



# Keratin 7 immunohistochemistry in medical liver diseases

Stratigoula Sakellariou<sup>1</sup> & Dina G. Tiniakos<sup>2,3</sup>

<sup>1</sup>1<sup>st</sup> Dept of Pathology, <sup>2</sup>Dept of Pathology, Aretaieion Hospital,

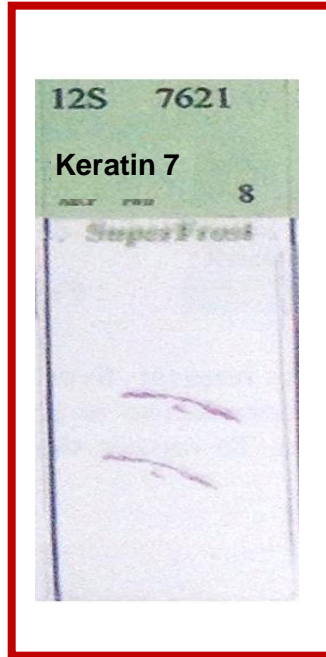
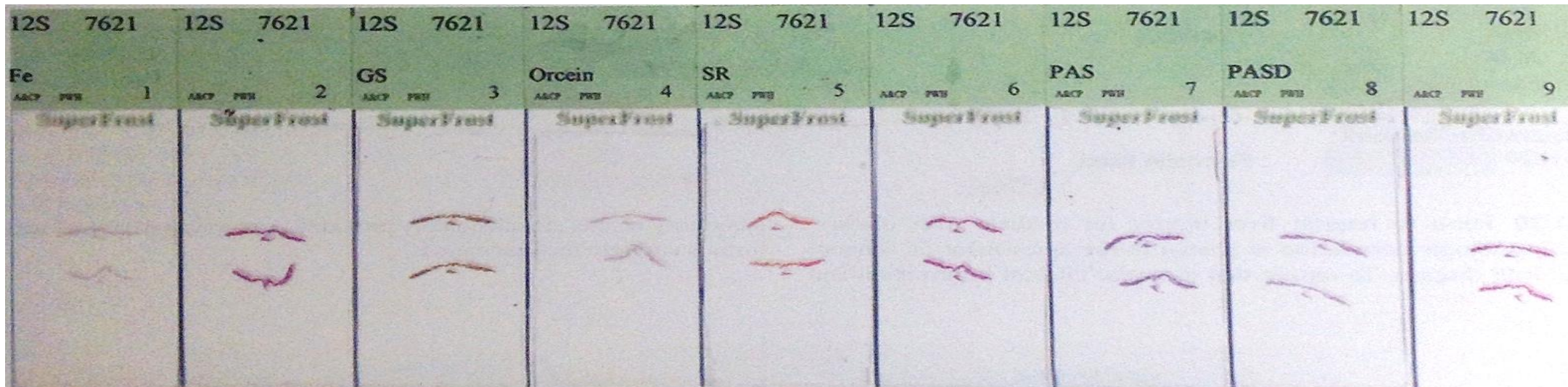
Medical School, National and Kapodistrian University of Athens, Greece

<sup>3</sup>Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, UK

# Layout

- Introduction
- Brief literature review
- Recent work “K7 expression in hepatic cholestatic diseases”
- Automated analysis of K7
- Take home messages

# The liver panel

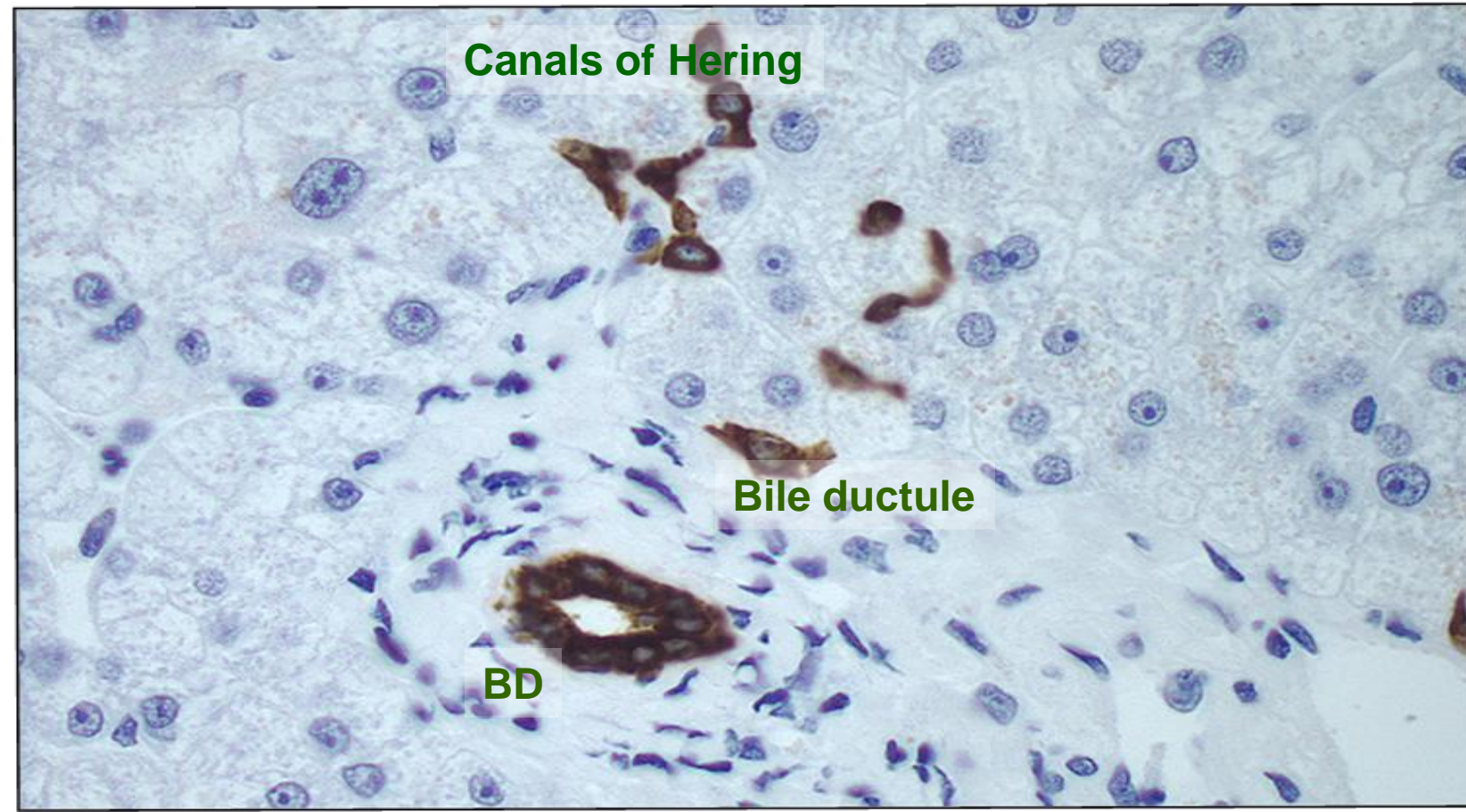


**Keratin 7 immunostain is one of the most valuable stains in medical liver biopsy diagnosis**

# Normal human liver

## Keratin 7 highlights

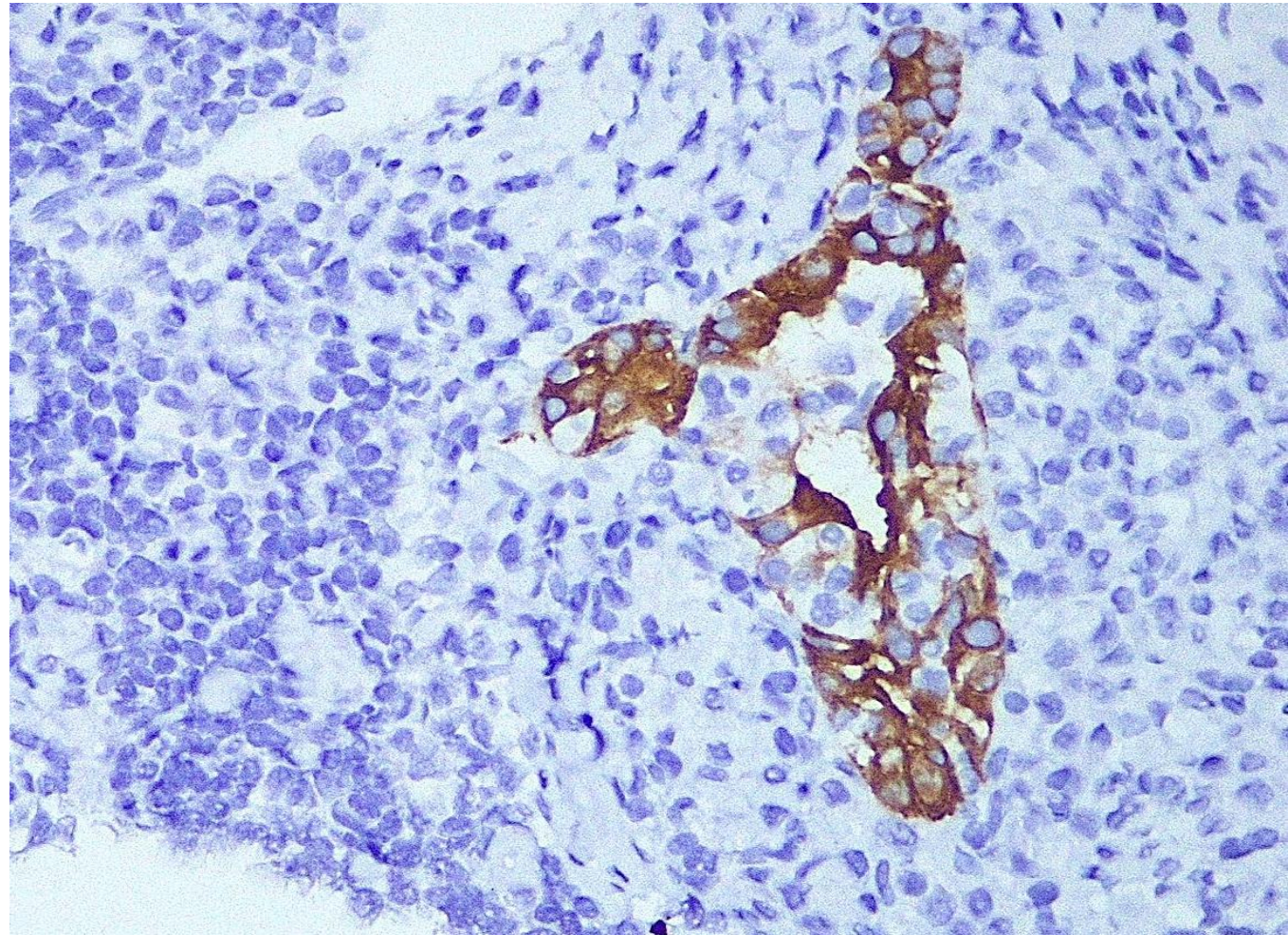
- Native bile ducts and ductules
- Canals of Hering



