham

Annual M… …Group

- First meetin… …d)
- Currently 15… …ia)

- Cases circul… …ular theme
  - 2013 & …
- Suggested d… …by local organiser

- Cases preser…
- Aim to reach…

- Most recent…
  - "Pathol… …2014

A CLASSIFICATION OF CHRONIC HEPATITIS

J. DE GROOTE
M.D., A.H.O. Leuven
PROFESSOR OF MEDICINE

V. J. DESMET
M.D., A.H.O. Leuven
PROFESSOR OF PATHOLOGY

ACADEMISCH ZIEKENHUIS ST. RAFAËL, LEUVEN, BELGIUM

P. GEDIGK
Dr.med. Innsbruck
PROFESSOR OF PATHOLOGY, UNIVERSITY OF BONN, GERMANY

G. KORB
Dr.med. Berlin
LECTURER IN PATHOLOGY, UNIVERSITY OF MARBURG, GERMANY

H. POPPER
M.D. Vienna, Ph.D. Illinois
PROFESSOR OF PATHOLOGY, MOUNT SINAI SCHOOL OF MEDICINE, NEW YORK, U.S.A.

H. POULSEN
M.D. Copenhagen
DIRECTOR, INSTITUTE OF PATHOLOGY, KOMMUNEHOSPITALET, COPENHAGEN, DENMARK

P. J. SCHEUER
M.D. Lond., M.C.Path.
SENIOR LECTURER IN MORBID ANATOMY, ROYAL FREE HOSPITAL SCHOOL OF MEDICINE, LONDON W.C.1

M. SCHMID
M.D. Basle
HEAD OF THE DEPARTMENT OF MEDICINE, STADTSPITAL WAID, ZÜRICH, SWITZERLAND

H. THALER
M.D. Vienna
UNIVERSITY LECTURER, WILHELMINENSPITAL, VIENNA, AUSTRIA

E. UEHLINGER
M.D. Zürich
PROFESSOR OF PATHOLOGY, UNIVERSITY OF ZÜRICH, SWITZERLAND

W. WEPLER
Dr.med.
DIRECTOR, INSTITUTE OF PATHOLOGY, STADTKRANKENHAUS, KASSEL, GERMANY

Gnomes 2014 - Villa Rufulo, Ravello

Gnomes in Ravello, May 2014
Balcony of Hotel Rufulo

Highlights from Gnomes Meeting 2014

- Selected slides from circulated cases

- Summarise current thoughts & future studies relating to cases that are diagnostically challenging
  - Well-differentiated hepatocellular neoplasms arising in non-cirrhotic livers (adenoma vs very well-differentiated HCC)

BASEL – B 2014
(Luigi Terracciano)

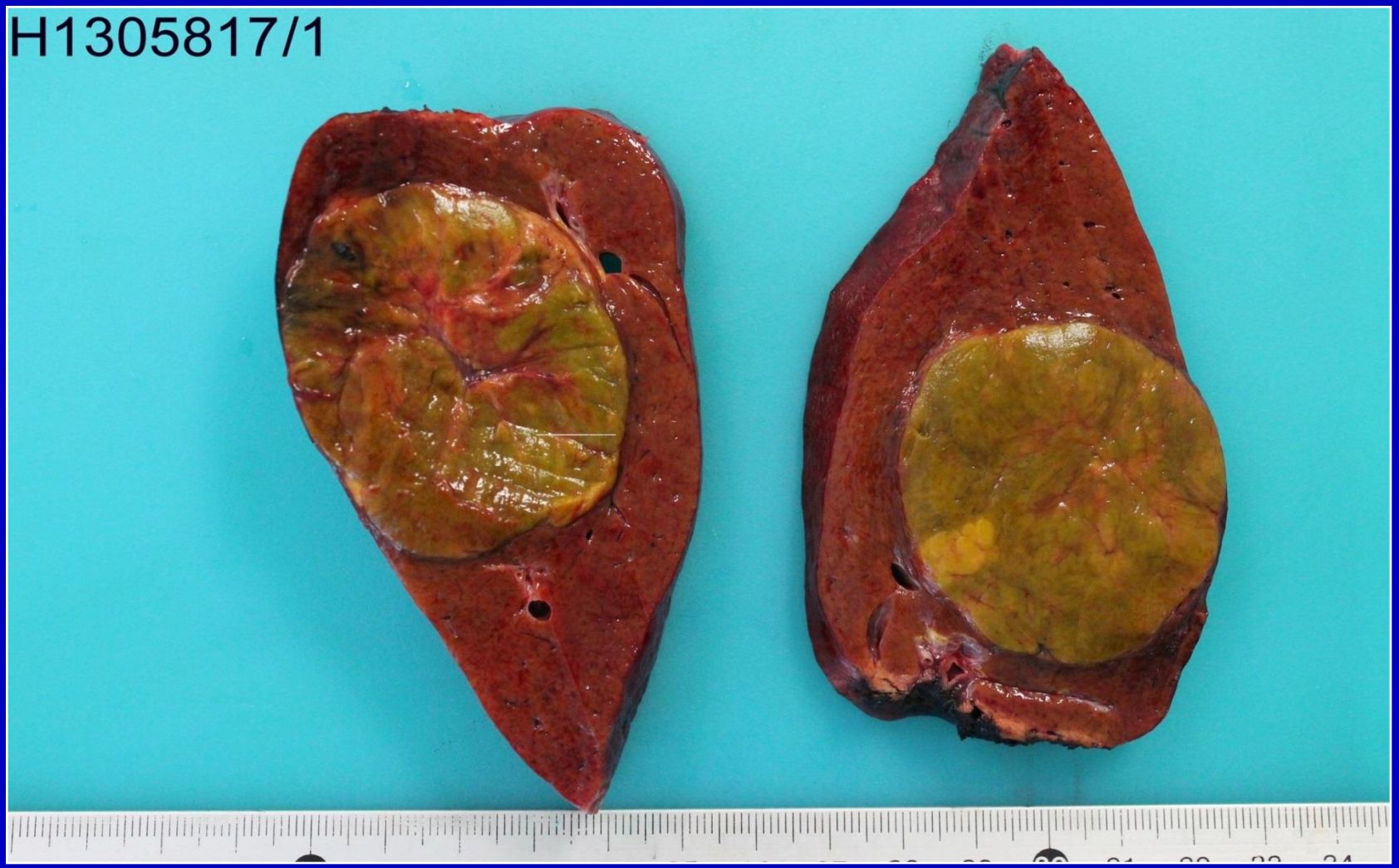
Well-differentiated HCC
with steatotic/inflammatory features

BASEL – B 2014

Clinical history

- A 75 year old woman with a liver mass 52 mm diameter in segments II and III.
- Search for causes of chronic liver disease was negative (HBV, HCV, hemochromatosis, autoantibodies).
- The patient underwent a bisegmentectomy (segments II and III).

BASEL - B, 2014



Well-circumscribed expanding tumor
(focal yellow areas)

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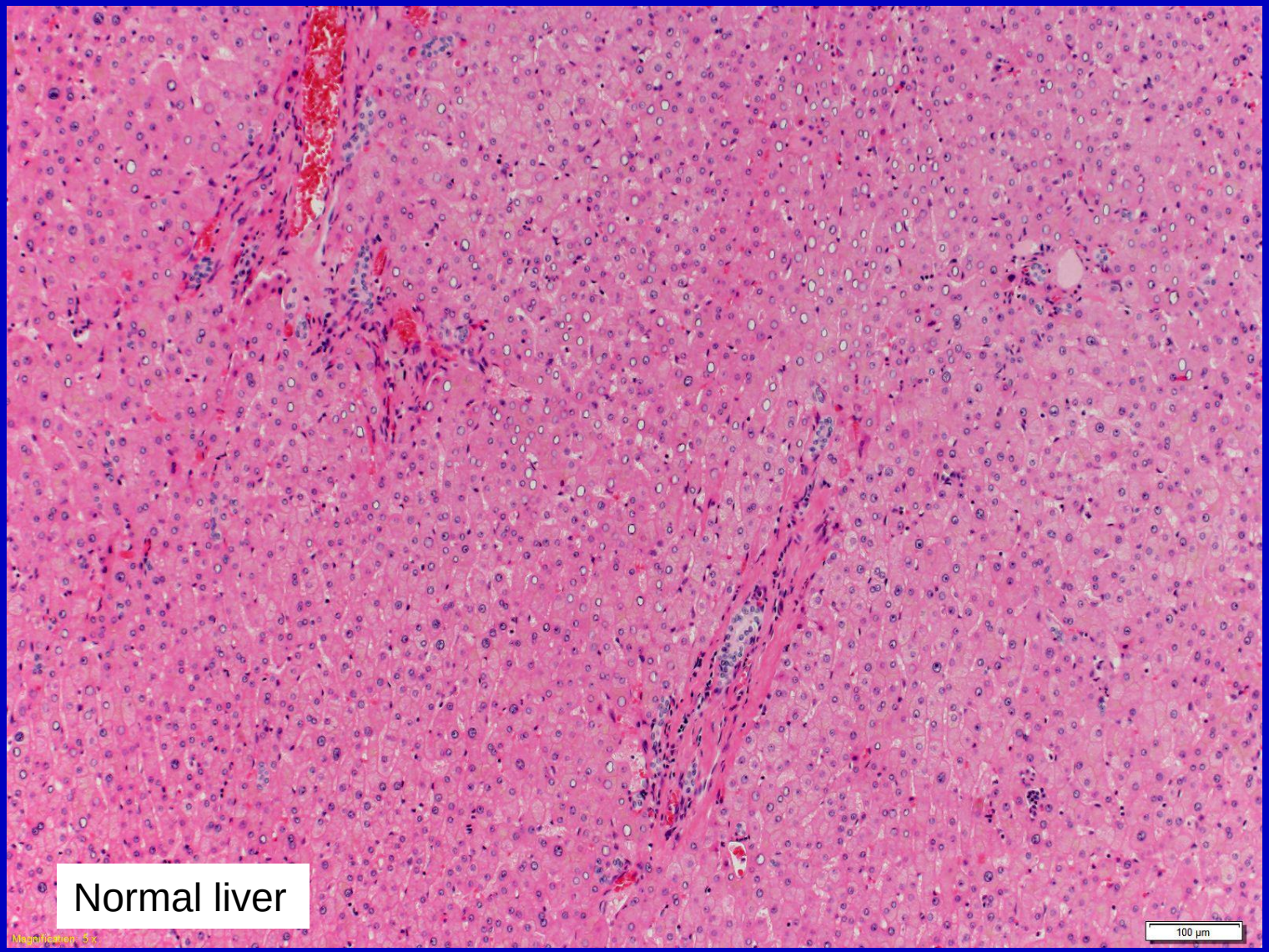

