



Festschrift for Prof. Hübscher - 13 May 2022



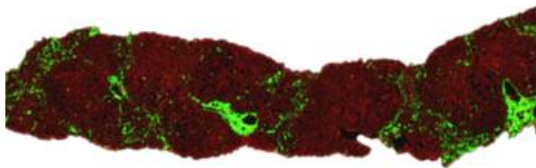
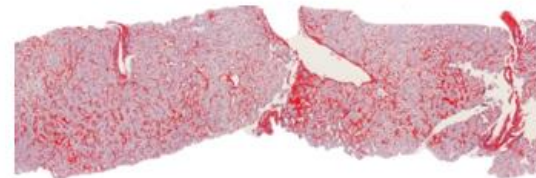
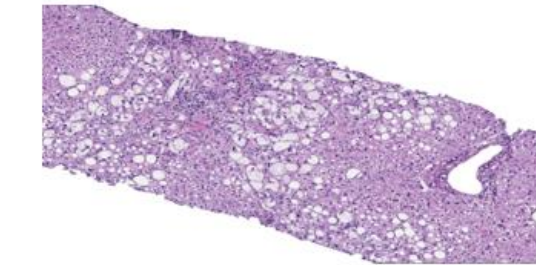
Artificial intelligence and evaluation of NAFLD pathology

Dina G. Tiniakos

*Translational & Clinical Research Institute,
Faculty of Medical Sciences, Newcastle University, UK*

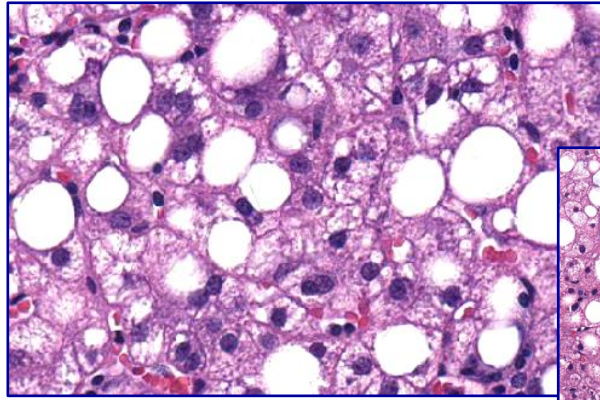


*Dept of Pathology, Aretaieion Hospital,
National & Kapodistrian University of Athens, Greece*

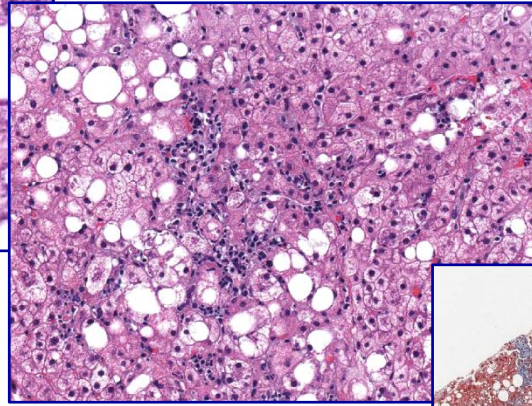




Histological spectrum of **NAFLD**

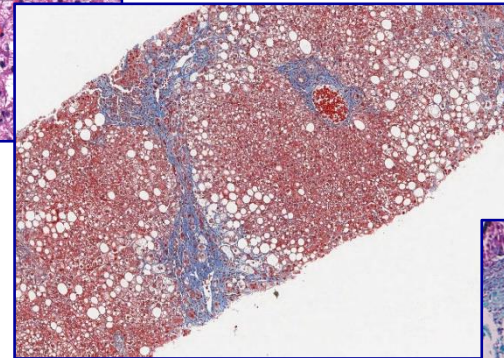


Steatosis **100%**

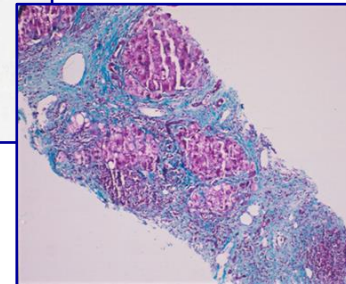


Steatohepatitis
(NASH)

7% Asia
30% North America
1.5-6.5% globally



Progressive fibrosis **40% of NASH**



Cirrhosis **3-5%**

Diehl & Day, NEJM 2017

Anstee, Nat Rev Gastro Hepatol 2019

• **HCC development**

- NASH/cryptogenic cirrhosis

- **non-cirrhotic NAFLD**

Incidence

2.6-11.3%

0.04-6%

Huang, Nat Rev Gastroenterol Hepatol 2021

Current concepts in NAFLD pathology

Liver biopsy is the “best” standard

- **diagnosis of NASH and classification of NAFLD**

EASL-ESD-EASO NAFLD Clinical Practice Guidelines 2016

AASLD NAFLD Practice Guidance 2018

Cotter & Rinella, Gastroenterology 2020

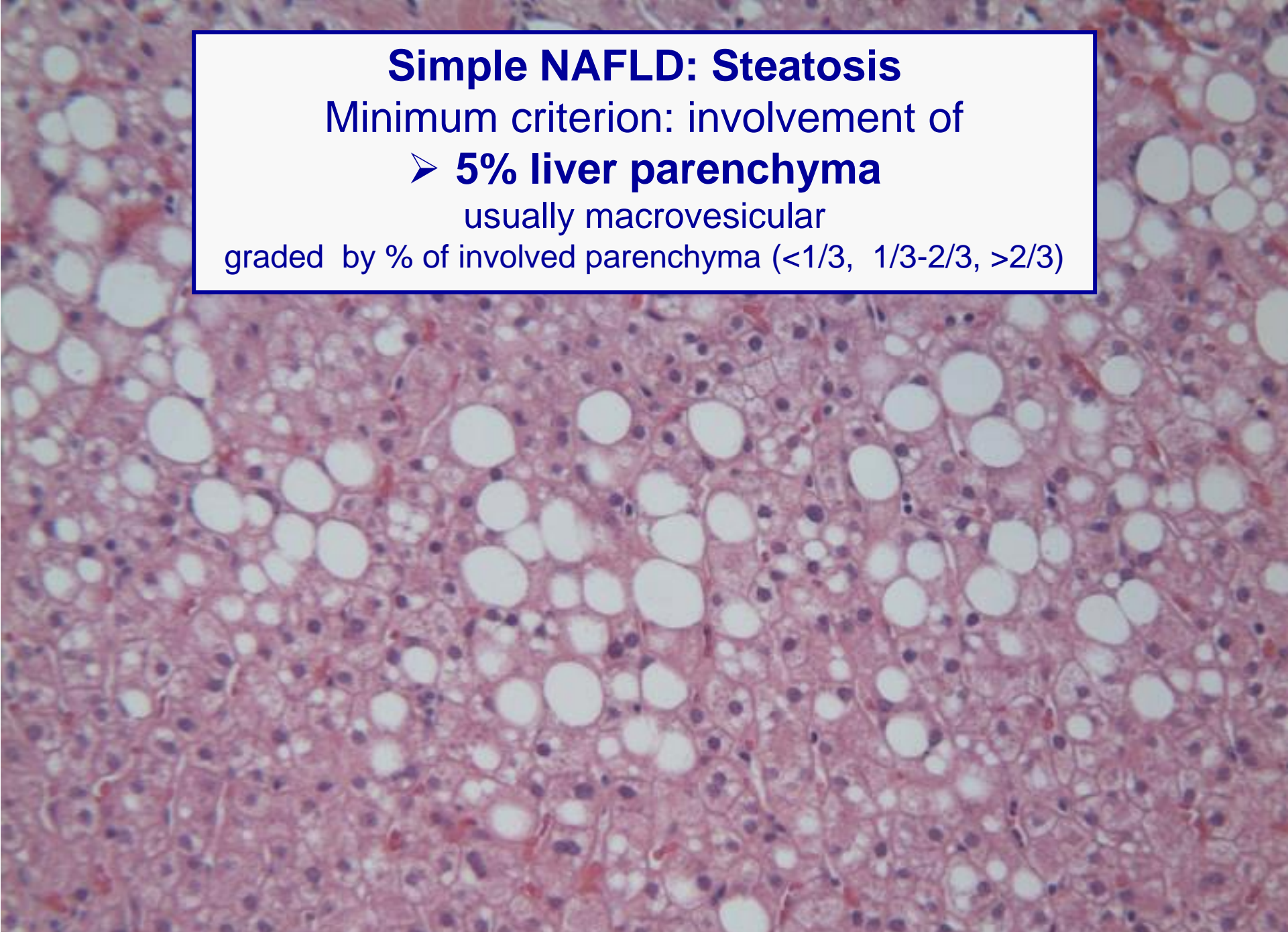
Simple NAFLD: Steatosis

Minimum criterion: involvement of

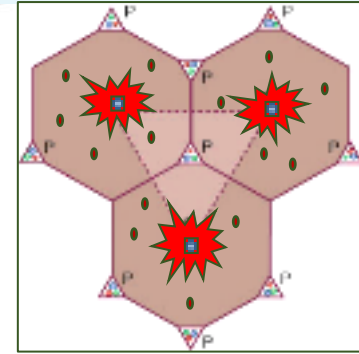
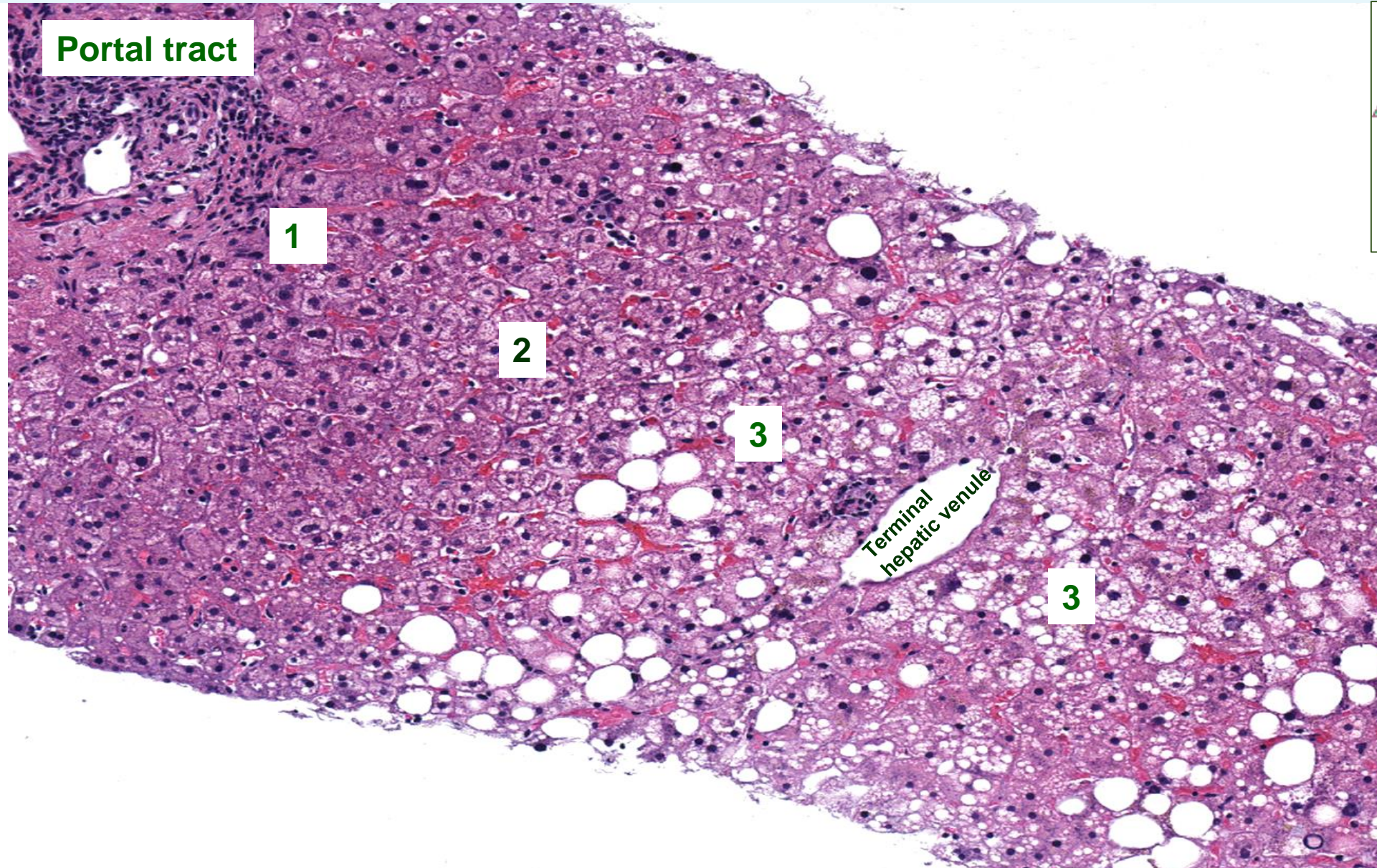
➤ **5% liver parenchyma**

usually macrovesicular

graded by % of involved parenchyma (<1/3, 1/3-2/3, >2/3)