# Liver diseases

## Keratin 7 reveals

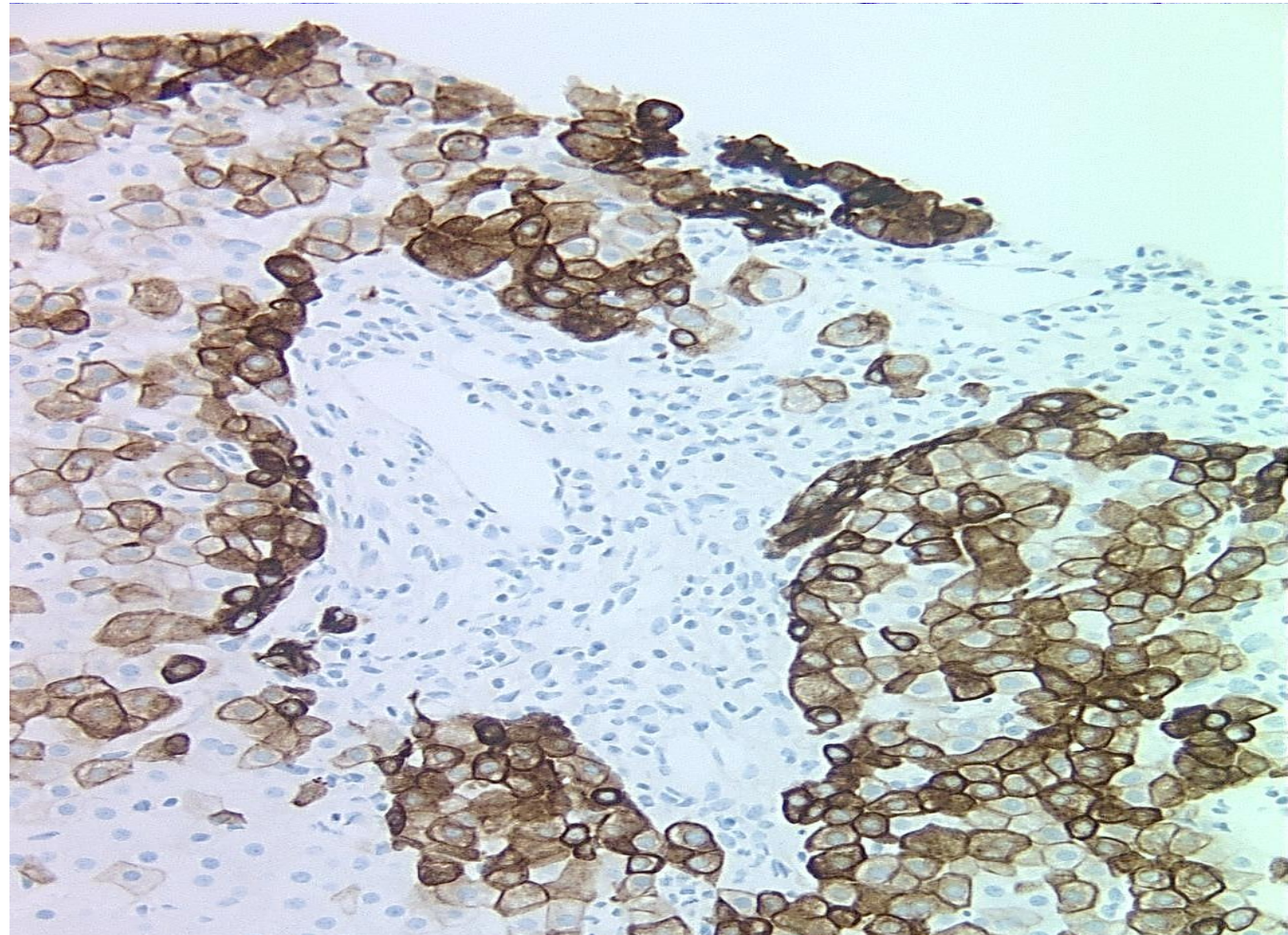
- Florid bile duct lesions



# Liver diseases

## Keratin 7 confirms

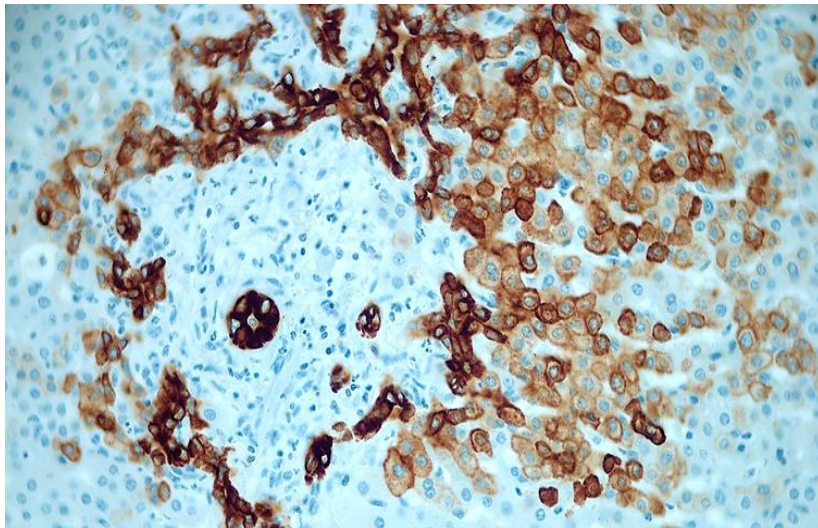
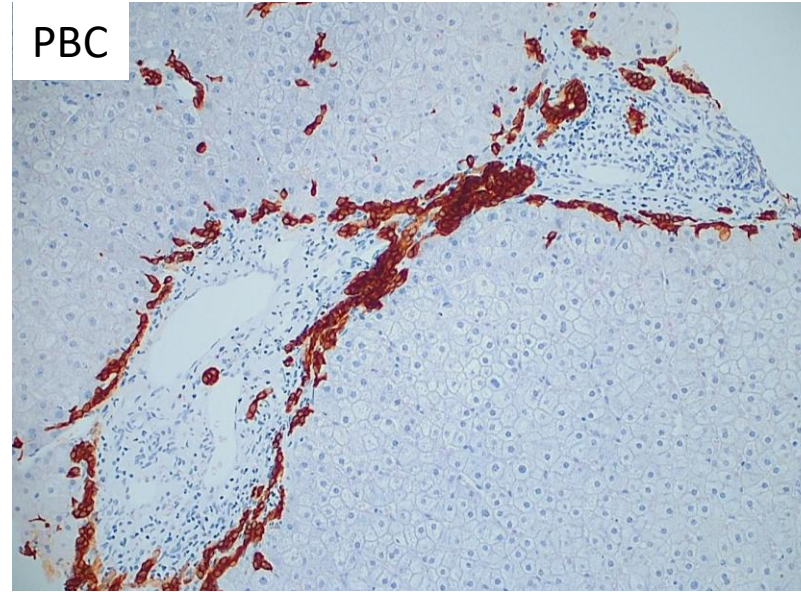
- Absence of native bile ducts



# Liver diseases

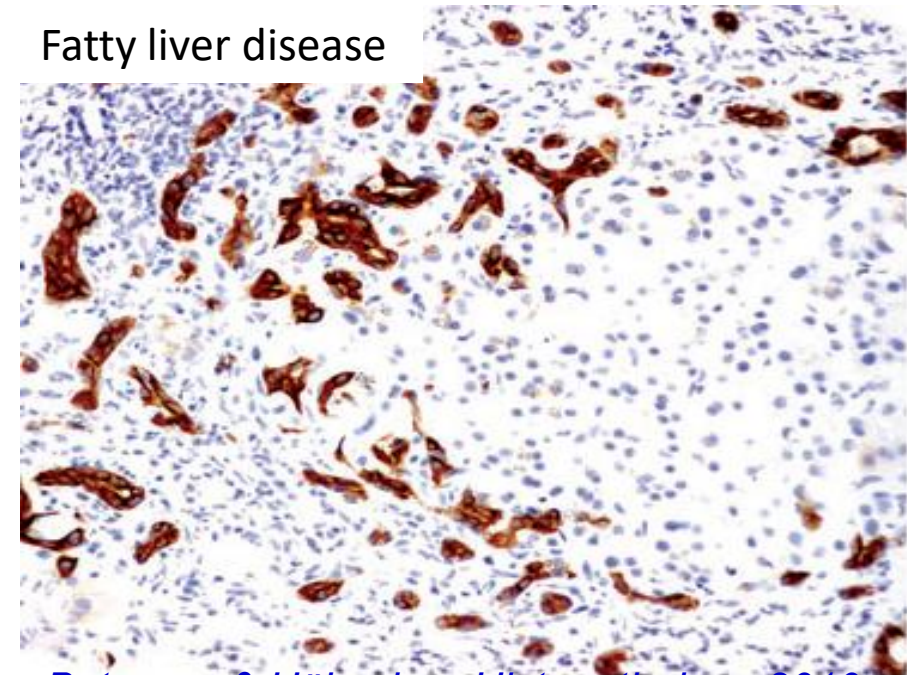
## Keratin 7 highlights

- Ductular reaction



Large bile duct obstruction

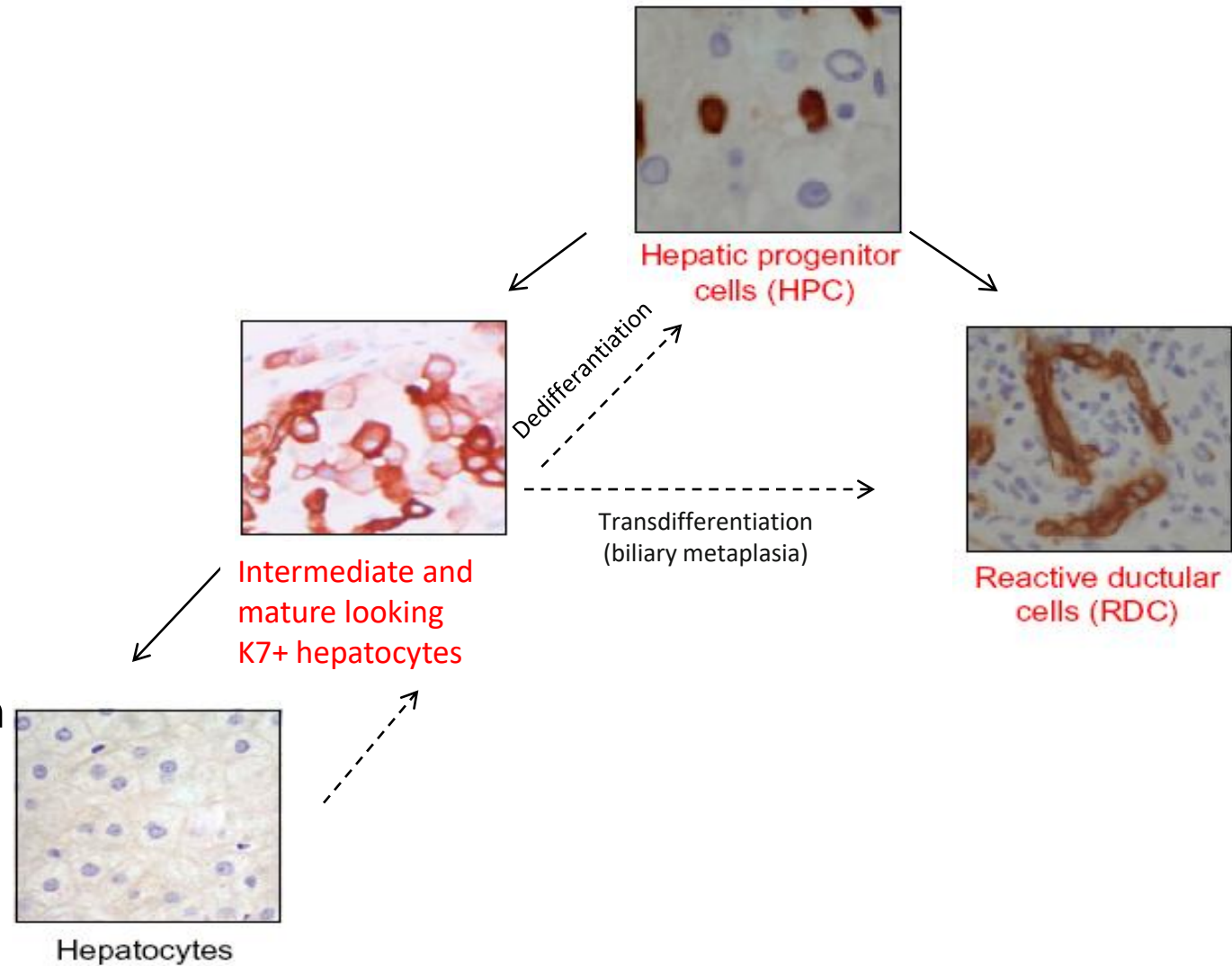
Fatty liver disease



# Liver diseases

## Keratin 7 highlights

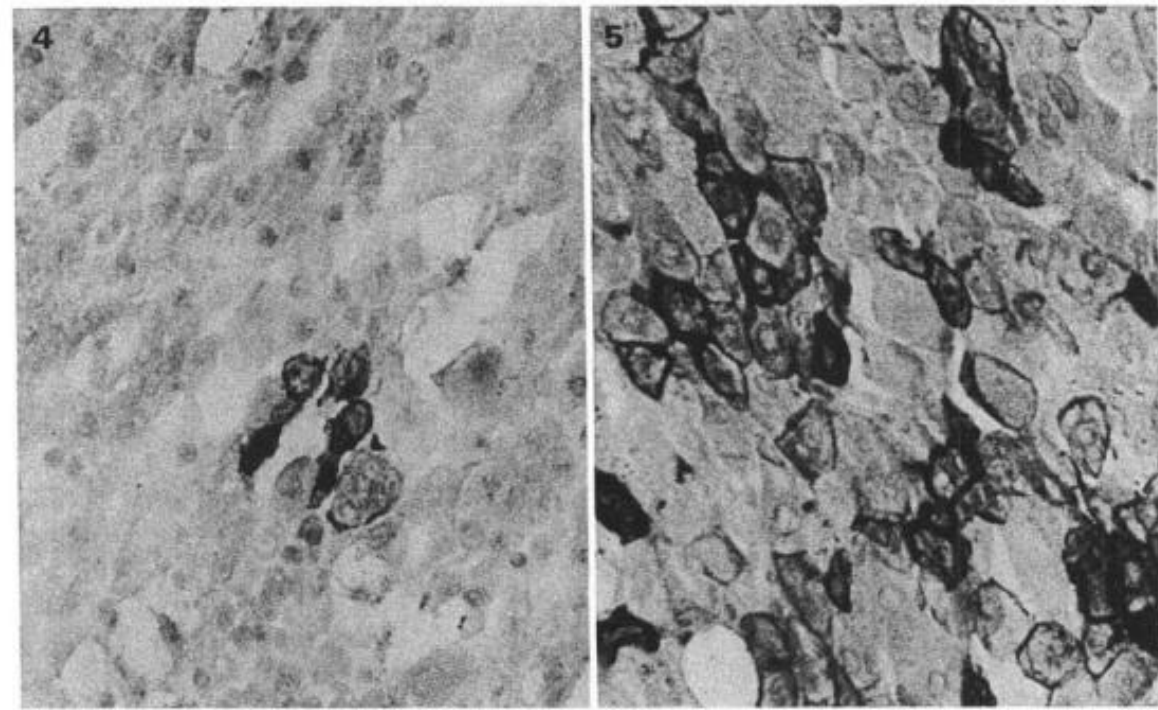
- Hepatic Progenitor Cells (HPC)
- Intermediate cells during differentiation of HPC to hepatocytes
- Intermediate cells during dedifferentiation of hepatocytes to HPC
- Hepatocytes as a metaplastic phenomenon in chronic cholestasis and hypoxia



# A cytokeratin immunohistochemical study of alcoholic liver disease: evidence that hepatocytes can express 'bile duct-type' cytokeratins

P.VAN EYKEN, R.SCIOT & V.J.DESMET

*Laboratory for Histo- and Cytochemistry, Pathology Department II,  
UZ St Rafaël, Catholic University of Leuven, Belgium*

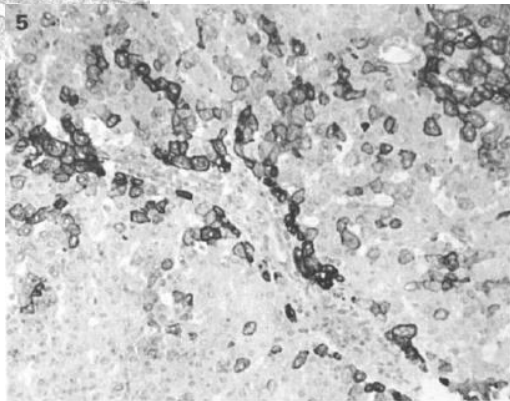
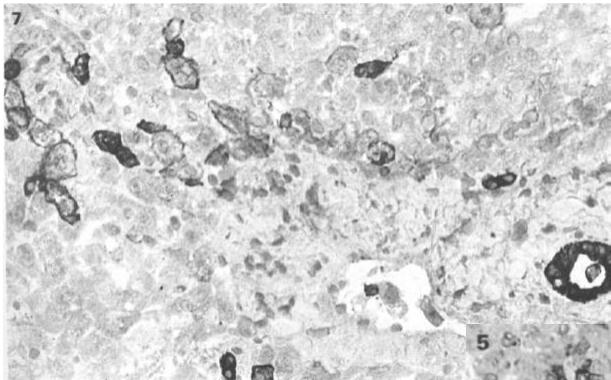


- In steatosis few K7 (+) hepatocytes in zone 3
- In alcoholic hepatitis K7 (+) hepatocytes in zones 3 and 1
- K7 (+) hepatocytes in acinar zone 3 greatly exceed zone 1 positive cells
- In cirrhosis in the center and the periphery of nodules

# A cytokeratin immunohistochemical study of cholestatic liver disease: evidence that hepatocytes can express 'bile duct-type' cytokeratins

P.VAN EYKEN, R.SCIOT & V.J.DESMET

*Histopathology* 1989.