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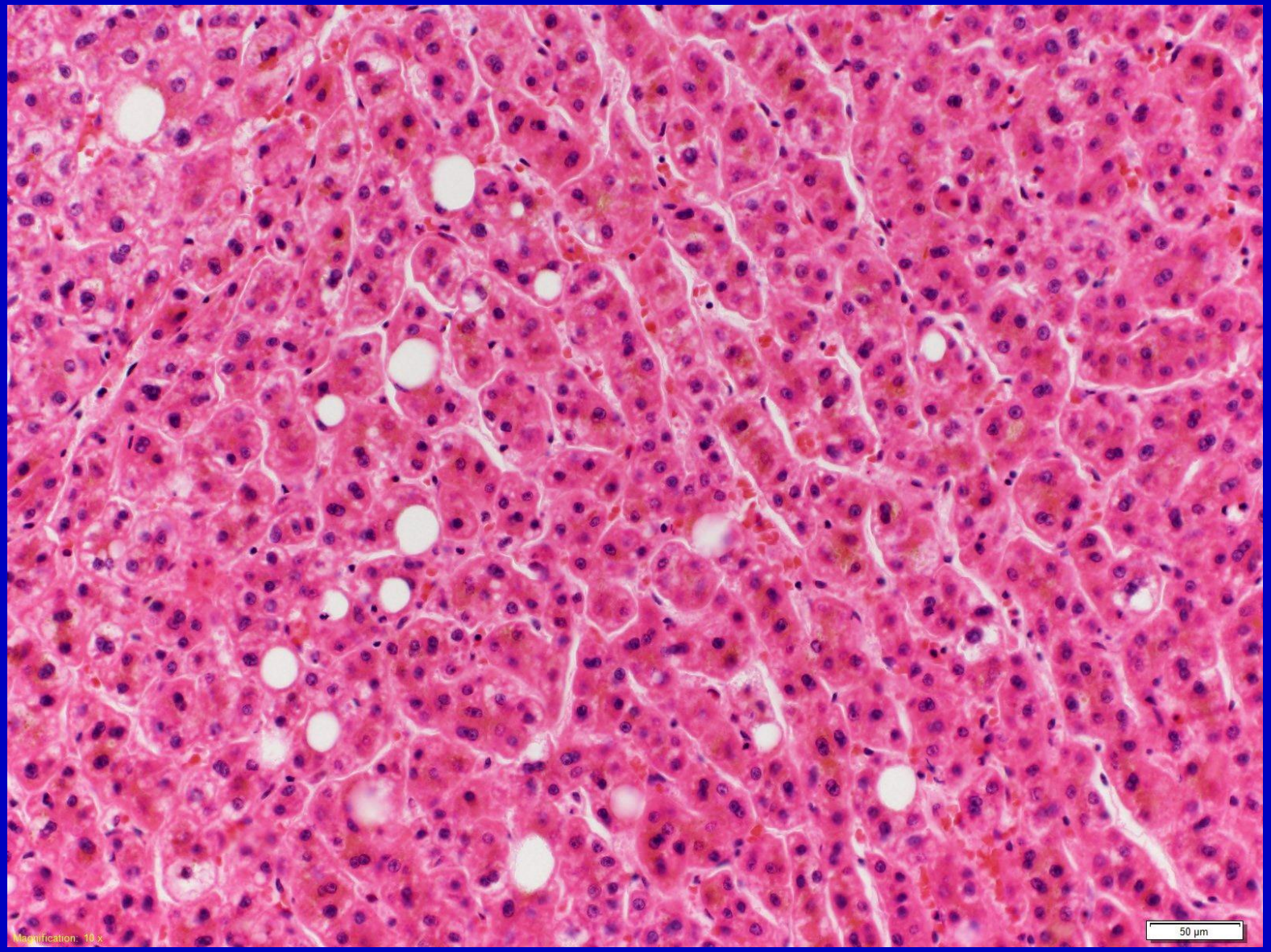

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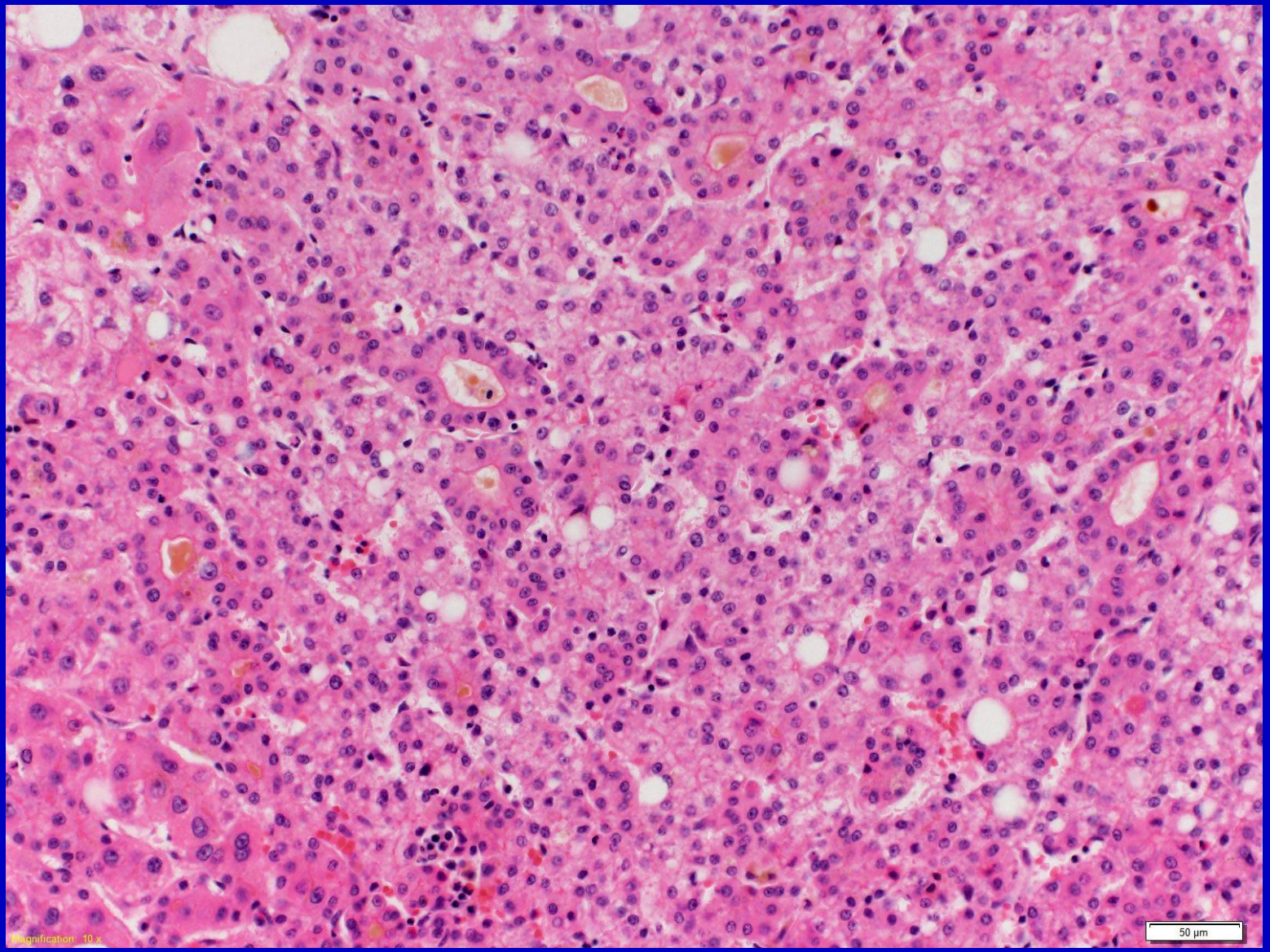
Trabecular pattern - minimal cytological atypia

BASEL - B, 2014



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Acinar pattern with bile formation

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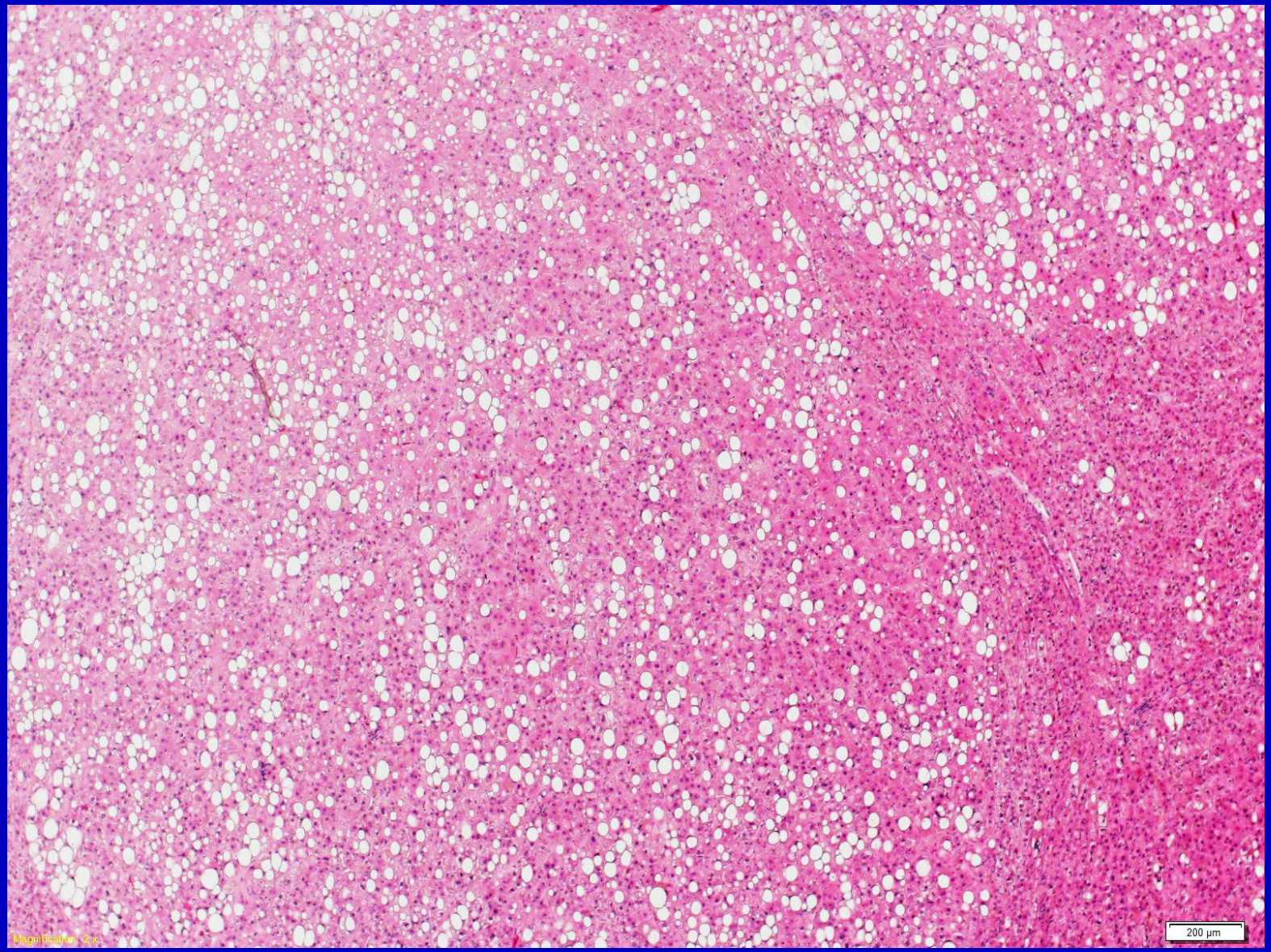

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Steatotic area

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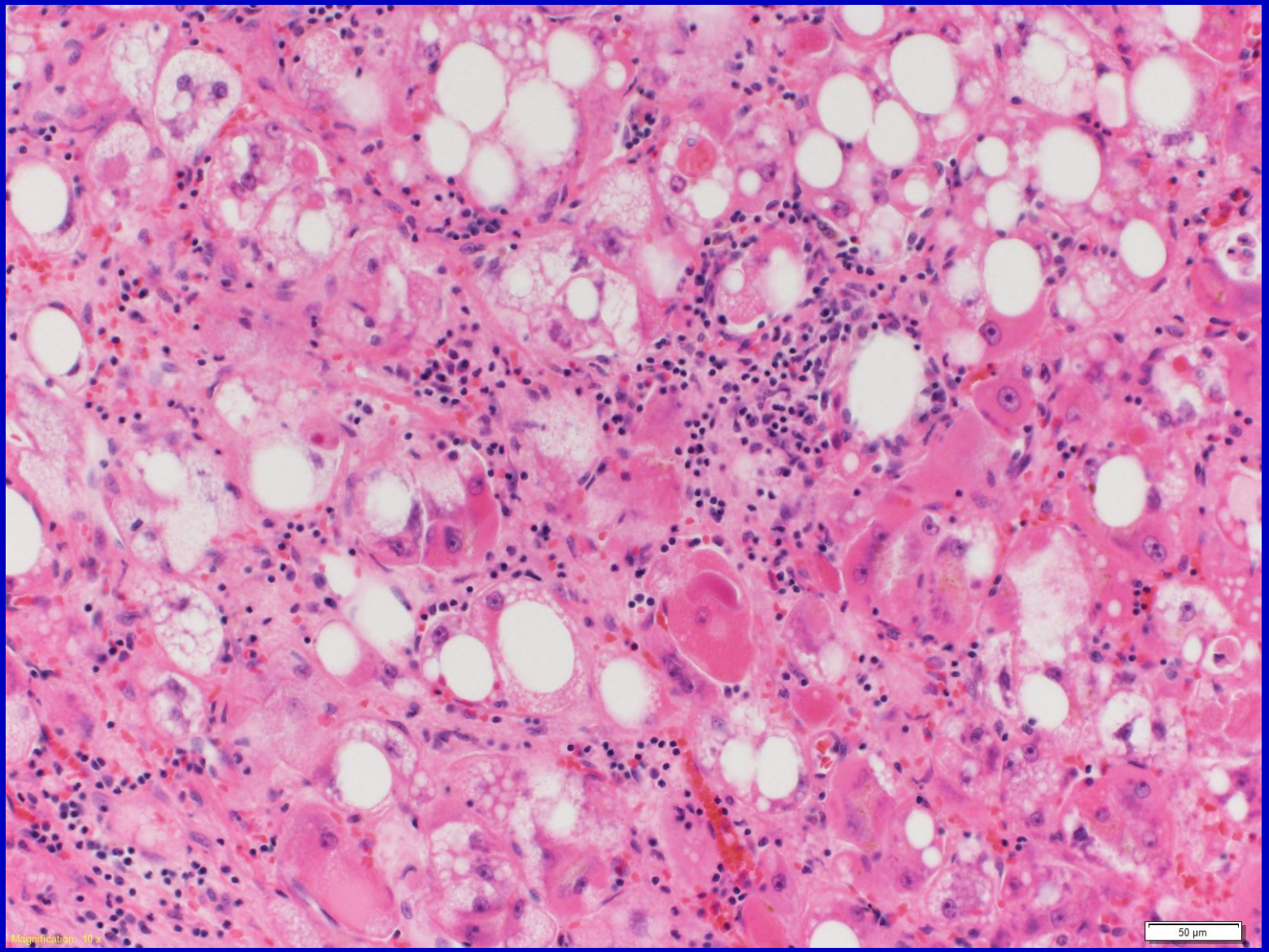