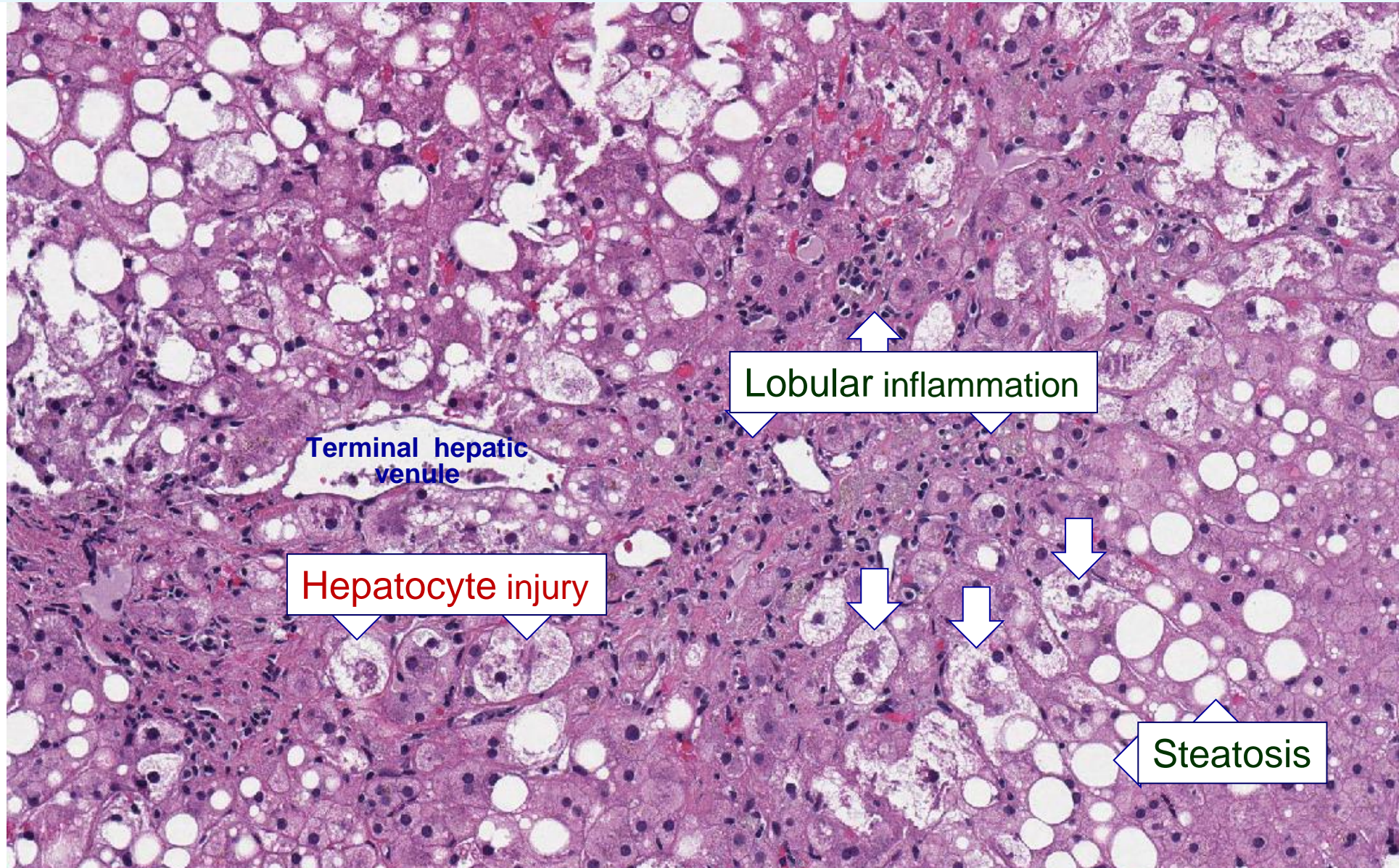


Adult NASH: Zone 3 predominance of the lesions



Minimal criteria for diagnosis of adult NASH

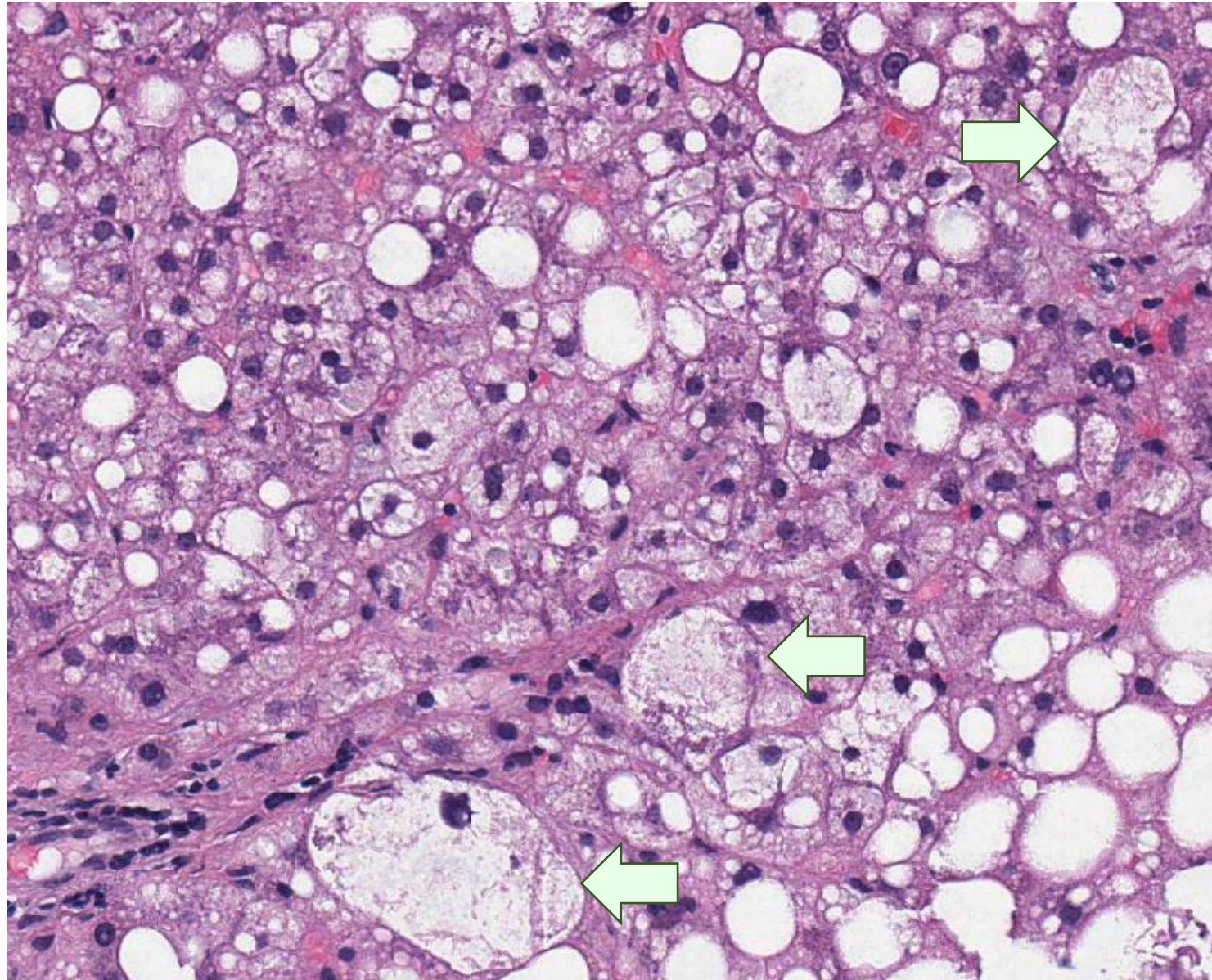
Constellation of findings



NASH: Hepatocyte Ballooning Feature required for the diagnosis

Zone 3

**Terminal
hepatic
venule**



Current concepts in NAFLD pathology

Liver biopsy is the “best” standard

- diagnosis of NASH and classification of NAFLD
- **assessment of necroinflammatory injury – grading NAFLD activity**

NAFLD Activity Score 0-8

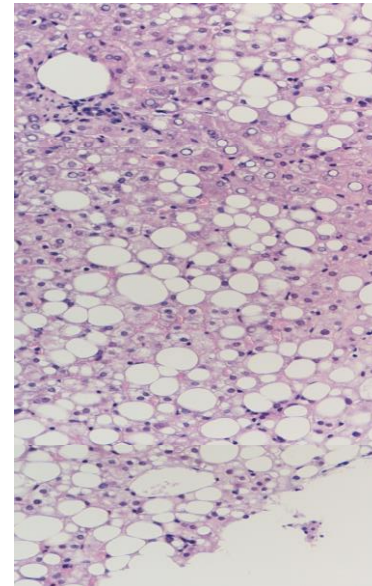
Steatosis+Lobular Inflammation+Ballooning

Kleiner D et al, Hepatology 2005

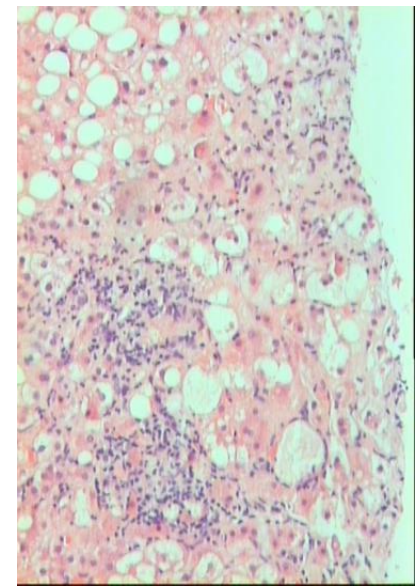
SAF activity score 0-4

Lobular inflammation+Ballooning

Bedossa P et al, Hepatology 2012



NAS 3
SAF A1



NAS 7
SAF A4

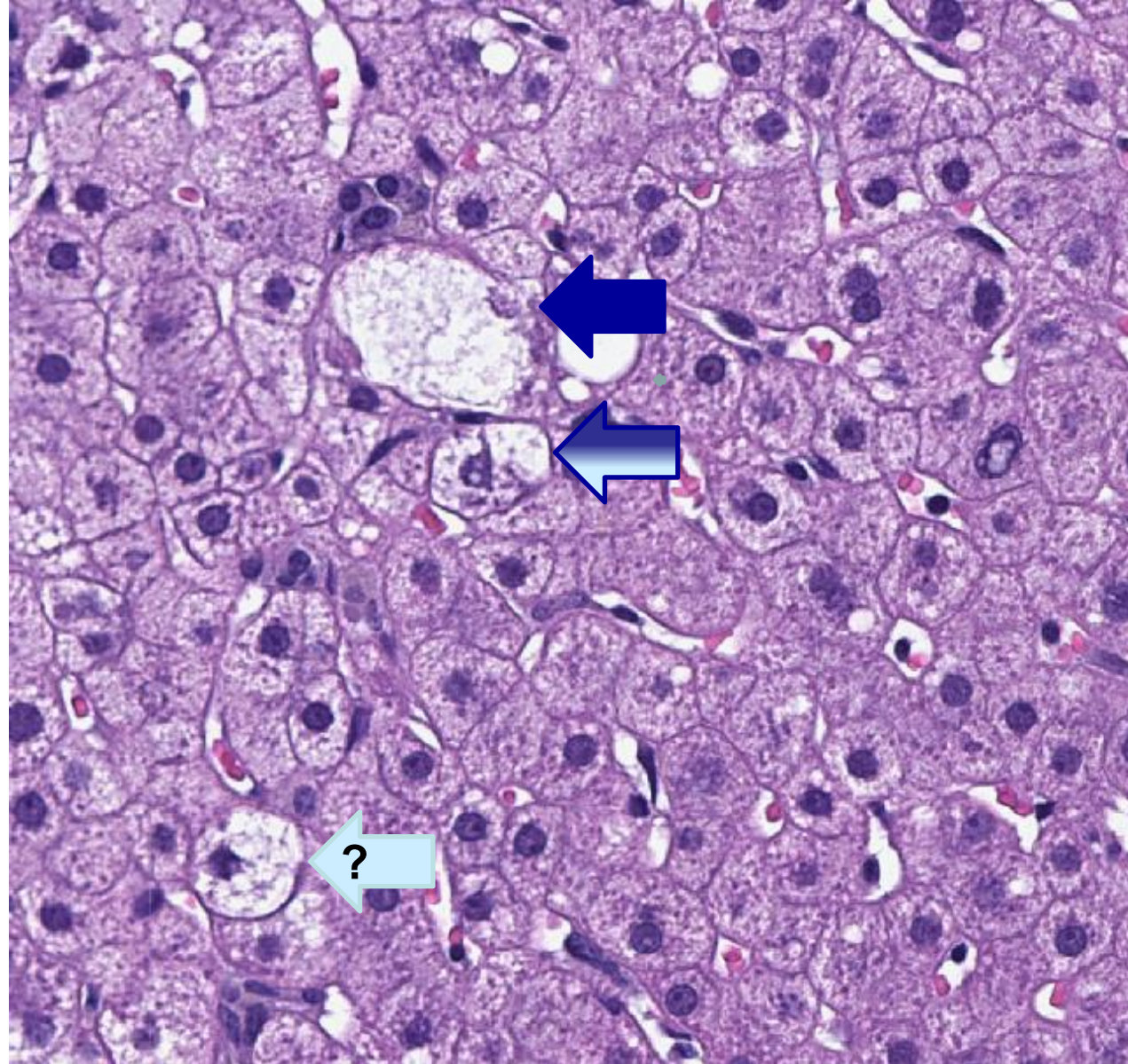
Reproducibility studies in adult NAFLD pathology

Assessment of inter-observer variability

- **Moderate to Excellent agreement** Kappa (κ)
 - ◆ grade of steatosis 0.61-0.80
 - ◆ classical hepatocellular ballooning 0.50-0.80
 - ◆ diagnosis of NASH 0.40* 0.61-0.85
- **Poor agreement:**
 - ◆ lobular inflammation 0.21-0.72

*Younossi, Mod Pathol 1998; Kleiner, Hepatology 2005; Fukusato, Hepatology Res 2006; Juluri, Clin Gastroenterol 2011; Gawrieh, Ann Diagn Pathol 2011; Bedossa, Hepatology 2014; Pournik, Hepatol Mon 2014; Davison, J Hepatol 2020**

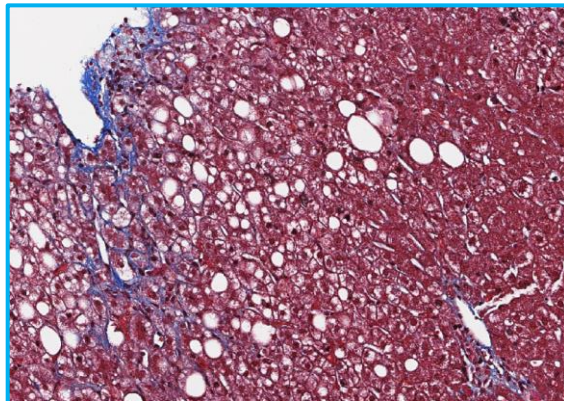
Different types of Hepatocyte Ballooning: Does ballooning have a spectrum? Inter-observer variability?