Diagnosis (no. of cases)	Hepatocytes positive with monoclonal anti-cytokeratin no. 7†			Hepatocytes positive with monoclonal anti-cytokeratin no. 19†		
	PV	PP/PS	Both	PV	PP/PS	Both
Primary biliary cirrhosis						
stage I (3)	0	0	0	0	0	0
stage II (2)	0	0	2	0	0	0
stage III (4)	0	1	3	1	0	0
stage IV (4)	0	0	4	0	1	0
Primary sclerosing cholangitis (3)	0	0	3	0	3	0
Extrahepatic biliary obstruction (8)	0	2	3	0	0	0
Toxic/drug-induced liver disease						
'simple cholestasis' (10)	1	0	2	0	0	0
hepatitis (4)	0	0	2	0	0	0

PV =perivenular (acinar zone 3); PP=periportal (acinar zone I); PS=periseptal;

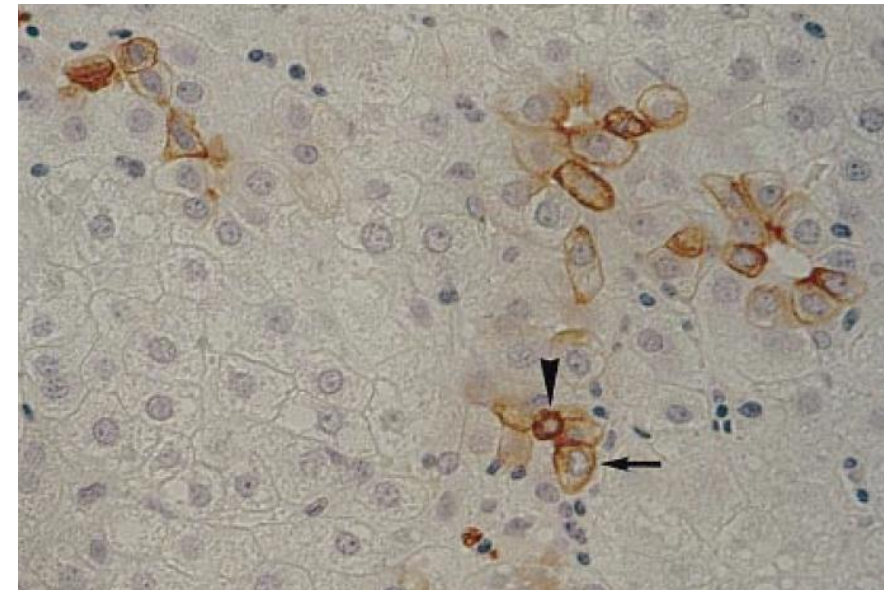
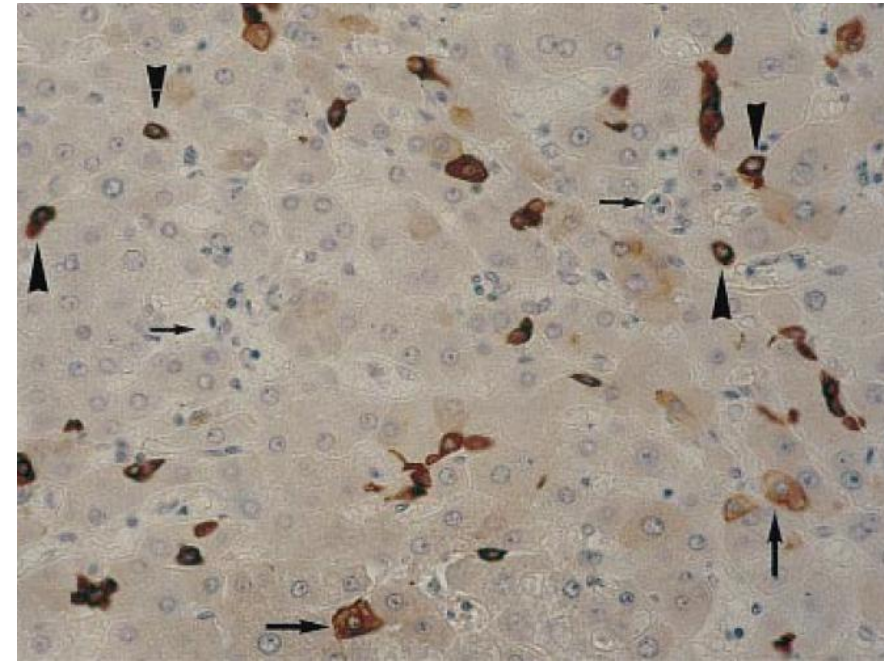
Original Paper

**Deep intralobular extension of human hepatic ‘progenitor cells’ correlates with parenchymal inflammation in chronic viral hepatitis: can ‘progenitor cells’ migrate?**

Louis Libbrecht\*, Valeer Desmet, Boudewijn Van Damme and Tania Roskams

**K7(+)** intermediate hepatocyte-like cells

- scattered throughout the parenchyma surrounding HPC
- differentiation towards hepatocytes
- association with moderate/severe lobular inflammation



## Hepatic progenitor cells in chronic hepatitis C: a phenomenon of older age and advanced liver disease

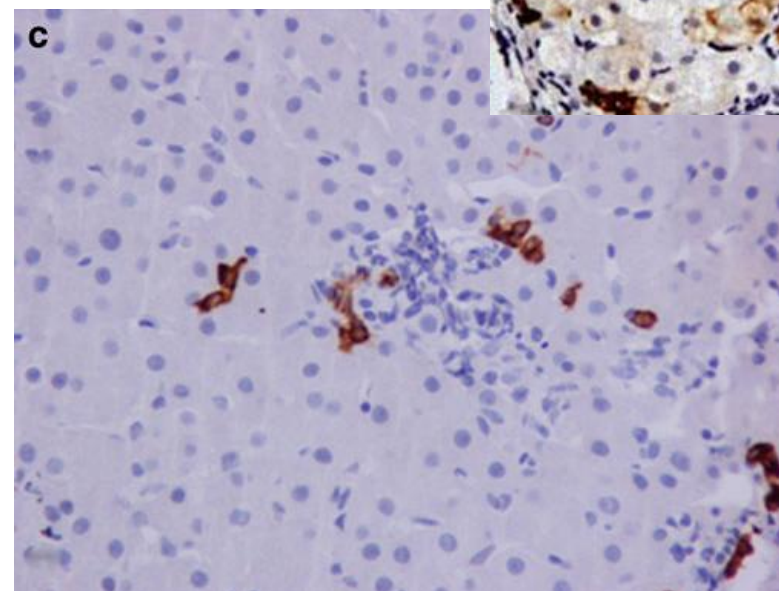
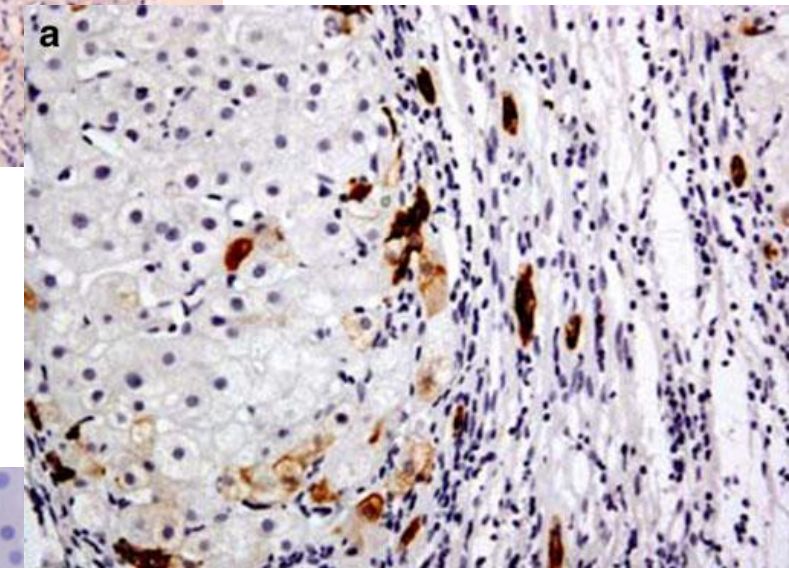
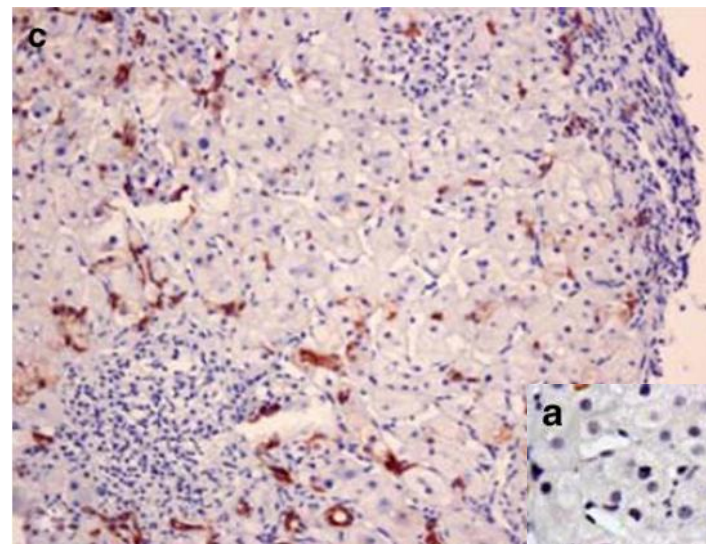
Johanna Delladetsima · Paraskevi Alexandrou ·  
Konstantinos Giaslakitiotis · Mina Psychogiou ·  
Gregory Hatzis · Vana Sypsa · Dina Tiniakos

### Periportal pattern

- HPC, ductular structures, K7(+) hepatocytes
- correlation with older age, advance stage

### Lobular pattern

- HPC, ductular structures around focal necrosis, few K7 (+) hepatocytes
- correlation with younger age, lower stage



## Progenitor cell expansion: an important source of hepatocyte regeneration in chronic hepatitis

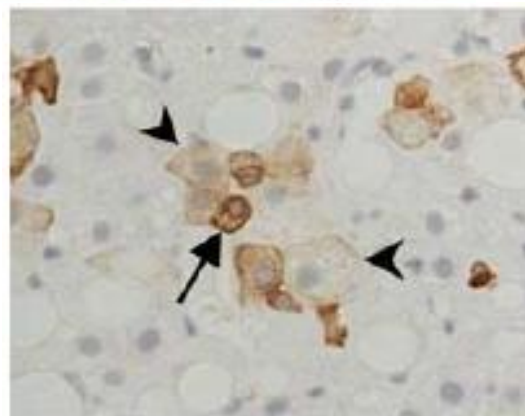
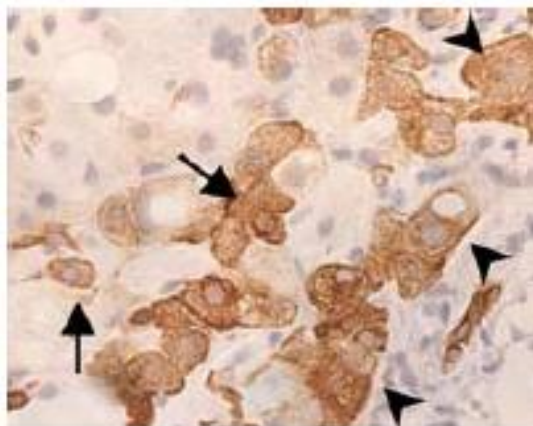
Jennifer A. Eleazar, Lorenzo Memeo, Jeffrey S. Jhang, Mahesh M. Mansukhani, Steven Chin, Soo Mi Park, Jay H. Lefkowitz, Govind Bhagat\*

Journal of Hepatology 2004

## Progenitor cell activation in chronic viral hepatitis

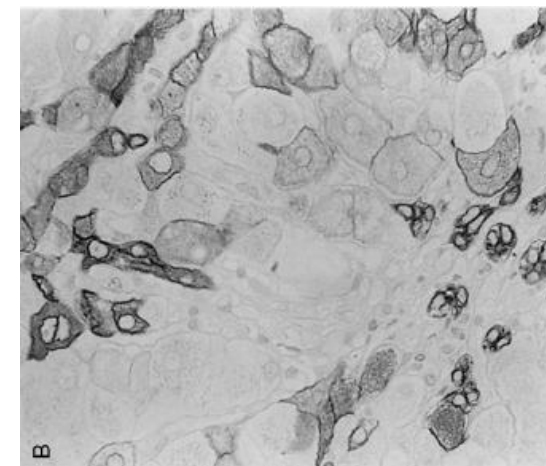
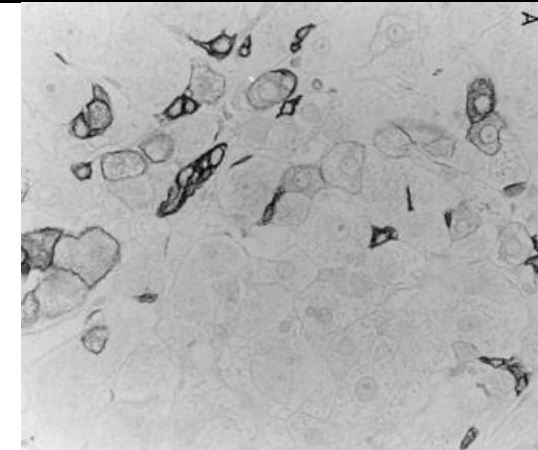
Fotiadu A, Tzioufa V, Vrettou E, Koufogiannis D, Papadimitriou CS, Hytioglou P.