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Steatohepatitic areas

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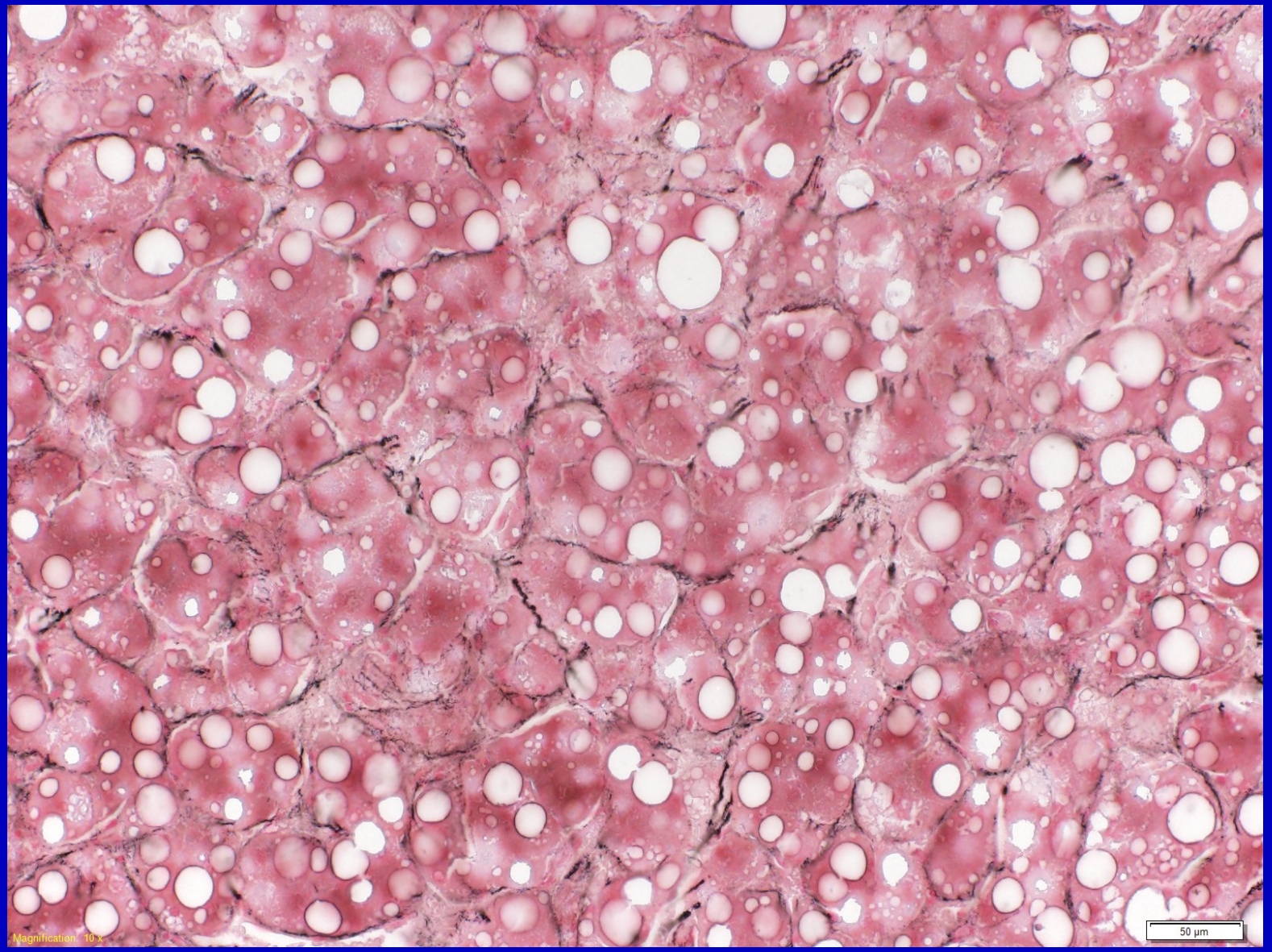

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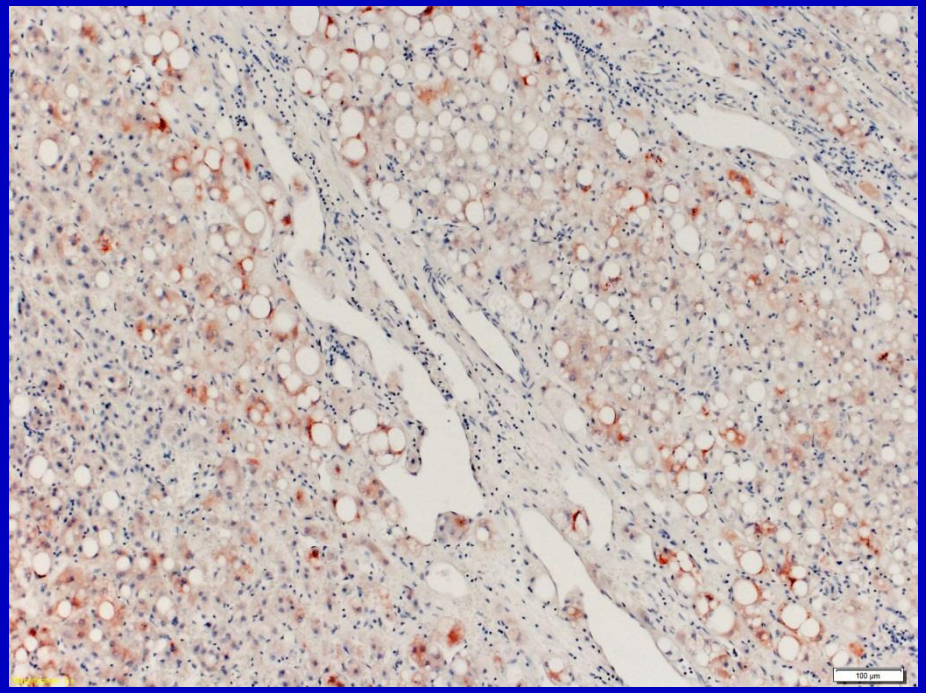
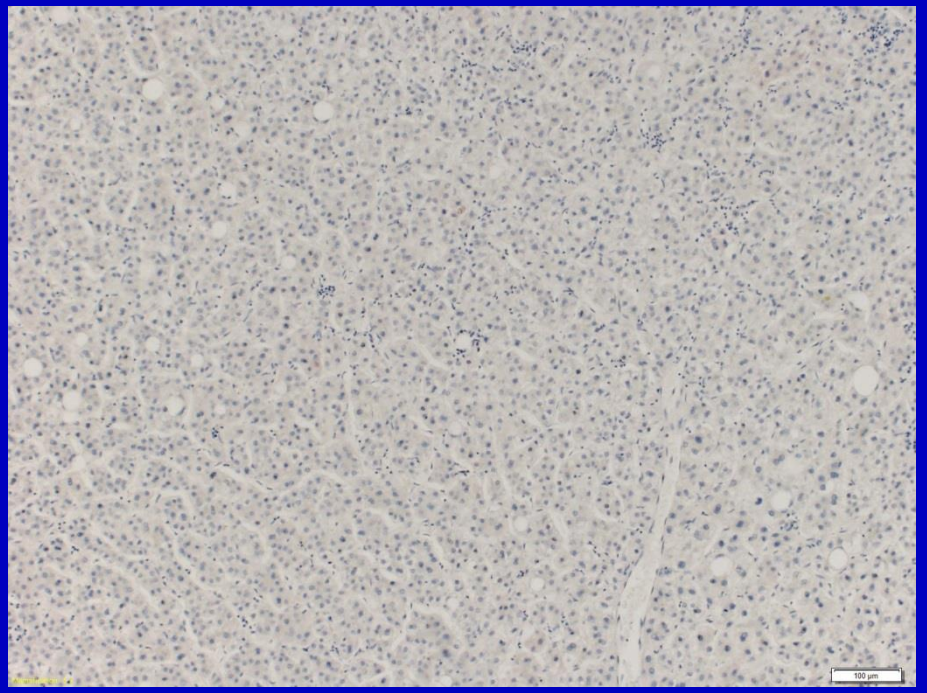
Loss of reticulin fibers

BASEL - B, 2014



Steatotic area



Trabecular area

Serum Amyloid A

Universitätsspital Basel

BASEL - B 2014

DIAGNOSIS

Well-differentiated hepatocellular carcinoma
(with focal steatotic/steatohepatitic features)

Discussion Points

1. HCC with features of steatosis/steatohepatitis
   (see “masterclass discussion”, circulation K1, case 449)

2. SAA positivity in hepatocellular lesions

SAA-positive Hepatocellular Nodules

1. **Inflammatory hepatocellular adenoma**
   - Commonest subtype of HCA (50-60%)
   - IL-6 /STAT3 activation (also express CRP)
   - Telangiectasia, inflammatory infiltrates and ductular reaction (FNH-like)
   - Associated with metabolic syndrome & steatosis in background liver

SAA-positive Hepatocellular Nodules

2. **SAA positive nodules in cirrhotic livers** (Sasaki Modern Pathology 2012)
   - 17 nodules in 7 patients with ALD – 15 had diffuse SAA expression
   - Resemble inflammatory HCA histologically
     - telangiectasia, inflammatory infiltrates and ductular reaction
     - no definite evidence of malignancy
   - 27/54 HCCs arising in cirrhotic livers were SAA-positive
     - Only 2 cases (both ALD cirrhosis) had diffuse SAA staining similar to that seen in inflammatory HCA – like nodules

   - Follow-up study showed STAT3 mutations in 2/17 SAA-positive hepatocellular lesions (Sasaki Histopathology 2014, in press)

SAA-positive Hepatocellular Nodules

3. SAA positivity in HCC arising in non -cirrhotic livers

- 4/74 cases of non-cirrhotic HCC had diffuse SAA-positivity **and** histological features in keeping with inflammatory HCA (Liu 2013)
- 4/19 cases of well-differentiated non-cirrhotic HCC were diffusely SAA-positive (Evason 2013)
- 4/14 cases of HCC arising from HCA were SAA positive – both in adenoma and HCC areas (Kakar 2014)

CONCLUSION:

- SAA immunostaining helpful in sub-typing cases of known HCA
- Not reliable in distinguishing HCA from well-differentiated HCC arising in non-cirrhotic liver.

