Pathology of NAFLD/NASH Revisited

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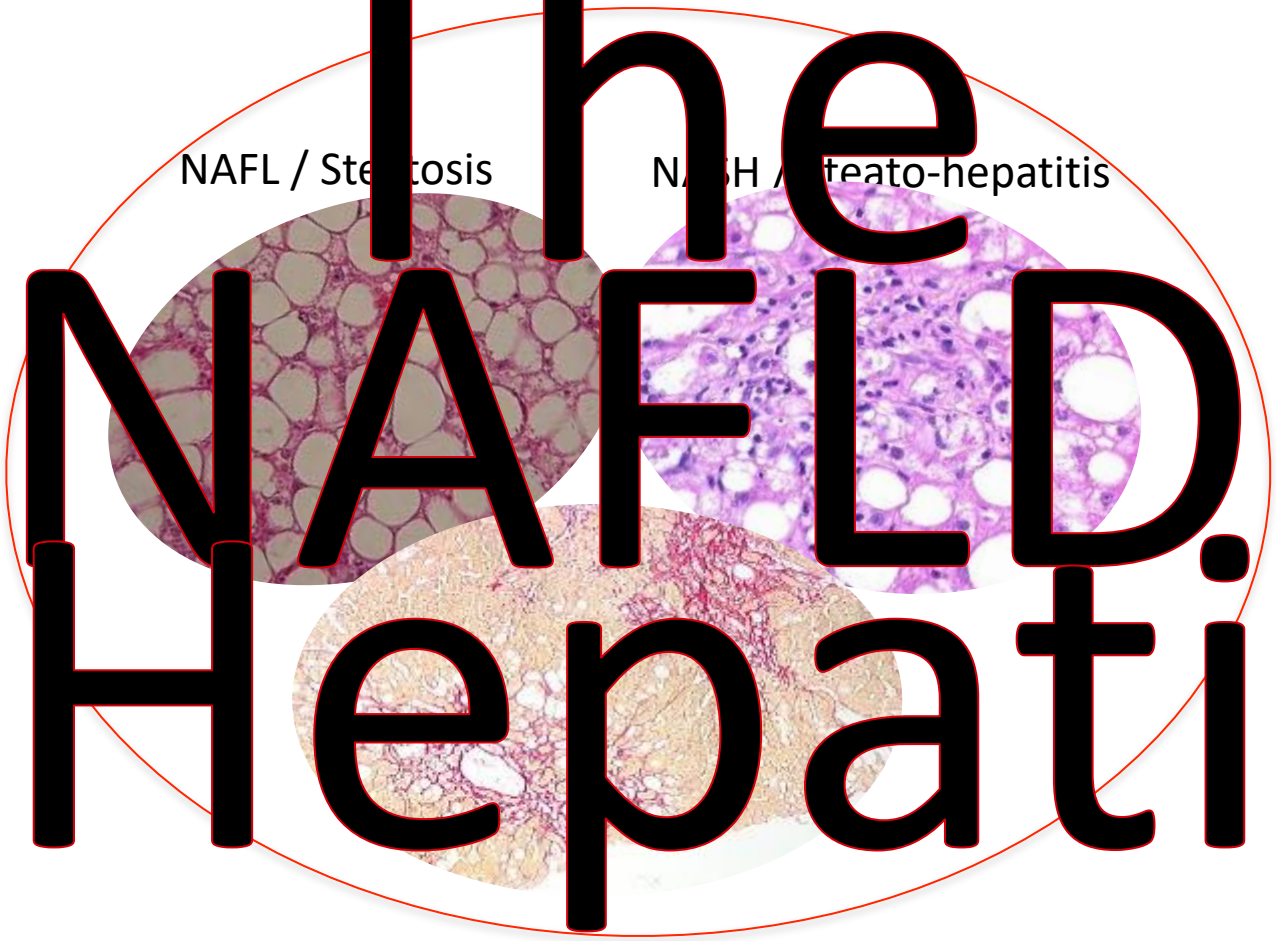
The NAFLD Hepati



NAFLD : A Diagnosis more and more observed

Epidemiology of chronic liver diseases in the USA in the past three decades

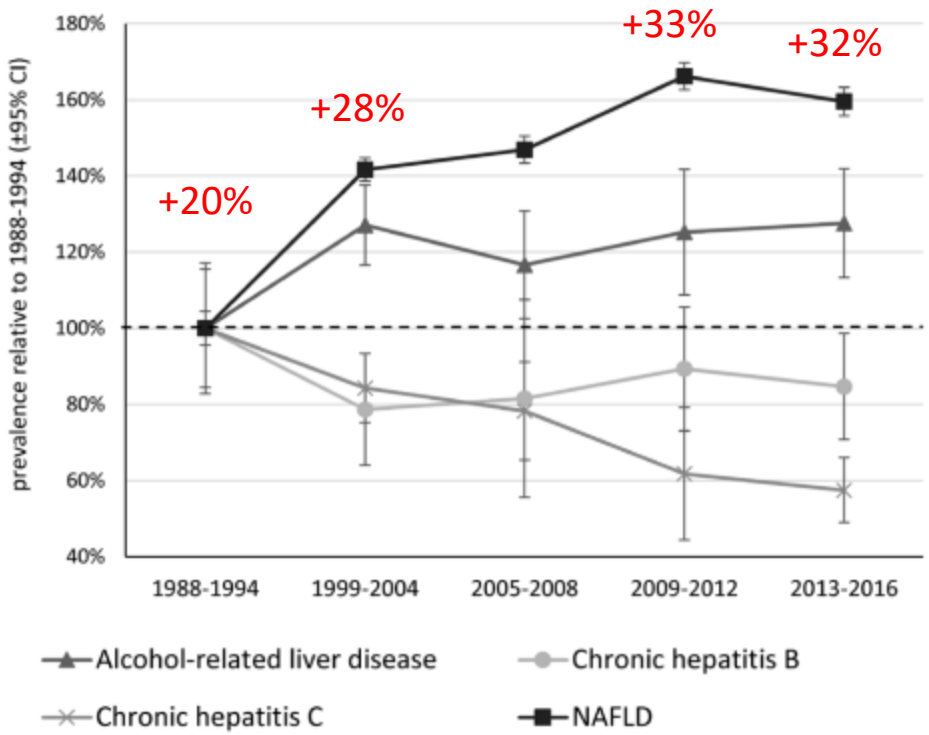


Figure 2 Relative changes in the prevalence of chronic liver disease aetiologies (reference: 1988–1994 cycle). NAFLD, non-alcoholic fatty liver disease.