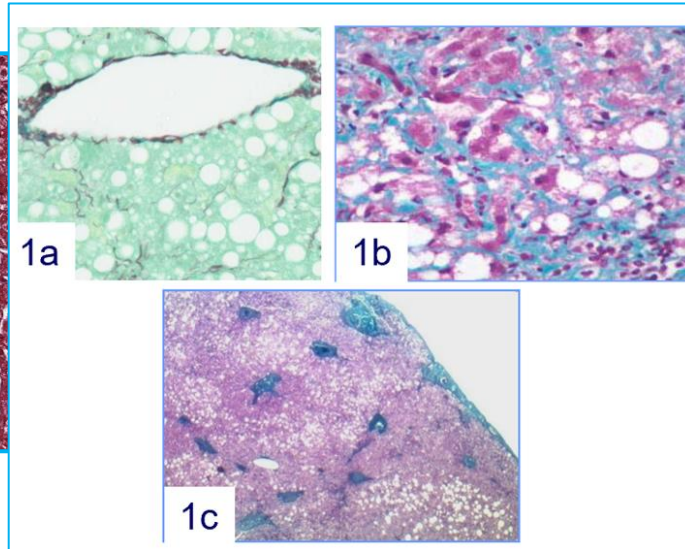
Current concepts in NAFLD pathology

Liver biopsy is the “best” standard

- diagnosis of NASH and classification of NAFLD
- assessment of necroinflammatory injury – grading NAFLD activity
- **evaluation of extent of fibrosis and architectural remodeling – staging fibrosis**



F1 Zone 3 sinusoidal/
pericellular
fibrosis-Z3PCF



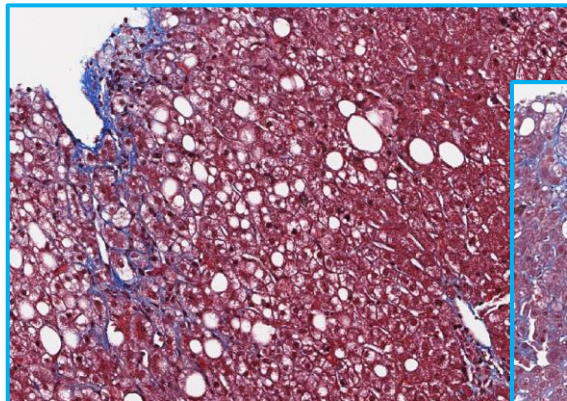
Semi-quantitative staging systems
Brunt EM et al, Am J Gastroenterol 1999
Kleiner D et al, Hepatology 2005

Current concepts in NAFLD pathology

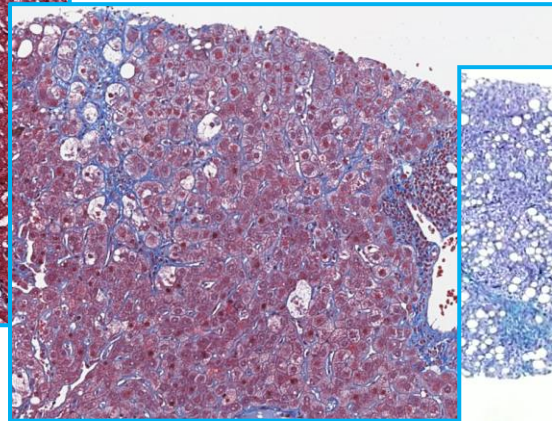
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- diagnosis of NASH and classification of NAFLD
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- evaluation of extent of fibrosis and architectural remodeling – **staging fibrosis**

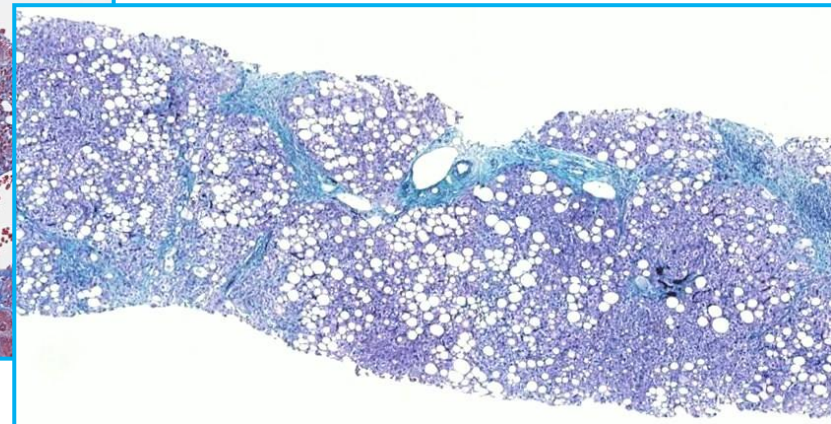
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Brunt EM et al, Am J Gastroenterol 1999
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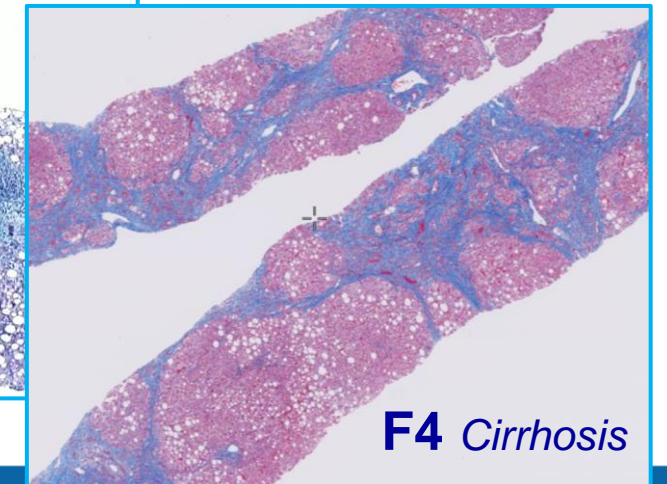
F1 Zone 3 sinusoidal/
pericellular
fibrosis-Z3PCF



F2 Z3PCF &
periportal fibrosis



F3 Bridging fibrosis



F4 Cirrhosis

Current concepts in NAFLD pathology

Liver biopsy is the “best” standard

- diagnosis of NASH and classification of NAFLD
- assessment of necroinflammatory injury – grading NAFLD activity
- evaluation of extent of fibrosis and architectural remodeling – **staging fibrosis**

Semi-quantitative staging systems
Brunt EM et al, Am J Gastroenterol 1999
Kleiner D et al, Hepatology 2005

Fibrosis stage is a key independent prognostic marker for all-cause and liver-related mortality in NAFLD patients

Taylor R et al, Gastroenterology 2020 Meta-analysis



Reproducibility studies in adult NAFLD pathology

Assessment of inter-observer variability

- **Moderate to Excellent agreement**

- ◆ stage of fibrosis κ 0.48*-0.84

*Younossi, Mod Pathol 1998; Kleiner, Hepatology 2005; Fukusato, Hepatology Res 2006; Juluri, Clin Gastroenterol 2011; Gawrieh, Ann Diagn Pathol 2011; Bedossa, Hepatology 2014; Pournik, Hepatol Mon 2014; Pavlides, Am J Clin Pathol 2017; Davison, J Hepatol 2020**

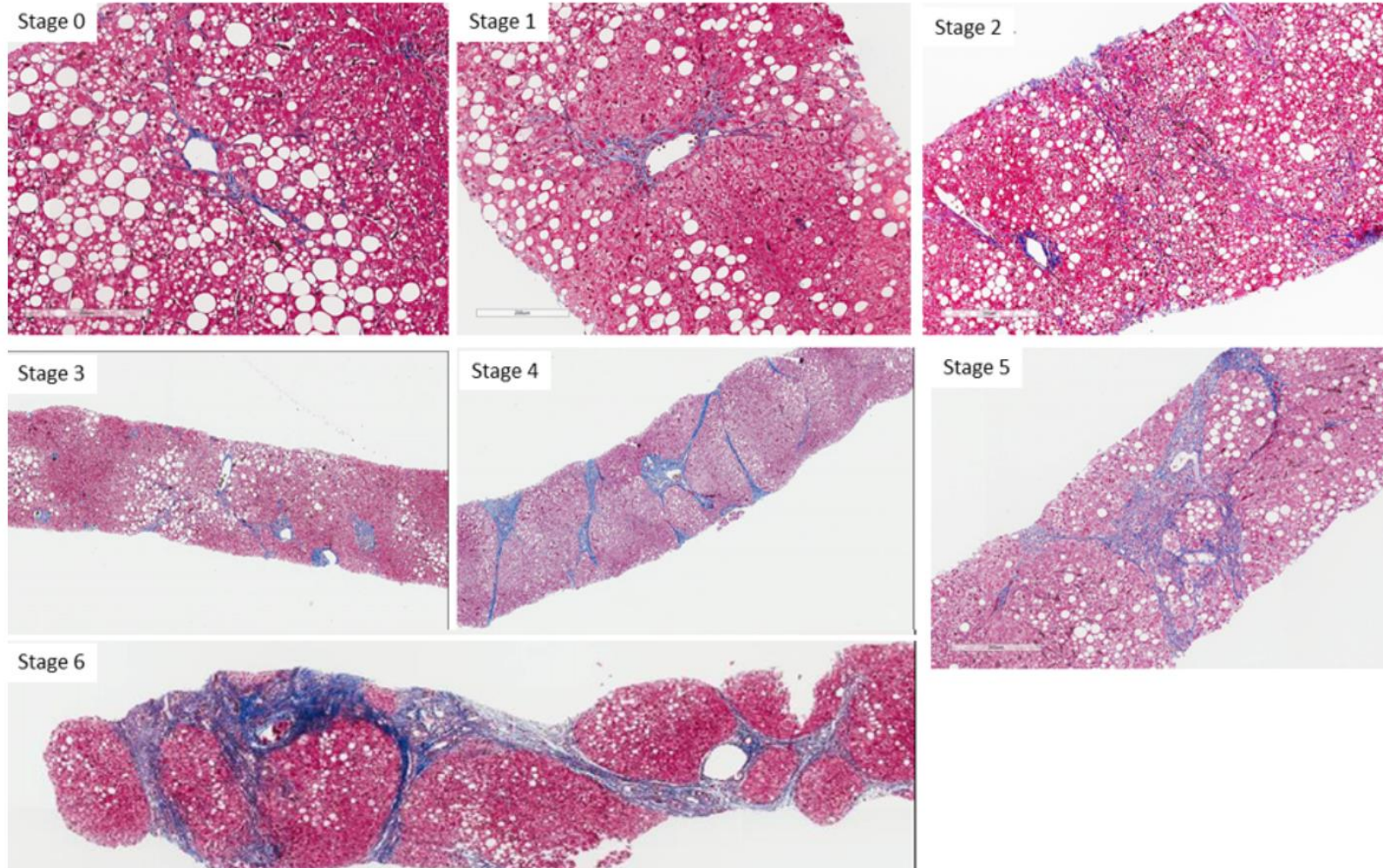
**The expertise of the pathologist plays significant role:
least variation/excellent agreement observed between experts**

Semi-quantitative staging in NAFLD – Need for increased granularity



The EPoS staging system is a reproducible 7-tier fibrosis score for NAFLD adapted both to glass slides and digitized images (e-slides)

P. BEDOSSA¹, J. AROLA², S. DAVIES³, A.S.H. GOUW⁴, M. GUIDO⁵, C. LACKNER⁶, P. SCHIRMACHER⁷, L. TERRACCIANO⁸, Q.M. ANSTEE⁹, V. RATZIU¹⁰, D.TINIAKOS^{9,11}



Clinical trial end-points in NAFLD: The need for liver biopsy

Table 2. Suggested endpoints for NASH trials across the development spectrum.

	Precirrhotic NASH	Cirrhotic NASH	Pediatric NASH
Early phase development	ALT, AST Hepatic fat (MRI-PDFF) Note: Consider mechanism of action of drug when choosing	Safety Hepatic impairment studies Proof of mechanism studies; endpoints will depend on mechanism of action	No clear recommendation
Phase 2b	NASH resolution without worsening of fibrosis or at least one stage reduction in fibrosis At least a 2-point reduction in NAFLD NAS: At least a 1-point reduction in either lobular inflammation or hepatocellular ballooning AND no worsening of fibrosis stage	Increase of MELD score from <12 to ≥15 At least a 1-point improvement in fibrosis with no worsening of NASH Reduction in HVPG of ≥2 mmHg	No clear recommendation
Phase 3	NASH resolution* with no worsening of fibrosis AND/OR At least a 1-point improvement in fibrosis with no worsening of NASH	MELD progression from <12 to ≥15 Fibrosis reduction by at least one stage without worsening of NASH Hepatic decompensation events - Ascites - Variceal hemorrhage	No clear recommendation

Signs of fibrosis regression, changes in the extent of sinusoidal fibrosis: Histological end-points in clinical trials?

How to further improve evaluation of activity grade and fibrosis stage in NAFLD?

Digital evaluation of fibrosis

- **Collagen Proportionate Area (CPA)**

Munsterman, Histopathology 2018

*Buzzetti, Alim Pharmacol Ther 2019**

Gawrieh, Ann Diagn Pathol 2020

Soon & Wee, Clin Mol Hepatol 2021 (Review)

- **Automated Quantitative image analysis tools**

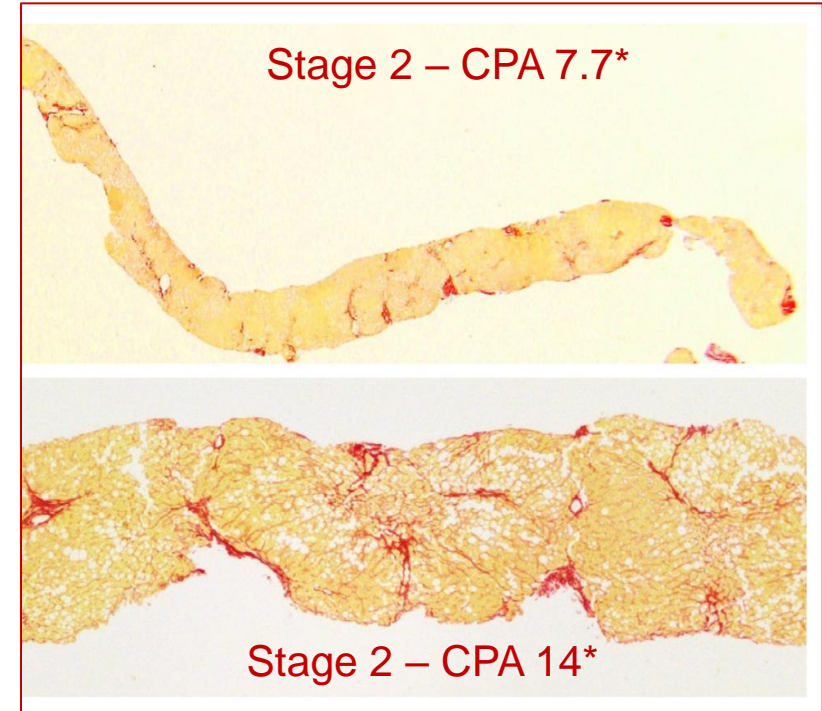
Biocellvia software

De Rudder, Lab Invest 2020

FibroNest

Briand, Metabolism 2021

2022 and beyond: Quantitation not only of fibrosis but all NAFLD activity score components