Birmingham A/2014
(Stefan Hübscher)

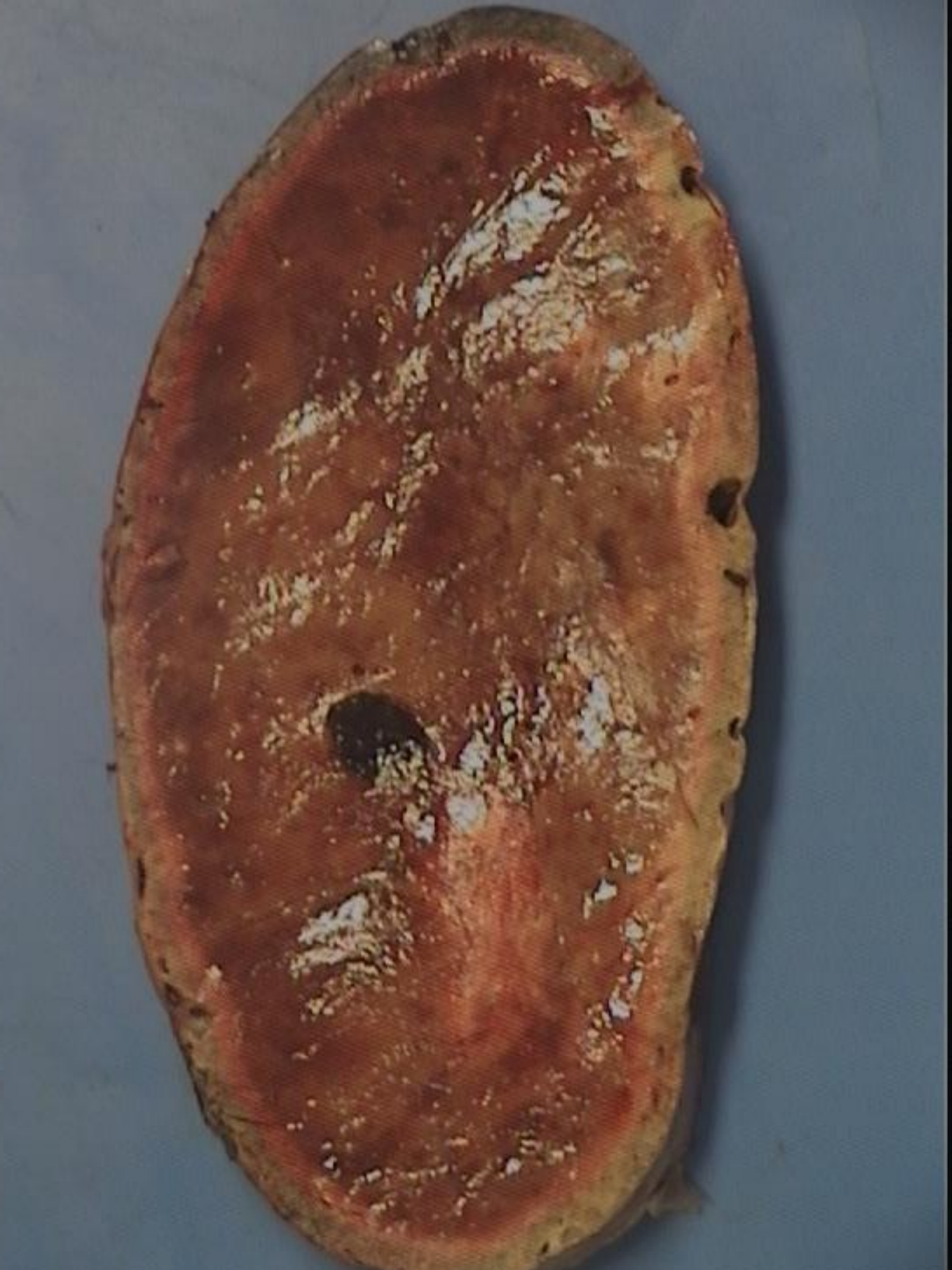
HCC arising from HCA

Birmingham A/2014 - Clinical Summary

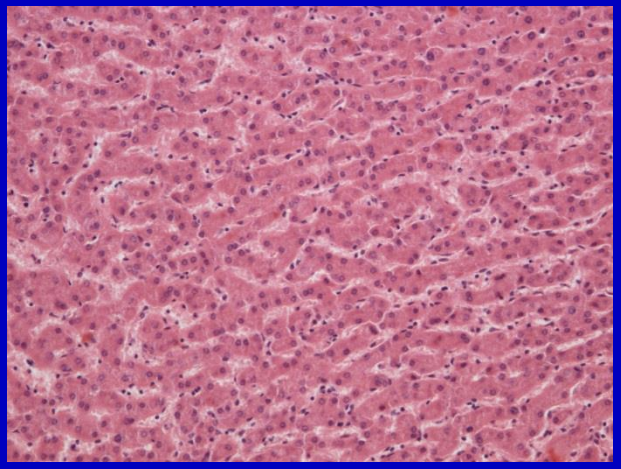
Female, age 27.

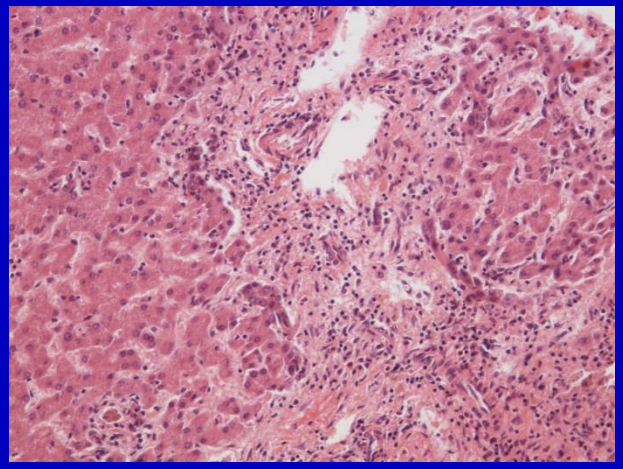
- Incidental liver mass discovered during early pregnancy assessment scan. Subsequently had a miscarriage at 6 weeks.
- Previous oral contraceptive pill use for 14 years.
- Nature uncertain radiologically - ? haemangioma, ?adenoma.
- Non-anatomical resection of large right lobe mass in segment 5/6.

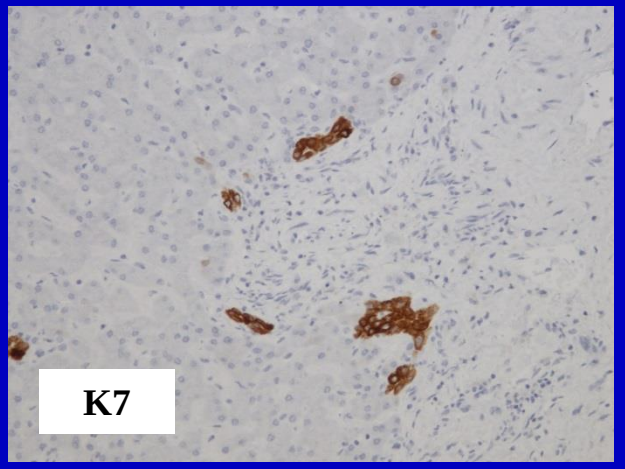
- Macroscopy showed a polypoid liver mass 17cm maximum dimension, weight 1030g, with prominent vascularity on the surface.
- Slicing revealed a clearly demarcated central haemorrhagic nodule 1.5cm diameter.