Liver International 2004



### K7(+) hepatocytes

- usually in association with intermediate cells
- correlation with grade and stage



# **Portal Tract Eosinophils and Hepatocyte Cytokeratin 7 Immunoreactivity Helps Distinguish Early-Stage, Mildly Active Primary Biliary Cirrhosis and Autoimmune Hepatitis**

*Neal S. Goldstein, MD,<sup>1</sup> Anjina Soman, MD,<sup>1</sup> and Stuart C. Gordon, MD<sup>2</sup> Am J Clin Pathol 2001*

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## **K7(+) periportal hepatocytes**

- 75% of PBC biopsies
- 12% of AIH biopsies (<10% of portal tracts)
- **supportive of PBC when present in moderate to extensive degrees**
- **absence does not exclude PBC and is not diagnostic of AIH**

## REVIEW

# Cytokeratin expression as an aid to diagnosis in medical liver biopsies

Adrian C Bateman & Stefan G Hübscher<sup>1</sup>

*Department of Cellular Pathology, Southampton General Hospital, Southampton and <sup>1</sup>Department of Pathology, University of Birmingham, Birmingham, UK*

### **Chronic viral and autoimmune hepatitis**

- DR and HPC increase in parallel with disease grade and stage
- K7 (+) hepatocytes may occur, much less widespread than in biliary tract disease

### **Fatty liver disease**

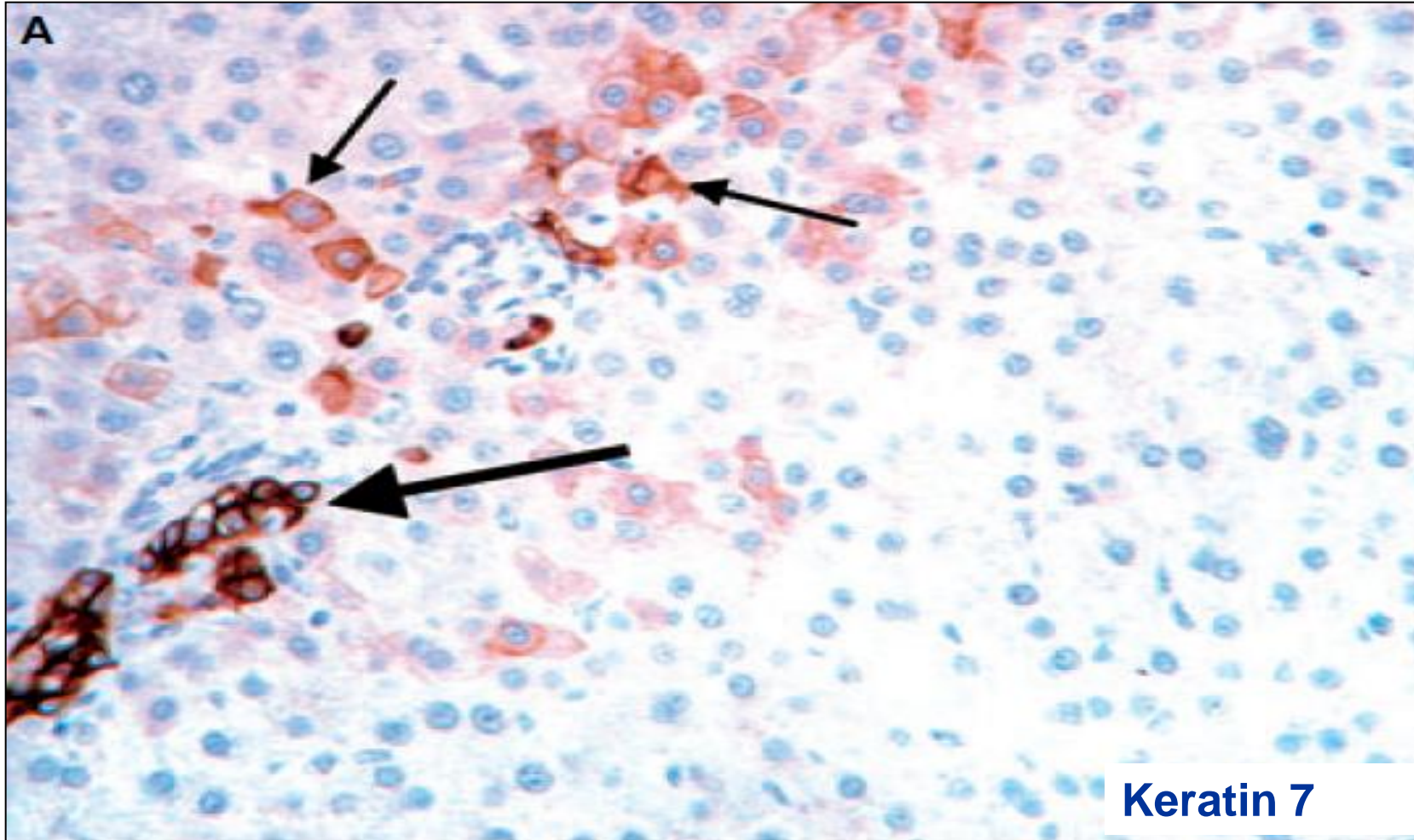
- DR due to replicative senescence of steatotic hepatocytes

### **Chronic biliary tract disease**

- DR associated with numerous K7+ periportal intermediate cells
- **K7 useful adjunct to histochemical stains**

# Intermediate hepatocytes with hepatobiliary phenotype

May point to biliary disease when ductular reaction  
is not well formed



Early  
PBC



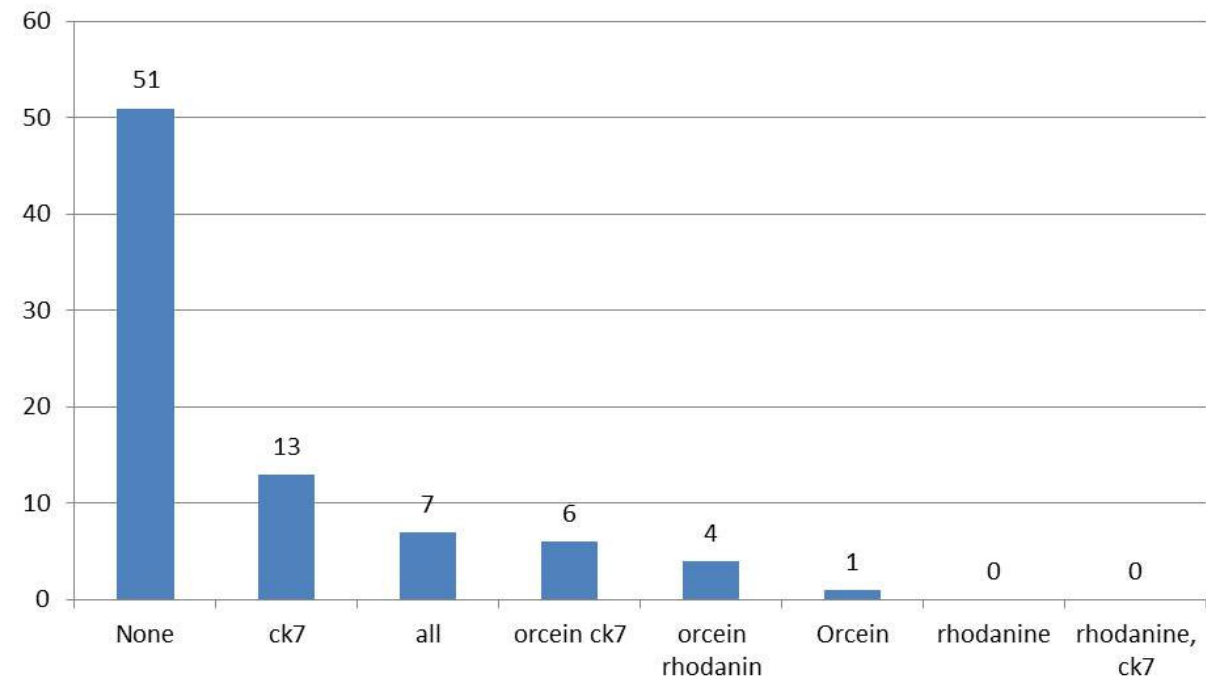
Correspondence

## Copper, copper-binding protein and cytokeratin 7 in biliary disorders

Alberto Quaglia, Prithi S Bhathal

“Adding K7 to a routine set of histochemical stains including orcein, should ensure that all signs of chronic cholestasis are detected”

### Cytokeratin 7 , orcein and rhodanine stain in 82 portal tracts from 12 core needle biopsies

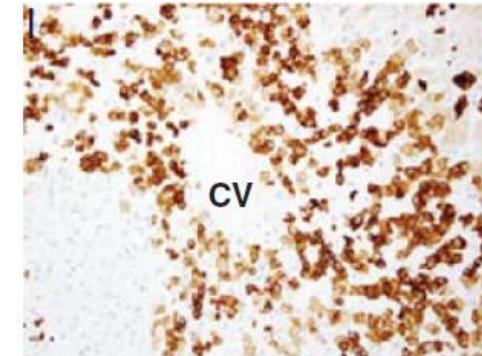
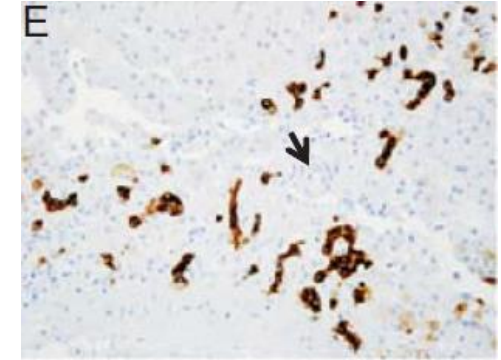


# Chronic venous outflow obstruction and ischemic liver lesions

## Chronic venous outflow obstruction

- Mimicry of zone 3 (centrilobular area) as portal tract with presence of immature K7+ ductules, intermediate-phenotype hepatocytes and arterioles