Digital evaluation of fibrosis: Collagen Proportionate Area (CPA) in NAFLD

- **Objective**
- **Accurate**
- **Measures fibrosis as continuous variable**
- **Correlates well with histological stage and liver stiffness**

Digital evaluation of fibrosis: Collagen Proportionate Area (CPA) in NAFLD

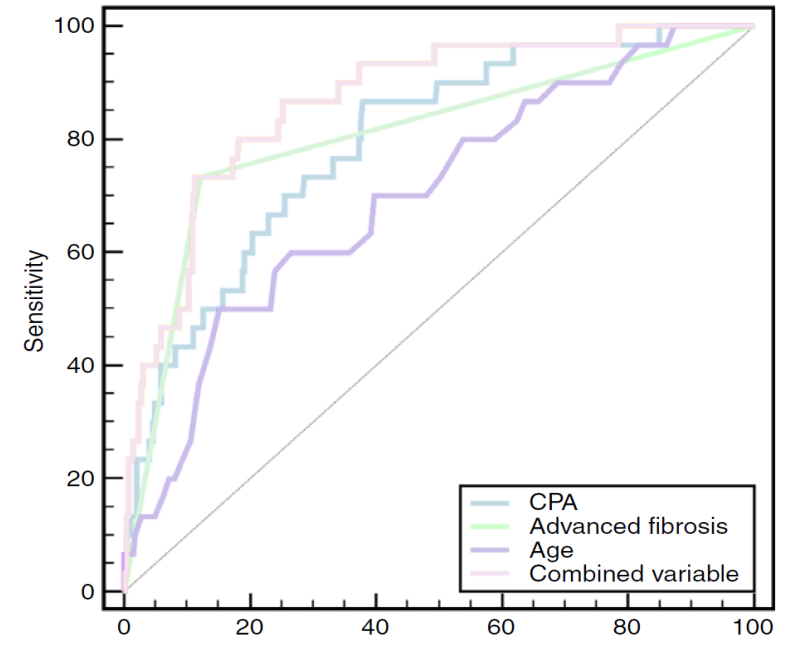
- Objective
- Accurate
- Measures fibrosis as continuous variable
- Correlates well with histological stage and liver stiffness

- **Independent predictor of long term-outcome**

BUT

Buzzetti, Alim Pharmacol Ther 2019

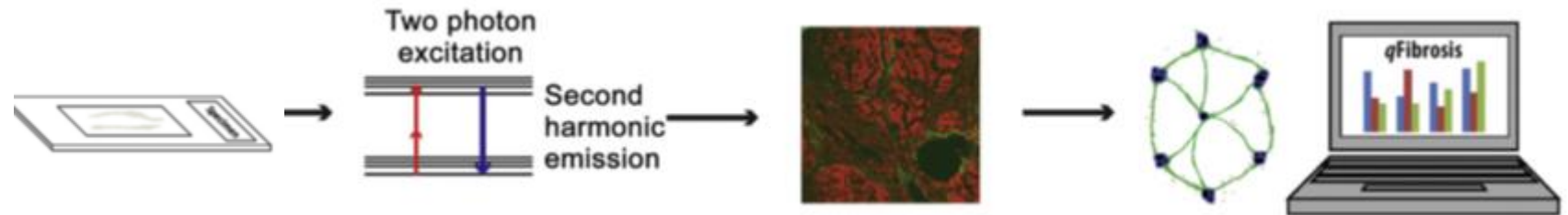
- no information on architectural remodelling or fibrosis dynamics
- technical issues (staining quality, operator experience, software)
- sampling variability



How to further improve evaluation of activity grade and fibrosis stage in NAFLD?

Digital evaluation of fibrosis

- Collagen Proportionate Area (CPA)
- **Label-free (stain-free) microscopy**
Second harmonic generation and two-photon excitation microscopy



Pirhonen, PLoS ONE 2016

Liu, Am J Pathol 2017

Roth, World J Gastroenterol 2018

Chang, PLoS ONE 2018

Leow, Diagnostics 2020

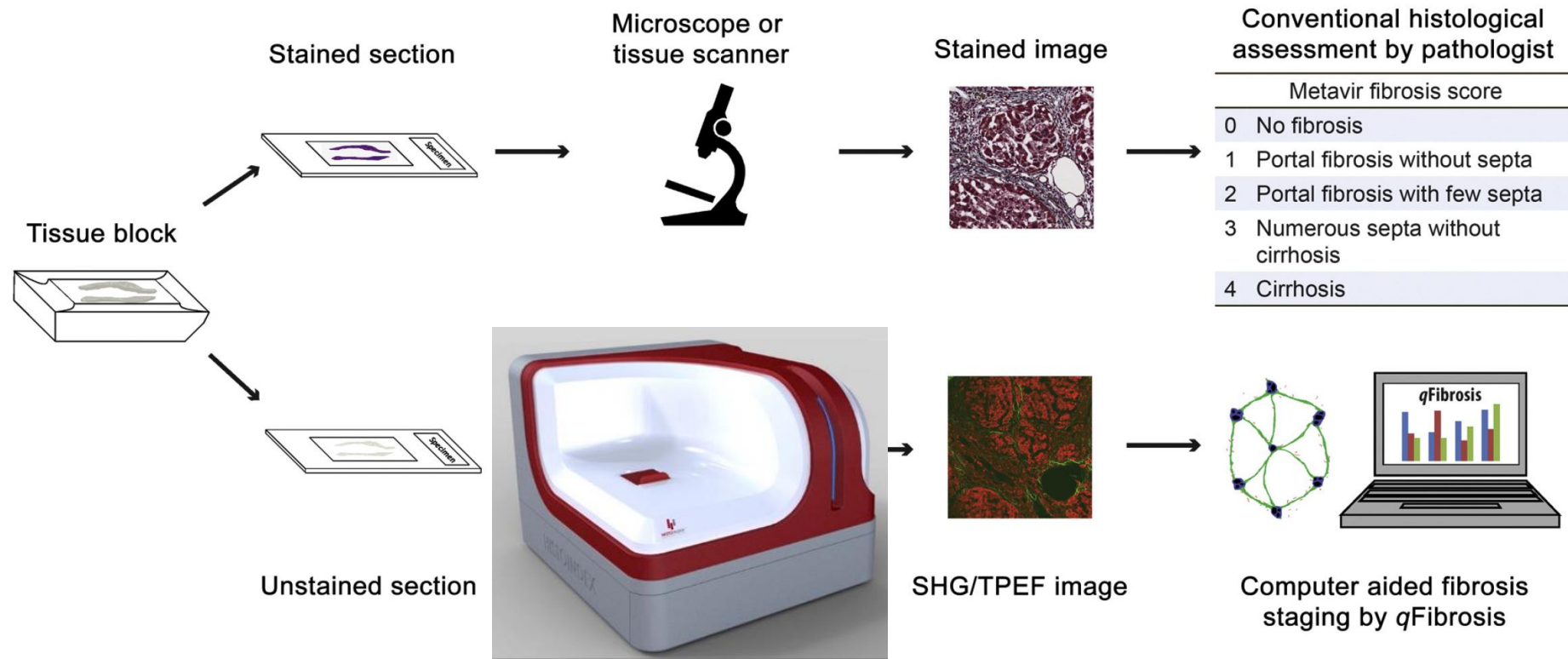
Liu, Hepatology 2020

Soon & Wee, Clin Mol Hepatol 2021 (Review)

© Xu, J Hepatol 2014

Non-linear optical microscopy

Second harmonic generation and two-photon excitation microscopy



Xu, J Hepatol 2014

Fully-automated, computer-aided liver fibrosis staging

Fibrosis stage
NASH CRN

F0

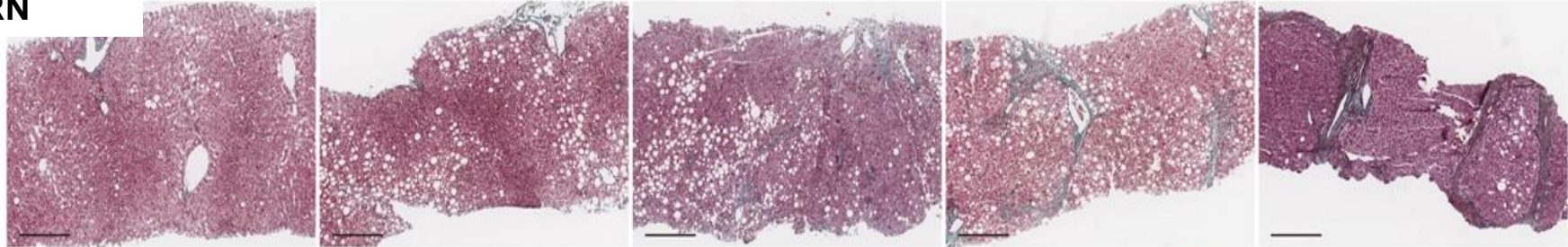
F1

F2

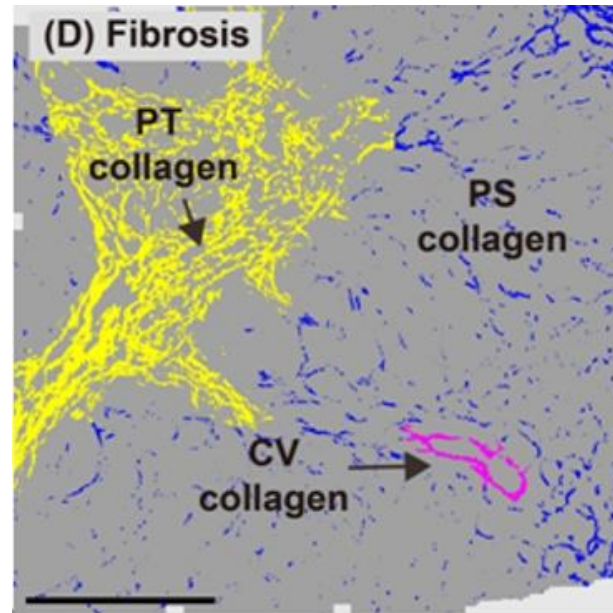
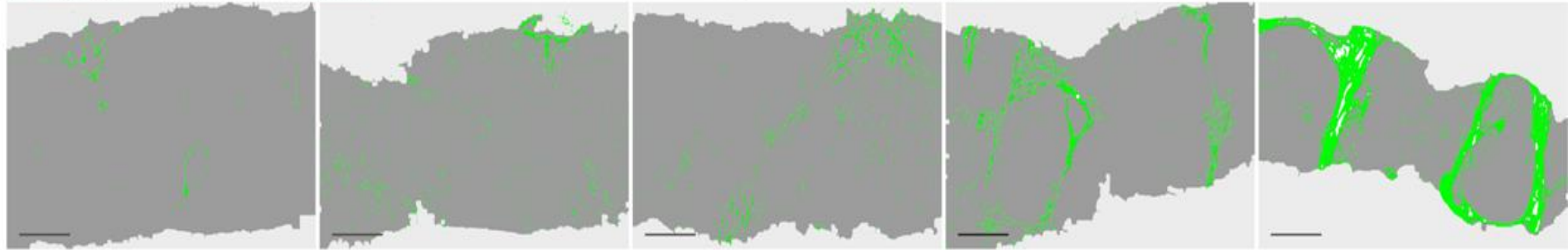
F3

F4

Masson
Trichrome



qFibrosis



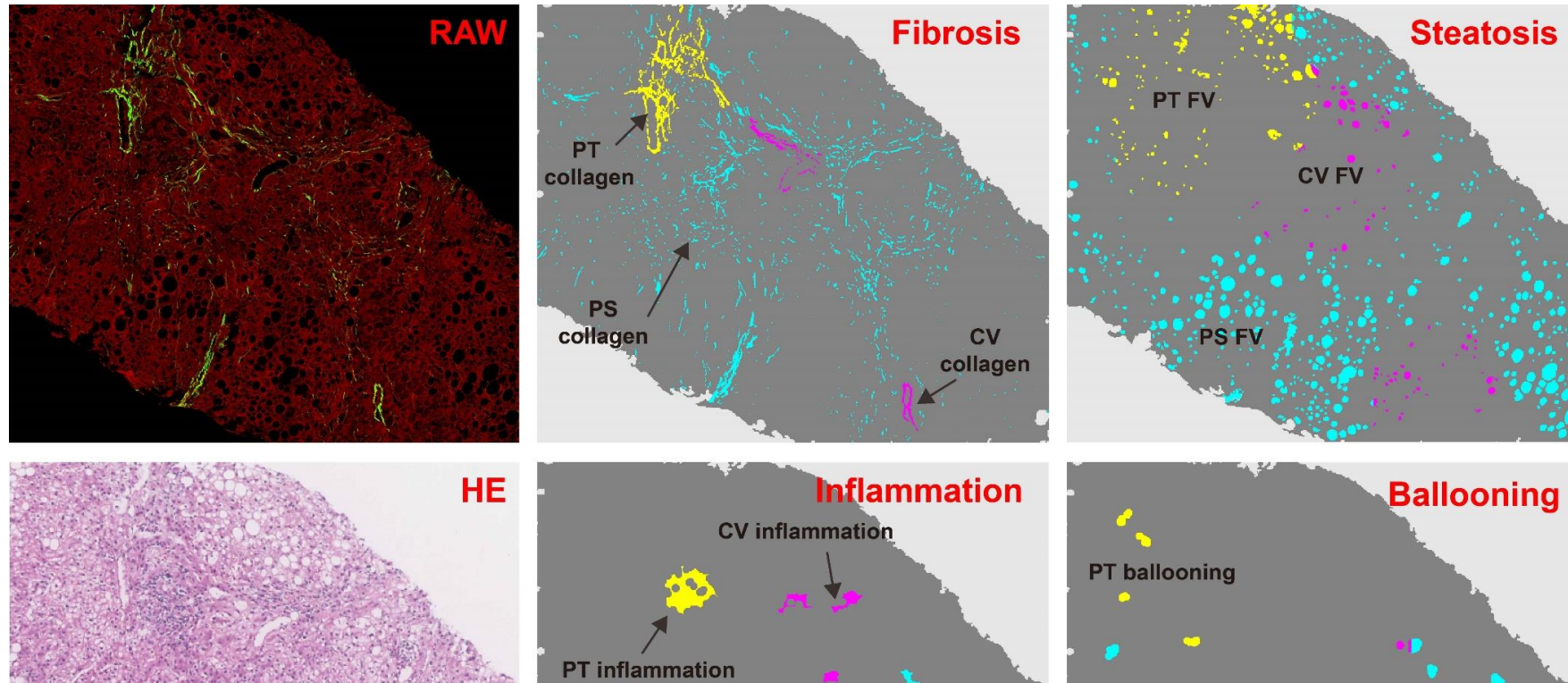
qFibrosis

**Accurate and reproducible method
for fibrosis evaluation in NAFLD
(quantitative and continuous)**

Already used in NASH clinical trial setting

Second Harmonic Generation/Two-Photon excitation microscopy on unstained (label-free) tissue sections

Algorithms for inflammation (qInflammation), ballooning (qBallooning), steatosis (qSteatosis)



**qFIBS: May aid reproducibility and standardization of liver biopsy
assessments required for NASH therapeutic clinical trials**

How to further improve evaluation of activity grade and fibrosis stage in NAFLD?