Yonoussi ZM Gut 2019

NAFLD for the General Pathologist

1. Hepatic fat accumulation (> 5% steatosis) : a prerequisite

2. 2 pathologically distinct conditions
   - NAFL (steatosis) and NASH (steato-hepatitis)

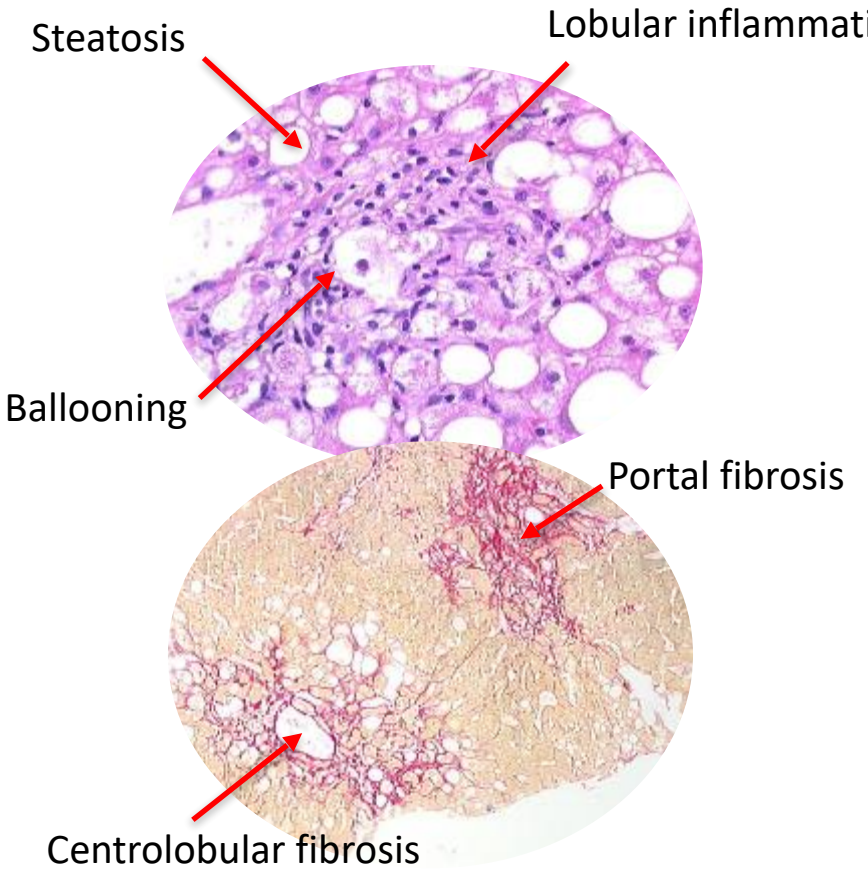
3. NASH : a wide spectrum of disease severity
   - Fibrosis, Cirrhosis, and Hepatocellular carcinoma

4. Liver biopsy required for NASH diagnosis
   - Clinical, biochemical or imaging measures cannot distinguish NASH from steatosis

EASL-EASD-EASO Clinical Practice Guidelines for the management of NAFLD
J Hepatol 2016

Liver biopsy for NAFLD: « The reference standard »

NASH in « a glance »

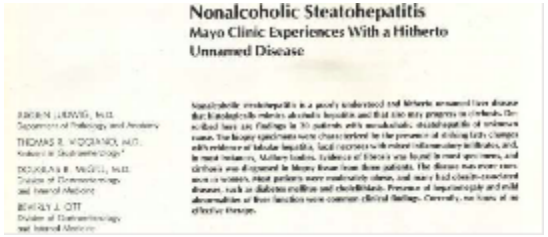


Issues

1. Confirm a diagnosis of NAFLD
2. Assess the severity of the disease
   - Activity (SH), Stage (Fibrosis)
3. Identify potential comorbidity risk factors
4. Support the inclusion in clinical trials
5. Evaluate the treatment response

Pathology of NAFL/NASH Already Revisited

Pathology of NAFL/NASH : Already Revisited


Nonalcoholic Steatohepatitis
Mayo Clinic Experiences With a Hitherto Unnamed Disease

1980

Acute Alcoholic Hepatitis-like
(Ludwig J, et al. Mayo Clin Proc. 1980)

- Steatosis + Mallory hyaline + Polymorphonuclears

1999

NonAlcoholic Steatohepatitis
A proposal for grading and staging
(Brunt E. Am J Gastroenterol 1999)

- Steatosis + ballooning + mixed lobular & portal inflammation + zone 3 perisinusoidal fibrosis

NASH
CRN

2005

NAS score (CRN)
Designed for use in CT
(Kleiner DE, Hepatology 2005)

- NAS (0-8) [Steatosis + lobular inflammation + ballooning]
- Fibrosis (0-4)

2012

SAF (FLIP consortium)
Diagnostic algorithm
(Bedossa P, Hepatology 2008)

- $S_{0-3}A_{0-4}F_{0-4}$
- Activity [Inflammation + ballooning]
- Fibrosis (0-4)

Future Steps ?

Endless challenges

- Adequacy of the biopsy
- Observer variability

New challenges

- Improve NASH stratification
- Refine Fibrosis staging

Future Steps ?

Endless challenges

- Adequacy of the biopsy
  - 1:50,000 to 1:65,000 of the liver
  - Operator-dependent invasive exam
- Observer variability

New challenges

- Improve NASH stratification
- Refine Fibrosis staging

Liver Biopsy: The « Reference » Standard

Recommandations

- Needle over wedge biopsy
  - Fibrosis overestimated
- ↘Sampling errors
  - Larger Gauge needles (14 G)
  - Longer (> 1.5 cm) or more than 1 core
- Optimal biopsy
  - 15-20 mm long, > 10 portal tracts
  - No fragmentation

Comments

- NAFLD is a « zonal disease »
  - Starting and predominating in centrilobular (CL) areas
  - May weaken the issue of sampling variability
  - Report the number of CLV would be informative
- Severity of the disease is heterogeneous
  - Analyze serial sections with ≠ stainings
  - Revisit the dogma on the size of liver biopsy
    - « The longer, the better »
    - « The less injuried, the more sampled »

Future Steps ?