*Krings et al , Am J Surg Pathol 2014*



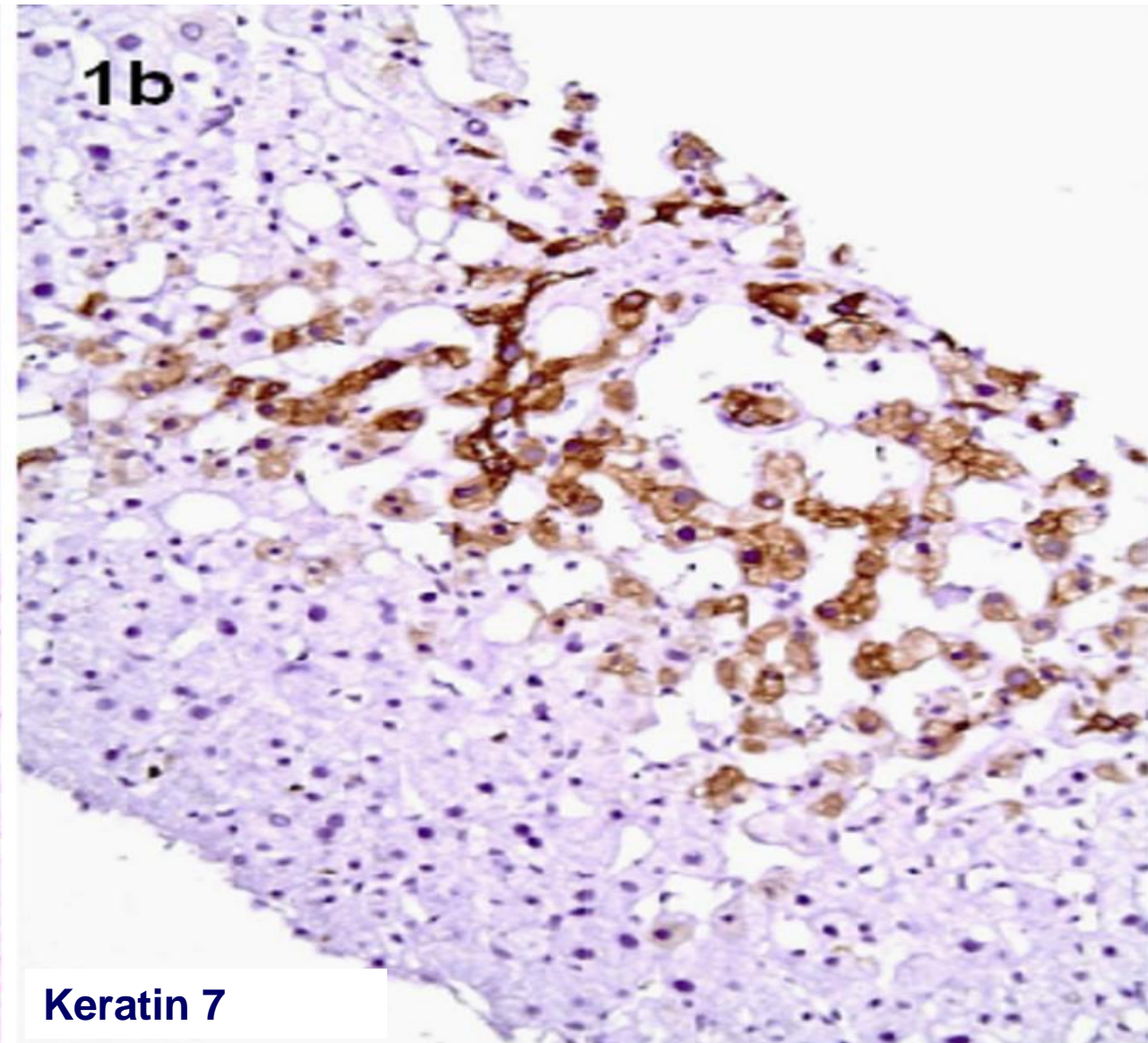
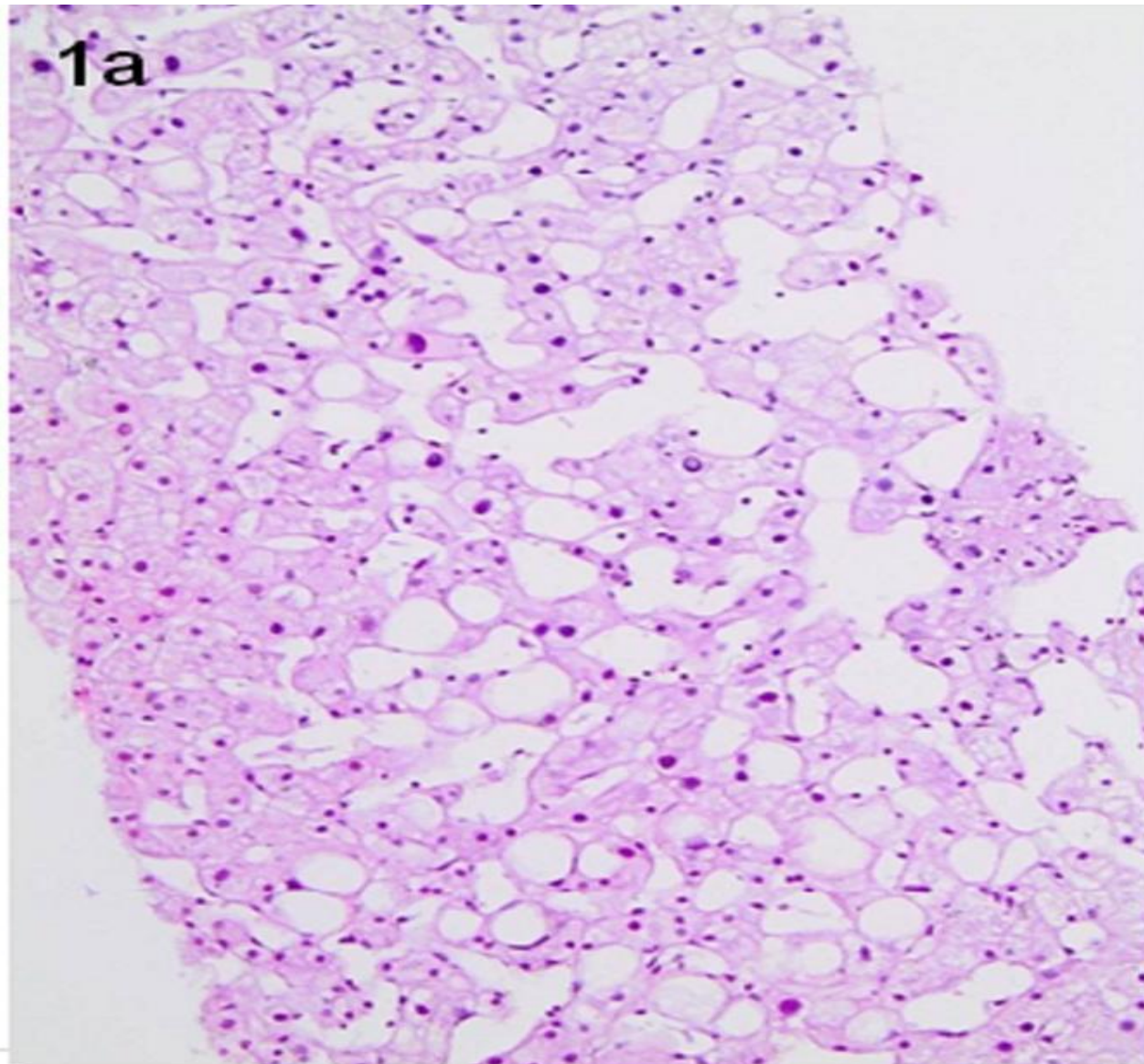
## Atrophic hepatocytes in ischemic liver lesions express K7

- K7+ hepatocytes are an early feature of parenchymal ischemia
- K7+ hepatocytes aid the diagnosis of genuine venous stasis and early Nodular Regenerative Hyperplasia

*Delladetsima et al, Histol Histopathol 2016*

# Venous congestion

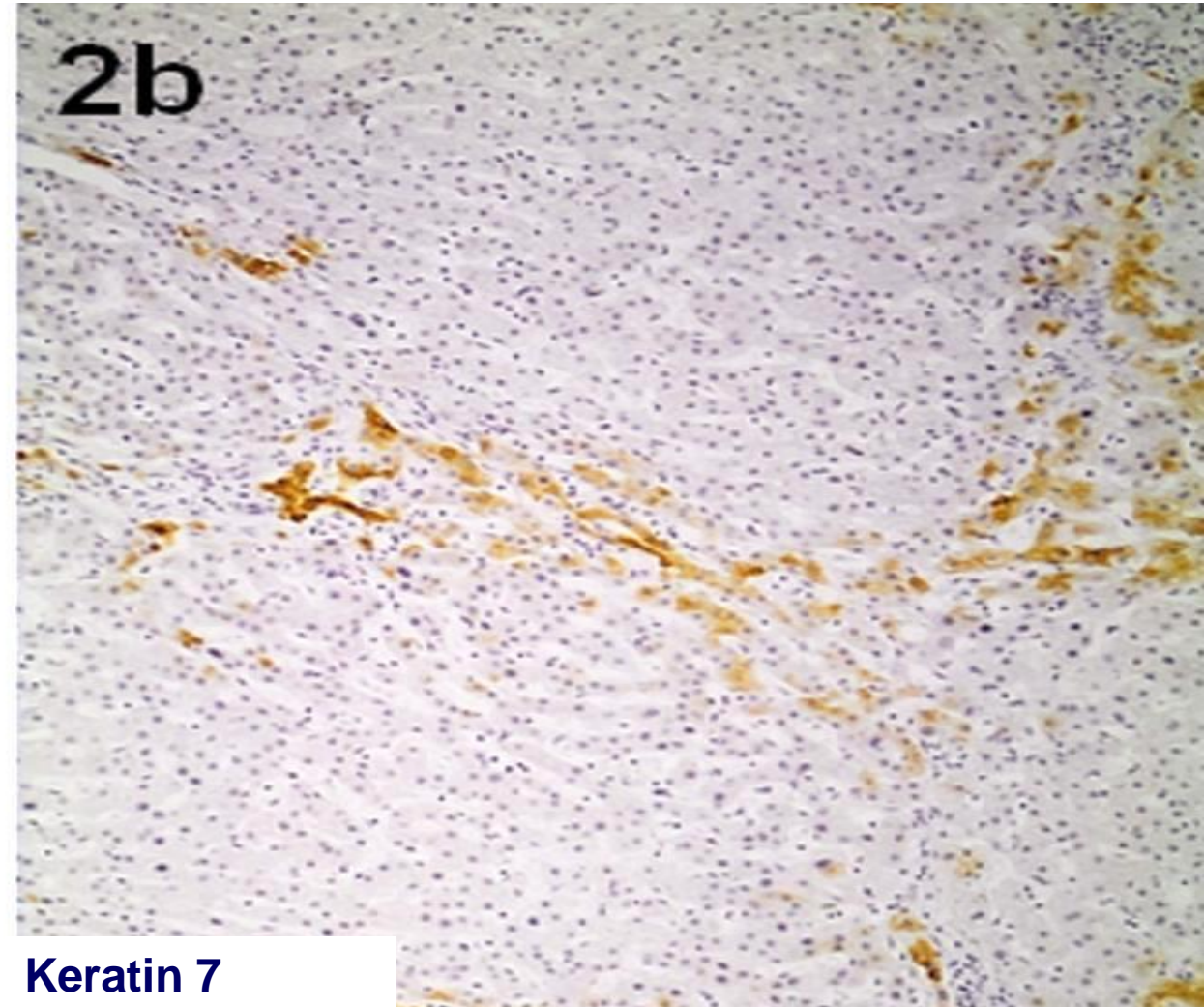
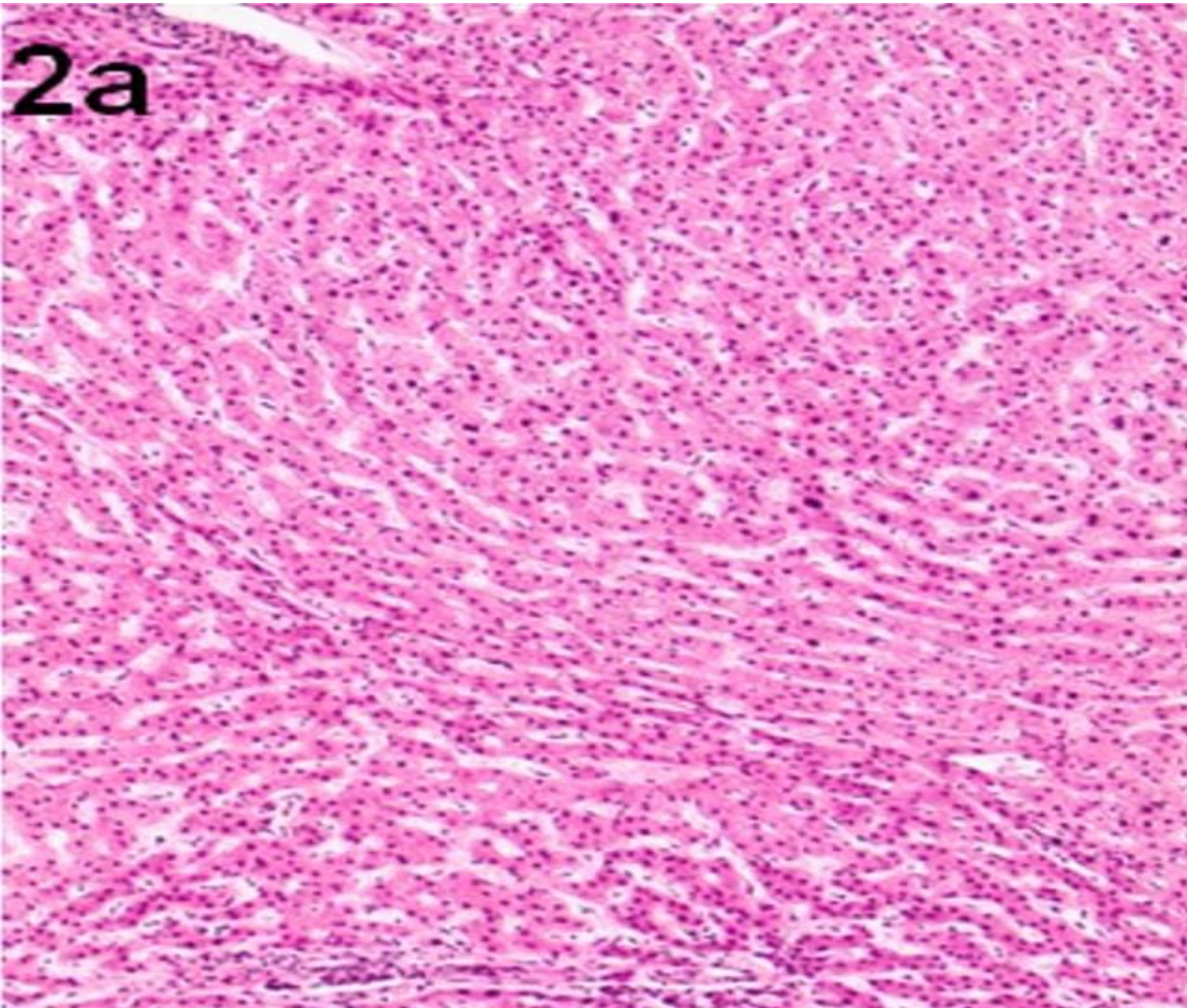
**K7+ indicates stasis vs “artifact”**



**Keratin 7**

# Nodular Regenerative Hyperplasia

**K7+ atrophic hepatocytes at the periphery of regenerative nodules**



## In hepatic venous outflow obstruction, alcoholic liver disease, and nonalcoholic fatty liver disease, centrilobular scars, CD34+ vessels, and keratin 7+ hepatocytes are in close proximity