- Collagen Proportionate Area (CPA)
- Label-free microscopy
Second harmonic generation and two-photon excitation microscopy

■ Artificial intelligence (AI) methods

Supervised machine learning Convolutional neural networks (CNN)

Vanderbeck, Hum Pathol 2014 & 2015

Heinemann, Sci Rep 2019 (animal model NAFLD)

Liu, Hepatology 2020; Leow, Diagnostics 2020

Forlano, Clin Gastroenterol Hepatol 2020

Taylor-Weiner, Hepatology 2021

Bosch, Hepatology 2021

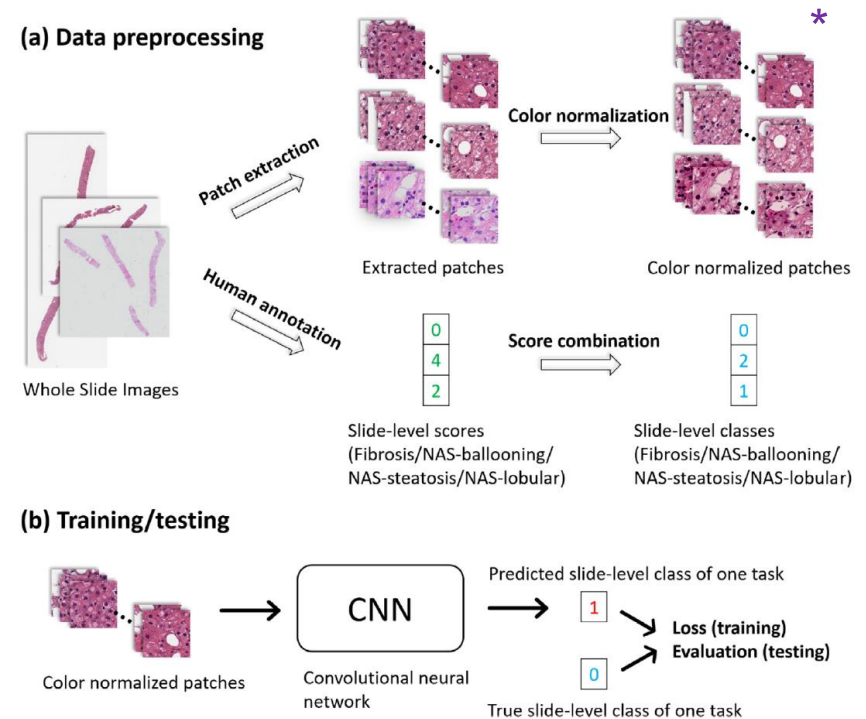
Teramoto, Comp Meth Program Biomed 2021

*Qu, Comp Meth Program Biomed 2021**

Dinani, Hepatology 2021 (Review)

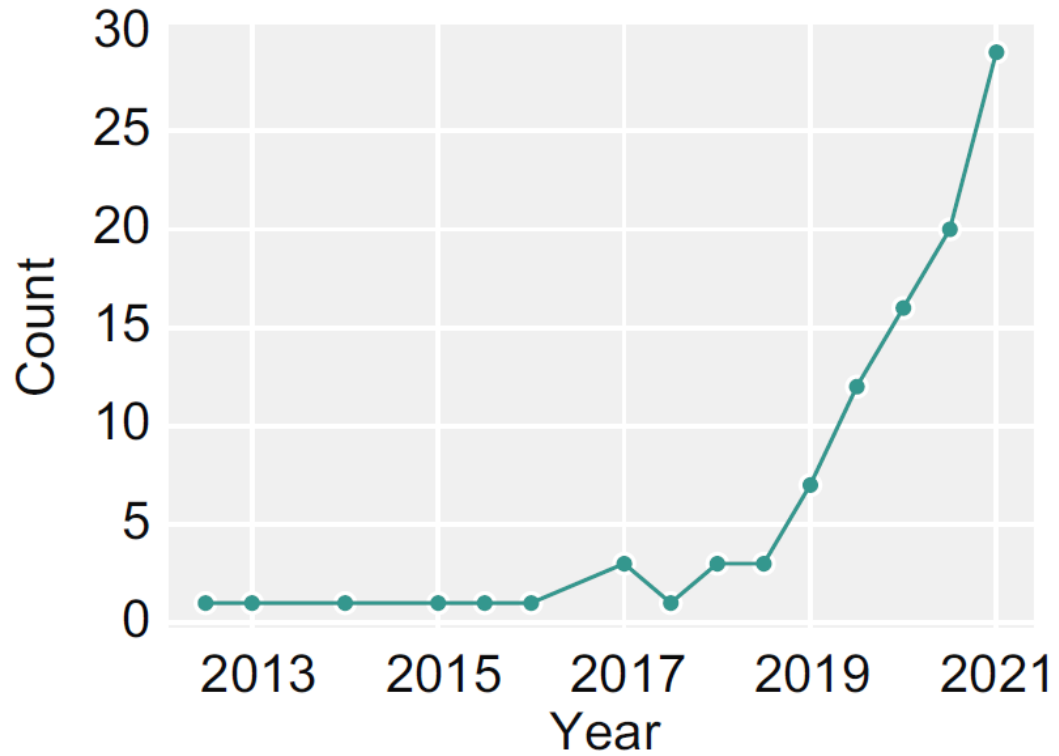
Brunt, J Hepatol 2022

Nam, JHEP Reports 2022 (Review)