Endless challenges

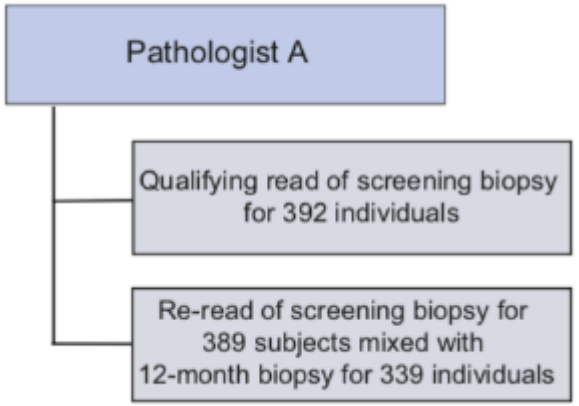
- Adequacy of the biopsy
- Observer variability
  - Intra- & inter-observer
  - Feature-dependent
    - Higher reproducibility rates observed for Fibrosis & Steatosis than for Ballooning & Lob. Inflammation

New challenges

- Improve NASH stratification
- Refine Fibrosis staging

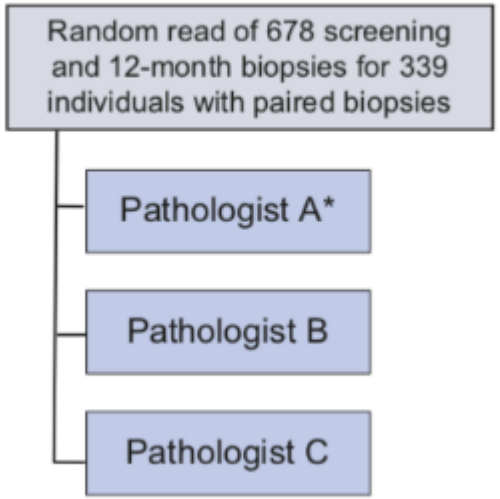
Suboptimal reliability of liver biopsy evaluation has implications for randomized clinical trials

EMMINENCE phase II study (insulin sensitizer: MSDC-0602K), 339 patients / 678 biopsies (digitized slides)



Qualifying vs re-reading	Weighted κ
Inflammation	0.227
Ballooning	0.487
Steatosis	0.666
NAS	0.372
Fibrosis	0.679

➢ Re-reading scores lower compared to the baseline scores
Pressure for enrollment from the clinician towards the pathologist ?



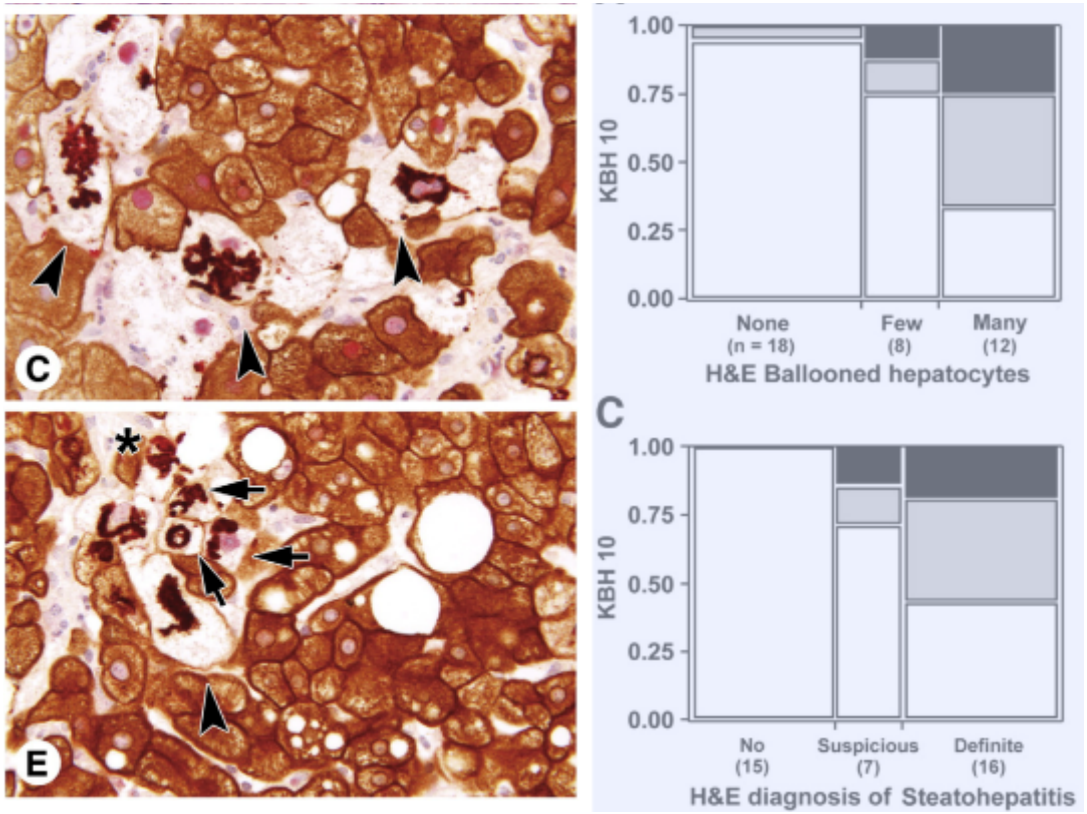
Overall inter-reader comparison	Weighted κ
Inflammation	0.328
Ballooning	0.517
Steatosis	0.609
NAS	0.495
Fibrosis	0.484

➢ Full agreement for qualifying patients achieved in ≈ half of cases
More objective features ?

Davison BA J Hep 2020

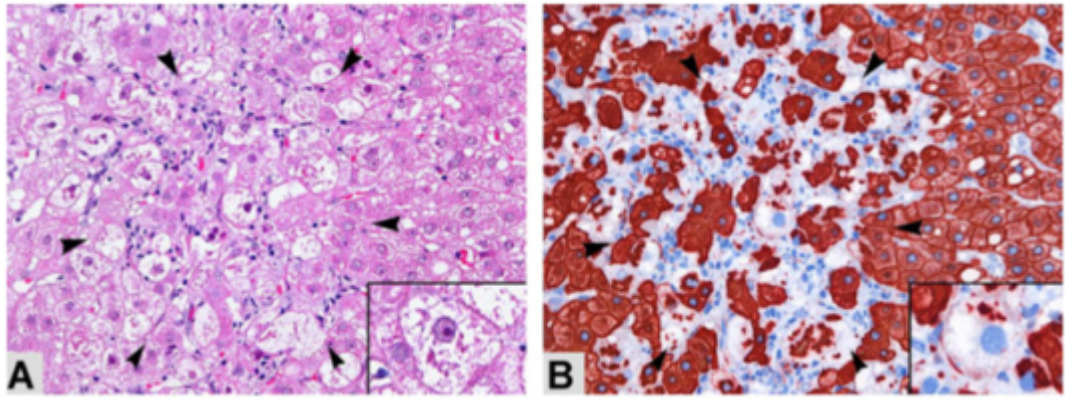
Loss of CK8/18
An objective marker of hepatocyte injury

Costaining for keratins 8/18 plus ubiquitin improves detection of hepatocyte injury in nonalcoholic fatty liver disease☆



Guy CD, Human Pathol 2011

Ballooned hepatocytes in steatohepatitis: The value of keratin immunohistochemistry for diagnosis☆