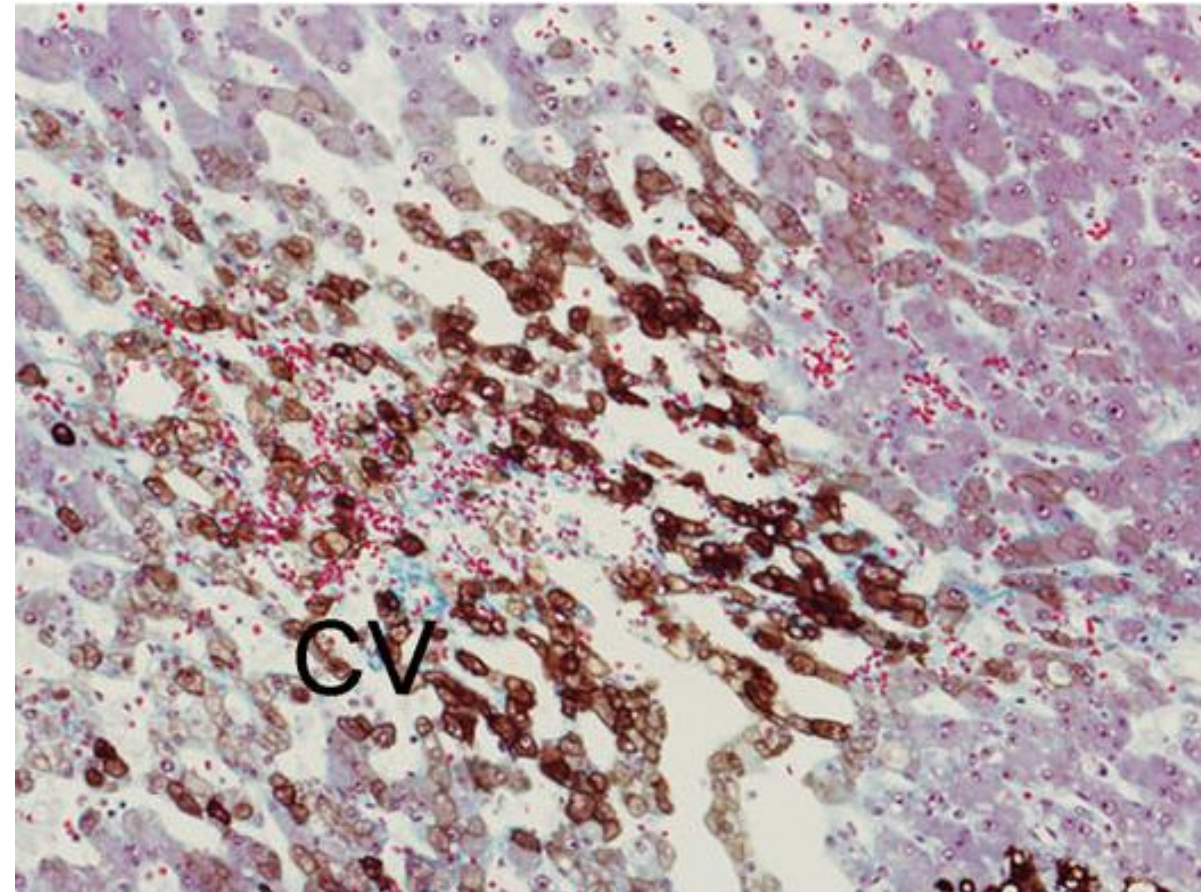
Susumu Matsukuma<sup>1,2</sup> · Hiroaki Takeo<sup>1</sup> · Yoshitaka Utsumi<sup>1</sup> · Kimiya Sato<sup>1</sup>

### Centrizonal injury disease

- hepatic venous outflow obstruction
- alcoholic/nonalcoholic fatty liver disease



- Frequent centrizonal K7 (+) hepatocytes
- Association with centrizonal scar



# Keratin 7 expression in hepatic cholestatic diseases

Virchows Archiv (2021)

S. Sakellariou<sup>1</sup> • C. Michaelides<sup>1</sup> • T. Voulgaris<sup>2</sup> • J. Vlachogiannakos<sup>2</sup> • E. Manesis<sup>3</sup> • D. G. Tiniakos<sup>4,5</sup>  
I. Delladetsima<sup>1</sup>

- Retrospective study of K7 hepatocellular expression and ductular reaction (DR)
- 92 patients, inclusion criterion known cholestasis (biochemical and/or histological)

## **Group 1: Cholestasis due to biliary obstruction/destruction**

**56 (58%) patients** (PBC 35, PSC 10, VBDS 3, complete large bile duct obstruction 8)

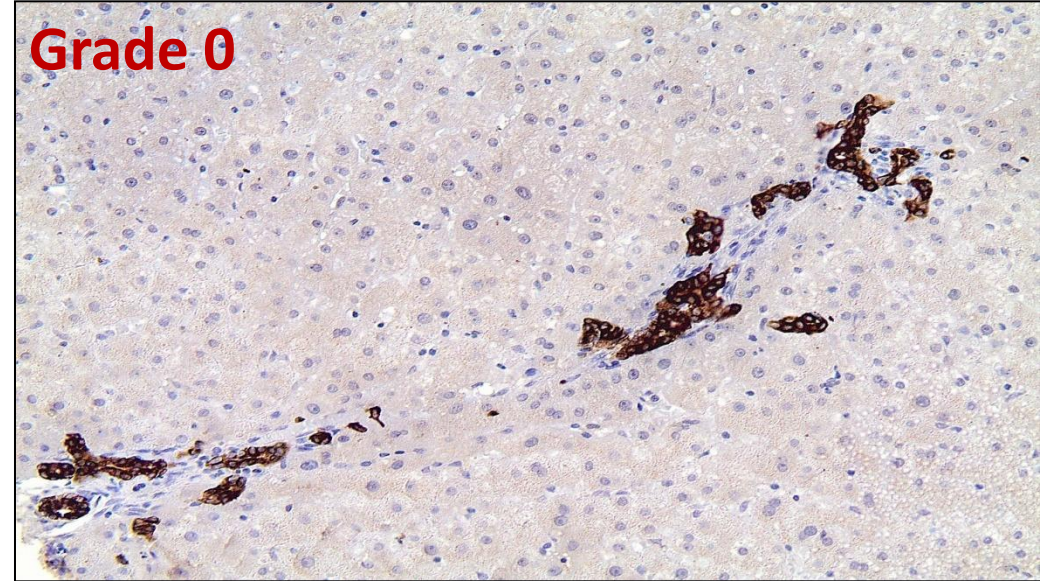
## **Group 2: Parenchymal cholestasis**

**36 (42%) patients** (acute hepatitis: 13 drug-induced, 7 autoimmune, pure/mixed cholestasis 16, all drug-induced)

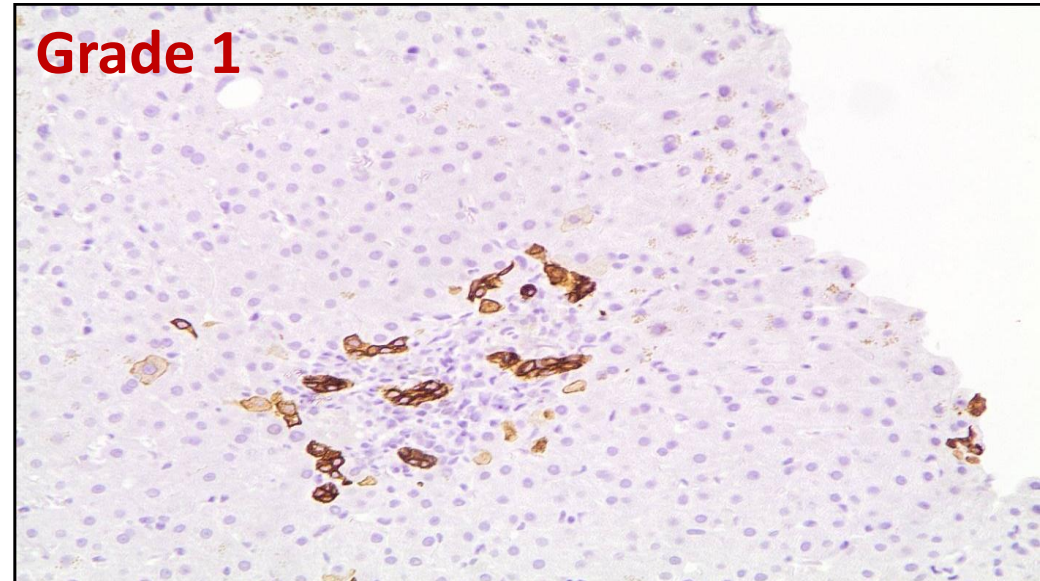
## Immunohistochemical assessment of K7 hepatocellular expression in zones 1 (periportal)

### Semiquantitative assessment in zone 1 (grade 0-3)

Grade	K7 (+) periportal hepatocytes
<b>0</b>	Absent
<b>1</b>	<b>1- 10</b> in at least 1 portal tract
<b>2</b>	<b>&gt;10</b> and <b>up to 50%</b> in at least 1 portal tract or <b>&gt;50%</b> in <50% of portal tracts
<b>3</b>	<b>≥ 50%</b> in <b>≥ 50%</b> of portal tracts



**Grade 0**  
**Acute cholestatic hepatitis(drugs/toxins)**

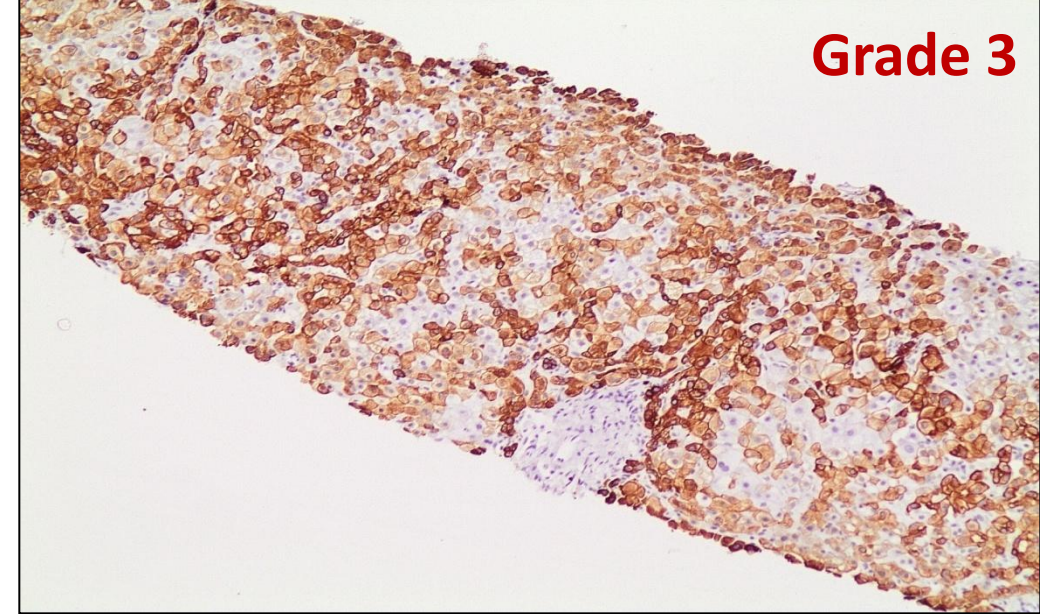


**Grade 1**  
**Mild cholestatic hepatitis (drugs/toxins)**

## Immunohistochemical assessment of K7 hepatocellular expression in zones 1 (periportal)

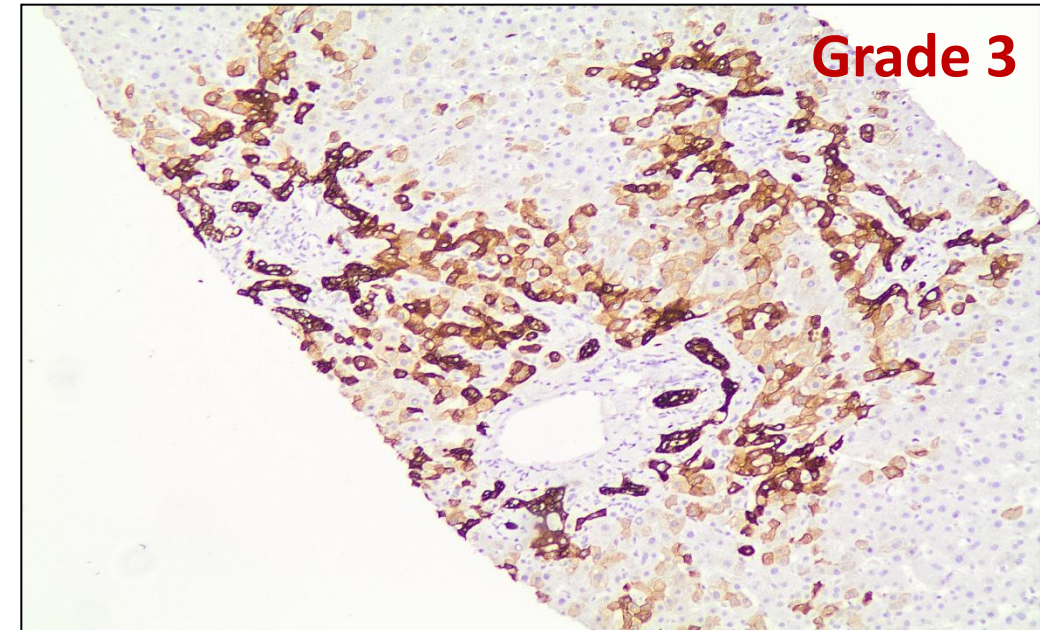
### Semiquantitative assessment in zone 1 (grade 0-3)

Grade	K7 (+) periportal hepatocytes
0	Absent
1	1- 10 in at least 1 portal tract
2	>10 and <b>up to 50%</b> in at least 1 portal tract or > <b>50%</b> in <50% of portal tracts
3	≥ <b>50%</b> in ≥ 50% of portal tracts