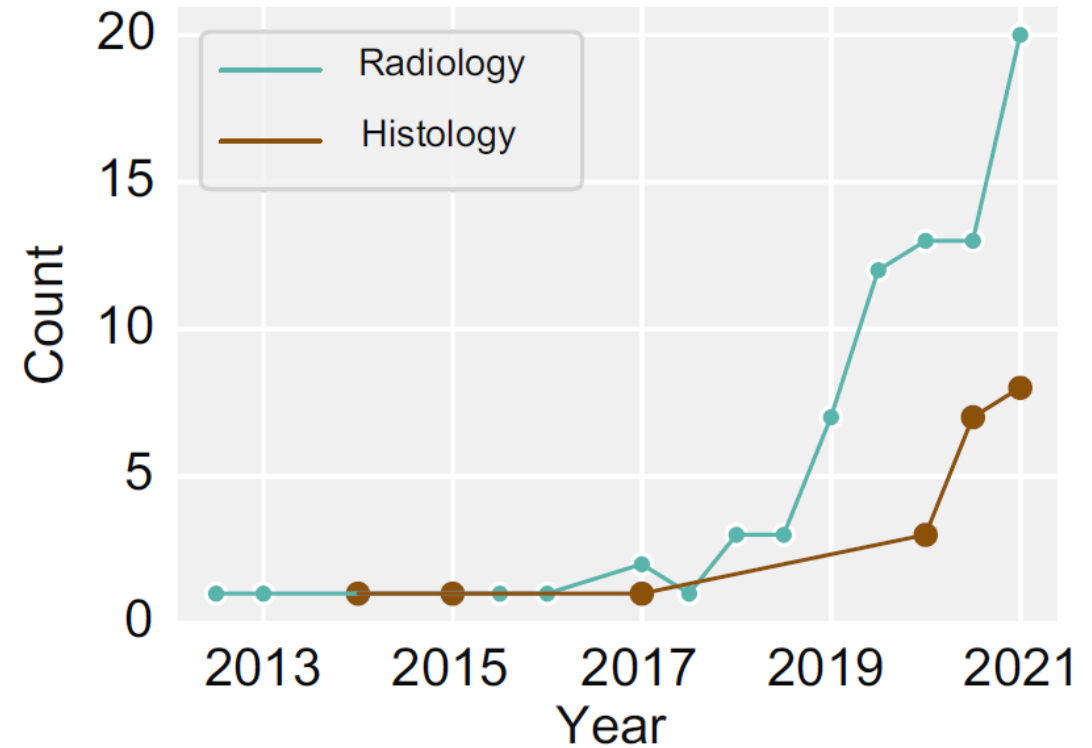


Artificial intelligence (AI) applications in Hepatology

Number of published original studies

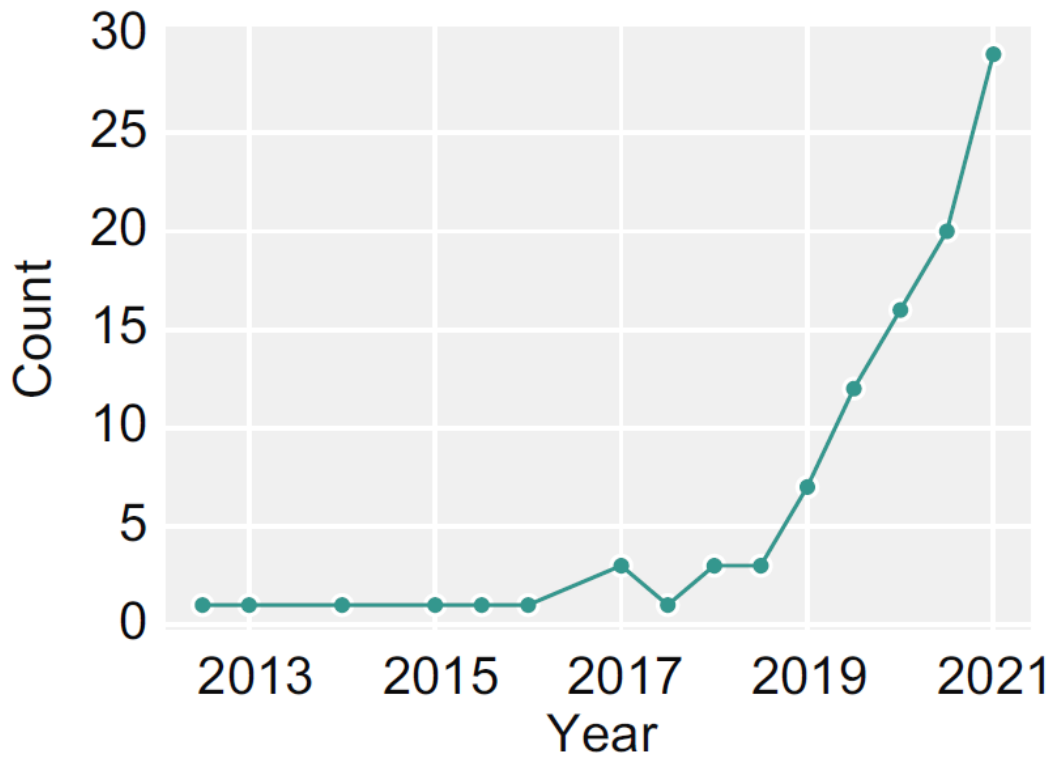


Number of studies by research field

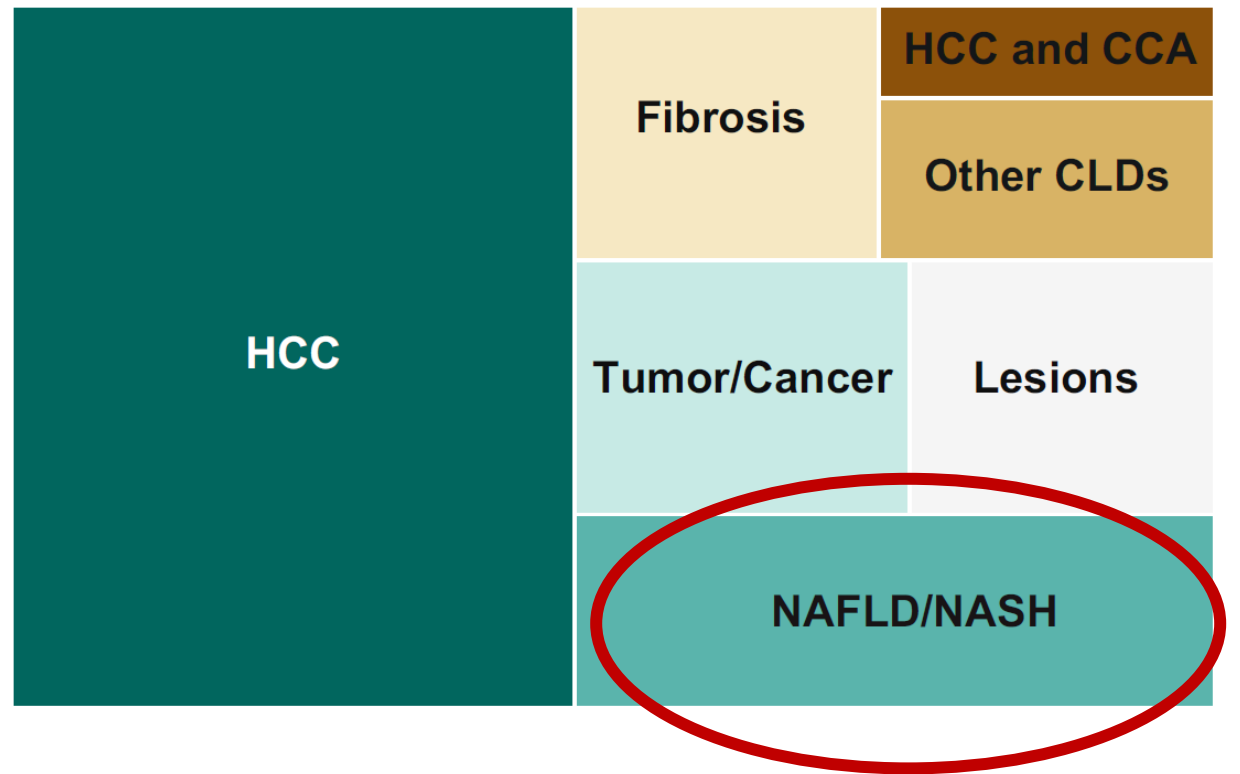


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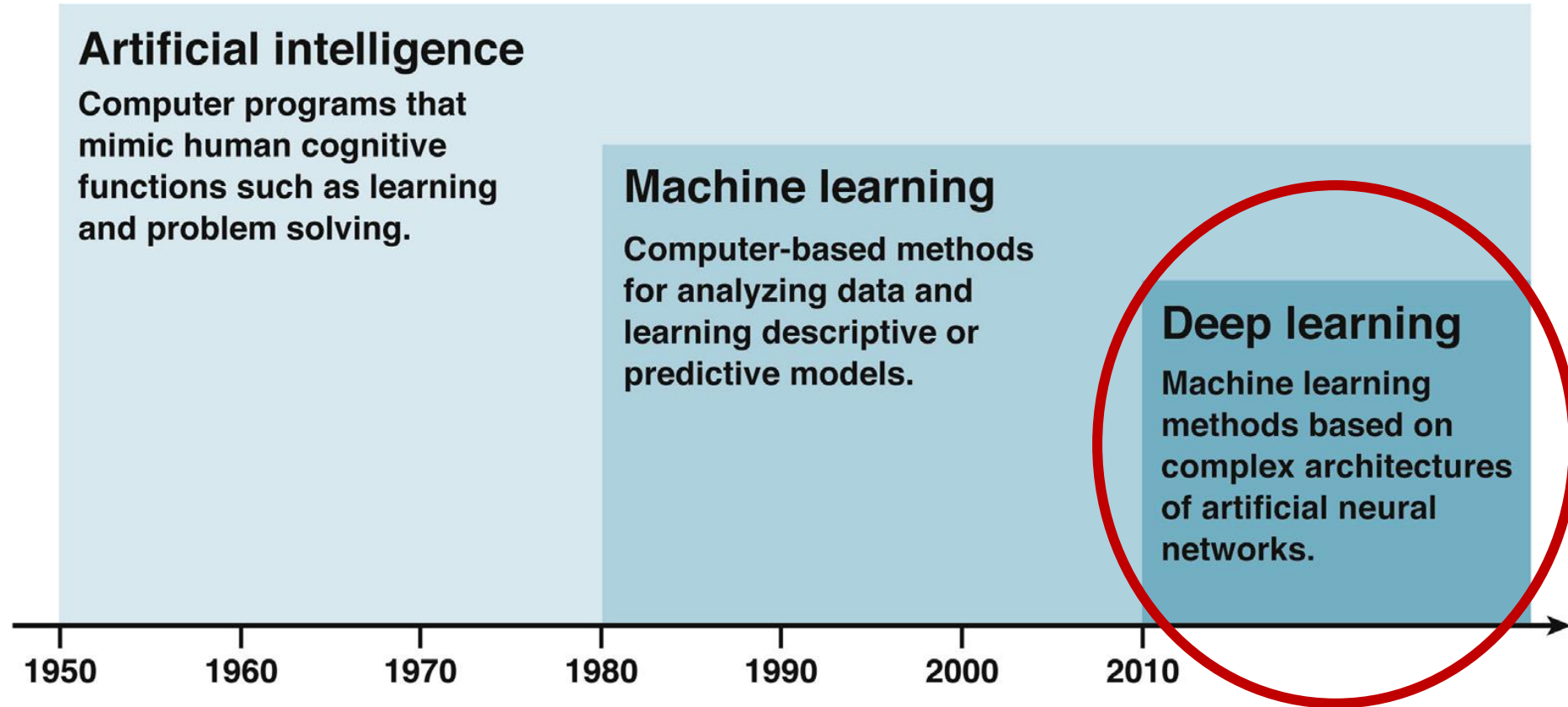