Not a specific feature

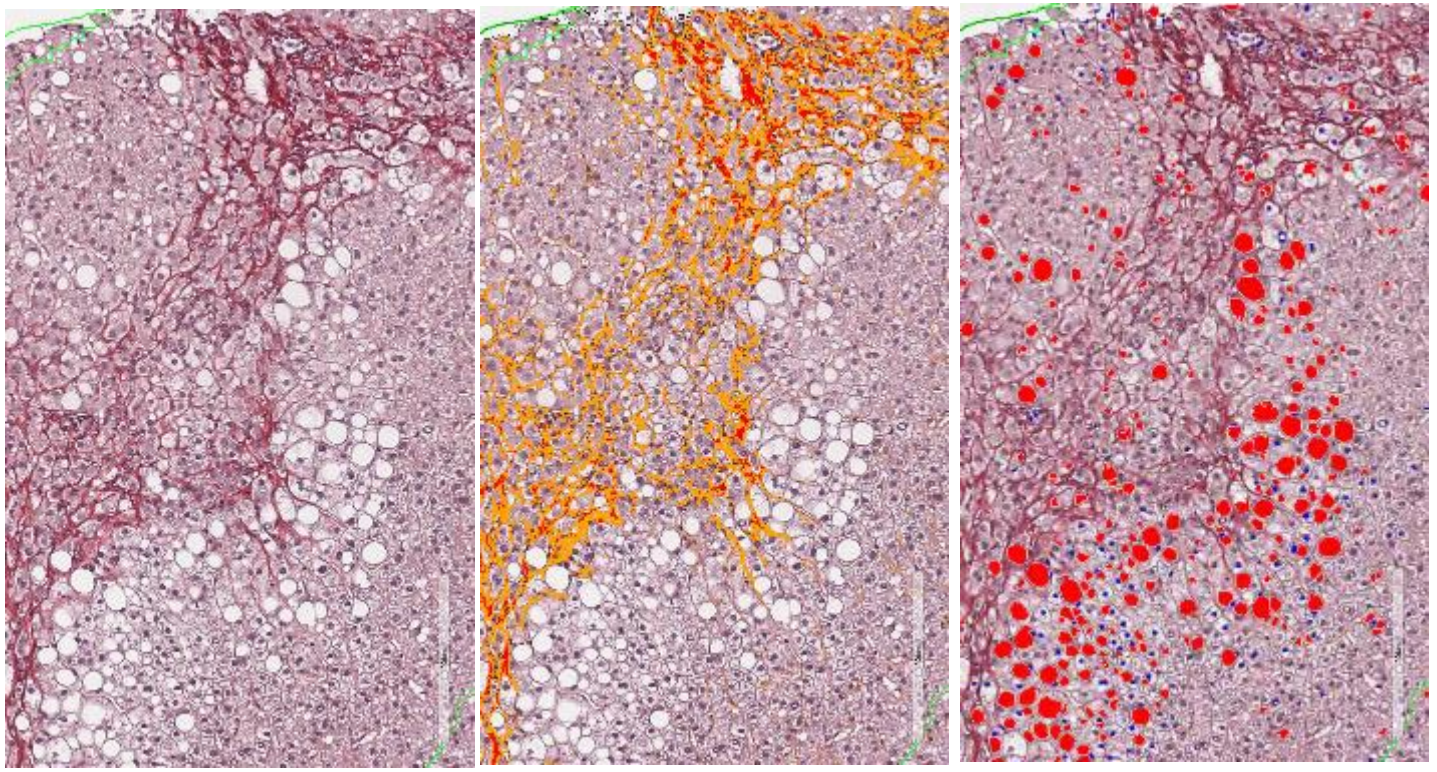
Observed in NAFL, Alcoholic liver diseases
Chronic cholestasis
Ischaemic/reperfusion in liver grafts

Lackner C, J Hep 2008

Digital Pathology : An alternative tool

From a semi-quantitative & subjective to a quantitative & objective analysis

- More objective
  - Rule-out pathologist's subjectivity
- More accurate & more sensitive
  - Better assessment of more subtile changes
  - Faster detection of treatment benefit
  - Further characterisation of phenotypical traits of fibrosis (>50 quantitative parameters)
- Machine learning image-based approaches
  - Recognize elementary morphological features from routine stained slides



Sirius red | CPA | Steatosis

High-Throughput, Machine Learning–Based Quantification of Steatosis, Inflammation, Ballooning, and Fibrosis in Biopsies From Patients With Nonalcoholic Fatty Liver Disease

246 biopsies [test (100) validation (146)]

Forlano R Clinical GastroEnterol & Hepatol 2020

A Machine Learning Approach Enables Quantitative Measurement of Liver Histology and Disease Monitoring in NASH

> 5,000 biopsies [STELLAR-3 &4, ATLAS]