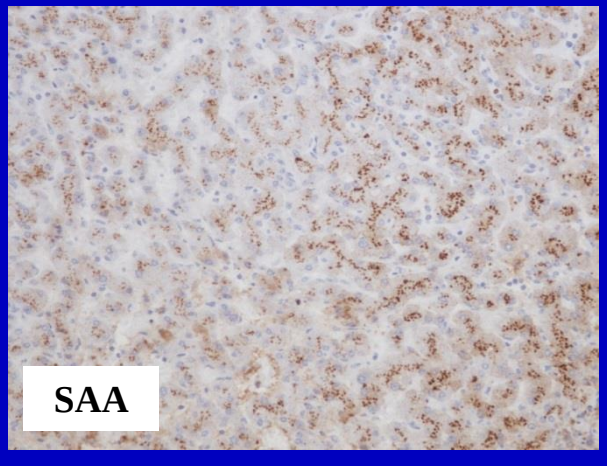


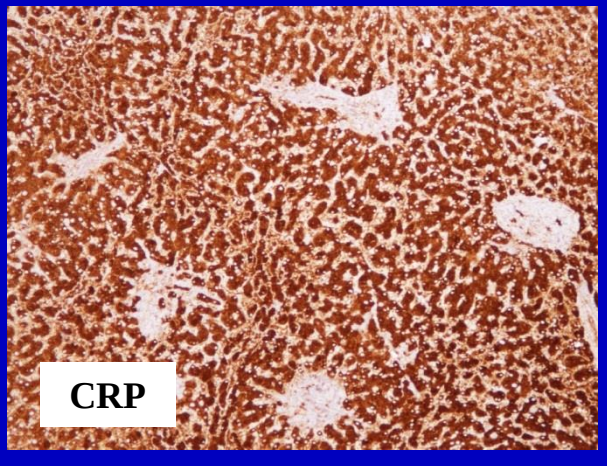
Microscopy – Main Lesion





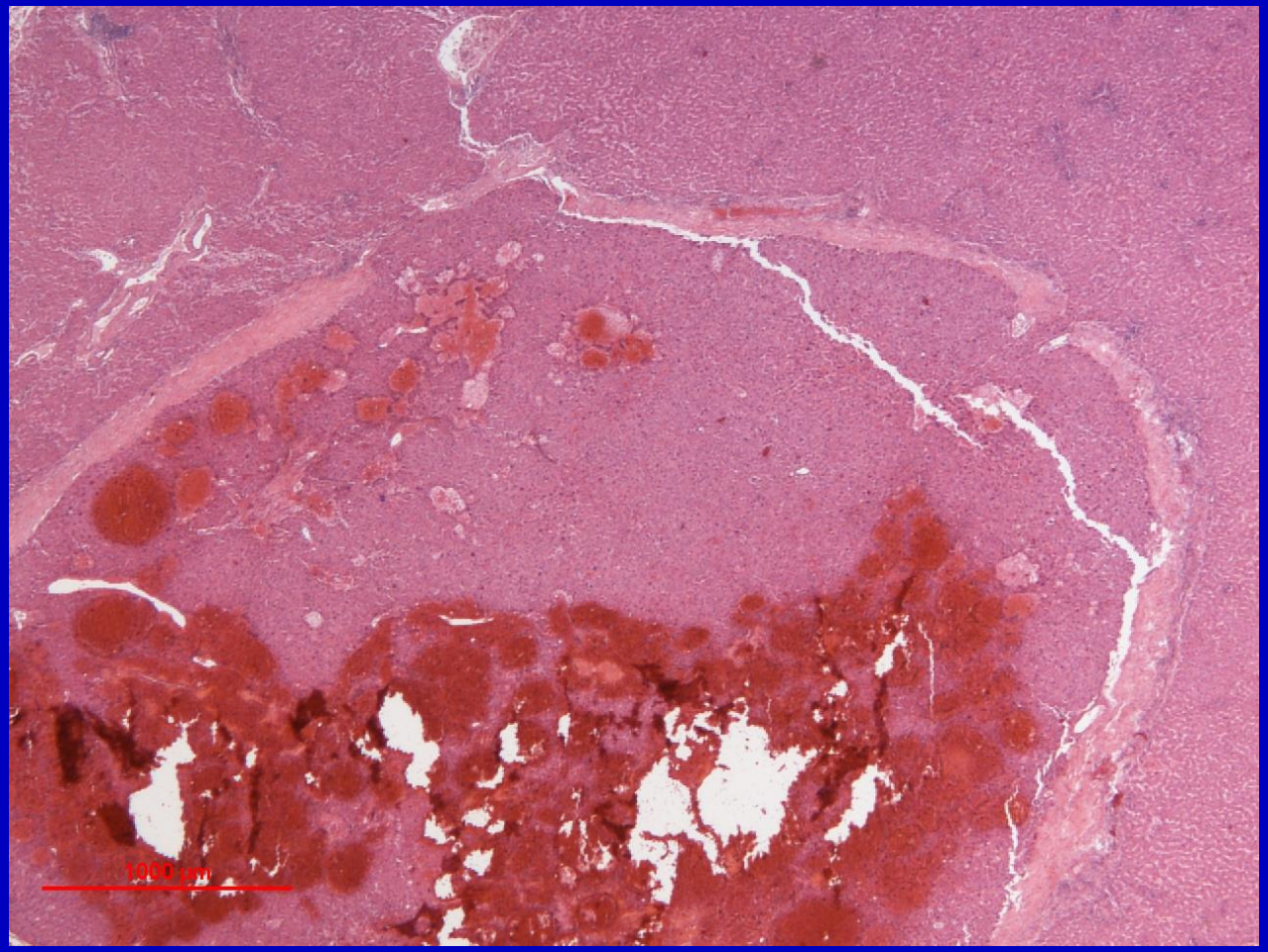






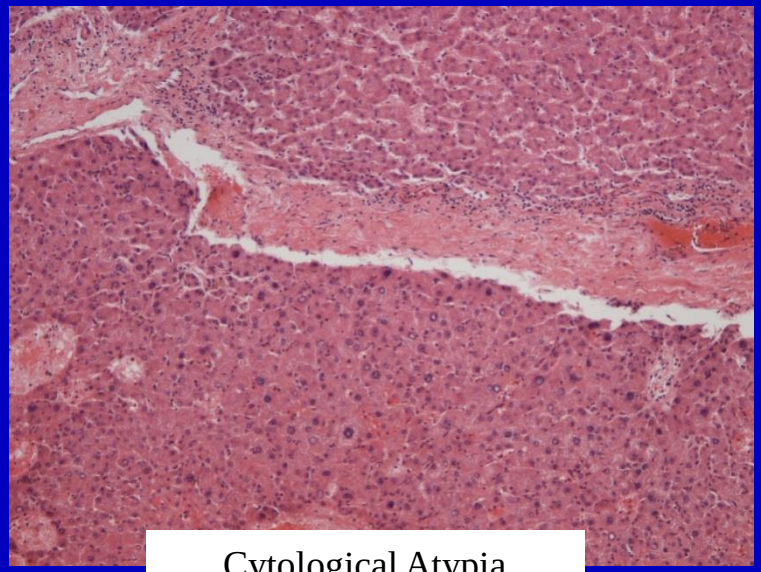
- Well-differentiated hepatocellular neoplasm
- Fibrovascular structures containing inflammatory cells and ductules
- Immunostains for SAA and CRP positive
- No cytological or architectural atypia

Microscopy – Central Nodule

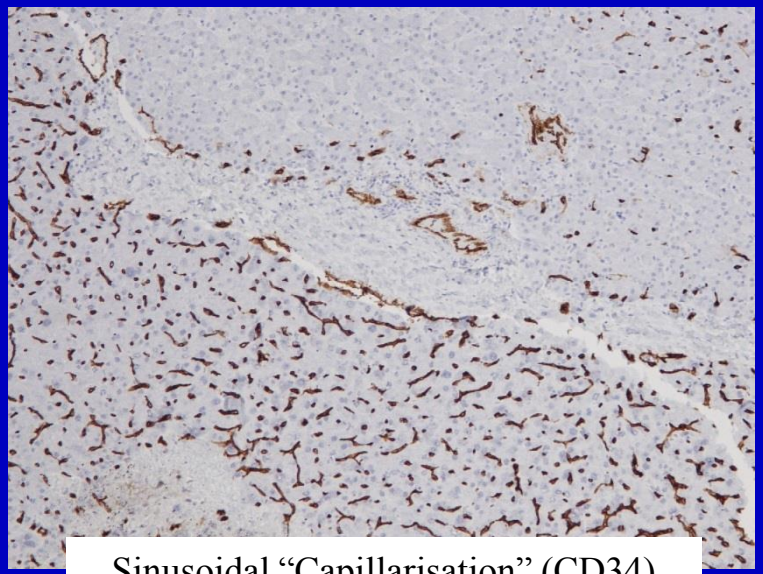


- Well-circumscribed, partly encapsulated
- Large blood-filled spaces (peliosis/haemangioma like)

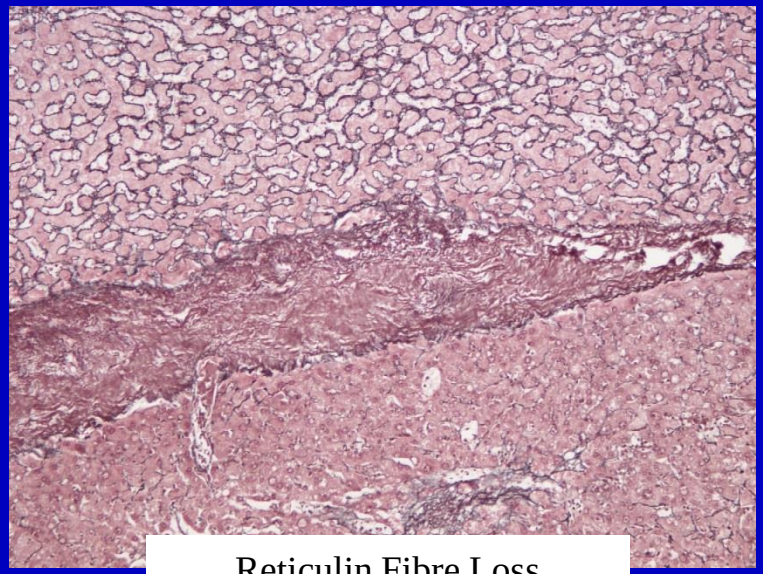
Microscopy – Central Nodule. Features Suggestive of Malignancy



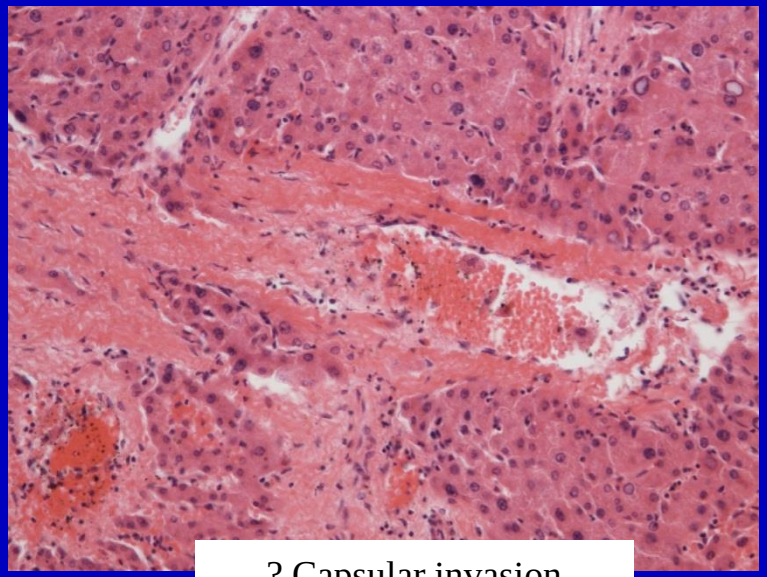
Cytological Atypia



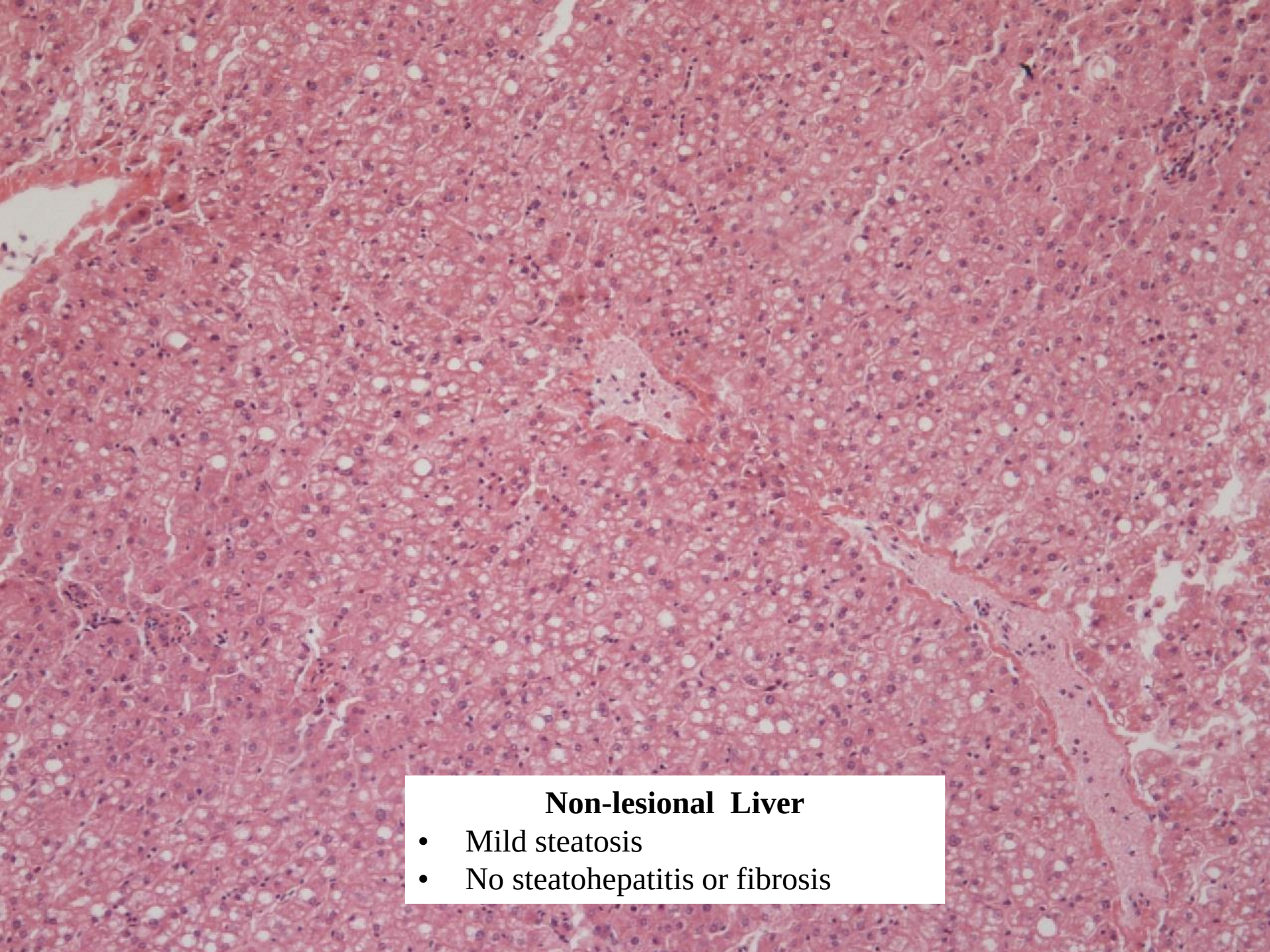
Sinusoidal “Capillarisation” (CD34)



Reticulin Fibre Loss



? Capsular invasion


Non-lesional Liver
• Mild steatosis
• No steatohepatitis or fibrosis

Birmingham A-2014 - Conclusion

- Well-differentiated HCC arising from inflammatory hepatocellular adenoma
  - No IHC evidence of beta-catenin mutation (HCC or adenoma)
- Background liver shows mild steatosis (presumed NAFLD – BMI=27)

Malignant Transformation of Hepatocellular Adenoma (HCA)

Prevalence :

- 68/1635 (4.2%) published cases of hepatocellular adenoma (Stoot 2010)
- 23/218 (10.5%) cases resected in Paris (Farges 2011)
- 4/57 (7%) cases resected in Edinburgh (Bellamy 2012)

Risk Factors

Tumour Size	> 90% occur in lesions > 5cm diameter (Stoot 2010, Farges 2011, Bellamy 2012)
Gender	Higher risk of malignant transformation in men (47%) than women (4%) (Farges 2011)
Beta-catenin mutation	30-40% β -cat mutated adenomas have features of malignant transformation (versus < 5% for non β -cat mutated HCA) (Zucmann-Rossi 2006, Bioulac-Sage 2007) 16/25 (64%) of adenomas with foci of malignant transformation had features of β -cat mutation (versus 5-15% of all HCAs) (Farges 2011)

HCC Arising from HCA – Pathological Features (25 cases studied by Farges 2011)

Macroscopic Features

- Well defined nodules > 1cm diameter (n= 8)
- Randomly distributed microscopic foci (n=17)
  - Microscopic pattern more common in men

Microscopic Features

- Usually well-differentiated (22/25)
- Vascular invasion/satellite nodules rare (3/25)

Hepatocellular Adenoma vs Well-Differentiated HCC
Features Favouring Diagnosis of Malignancy

Feature	Comment	Present in Bham A/2014
Nuclear pleomorphism/ hyperchromatism		+
Increased N/C ratio		+
Reticulin fibre loss	May be occur in areas of steatosis (including steatotic adenomas)	+
CD 34 – diffuse sinusoidal staining	Pan-lesional CD 34 in 17/64 adenomas (Bellamy 2013)	+
Glypican 3 expression	Present in 257/330 (78%) HCC vs 0/100 (0%) HCA (Coston 2008, Ligato 2008, Shafizadeh 2008,Wang 2008, Lagana 2013)	-
HSP 70 expression	Present in 14/ 30 (47%) HCC vs 0/18 HCA (Lagana 2013)	-
Chromosomal abnormalities (CGH)	Present in 11/12 (92%) HCC vs 0/12 HCA (Kakar 2009)	N/A

Does Non-Cirrhotic HCC Arise From Hepatocellular Adenoma?