Number of published studies by liver disease type

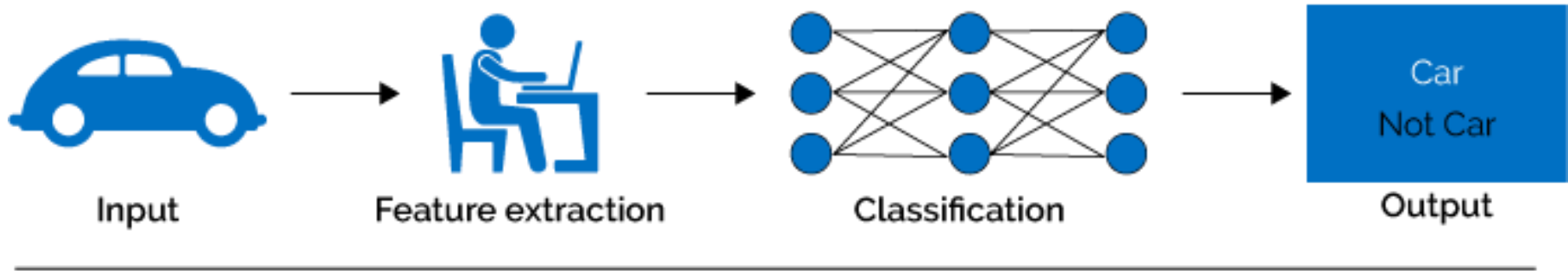


Artificial intelligence (AI) methods for quantitation of NAFLD histology

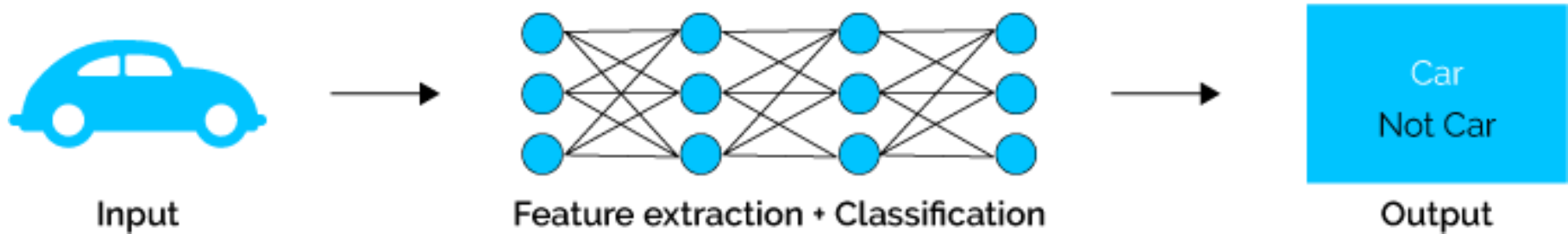
Timeline of AI main concepts



Machine Learning

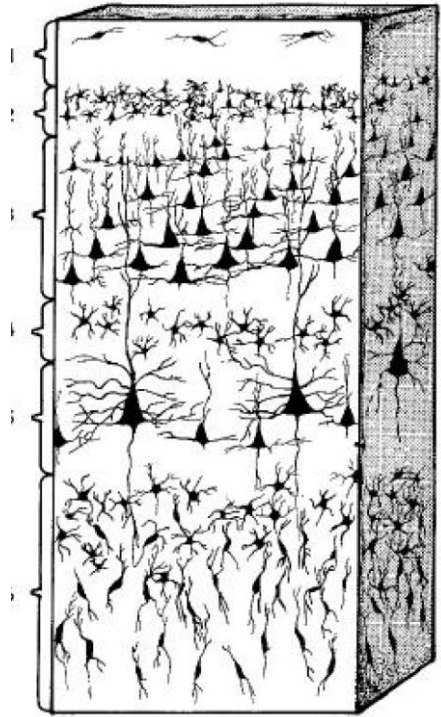


Deep Learning

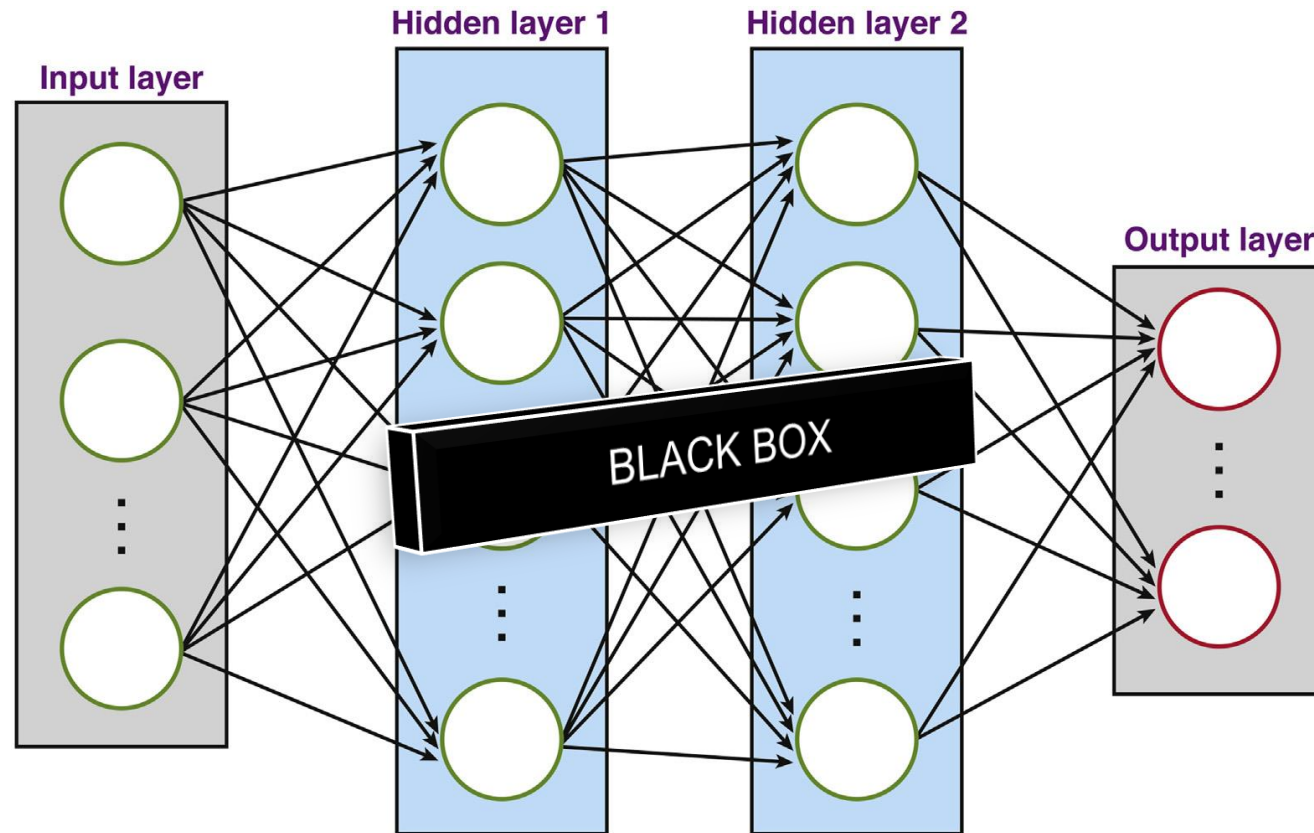


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Artificial Neural Networks

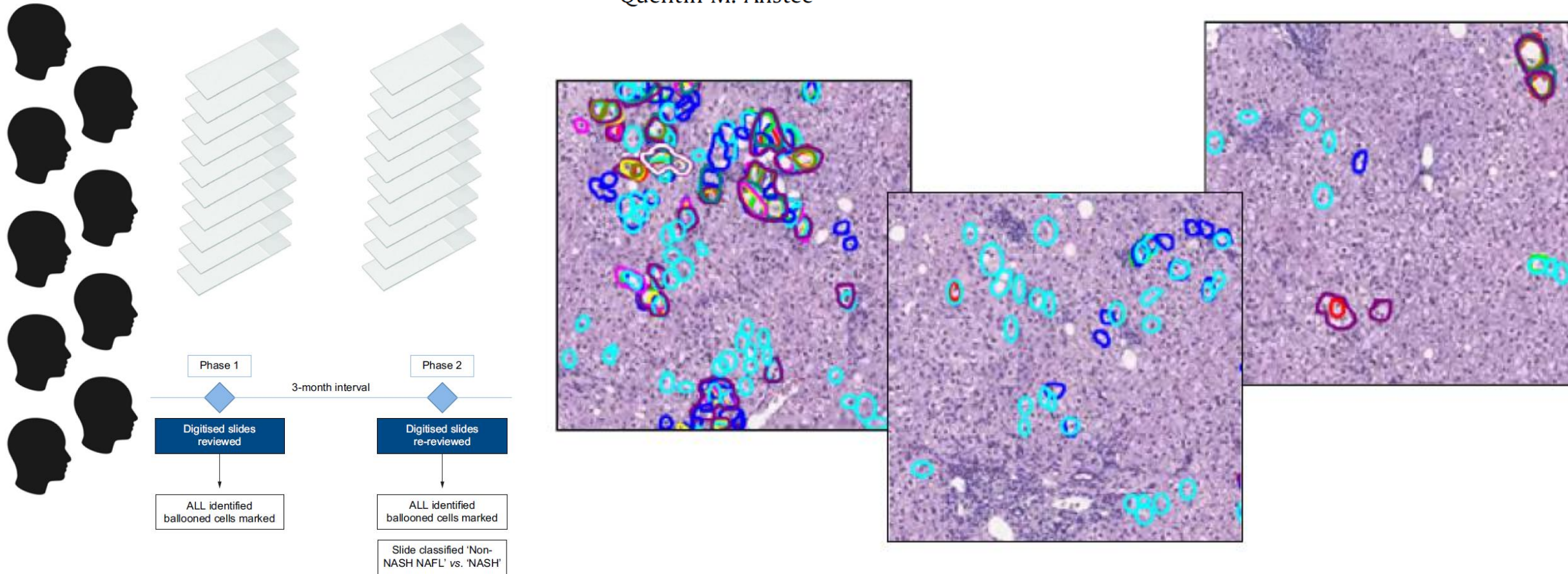


Human brain neuronal networks



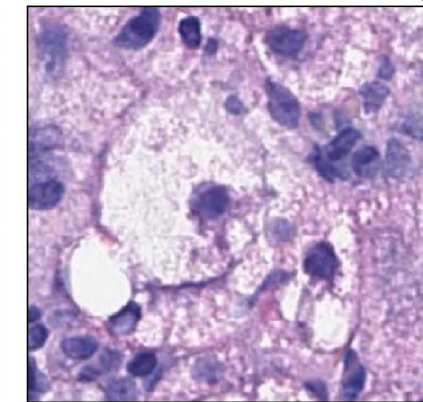
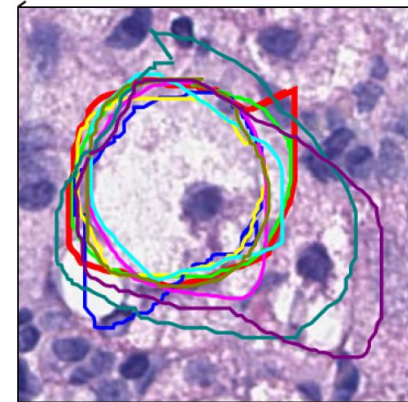
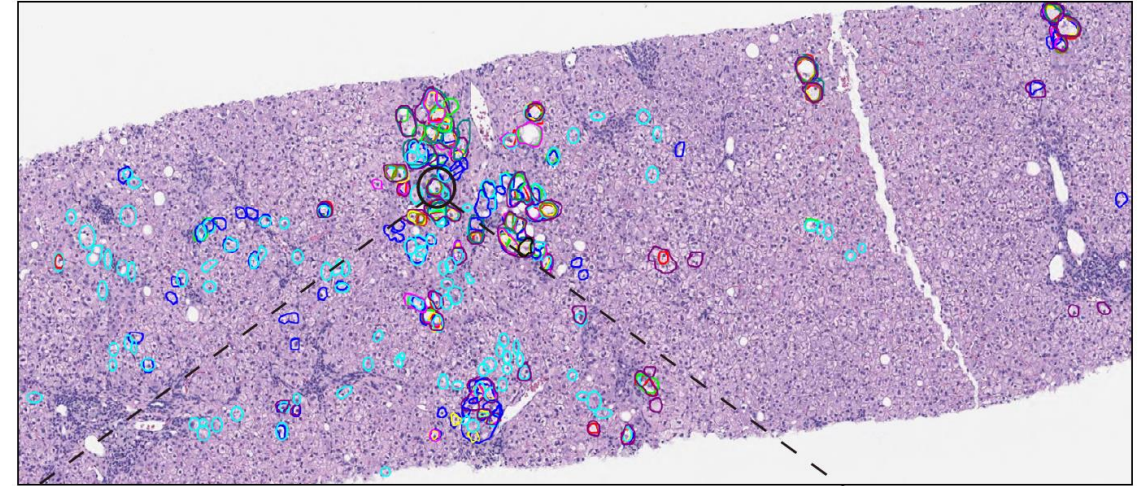
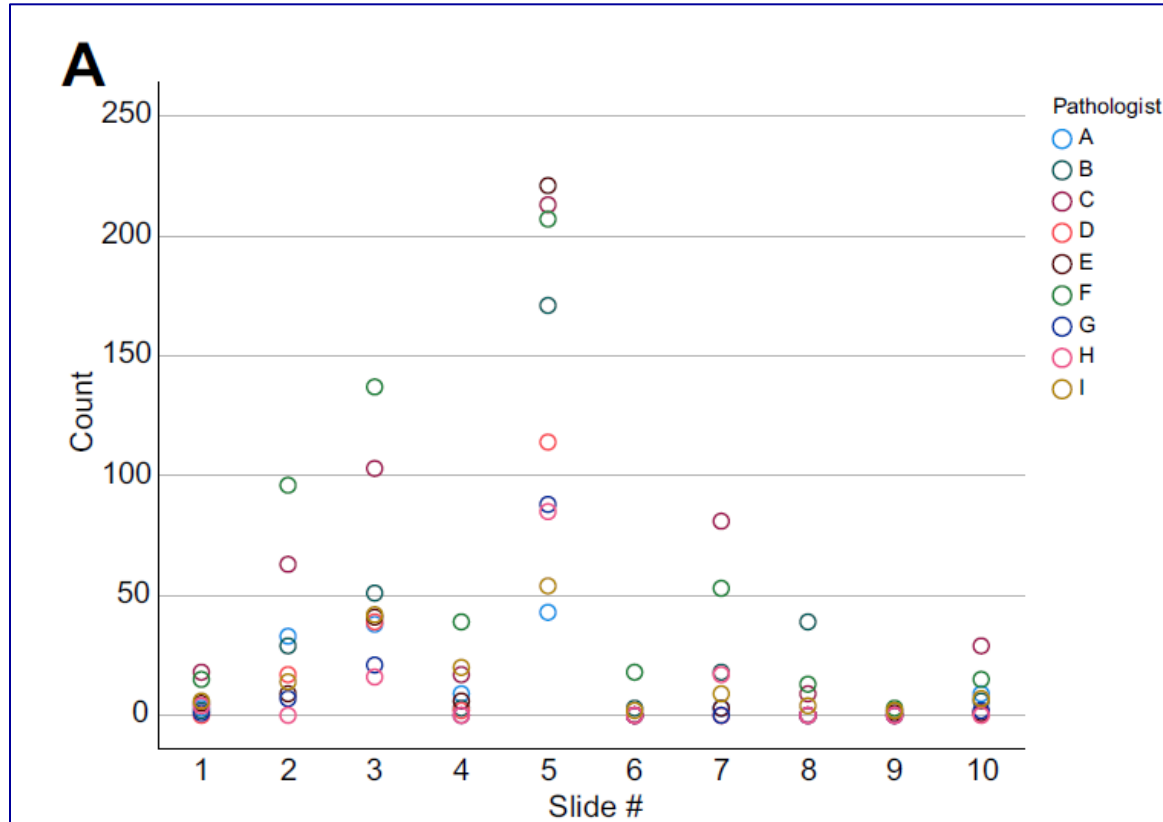
Complexity of ballooned hepatocyte feature recognition: Defining a training atlas for artificial intelligence-based imaging in NAFLD

Elizabeth M. Brunt^{1,*,\dagger}, Andrew D. Clouston², Zachary Goodman³, Cynthia Guy⁴, David E. Kleiner⁵, Carolin Lackner⁶, Dina G. Tiniakos^{7,8}, Aileen Wee⁹, Matthew Yeh¹⁰, Wei Qiang Leow¹¹, Elaine Chng¹², Yayun Ren¹², George Goh Boon Bee¹³, Elizabeth E. Powell^{14,15}, Mary Rinella¹⁶, Arun J. Sanyal¹⁷, Brent Neuschwander-Tetri¹⁸, Zobair Younossi¹⁹, Michael Charlton²⁰, Vlad Ratziu²¹, Stephen A. Harrison^{22,23}, Dean Tai^{11,*,\dagger}, Quentin M. Anstee^{7,24,*,\dagger}



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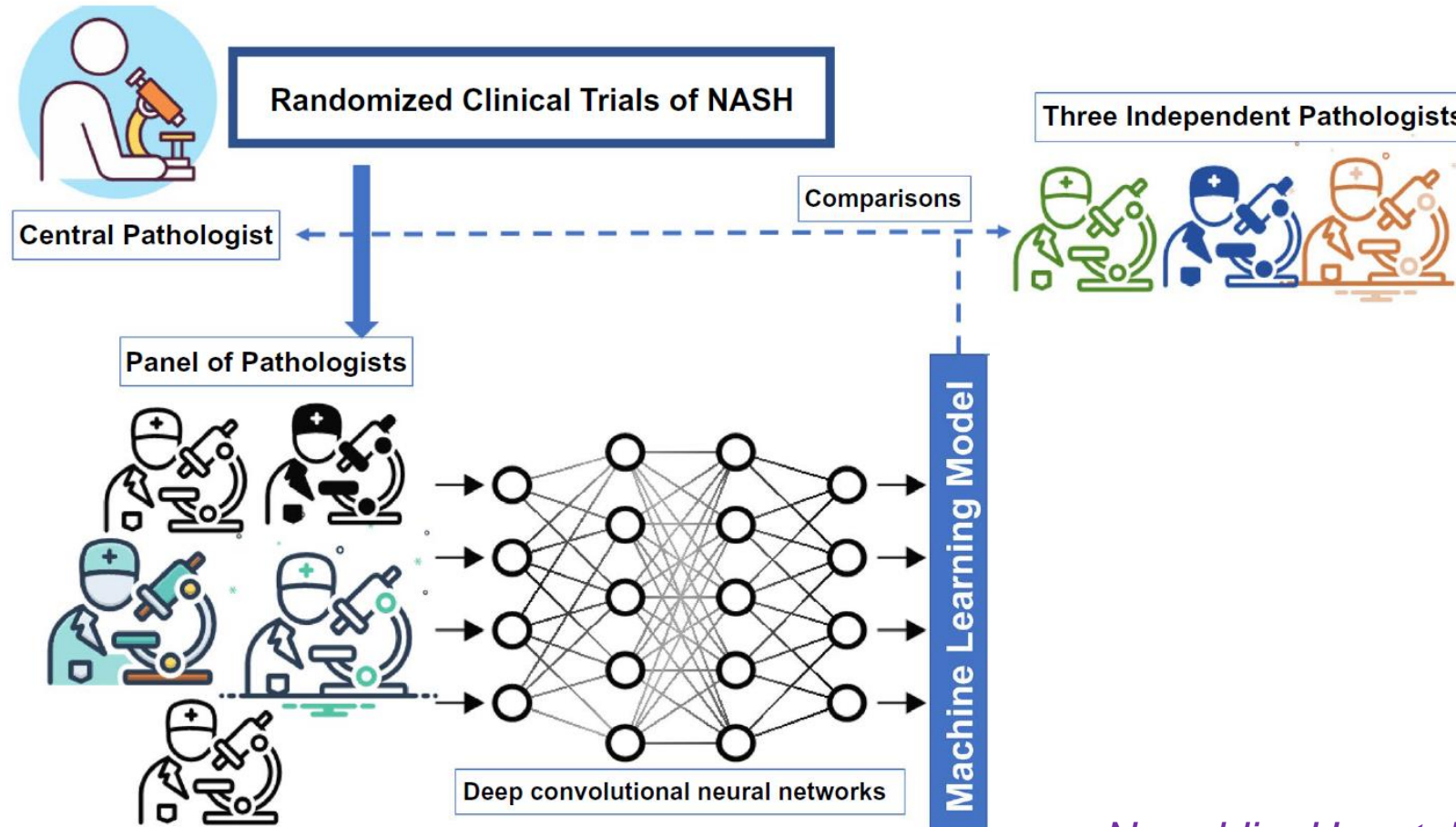


Artificial intelligence (AI) methods for quantitation of NAFLD histology

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



Taylor-Weiner A et al, Hepatology 2021



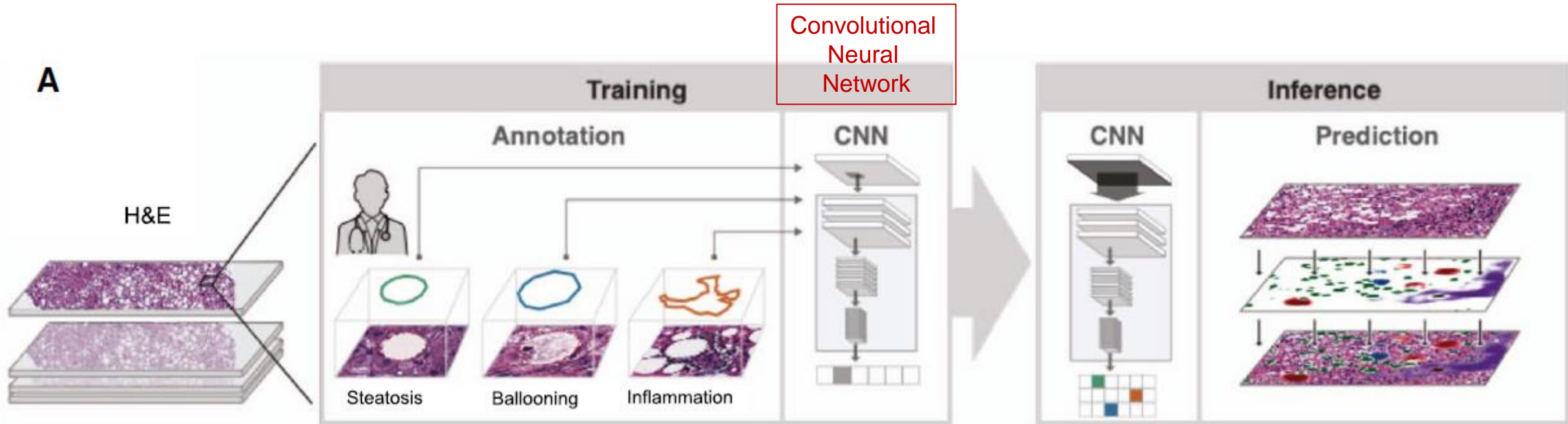
Nureddin, Hepatology 2021

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Training and validation sets: liver biopsies from the STELLAR 3 (n=388) and STELLAR 4 (n=256) clinical trial for patients with advanced NASH-fibrosis

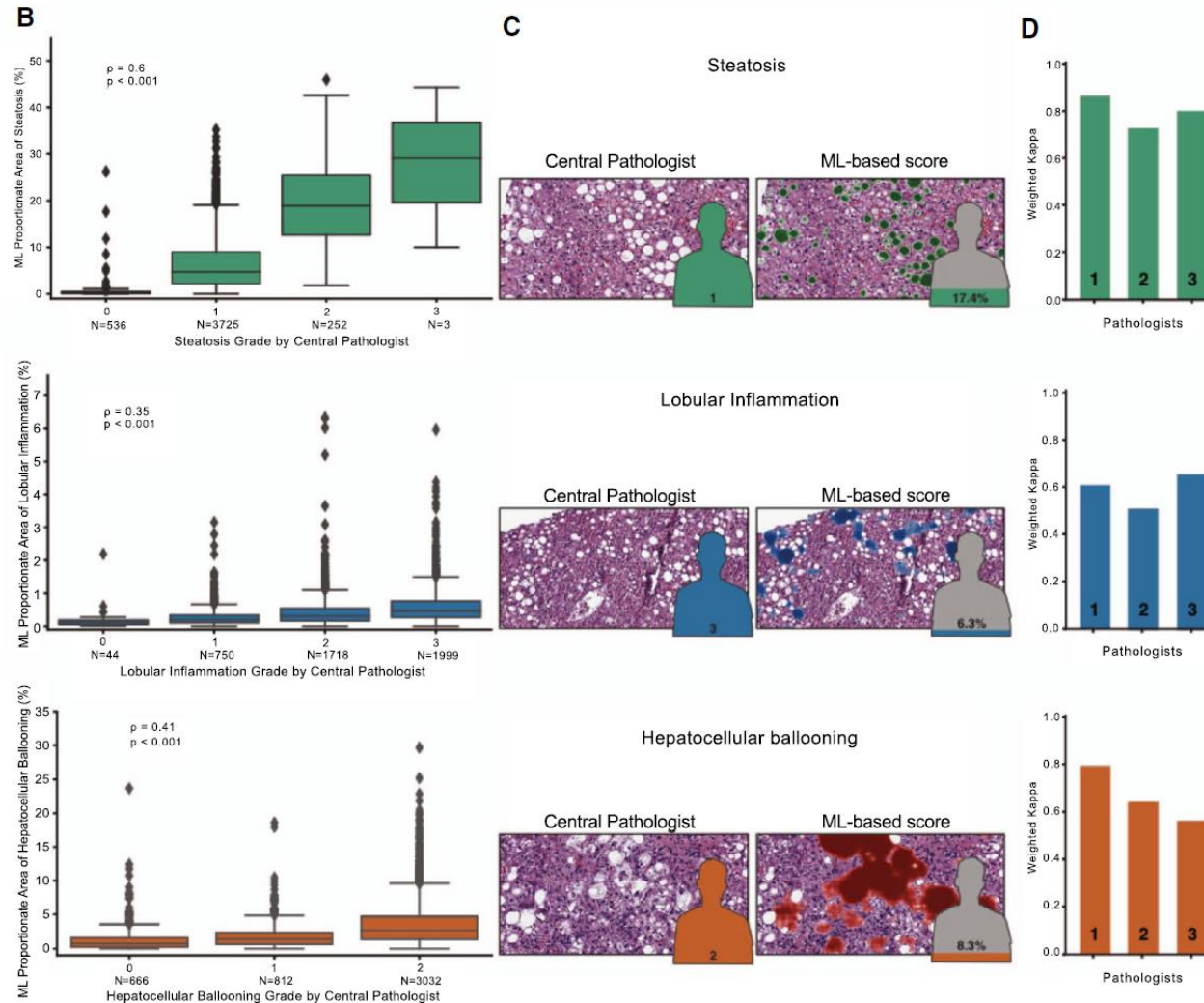
>65,424 annotations on whole slide images by 3 HPB pathologists used for supervised model training