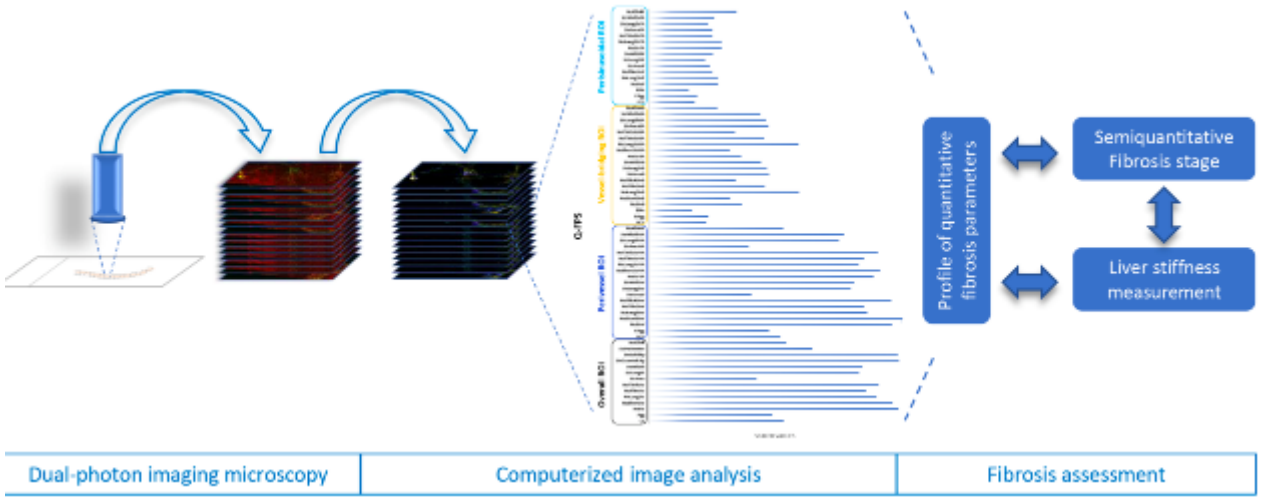
Pokkalla H Hepatology on line

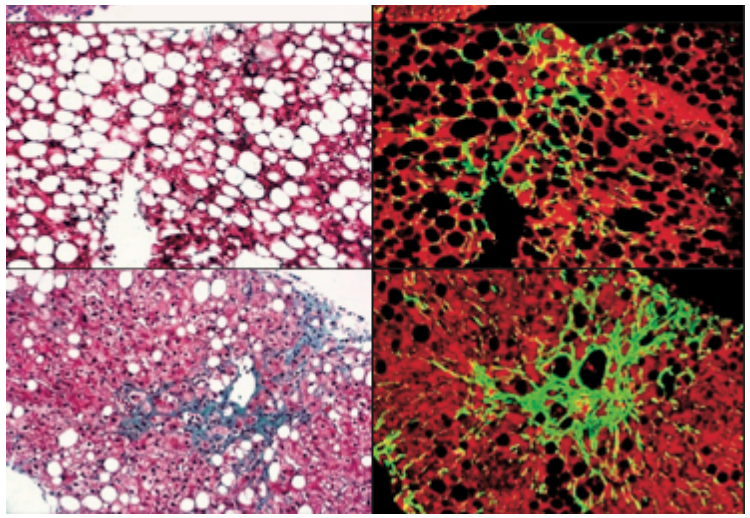
In Brief,

- Moderate to strong correlations between ½ quantitative and quantitative analysis
- Overestimation of steatosis by the pathologist [from 2.5% (grade 1) to 26.1% (grade 3)]
  - Reliability of quantitative pathological assessment ?
- Fibrosis stage increase follows an exponentional fashion
  - Meaning of 1 stage variation interpretated according to the extent of fibrosis (F3 ↔ F4 ≠ F1 ↔ F2) ?
- Prognostic utility and the potential to monitor response to therapy

Dual Photon Imaging Microscopy

- Unstained FFPE 4 µm slides, dedicated equipment
- Automated quantification of a panel of fibrosis parameters (collagen distribution, morphology and location)





- Numerical fibrosis systems scoring based on specific parameters
- Specific patterns of fibrosis in adult and pediatric patients
- May be applied to steatosis measurement

Wang Y Hepatology 2017 & Gut 2019, Liu F Am J Clin Pathol 2017 & Hepatology 2020

Future Steps ?