**Grade 3**

**Vanishing bile duct syndrome**



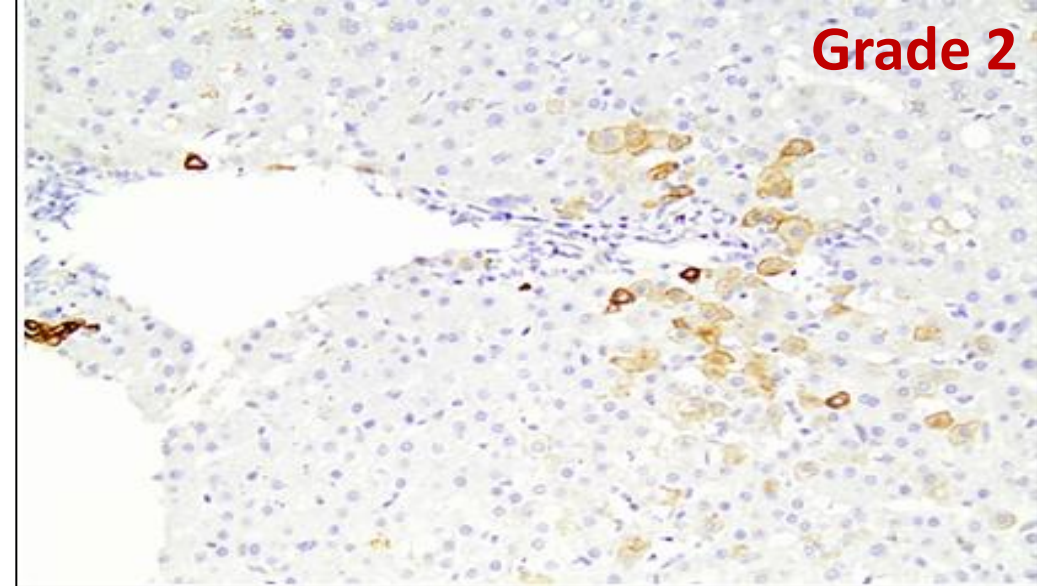
**Grade 3**

**Pure/mixed cholestasis**

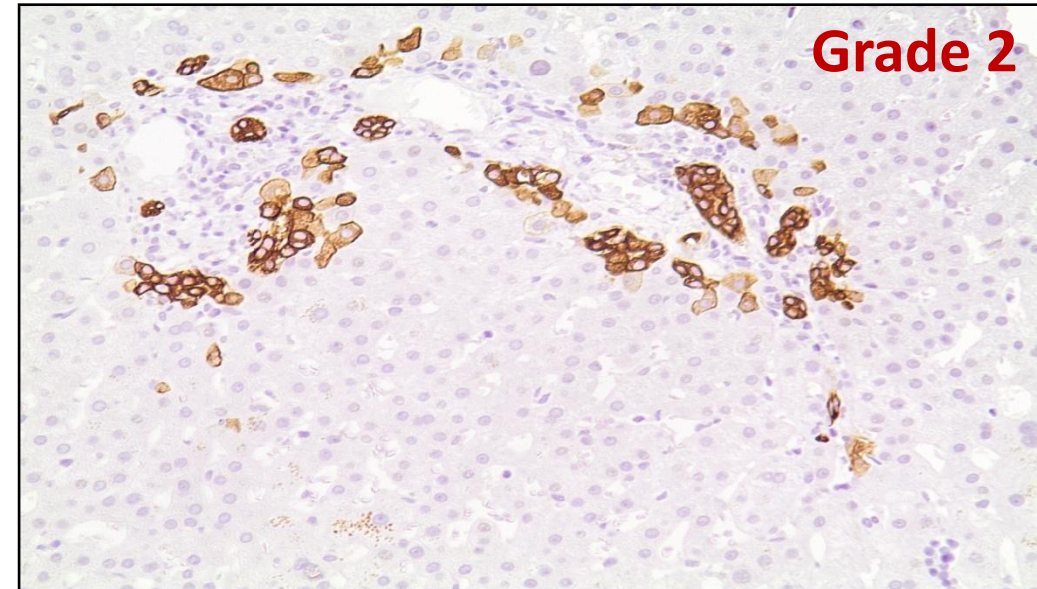
## Immunohistochemical assessment of K7 hepatocellular expression in zones 1 (periportal)

### Semiquantitative assessment in zone 1 (grade 0-3)

Grade	K7 (+) periportal hepatocytes
<b>0</b>	Absent
<b>1</b>	<b>1- 10</b> in at least 1 portal tract
<b>2</b>	<b>&gt;10</b> and <b>up to 50%</b> in any portal tract or <b>&gt;50%</b> in <50% of portal tracts
<b>3</b>	<b>≥ 50%</b> in <b>≥ 50%</b> of portal tracts



**PSC**

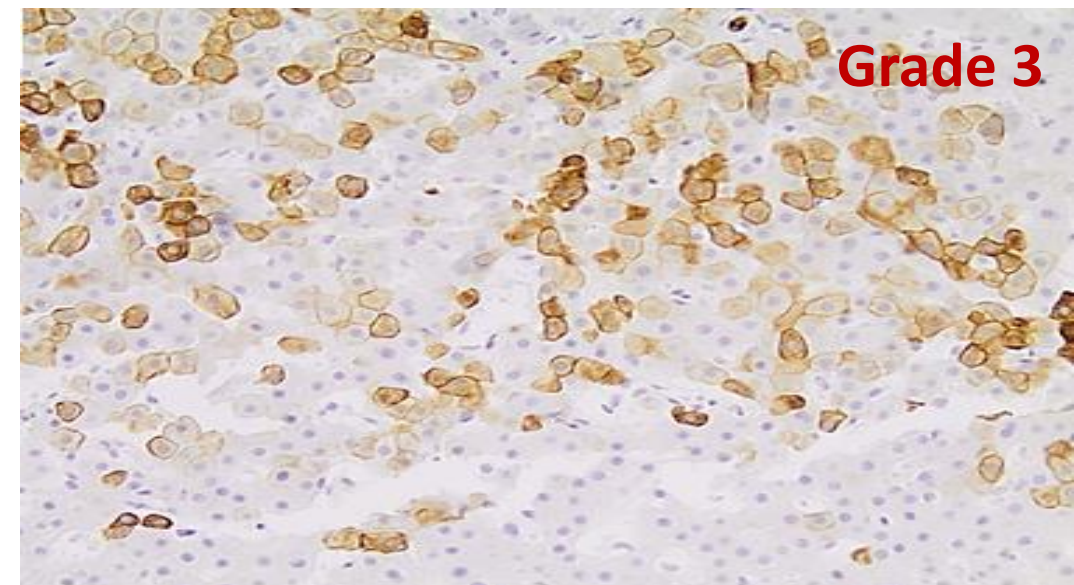
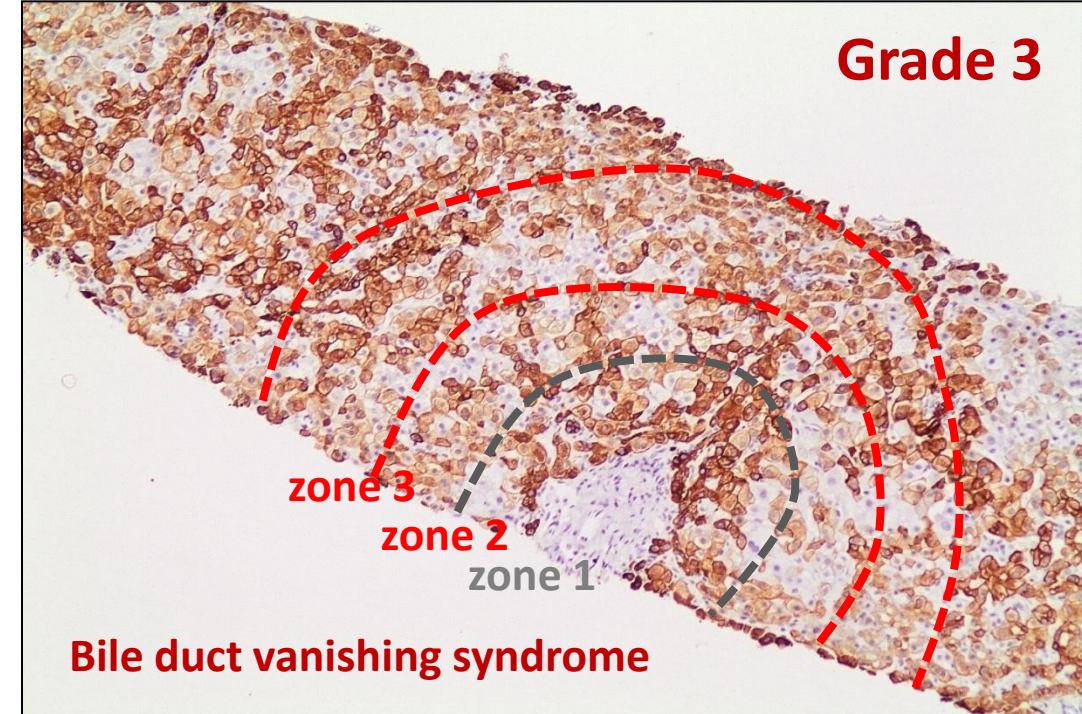


**Acute cholestatic hepatitis(drugs/toxins)**

## Immunohistochemical assessment of K7 hepatocellular expression in zones 2 και 3

### Semiquantitative assessment in zones 2 and 3 (grade 0-3)

Grade	K7 (+) periportal hepatocytes
0	Absent
1	≤10% in at least 1 acinus
2	11–30% in at least 1 acinus
3	>30% in at least 1 acinus



**Acute cholestatic hepatitis(drugs/toxins)**

S. Sakellariou<sup>1</sup> · C. Michaelides<sup>1</sup> · T. Voulgaris<sup>2</sup> · J. Vlachogiannakos<sup>2</sup> · E. Manesis<sup>3</sup> · D. G. Tiniakos<sup>4,5</sup>  
 I. Delladetsima<sup>1</sup>

**Table 1** Hepatocellular keratin 7 expression in all diagnostic categories and subgroups

	K7 hepatocellular expression					
	All zones		Zone 1		Zones 2 & 3	
	Score $\geq 1$ n (%)	Score $\geq 2$ n (%)	Score $\geq 1$ n (%)	Score $\geq 2$ n (%)	Score $\geq 1$ n (%)	Score $\geq 2$ n (%)
All patients (n=92)	81 (87)	55 (60)	79 (86)	54 (60)	67 (73)	30 (32.6)
Acute hepatitis (n=20)	16 (80)	5 (25)	15 (75)	4 (20)	11 (55)	5 (25)
Pure/mixed cholestasis (n=16)	12 (75)	6 (37.5)	11 (68.8)	6 (37.5)	12 (75)	3 (18)
iBDO (n=48)	46 (96)	38 (79)	46 (96)	38 (79)	38 (79)	17 (35.4)
PBC (n=35)	34 (97.1)	26 (74.35)	34 (97)	26 (74.3)	26 (74.3)	10 (28.6)
PSC (n=10)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	5 (50)
VBDS (n=3)	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)
cBDO (n=8)	7 (87.5)	6 (75)	7 (87.5)	6 (75)	6 (75)	5 (62.5)

## **K7 hepatocellular expression**

- **80% Acute hepatitis**
- **75% Pure/Mixed cholestasis**
- **96 % Incomplete bile duct obstruction  
(PBC, PSC, VBDS)**
- **87.5% Complete bile duct obstruction**

## **High K7 hepatocellular expression**

- **25% Acute hepatitis**
- **37.5% Pure/Mixed cholestasis**
- **79 % Incomplete bile duct obstruction  
(PBC, PSC, VBDS)**
- **75% Complete bile duct obstruction**

# Bile duct obstruction vs parenchymal cholestasis

- K7 hepatocellular expression(all zones): 94% vs 77%
- **High** K7 hepatocellular expression (all zones): **78.6% vs 30%**
- K7 hepatocellular expression(zone 1): 94% vs 72%
- **High** K7 hepatocellular expression(zone 1): **78.6% vs 27.8%**

# All disease groups

- **Significant correlation of K7 expression with bile duct loss and fibrosis**
- **No correlation with necroinflammation and bilirubinostasis**
- HepPar1 expression well preserved irrespective of K7 expression grade
- Hepatocellular Ki67 LI low (0–5%) in all disease groups except AH