NO

52 HCCs resected from non-cirrhotic liver (Rotterdam – Witjes, Gut 2012)

- no evidence for adenoma component

POSSIBLY

22/74 (30%) cases of non-cirrhotic HCC had features suggesting possible derivation from adenoma (St Louis - Liu, Mod Pathol 2013)

HCC Arising in HCA
(Kakar Mod Pathol, April 2014)

11 cases of HCC ex HCA

- 7 cases had atypical morphological features present in adenoma areas
  - Small cell change (7), pseudoacinar architecture (2), cytologic atypia (2), focal reticulin loss (3)

- 9 cases had similar immunohistochemical and cytogenetic features in adenoma and carcinoma portions of tumour
  - Nuclear beta catenin (4), diffuse GS staining (6), HSP positivity (4), chromosomal abnormalities (5)

- Adenoma-like areas may represent “extremely well-differentiated HCC that has progressed to a morphologically overt HCC leading to an appearance of HCC arising in an adenoma”

GRONINGEN CASE A
(Annette Gouw)

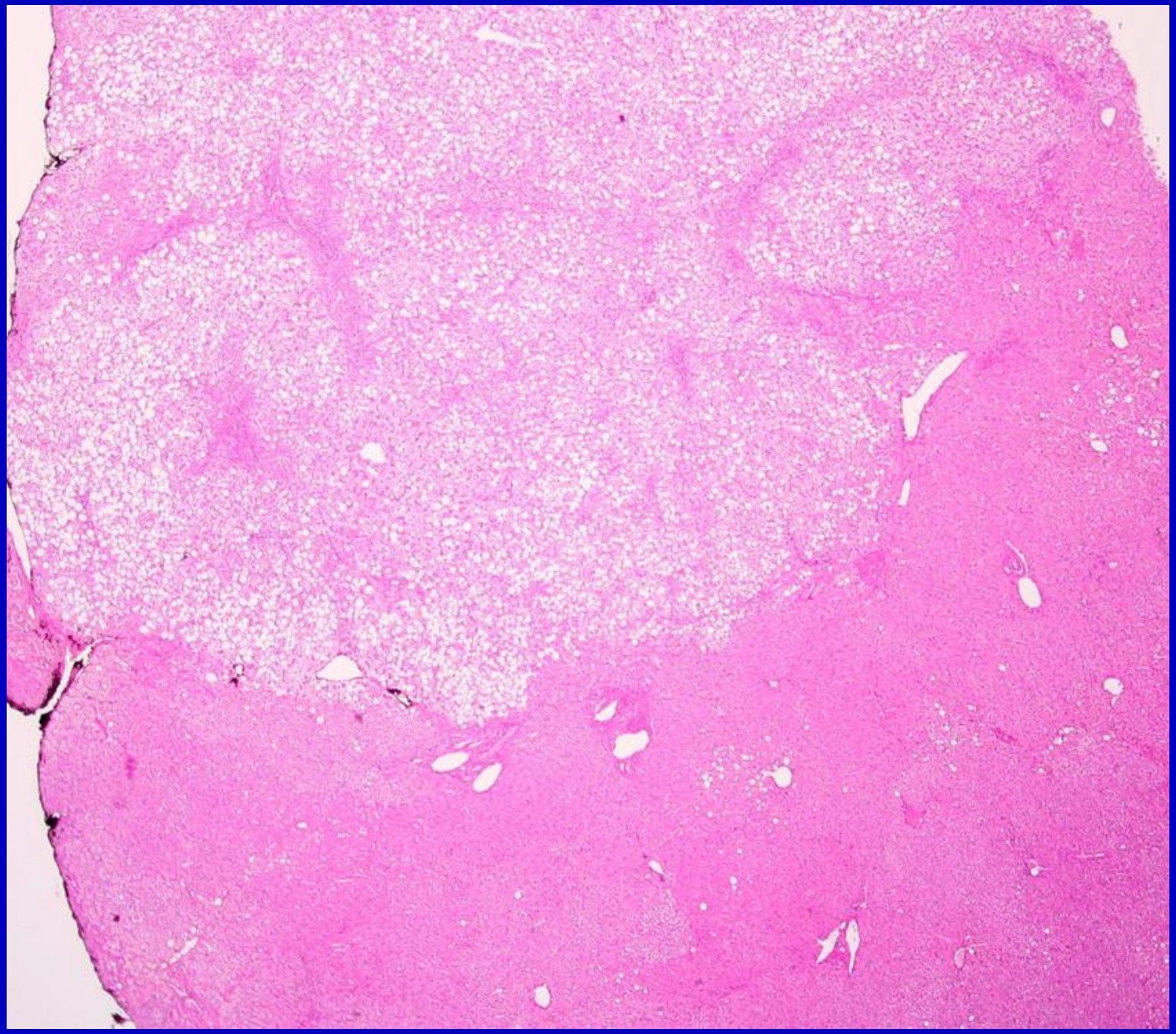
Is there an age limit for hepatocellular adenoma?