Endless challenges

➢ Adequacy of the biopsy

➢ Observer variability

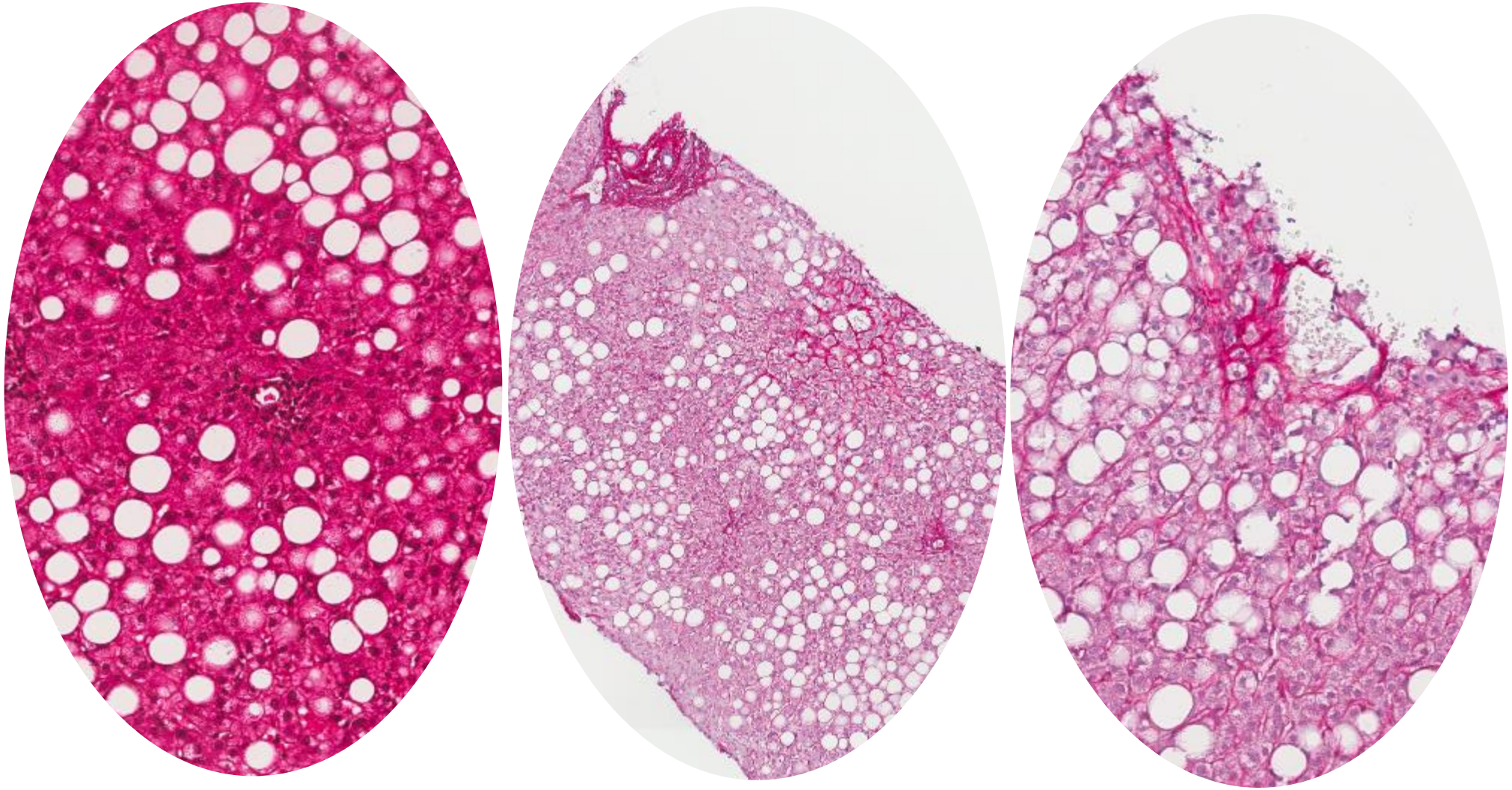
New challenges

➢ Improve NASH stratification

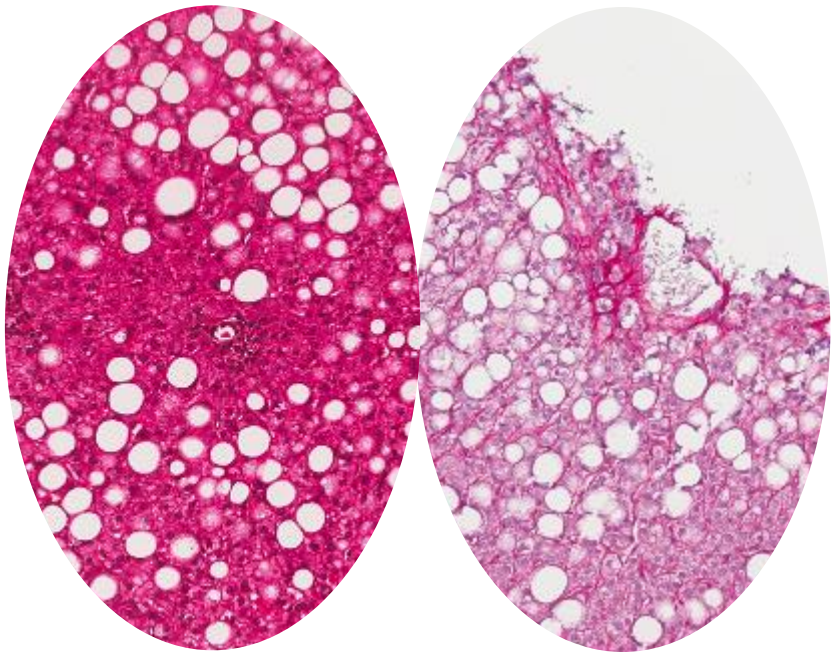
- Input of other morphological features ?

➢ Refine Fibrosis staging

Steatosis + Lob. Inflammation + Perisinusoidal fibrosis
Not enough for « Definite NASH »



Steatosis + Lob. Inflammation + Perisinusoidal fibrosis
Why Not NASH ?

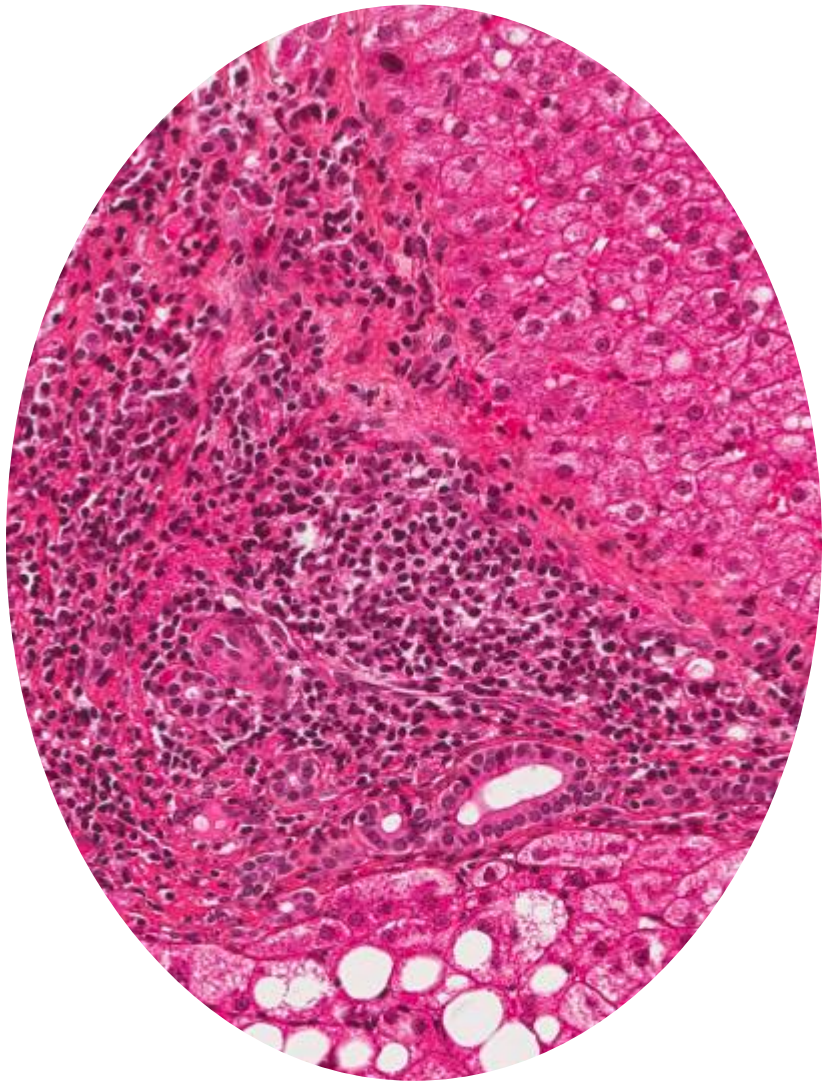


- NAFLD is a dynamic process
  - Early development of perisinusoidal fibrosis
  - Fibrosis considered as a consequence of disease activity
  - Perisinusoidal fibrosis as an early marker of agressive disease (triggered by lob. inflammation ± Ballooning)
    - Consider perisinusoidal fibrosis in NASH diagnosis ?
    - Ballooning : poor reproducible feature, unknown fate

- Diagnosis of NASH *
  1. Steatosis (any degree) + CL ballooning (± MDB)
  2. Steatosis (any degree) + CL fibrosis or bridging fibrosis

* Younossi ZM, ... Goodman Z, Hepatology 2011

Portal Inflammation



Portal Chronic Inflammation in Nonalcoholic Fatty Liver Disease (NAFLD): A Histologic Marker of Advanced NAFLD—Clinicopathologic Correlations from the Nonalcoholic Steatohepatitis Clinical Research Network

Portal inflammation

- « More than mild *» in 23 % of adult patients
- Correlated with features of progressive disease
  - Clinical features
  - Definite diagnosis of NASH
  - Advanced fibrosis

* More than mild (at least 2 PT with inflammation replacing a portion of the matrix)

Brunt E Hepatology 2009

Portal Inflammation: To be included in scoring ?

Original Investigation | Gastroenterology and Hepatology

Association of Histologic Disease Activity With Progression of Nonalcoholic Fatty Liver Disease

- Prospective cohort substudy (NASH CRN) to evaluate histological evolution and factors associated with changes over time
- 446 patients with 2 liver biopsies
- Portal inflammation associated with progression and regression changes

Kleiner DE JAMA Network Open 2019

Table 1. Necroinflammatory Grading System for Steatohepatitis

Mild, grade 1	Steatosis (predominantly macrovesicular) involving up to 66% of biopsy; may see occasional ballooned zone 3 hepatocytes; scattered rate intra-acinar pmn's ± intra-acinar lymphocytes; no or mild portal chronic inflammation.
Moderate, grade 2	Steatosis of any degree; ballooning of hepatocytes (predominantly zone 3) obvious; intra-acinar pmn's noted, may be associated with zone 3 pericellular fibrosis; portal and intra-acinar chronic inflammation noted, mild to moderate.
Severe, grade 3	Panacinar steatosis; ballooning and disarray obvious, predominantly in zone 3; intra-acinar inflammation noted as scattered pmn's, pms's associated with ballooned hepatocytes ± mild chronic inflammation; portal chronic inflammation mild or moderate, not marked.

Brunt E Am J GastroEnterol 1999

Future Steps ?