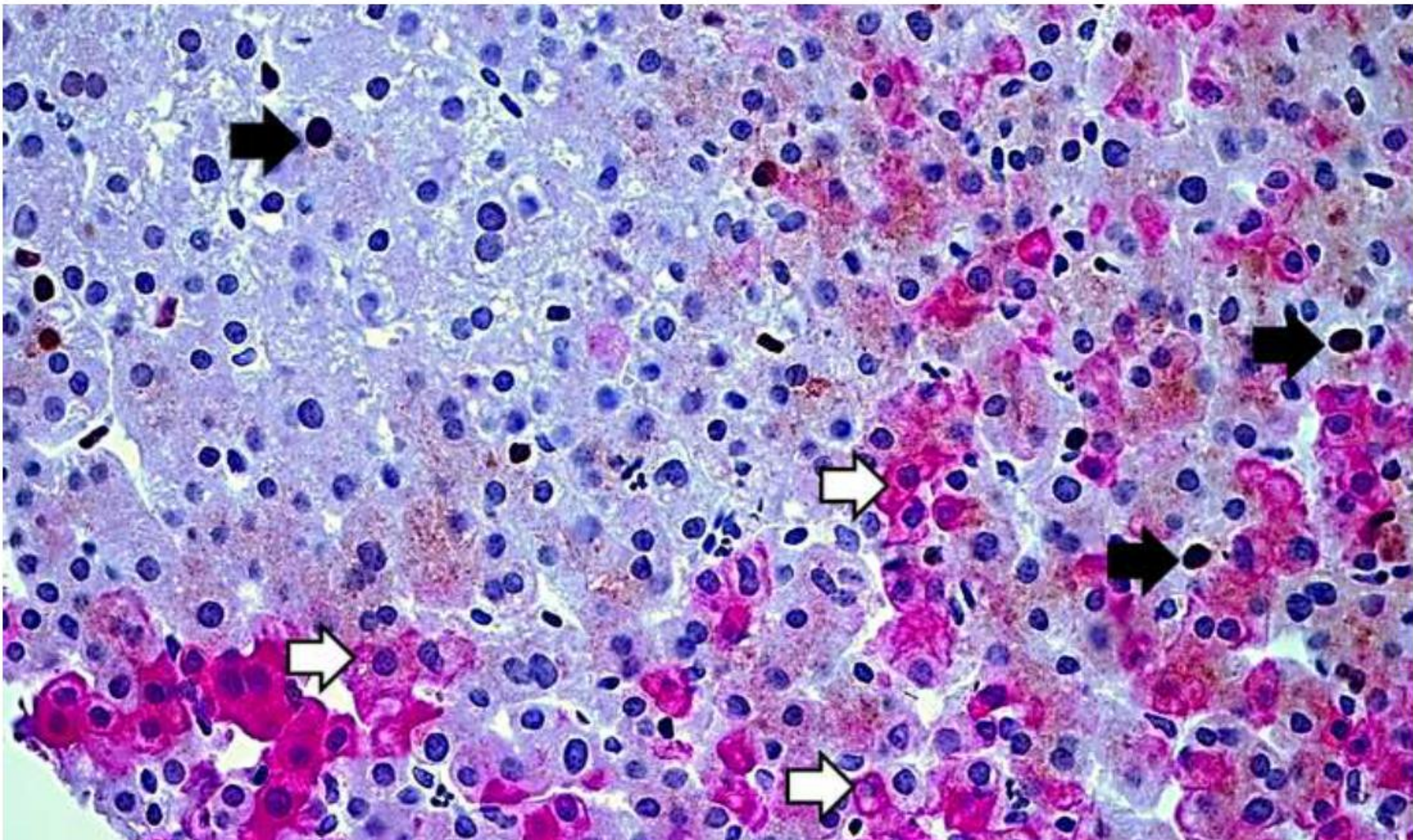
# Parenchymal cholestasis

## Acute hepatitis

- Inverse correlation of K7(+) hepatocytes with disease severity and ALT levels
- Inverse correlation between Ki67 and K7 grade

## Pure/mixed cholestasis

- Inverse correlation of K7(+) hepatocytes with ALT levels



**Ki67 nearly absent in K7 (+) hepatocytes, mainly detectable in K7 (-) hepatocytes**

# Incomplete bile duct obstruction/destruction

- K7(+) hepatocytes in all PSC and 97% PBC cases
- High K7 hepatocellular expression in **all PSC** even with **intact bile ducts**
- High K7 hepatocellular expression in **20% PBC** cases with **intact bile ducts**
- Correlation between K7 hepatocellular expression, younger age and GGT

# Conclusions

## K7 expression in hepatocytes


- **sensitive** though non-specific **marker of cholestasis**
- significant correlation with **bile duct loss and fibrosis**
- **predominates in bile duct obstruction/destruction group**
  - constant finding in all PSC (possible DDx tool vs PBC)
  - correlation with younger age and GGT

# Conclusions

## K7 expression in hepatocytes

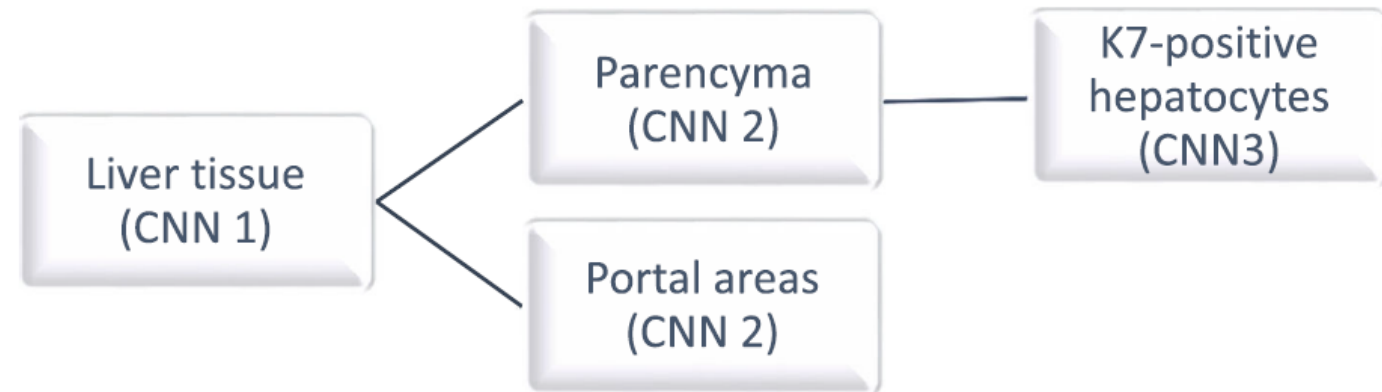
- **common finding in parenchymal cholestasis**
  - more extensive in milder parenchymal lesions
  - inverse correlation with cell proliferation and ALT

# Chronic cholestasis detection by a novel tool: automated analysis of cytokeratin 7-stained liver specimens

Nelli Sjöblom<sup>1\*</sup> , Sonja Boyd<sup>1</sup>, Anniina Manninen<sup>2</sup>, Anna Knuuttila<sup>2</sup>, Sami Blom<sup>2</sup>, Martti Färkkilä<sup>3†</sup>  
Johanna Arola<sup>1†</sup>

## Automated image analysis to locate and quantify K7+ hepatocytes in cholestatic diseases

Artificial Intelligence by  
convolutional neural networks (CNNs)

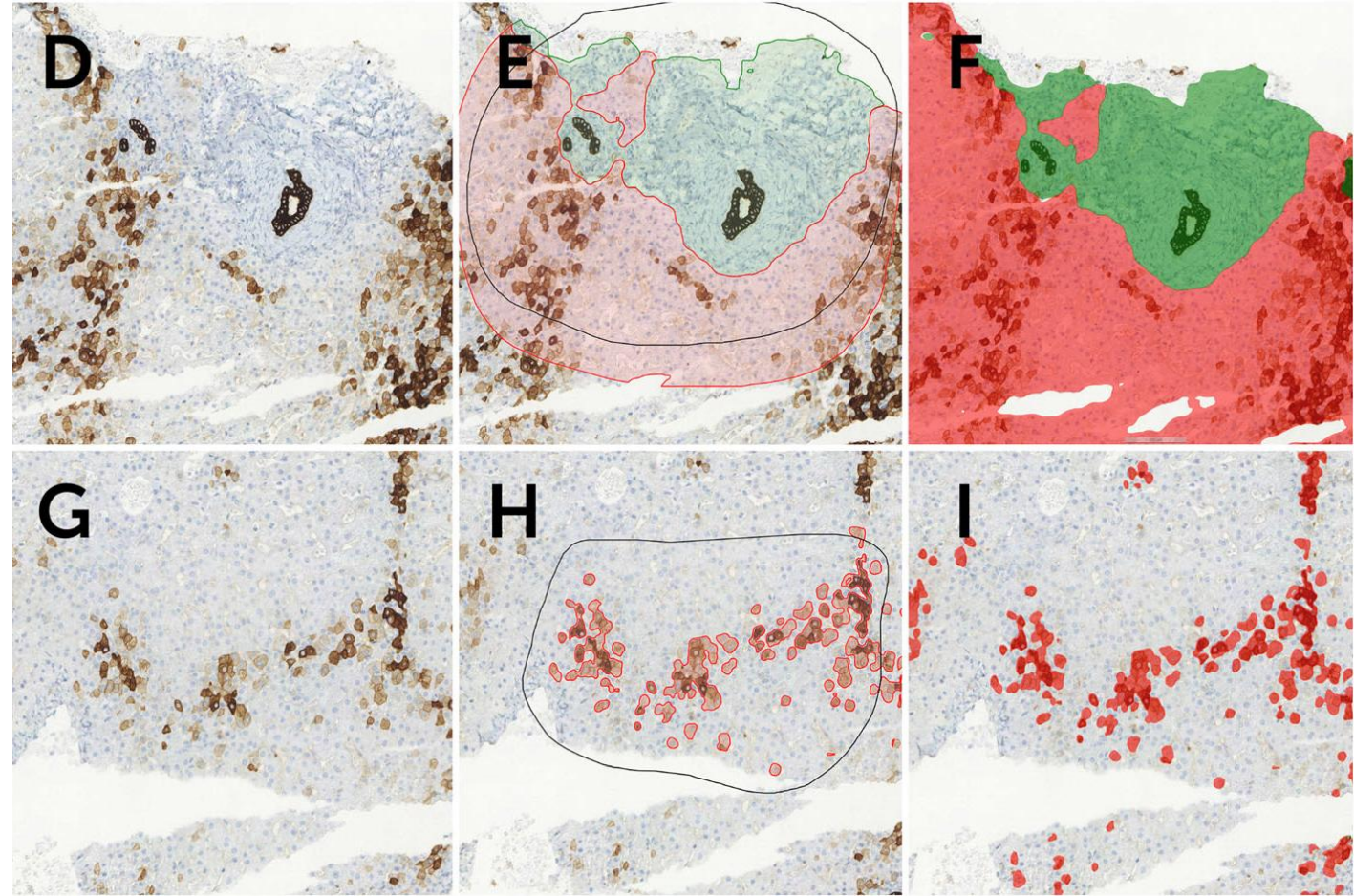


The first CNN model was trained to classify liver tissue. The second model was trained to segment liver parenchyma and portal areas (including BD, DR). The third model was trained to classify K7(+) hepatocytes.


# Training annotations drawn by a pathologist to teach the AI model

D-F: green areas are what the AI model considers as portal areas and red areas are liver parenchyma

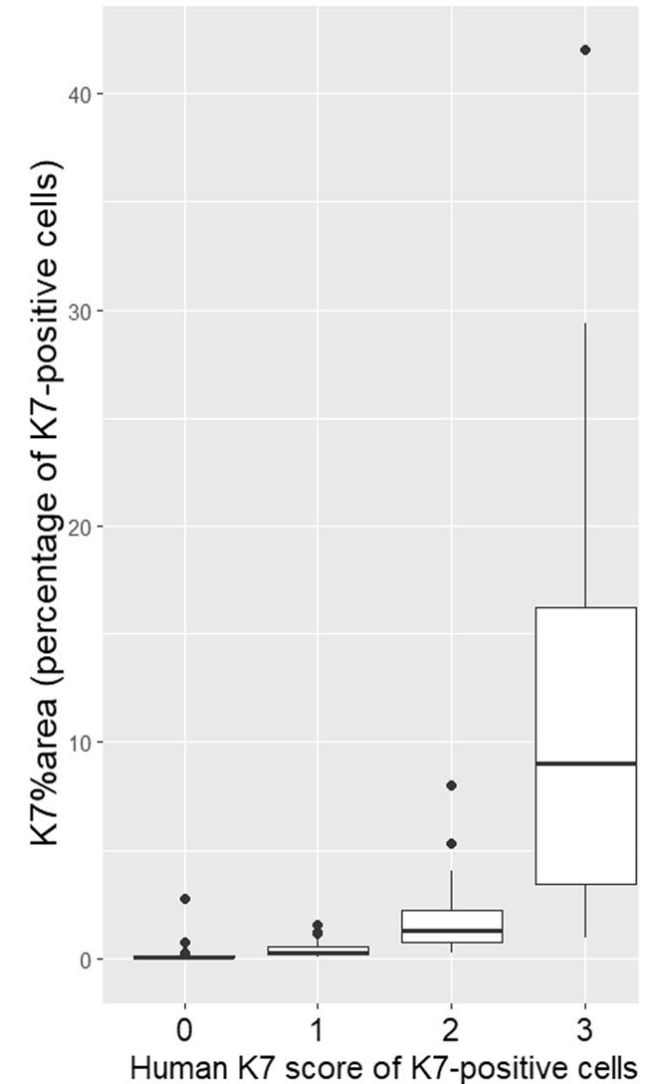
G-I: red areas are what the K7-AI model interprets as K7-positive hepatocytes after training



# Chronic cholestasis detection by a novel tool: automated analysis of cytokeratin 7-stained liver specimens

Nelli Sjöblom<sup>1\*</sup> , Sonja Boyd<sup>1</sup>, Anniina Manninen<sup>2</sup>, Anna Knuuttila<sup>2</sup>, Sami Blom<sup>2</sup>, Martti Färkkilä<sup>3†</sup>  
Johanna Arola<sup>1†</sup>

- K7-AI model results (K7%area) comparable to the human K7 score
- K7%area correlated with stage of PSC and plasma ALP levels
- Automated methods provide fast (9.75 s/specimen) analysis of K7 staining in chronic cholestatic diseases



# Take home messages

## **K7(+)** hepatocytes

- ✓ Not a feature of normal liver
- ✓ Limited in chronic viral, autoimmune hepatitis, and steatohepatitis

## **Chronic hepatitis**

- correlation with grade and stage
- close to HPC and intermediate cells

## **Steatohepatitis**

- centrilobular, in association with scarring

# Take home messages

## K7(+) hepatocytes

- ✓ May be widespread in **chronic venous outflow obstruction and ischemic liver lesions**
- early feature of parenchymal ischemia
- aids diagnosis of genuine venous stasis and early NRH

# Take home messages

## K7(+) hepatocytes

- ✓ May be widespread in **cholestatic diseases**
- more frequently in cholangiopathies/ obstruction, though not uncommon in parenchymal cholestasis
- correlation with bile duct loss and fibrosis
- inverse correlation with acute hepatitis severity
- zone1 high K7+ expression in all PSC but not all PBC cases