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Taylor-Weiner A et al, *Hepatology* 2021

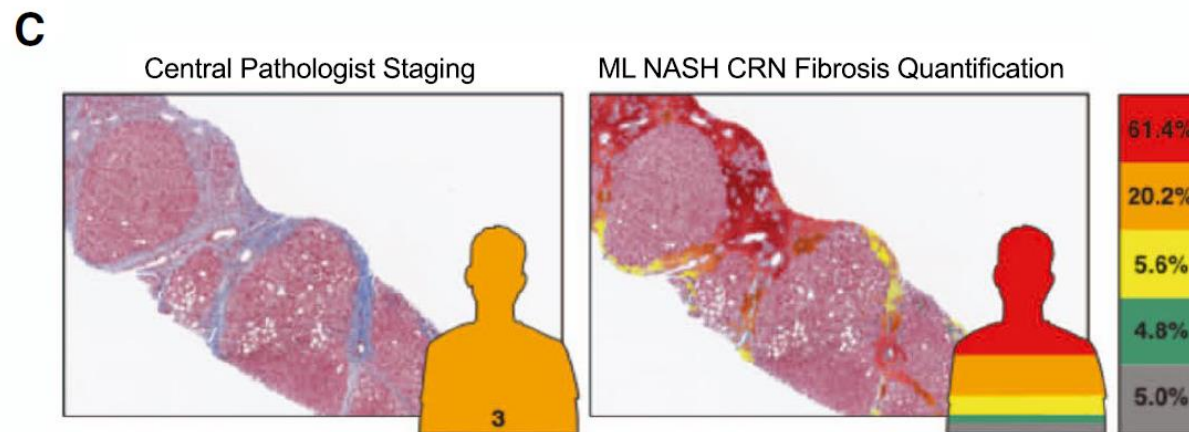
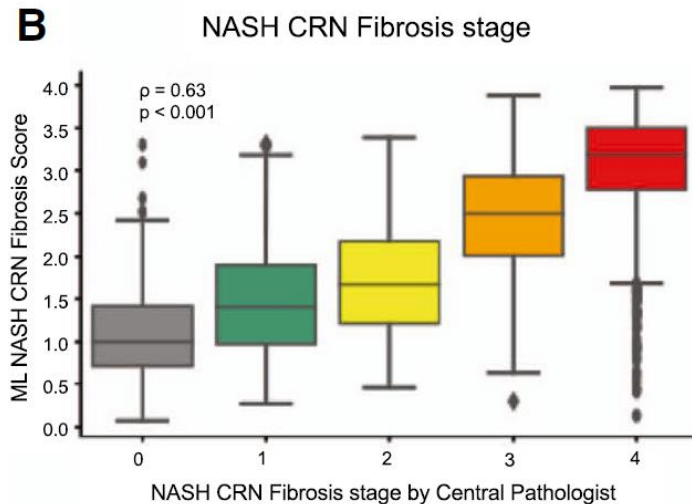
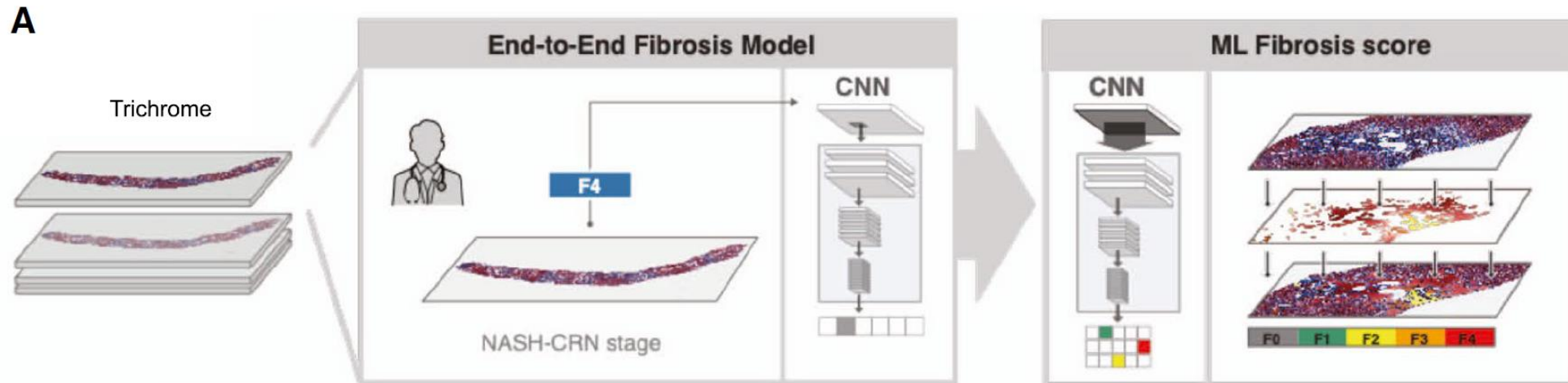


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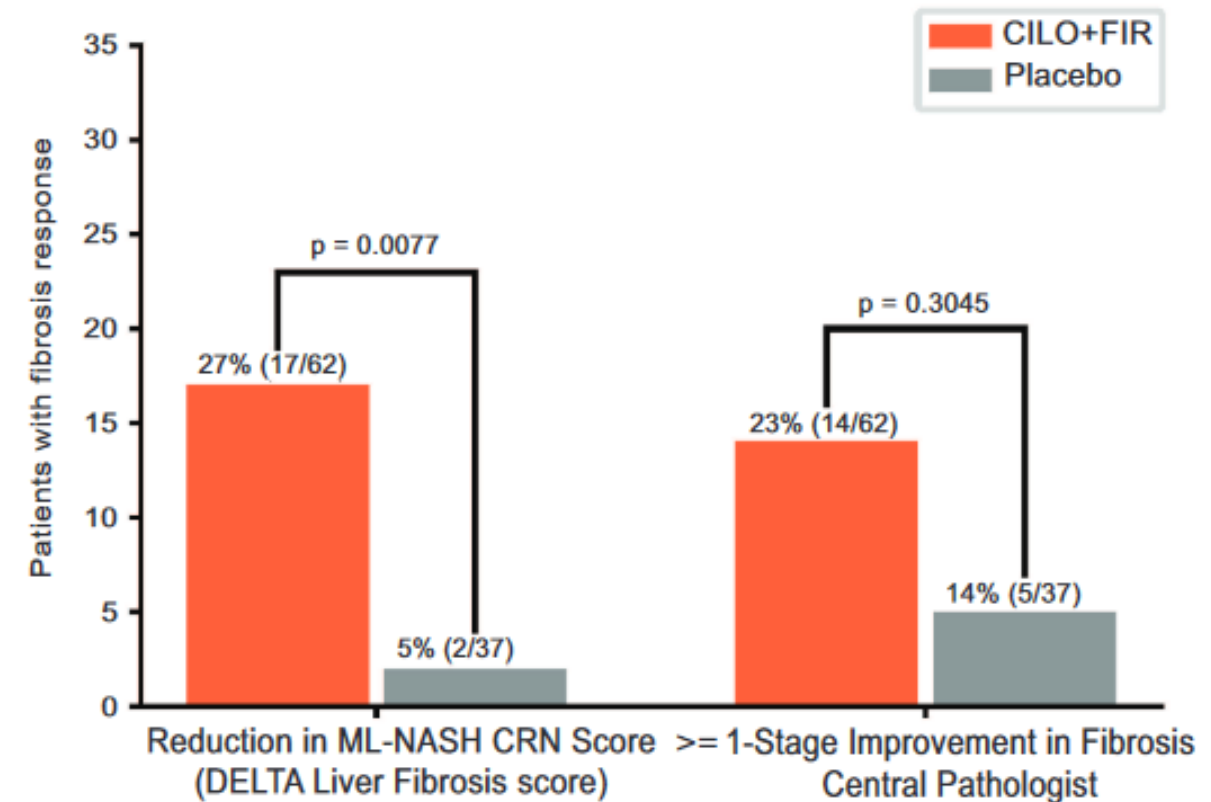
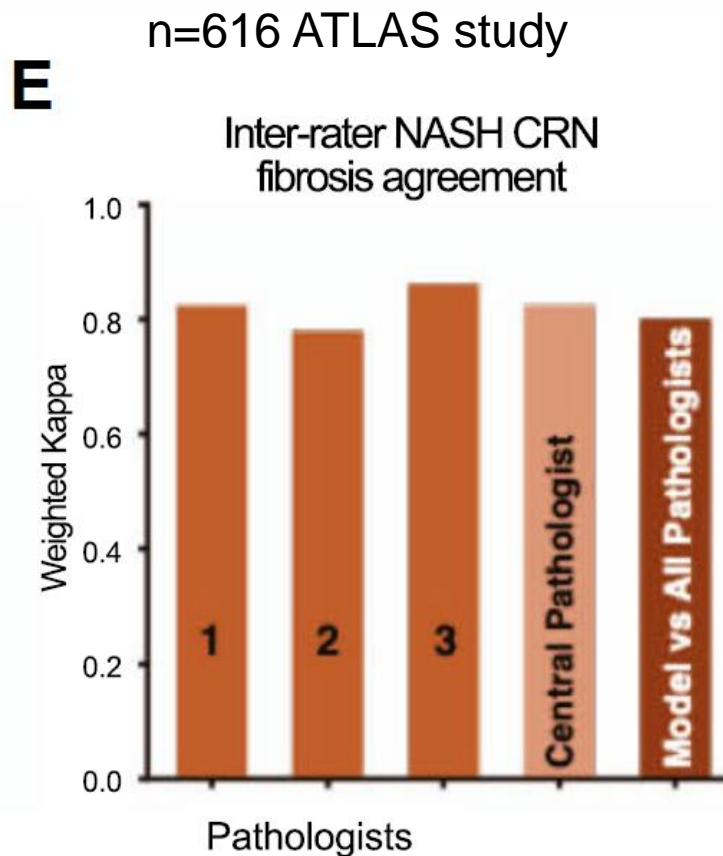


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A Machine Learning Approach Enables Quantitative Measurement of Liver Histology and Disease Monitoring in NASH



Taylor-Weiner A et al, Hepatology 2021



Artificial intelligence (AI) methods for quantitation of NAFLD histology

- Data to date are limited but AI-derived stage will likely be a standard component of NASH clinical trials in the future

Artificial intelligence methods for grading and staging of NAFLD

Can AI replace the pathologist?

- Data to date are limited but AI-derived stage will likely be a standard component of NASH clinical trials in the future
- Large validation studies in a range of populations are required
- Algorithm accuracy issues - depends on the quality of training data
 - may be hampered by human annotator bias
- “Black-box” type AI models (i.e. artificial neural networks) are not explainable
- Accountability and ethical issues
- Tissue sampling variability

AI in medicine must be explainable

AI algorithms used for diagnosis and prognosis must be explainable and must not rely on a black box.

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Quantitative Image Analysis and AI applications: Present regulatory framework

Quantitative Image Analysis (QIA)

- 33 quantitative image analysis applications for **various tissue markers** cleared by FDA (as of April 2020)
- most based on colour detection/threshold
- few on supervised machine-learning algorithms
- no independent interpretation
- **One FDA- approved software for diagnostic use (Paige Prostate – September 2021)**
- **results should be confirmed by a pathologist**

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Quantitative Image Analysis for Tissue Biomarker Use: A White Paper From the Digital Pathology Association

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Deep learning AI-based algorithms / applications

- Few algorithms for imaging applications already approved by FDA
- **Unanswered questions** for AI-based QIA
 - Role of health-care provider: pathologist as «special control»?
 - Good Machine Learning Practices?
 - Specifications for complex algorithms (i.e. for non-visible features)?

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2019

Proposed Regulatory Framework for Modifications to Artificial Intelligence/Machine Learning (AI/ML)-Based Software as a Medical Device (SaMD)

Discussion Paper and Request for Feedback



Summary

- **Liver biopsy:** still “best standard” for diagnosing NASH, grading activity and staging fibrosis in NAFLD in routine and clinical trial setting
- **Need for increased granularity in fibrosis evaluation to capture a wider range of changes in therapeutic trials**
 - staging systems with >4 stages i.e. F0-F6 (EPoS, modified Ishak)
 - inclusion of fibrosis regression features as end-points in clinical trials?
 - quantitative fibrosis evaluation:
 - digital Collagen Proportionate Area
 - second harmonic generation/two-photon excitation fluorescence imaging (SHG/TPE)
 - artificial intelligence (AI) tools

Summary

- **Advances in microscopy techniques** (SHG/TPE, other stain-free methods) may improve reproducibility and standardisation of liver biopsy assessment
- **Fully automated software applications for quantification of NAFLD** cardinal histology features exist and have been used in research setting and, recently, in few clinical trials alongside histopathology scoring
- **Pathology grading and staging of NAFLD/NASH in clinical trials in the near future may be supported by AI methods**

Summary

- **AI methods are rapidly emerging as promising methods for reproducible NAFLD histology scoring**
 - currently not officially approved for use in clinical trials
 - specific guidelines from regulatory agencies needed
 - cannot replace morphological diagnosis but could support histopathologists and enhance their capabilities