Endless challenges

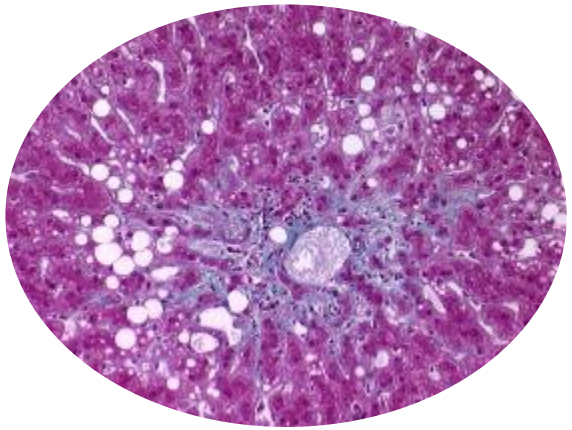
- Adequacy of the biopsy
- Observer variability

New challenges

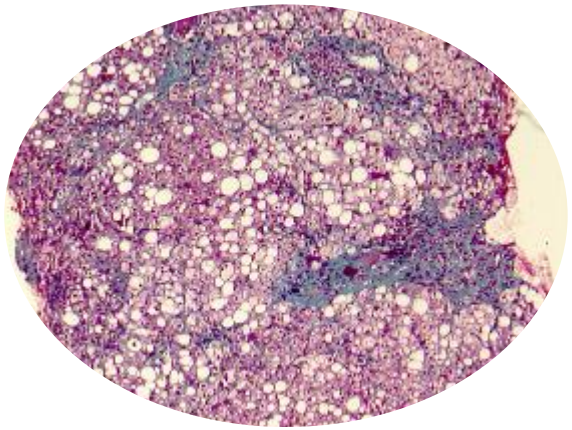
- Improve NASH stratification
- Refine Fibrosis staging
  - Towards a more granular system

NAFLD Staging (F)
The most relevant histological endpoint

Lobular Fibrosis



Portal Fibrosis



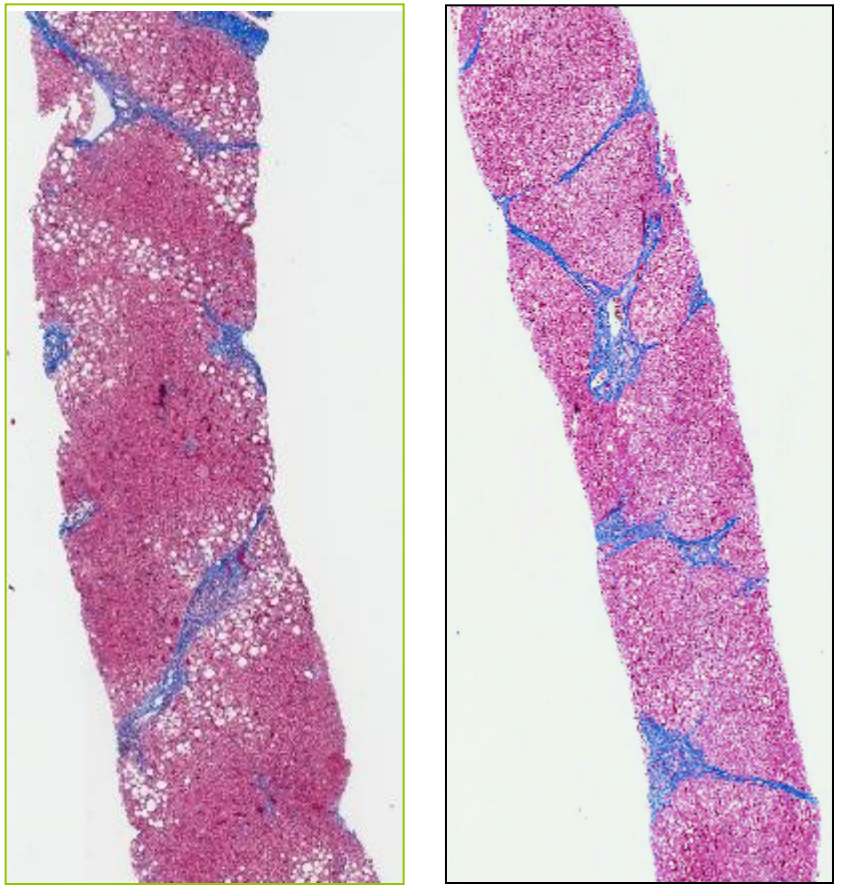
NASH CRN (Kleiner D Hepatology 2005)

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

- Staging is robust (very low inter-observer variability, κ 0.83)

(Kleiner D Hepatology 2005, Bedossa P Hepatology 2012 & 2014)

Lack of granularity



Stage 3

Proposal EPOS (FLIP consortium)

NASH CRN → EPOS	Comments
1a 1b 1c → 1	Lumping together because: - Poor reproducibility, Sampling error - No clinical relevance
2 → 2	Changing definition : Central or Portal fibrosis extending to the midzone or portal + central fibrosis
3 → 3 3 → 4	Increased granularity: Few septa (no more than 2 /10mm length of biopsy) Many septa without nodule formation
4 → 5 4 → 6	Increased granularity : Many septa with occasional nodules Cirrrhosis