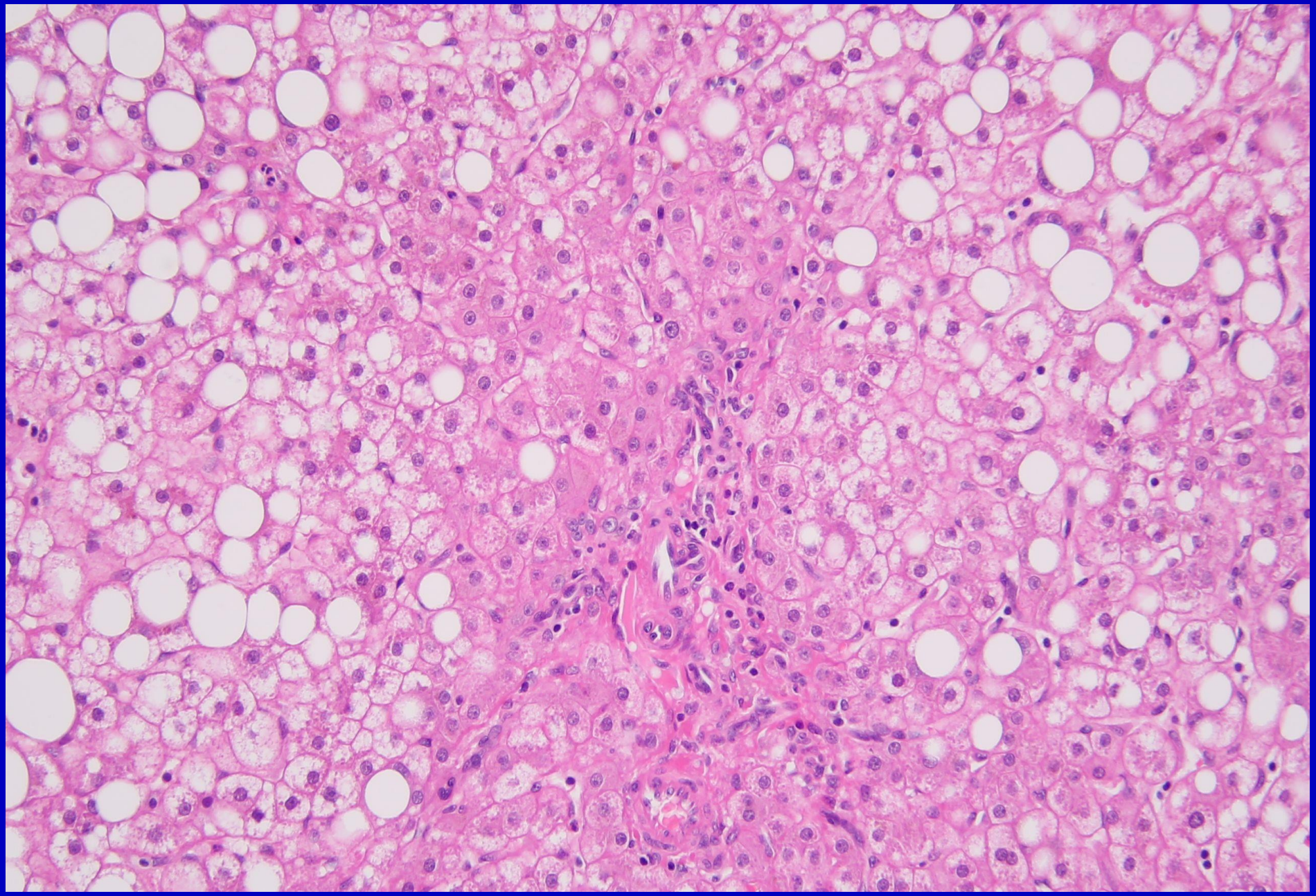
Case A: Clinical data

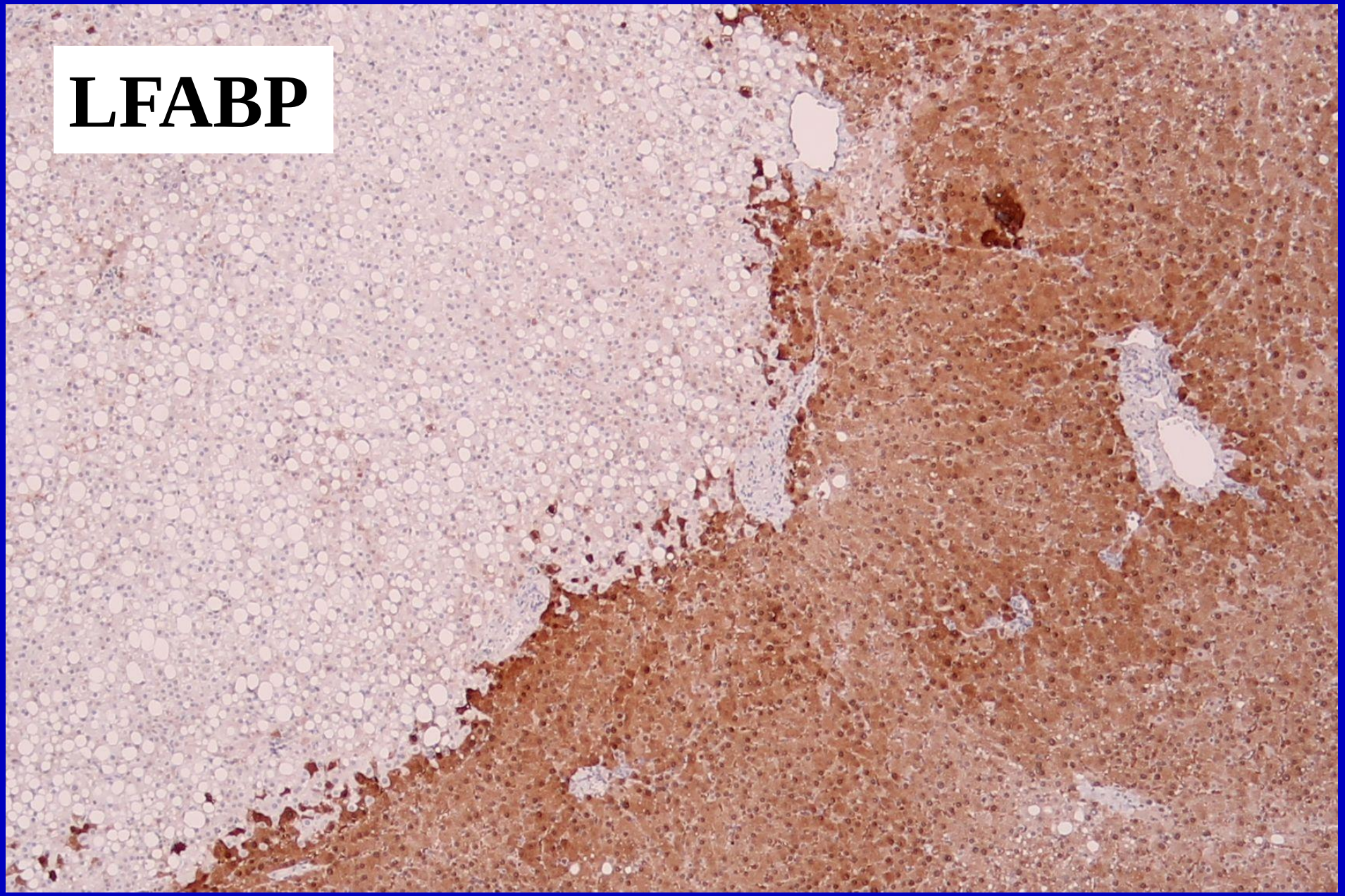
Female, 74 yrs

- Resection of liver segments II/III for a 3.4 cm lesion possibly CRC metastasis
- During surgery 3 other lesions identified (US), different aspect from the metastasis, located in segm IVB, VI and VII (all circa 1cm diameter)
- ***Local excision of lesion in segment IVB***
- Histopathology of the 3.4 cm lesion confirmed an intestinal type adenocarcinoma.

HE of the 1 cm lesion in segm IVB






LFABP

Diagnosis

- Small H-HCA (HNF1α mutated) without cytological/architectural atypia

The question of age and HCA

TABLE 2: Clinical data according to hepatocellular adenoma subtypes. (The patient with both H-HCA and IHCA is not included in the table.)

Characteristics	H-HCA	IHCA	b-HCA	Unclassified
Number of cases	9 (25 %)	20 (55.5%)	5 (14%)	2 (5.5%)
Age (years)	27–68	17–49	11–42	28, 32

(Fonseca, Int J Hepatol 2013)

Table 3. Final categorization of type in 64 adenomas

	Final adenoma type			
	Steatotic/LFABP↓	Inflammatory	GS-positive only	Unclassified
Patients (n = 36), no. (%)	12/36 (33)	17/36 (47)	4/36 (11)	3/36 (8)
Adenomas (n = 64), no. (%)	36/64 (56)	20/64 (31)	5/64 (8)	3/64 (5)
Male (n = 7), no. (%)	0/7*	5/7 (71)	1/7 (14)	1/7 (14)
Age (years), mean ± SD	48 ± 16	35 ± 6	44 ± 16	38 ± 11

(Bellamy C, Histopathology 2013)

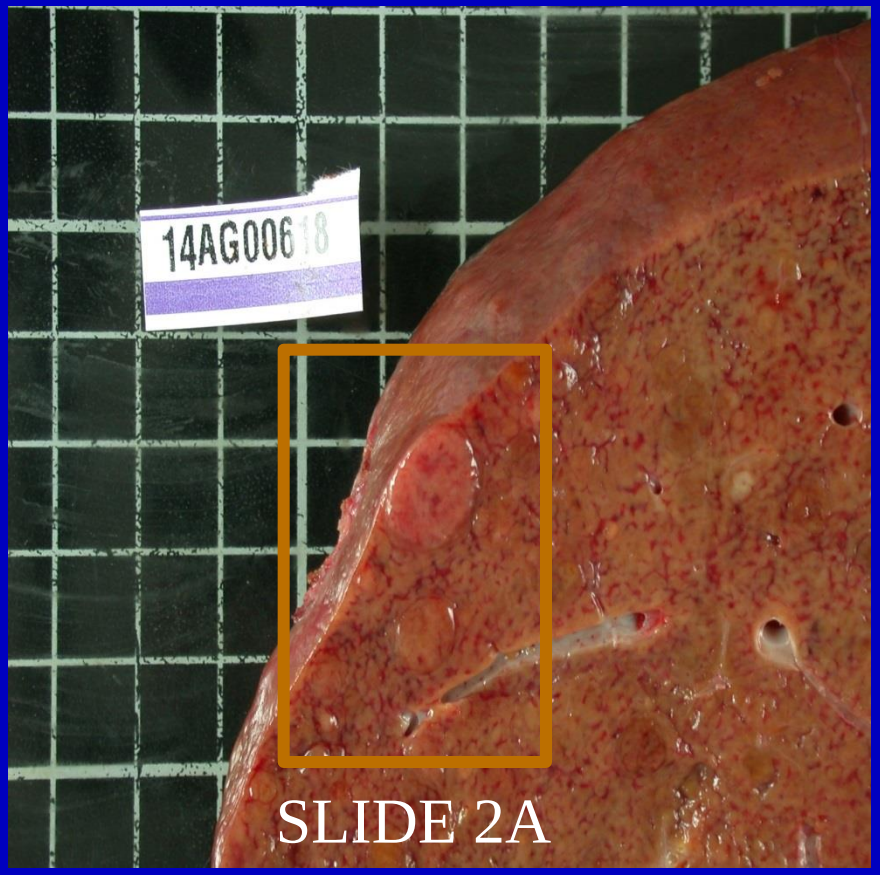
- hHCA can (probably) occur at any age

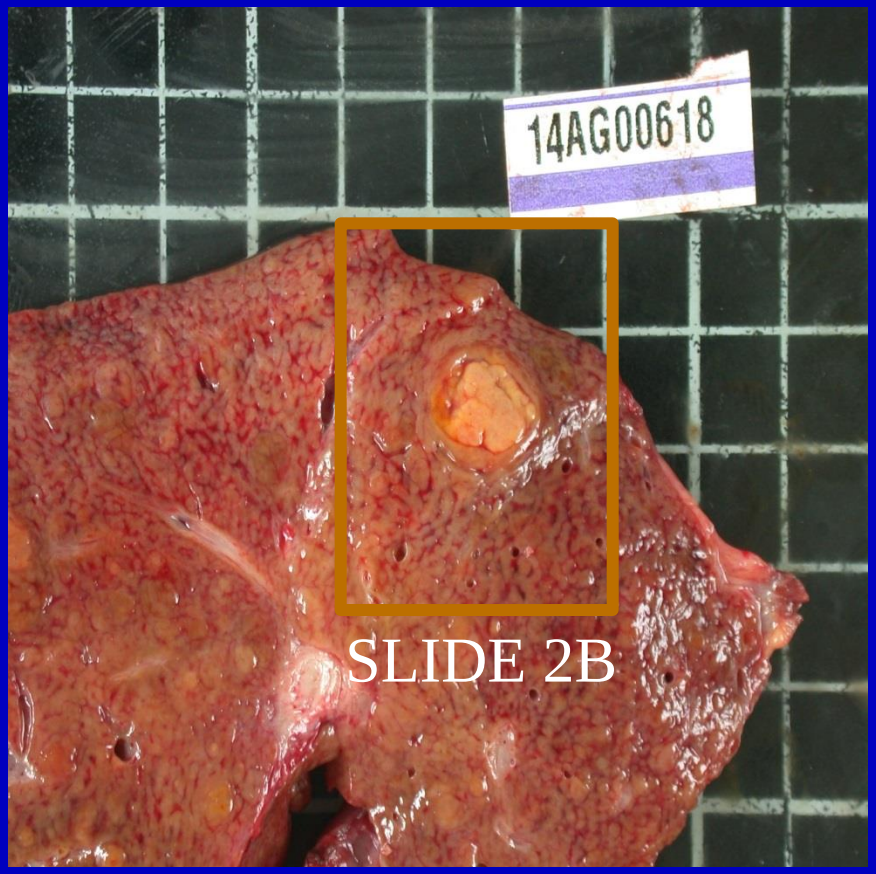
Paris – Case 2
(Pierre Bedossa)

Hepatocellular Nodules in Budd-Chiari Syndrome

Paris – Case 2

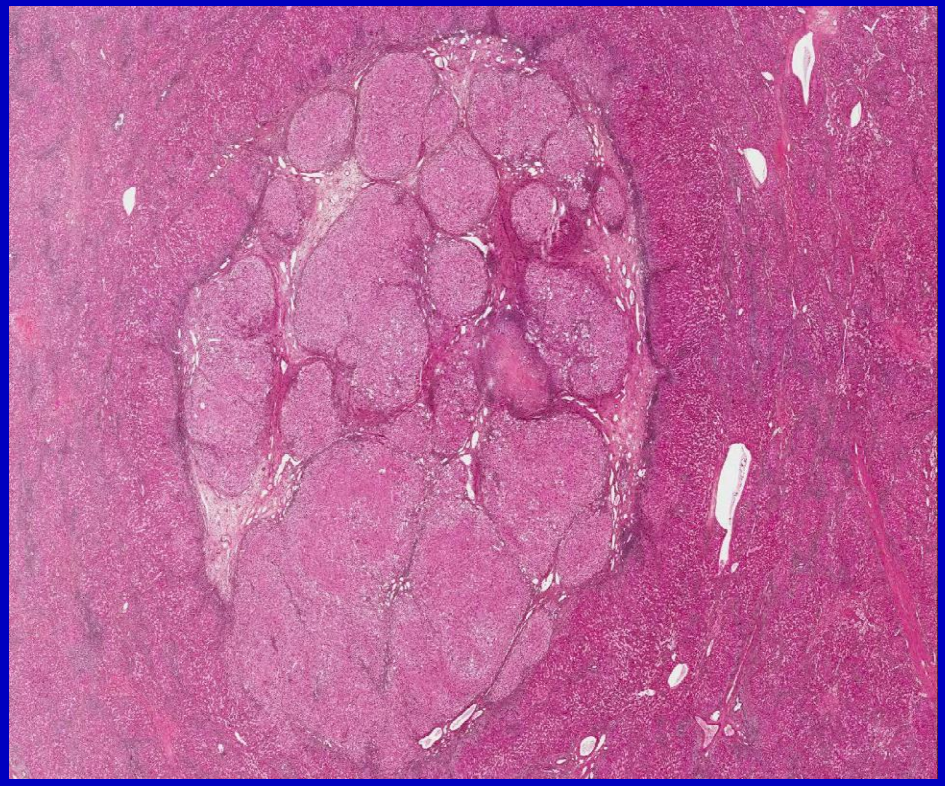
- A 32 years old lady with a history of chronic Budd Chiari syndrom.
- Severe portal hypertension treated initially with a Transjugular Intrahepatic Portosystemic Shunt
- On follow-up, she developed several nodules. Repeated Liver biopsies were suspicious for well-differentiated HCC
- A decision of transplantation was taken.
- On gross examination the liver contains many nodules of various size (Slide 2A and 2B)