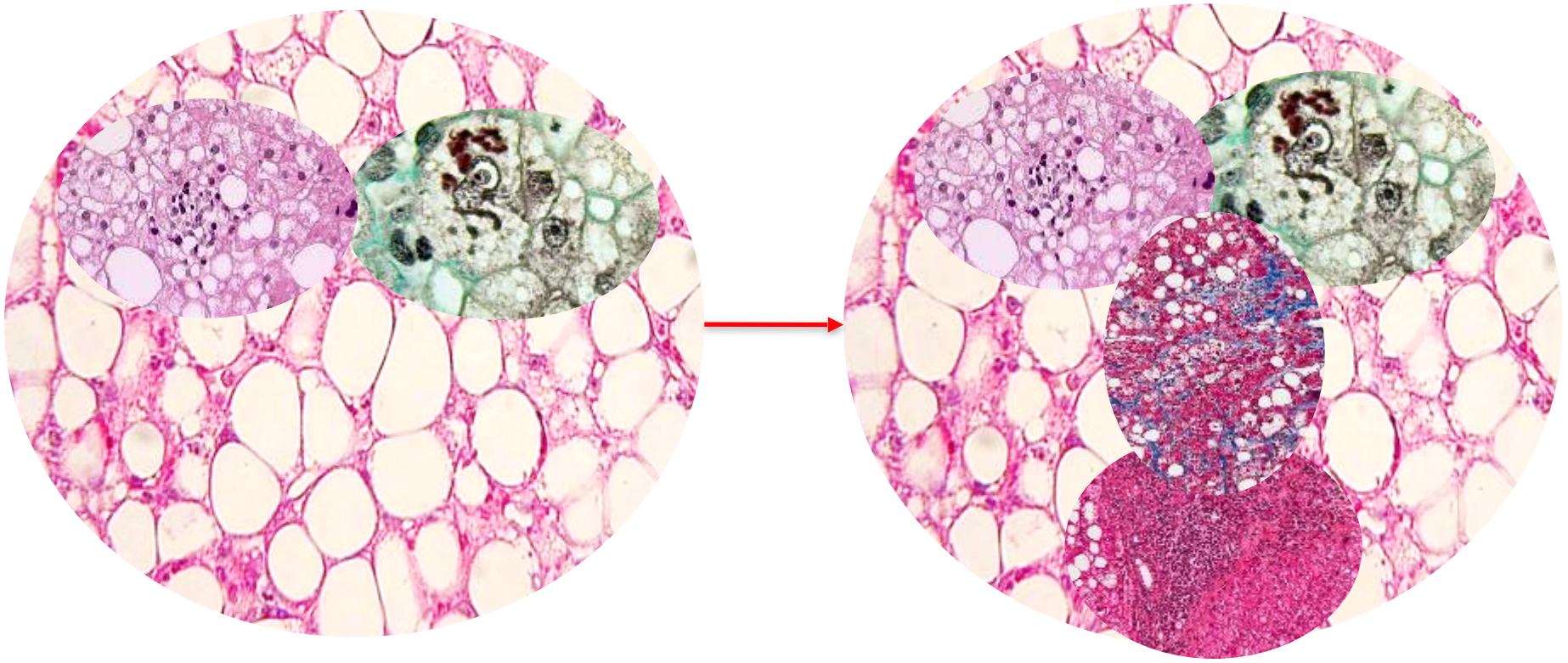
Bedossa P et al EASL 2018

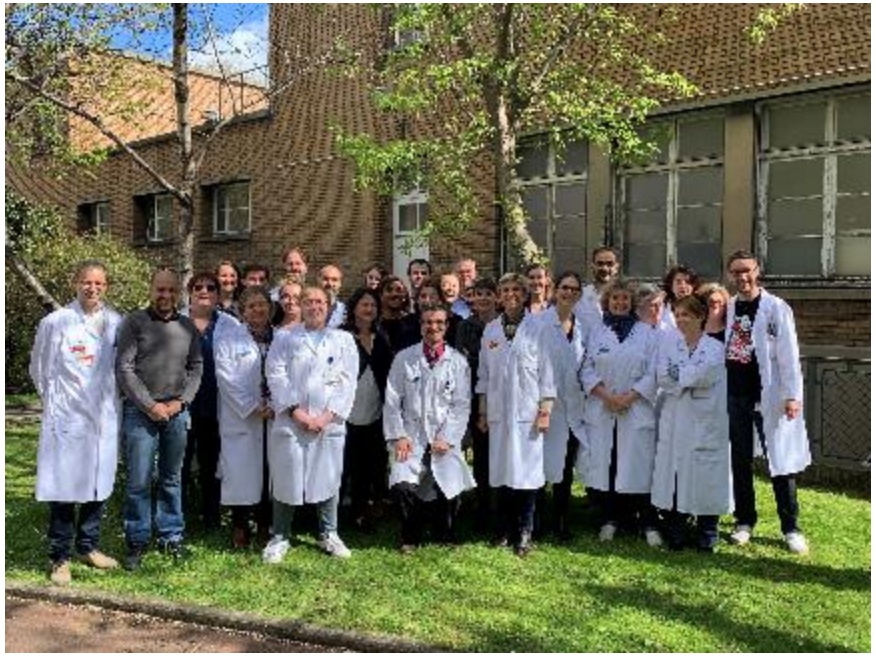
Take-Home Messages

- NASH diagnosis is based on histology
- Liver biopsy : “The reference standard”
  - Diagnosis assessment, grading & staging of the disease
  - Required for patient eligibility and drug evaluation (phase 3 CT)
- Liver biopsy is still challenged
  - Sampling variability: impact dependent to the severity of the disease
  - Observer reproducibility: Quantitative computerized approaches are developing with encouraging and promising results

Further Step
Refine Definition, Grading and Staging

Better identification of patients with NASH
for prognostic and theranostic issues





Inserm U 1149 / CRI

From inflammation to cancer in digestive diseases »

V Paradis

- A Couvelard, N Guedj, J Cros, V Rebours, A Beaufrère
- A Hammoutène (Post-doc)
- F Cauchy, S Frendi, E Gigante, L de Mestier (Doc)
- M Tabard (M2)
- S Laouirem, C de Flori, H Cazier, M Albuquerque (IE)

Beaujon hospital

- Pathology (V Paradis)
- Surgery (O Soubrane)
- Oncology (M Bouattour)
- Radiology (V Vilgrain)
- Hepatology (F Durand)

CONTRE LE CANCER
LA LIGUE
pour la vie

HÔPITAUX UNIVERSITAIRES PARIS NORD VAL DE SEINE
Beaujon

CRI
CENTRE de RECHERCHE sur l'INFLAMMATION

unity
Addressing Unmet Needs through Innovation in hepaTology and gastroenterologY

Université de Paris

Some (non anecdotic) nuances

NAS

- All features combined
- Not diagnostic score

SAF

- Separately assessment
- Diagnostic score

Steatosis (From 0 to 3)

Ballooning

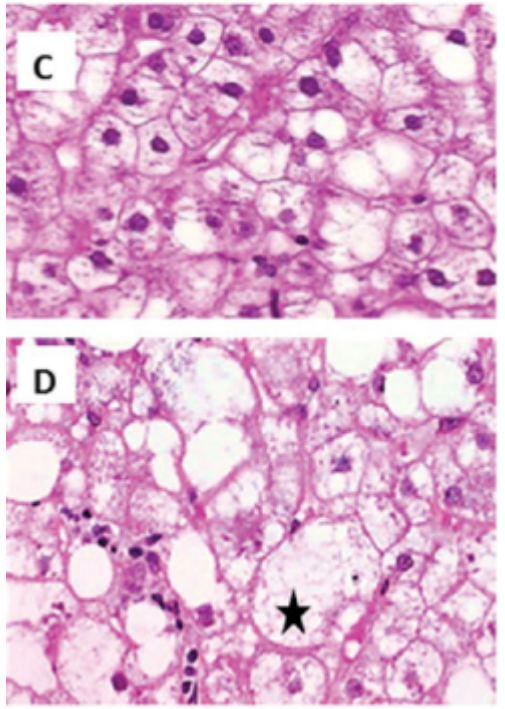
(1) Few / (2)Many (1) Normal size / (2) Large

Lobular inflammation

From 1 to 3 From 1 to 2

NAS and SAF scores not interchangeable

SAF Ball (1)



SAF Ball (2)

From Bedossa P Hepatology 2012