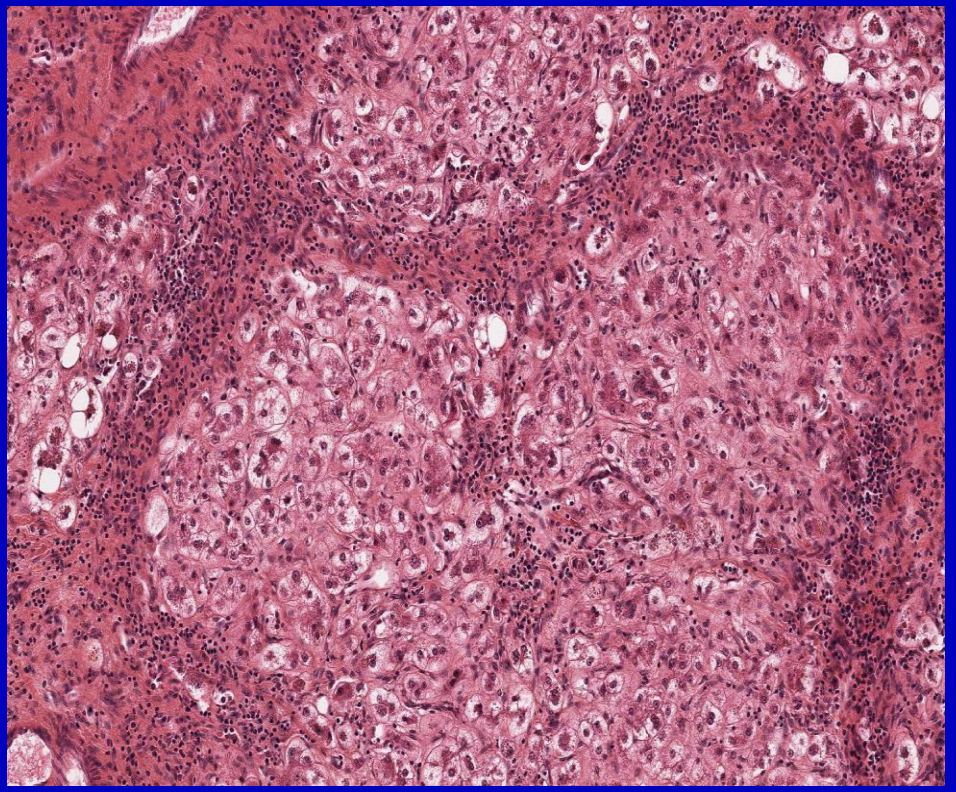




Slide 2A – typical features of FNH (2 nodules)
Slide 2B – more difficult to classify

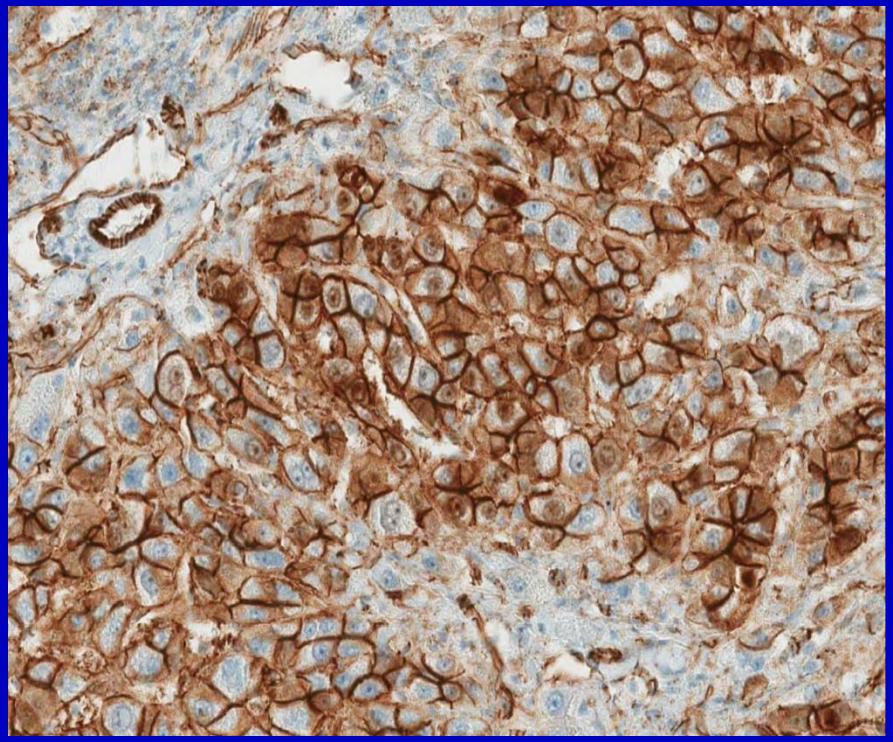
SLIDE 2B



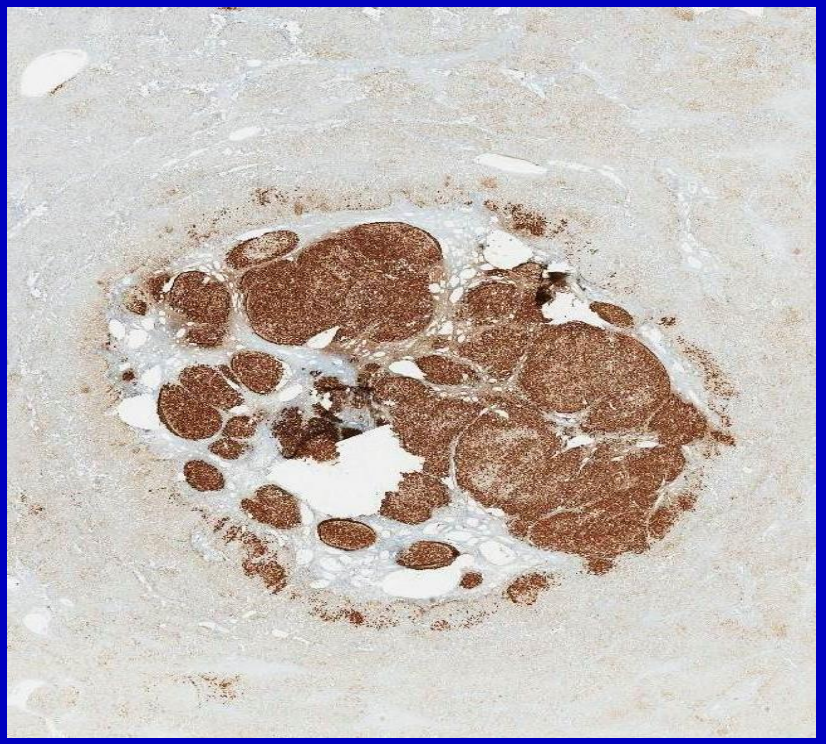


- Well-differentiated multinodular hepatocellular lesion
- Fibrous septa contain inflammatory cells, but no ductules

SLIDE 2B – Features Suggestive of Beta-Catenin Activation/Mutation



Nuclear beta-catenin expression



Diffuse glutamine synthetase expression

- **Paris Case 2**

  DIAGNOSIS :

  Chronic Budd Chiari syndrome with FNH-like nodules and multiple adenoma-like nodules with beta-catenin activation of uncertain malignant potential (HCC ?)

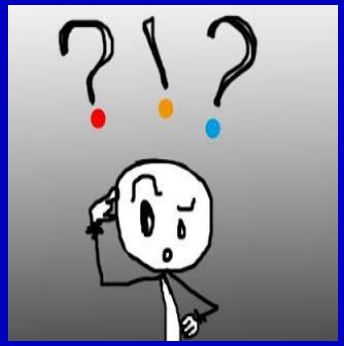
Nodules in chronic Budd Chiari Syndrom
Hôpital Beaujon experience (Moucari,Gut 2008)

- 97 consecutive patients with BCS and follow-up > 1 year (median 5 years).
- Detection of liver nodules in 43 patients (45%)
  - 11 HCC, histologically-proven
  - 32 benign nodules (10 histologically confirmed)

- Histology of benign nodules
  - FNH-like +++
  - Regenerative nodule without dysplasia

  - (Adenoma with Beta-Catenin activation - uncertain malignancy)

Questions



- Is Glutamine synthase diffuse expression enough specific to conclude for Beta-catenin activation ?
- If yes, is beta catenin activation related to a genetic mutation ? Should it be epigenetic or environmentally related (hypoxia) ?
- Should GS-diffusely positive nodules be called adenomas ?
- Is malignant potential similar to adenomas on normal livers ?

Well-differentiated hepatocellular neoplasms in which distinction between HCA and HCC is difficult.

How are these best classified?

"Atypical hepatocellular adenoma-like neoplasms"

(Evason, Human Pathology 2013; 44: 750-758)

Tumours that resembled HCA morphologically, but had one or both of the following atypical features:

1. **Atypical age/sex**
   - Men (any age)
   - Women aged ≥ 50
2. **Atypical morphology**
   - Focal cytologic or architectural atypia (small cell change, pseudogland formation, nuclear atypia) involving <5% of the tumour

Compared with typical HCA

- More frequently beta-catenin activated (35% vs 10%)
- More frequently have chromosomal abnormalities (59% vs 0%)

Outcome uncertain

- 4/14 with follow-up data had features suggestive of malignancy

"Well-differentiated hepatocellular neoplasm of uncertain malignant potential" (HUMP)

(Bedossa, Human Pathology 2014;45:658-60)

- Arose from discussions at 2013 Gnomes meeting (Noosa, Australia)

- 6/28 circulated cases difficult to classify:
  - All had histologic features compatible with HCA
  - Median diameter 83mm (range 30 -170)
  - 3 had features of pigmented ("black") adenoma , none steatotic
  - Focal areas of reticulin loss +/- slight nuclear crowding
  - No vascular invasion
  - No metastasis or recurrence after resection

"Well-differentiated hepatocellular neoplasm of uncertain malignant potential" (HUMP)

(Bedossa, Human Pathology 2014;45:658-60)

Table Proposed entities considered to represent well differentiated HUMP

1. Lesions with features of hepatocellular adenoma morphologically, but:

A. Focally histologically atypical
- Focal reticulin loss
- Focal cytological atypia (small cell change, nuclear atypia) in <5% of tumor (1) [a]
- Focal architectural atypia (pseudogland formation) in <5% of tumor (1) [a]

B. Genetically atypical
- β-Catenin activated tumors [b]

C. Clinically atypical
- Female >50y or <15y [a]
- Male

2. Lesions with features of hepatocellular carcinoma morphologically that can regress with treatment of underlying disease:

A. Anabolic steroid-induced tumors

[a] The precise degree of atypia and the age cut-offs are currently not known with certainty and require further study.
[b] Nuclear/cytoplasmic positivity for β-catenin without other features of atypia is of unknown significance at this time.

- Further letters in "Human Pathology" in press……

What Next?

- Collect cases of well-differentiated hepatocellular lesions difficult to classify ("HUMP")
  - Cases where whole lesion may be HUMP
  - Cases of HCC ex HCA (where background lesion may be HUMP)

- Control groups:
  - Typical HCA (hHCA, iHCA)
  - Well differentiated HCC (in non-cirrhotic liver)

- Morphological analysis
  - H&E, reticulin, trichrome
- Immunohistochemistry
  - CD34 beta-catenin, Glut Synthetase, GPC3 , L-FABP, SAA, HSP70, CRP, K19, K7
- Molecular analysis (L Terracciano, Basel)
  - high resolution array comparative genomic hybridization

And, finally……..

“The Ceremonial Transfer of the Gnomes Hat”

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UNIVERSITY OF BIRMINGHAM

Welcome to the Gnomes in Birmingham 2015 !

(Theme for circulated Cases – “Acute hepatitis/acute liver failure”)

University Hospitals Birmingham NHS Foundation Trust

Does Non-Cirrhotic HCC Arise From Hepatocellular Adenoma?

NO

52 HCCs resected from non-cirrhotic liver (Rotterdam – Witjes, Gut 2012)

- no evidence for adenoma component

POSSIBLY

22/74 (30%) cases of non-cirrhotic HCC had features suggesting possible derivation from adenoma (St Louis - Liu, Mod Pathol 2013)

1. Morphological features compatible with HCA and HCC in same lesion (n=10)
2. Steatotic tumour lacking LFABP staining (n=4)
3. Diffuse SAA expression and features compatible with inflammatory adenoma (n=4)
4. Nuclear/cytoplasmic beta-catenin and diffuse glutamine synthetase (n=4)

HCC Arising from HCA - Molecular Evidence for Adenoma-Carcinoma Sequence

(Pilati; Cancer Cell 2014; 25: 428-441)

Genomic profiling of 250 tumours from 195 patients

- 223 classical HCA (no suspicion of malignancy)
- 18 borderline lesions
- 9 HCCs derived from HCA

Progressive alteration seen in malignant transformation: