Liver Forum 12
Disease Assessment Strategies to Accelerate Drug Development
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Challenges with Histological System: *Pathologist Perspective*

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Overview

- **Observer-related bias** of semiquantitative histological interpretation of key features of NAFLD
  - Possible causes
  - Strategies for improvement
- **The International NAFLD Pathology Group** (INPG)
- **Histological/morphological assessment of disease regression** in NAFLD
  - Conventional semiquantitative and novel quantitative methods

Background

- **NASH is a potent driver of fibrosis** *(Singh S, Clin Gastroenterol Hepatol 2015)*
- **CRN stage is an independent predictor of outcome** *(Angulo P, Gastroenterology 2015, Ekstedt M, Hepatology 2015)*
- **Histological features are determining factors for clinical trials of NASH** *(FDA/EMA 2018)*
  - Patient selection & stratification
  - 1–2 years treatment with the intervention increases the proportion of patients with NASH resolution without worsening fibrosis and/or a >−1-stage fibrosis improvement without worsening of NASH.
- **Histological features are assessed using semiquantitative scoring systems CRN NAS/staging and/or the Steatosis, Activity, Fibrosis score (SAF)** *(Kleiner D, Hepatology 2005, Bedossa P, Hepatology 2014)*

Commonly used semiquantitative grading systems in NAFLD

NAFLD Activity Score (NAS)	Steatosis, Activity, and Fibrosis Score (SAF)
Steatosis (parenchymal involvement) Score 0: <5% Score 1: 5-33% Score 2: 33-66% Score 3: >66%	**Steatosis** (% of hepatocytes) Score 0: <5% Score 1: 5-33% Score 2: 34-66% Score 3: >67%
Lobular inflammation (overall, 200x field) Score 0: no foci Score 1: <2 foci Score 2: 2-4 foci Score 3: >4 foci	**Lobular inflammation** (200x, per lobule) Score 0: none Score 1: <2 foci Score 2: ≥2 foci
Hepatocellular ballooning Score 0: no ballooning Score 1: few ballooned cells Score 2: many ballooned cells	**Hepatocellular ballooning** Score 0: no ballooning **Score 1 : type 1** **Score 2: type 2 (classical)**
NAS = S+I+B (0-8)	**Activity = I+B (0-4)**
Kleiner, Hepatology 2005	*Bedossa, Hepatology 2014*

Commonly used semiquantitative staging systems in NAFLD

NAFLD CRN fibrosis staging
Stage (F)
F0: no fibrosis F1a: mild, zone 3, perisinusoidal/pericellular F1b: moderate, zone 3, perisinusoidal/pericellular F1c: portal / periportal fibrosis F2: perisinusoidal/pericellular and portal/periportal F3: bridging fibrosis F4: cirrhosis

Kleiner, Hepatology 2005

Steatosis, Activity and Fibrosis score
Stage (F)
F0: no fibrosis F1: centrilobular PCF and/or periportal fibrosis F2: centrilobular and periportal fibrosis F3: bridging fibrosis F4: cirrhosis
Disease severity • **Mild disease: A < 3 and F < 3** • **Severe disease: A ≥ 3 and/or F ≥ 3**

Bedossa, Hepatology 2014

Inter- and intra-observer agreement of the histologic interpretation of key features NAFLD grade			
	Reference	Kappa Coefficient	
		Inter-observer	Intra-observer
Steatosis	**Younossi 1998 (A)**	0.64	0.64
	Kleiner 2005 (B)	0.83	0.79
	Fukusato 2005 (C)	0.53	
	Gawrieh 2011 (D)	0.74	0.75
	Bedossa 2014 (E)	0.61	
	Davison 2020 (F)	0.61	0.75
Ballooning	A	0.50	0.51
	B	0.66	0.56
	C	0.14	
	D	0.18	0.56
	E	0.80	
	F	0.52	0.66
Lobular inflammation	A	0.33	0.62
	B	0.60	0.45
	C	0.10	
	D	0.20	0.48
	E	0.75	
	F	0.33	0.44

Kappa coefficient and strength of concordance: 0: none; <0.21: slight; 0.21-0.40: fair; 0.41-0.6: moderate; 0.61-0.80: substantial; >0.81 almost perfect

Inter- and intra-observer agreement of the histologic interpretation of NAFLD stage			
	Reference	Kappa Coefficient	
		Inter-observer	Intra-observer
Stage	A	0.60	0.73
	B	0.85	0.84
	C	0.55	
	D	0.56	0.75
	E	0.84	
	F	0.48	0.78

Kappa coefficient and strength of concordance:
0: none; <0.21: slight; 0.21-0.40: fair; 0.41-0.6: moderate; 0.61-0.80: substantial; >0.81 almost perfect

(A) Younossi ZM, Mod Pathol 1998;11(6):560-565
(B) Kleiner D, Hepatology 2005;41:1313-1321
(C) Fukusato T, Hepatology Res 2005;33:122-127
(D) Gawrieh S, Annals of Diagnostic Pathology 2011;15:19-24
(E) Bedossa P, Hepatology 2014;60:565-575
(F) Davison BA, J Hepatology 2020;

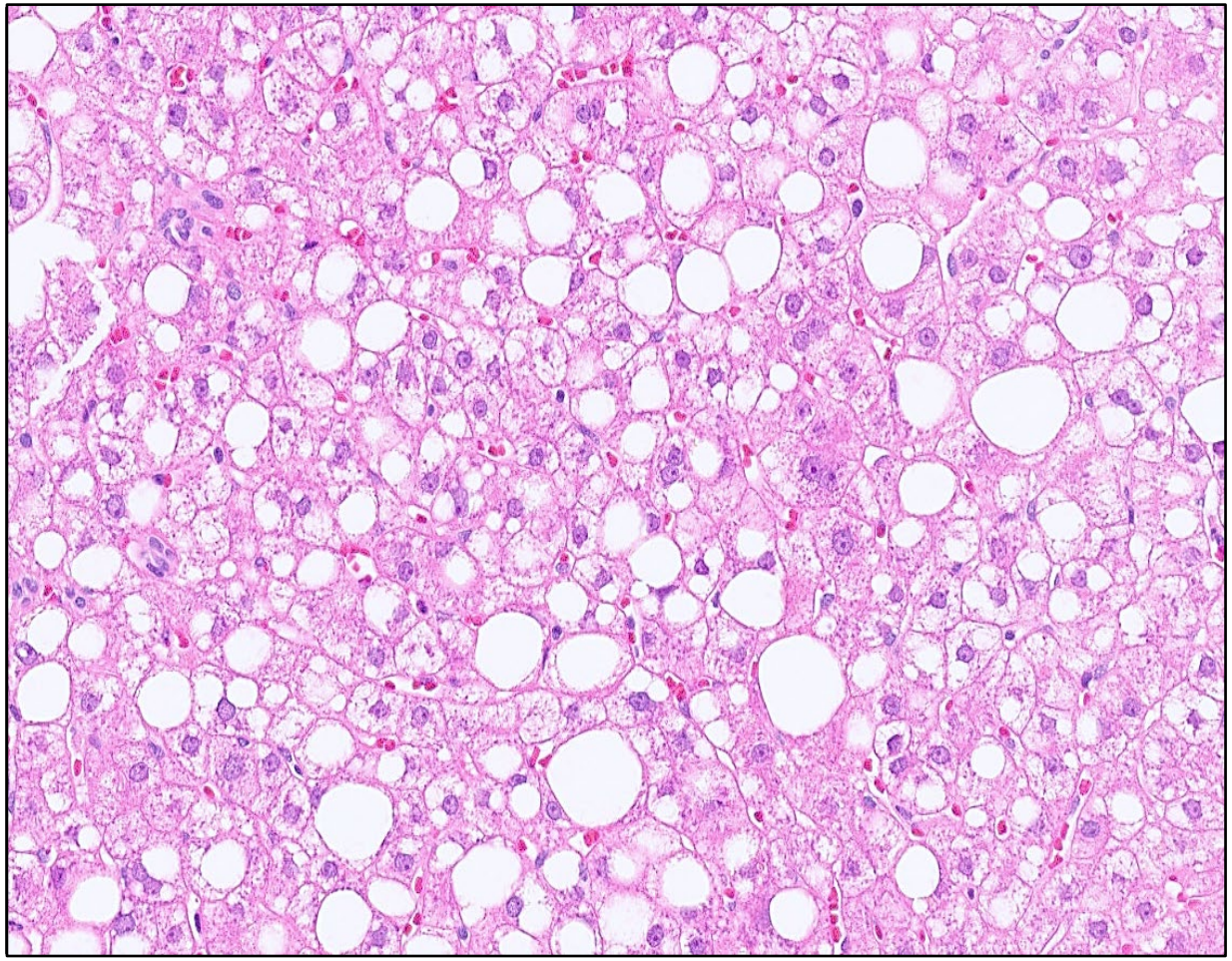
Intra- and inter-observer strength of concordance for the histological interpretation of key features of NAFLD range from slight to almost perfect

Reasons?

Possible explanations for high observer-related bias

- **Technical reasons**
  - Inadequate biopsy length and/or poor quality of the histology (thickness of sections, inadequate staining, fragmented and/or folded sections etc.)
- **Definitions of the scoring categories in the NAS and SAF offer a range of possible interpretations**
  - Variable rules for the application of semiquantitative assessments depending on opinions of individual as well as groups of pathologists
  - Variable histological definitions of key features of NAFLD in the literature

Example I: Definition and semiquantitative assessment of macrovesicular steatosis



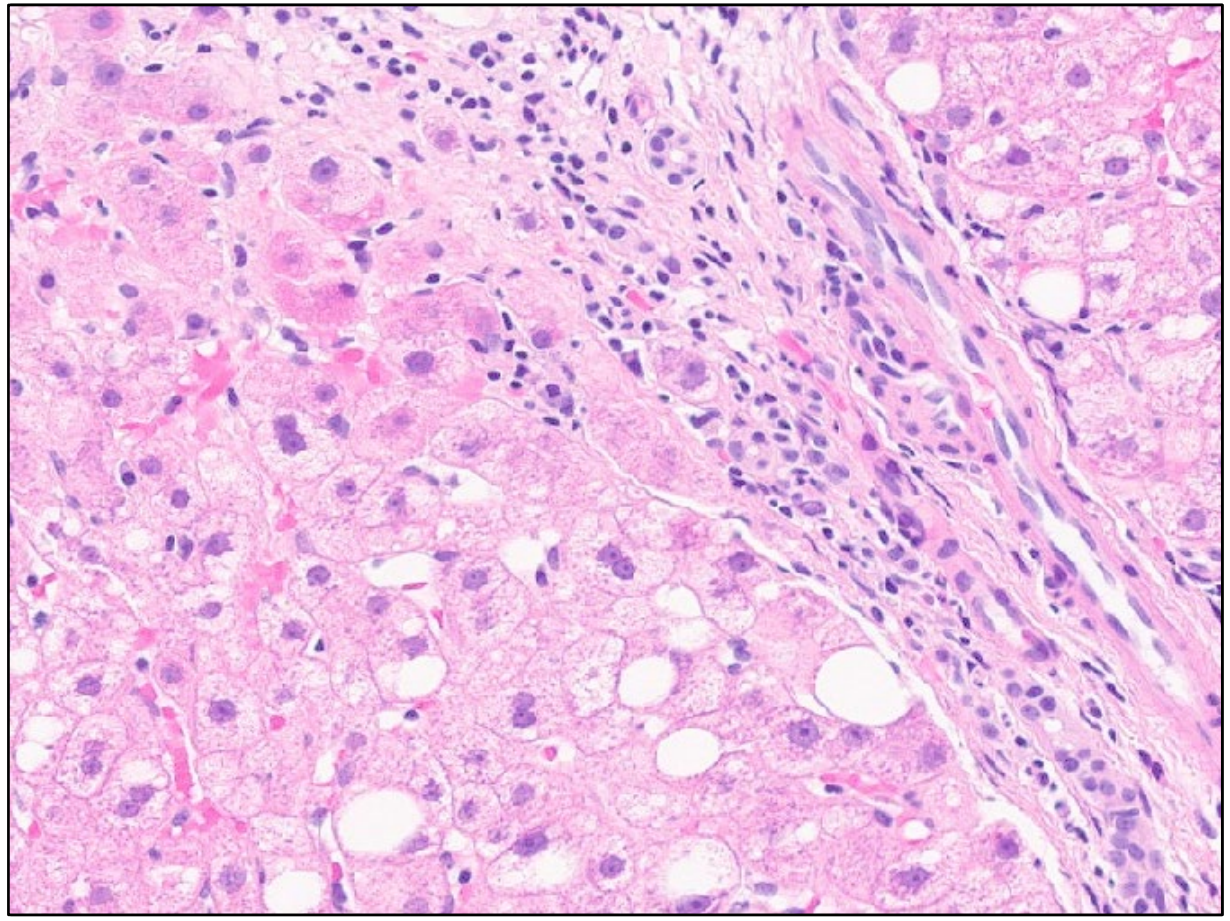
Definition of macrovesicular steatosis

- >50% of the cytoplasm of hepatocyte
- Larger than hepatocellular nucleus

Semiquantitative assessment

- Parenchymal area contributed by steatosis
- % of hepatocytes involved
- Magnification

Example II: Definition and semiquantitative assessment of lobular inflammation



Definition of inflammatory focus

- At least two inflammatory cells within parenchyma or sinusoids
- Focus has to be at least the size of a hepatocyte
- Macrophages with small fat droplets may/may not be a component of inflam focus

Semiquantitative assessment

- Average number of foci in 200x fields - by „gestalting“ or counting ?
- Assessment in areas with pericellular and/ or perivenular fibrosis
- Assessment in the vicinity of portal tracts

Suggestions for improvement of observer-related bias

- **Assessment of the key features of NAFLD**
  - **Standardized definitions** of morphological lesions
  - **Standardized rules** for semiquantitative assessment
- **However,**

  Application of standardized criteria after tutorials have yielded conflicting results *(Gawrieh S, Ann Diagn Pathol 2011, Bedossa P, Hepatology 2014)*

Inter- and intra-observer agreement of the histologic interpretation of key features NAFLD grade			
	Reference	Kappa Coefficient	
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Inter-rater concordance of the EPoS staging system for NAFLD

EPOS	OBS 1	OBS 2	OBS 3	OBS 4	OBS 5	OBS 6	OBS 7	OBS 8	OBS 9
OBS 1		0,88	0,86	0,85	0,87	0,87	0,77	0,78	0,89
								0,83	0,92
								0,8	0,91
								0,83	0,89
								0,75	0,88
								0,88	0,93
OBS 7	0,77	0,79	0,81	0,87	0,75	0,84		0,78	0,82
OBS 8	0,78	0,83	0,8	0,83	0,75	0,88	0,78		0,86
OBS 9	0,89	0,92	0,91	0,89	0,88	0,93	0,82	0,86	

Strategy to improve observer-related bias

- ✓ Seek & accept consensus definitions
- ✓ Apply consensus definitions

Kappa scores by pair of observers after assessment of 45 slides of NAFLD

Bedossa P, J Hepatol 2018;68:S553

The International NAFLD Pathology Group & Working groups for Delphi Statements

A. Technical and observer related issues & Definition of Steatohepatitis

1.	Venancio Alves	Brazil
2.	Cynthia Behling	USA
3.	Oscar Cummings	USA
4.	Archana Rastogi	India
5.	Valerie Paradis	France
6.	**Dina Tiniakos***	Greece/UK
7.	Hiro Yano	Japan

B. Grading

1.	Johanna Arola	Finland
2.	Alastair Burt	UK
3.	Zack Goodman	USA
4.	Stefan Hübscher	UK
5.	David Kleiner	USA
6.	**Carolin Lackner** *	Austria
7.	Young Nyun Park	South Korea
8.	Aileen Wee	Singapore

C. Staging

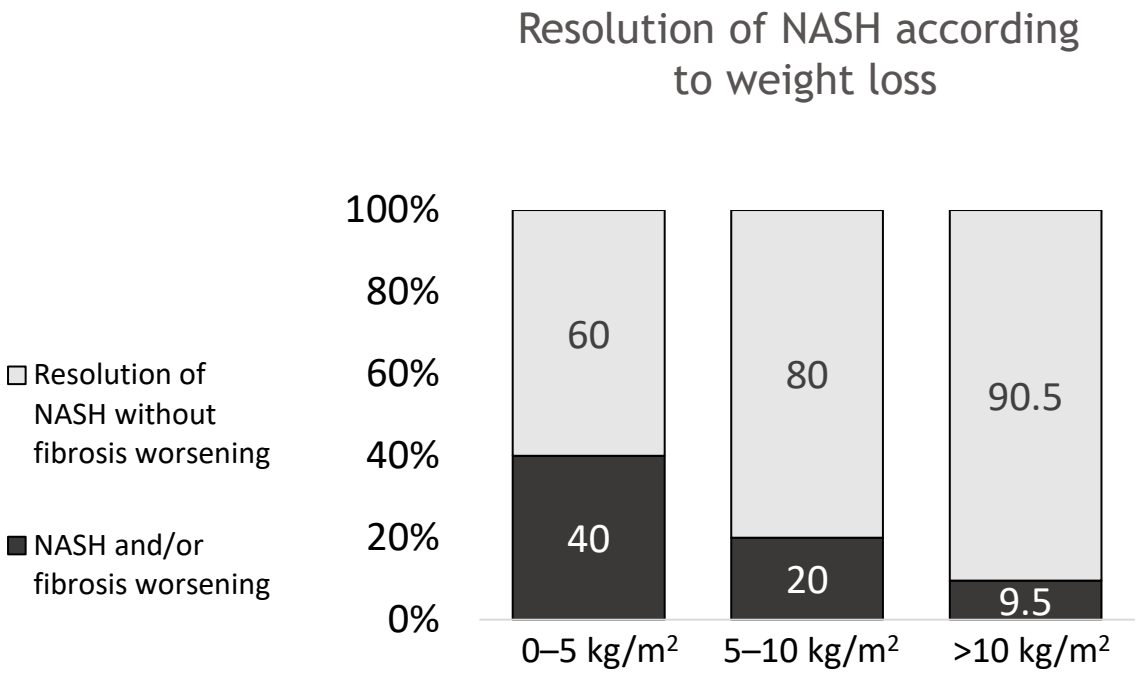
1.	Pierre Bedossa	France
2.	Andrew Clouston	Australia
3.	**Annette Gouw***	The Netherlands
4.	Cynthia Guy	USA
5.	Maria Guido	Italy
6.	Prodromos Hytiroglou	Greece
7.	Rish Pai	USA
8.	Peter Schirmacher	Germany

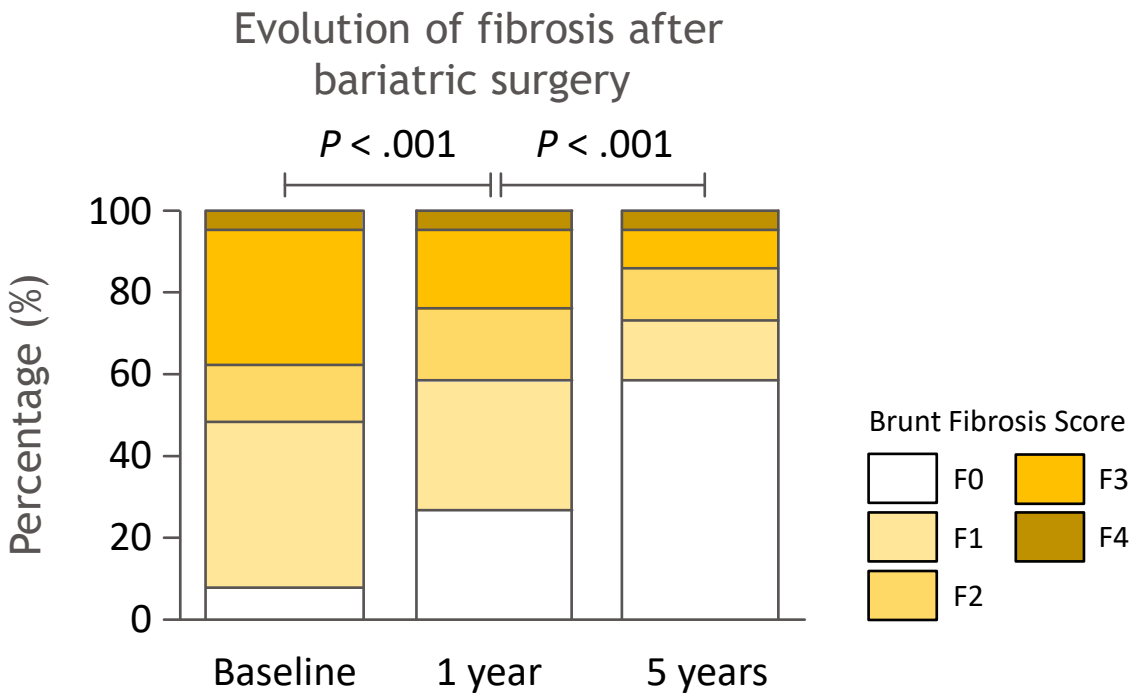
D. Regression

1.	Beth Brunt	USA
2.	Venancio Alves	Brazil
3.	Aileen Wee	Singapore
4.	Prodromos Hytiroglou	Greece
5.	Dina Tinakos	Greece
6.	**Annette Gouw***	The Netherlands
7.	Carolin Lackner	Austria
8.	Cythia Guy	USA
9.	Cythia Behling	USA

***....Coordinators**

Regression of NASH and fibrosis after bariatric surgery





Lassailly G, Gastroenterology, 2020;

Emerging role for quantitative assessment of histological features of NAFLD

Multiple laser-based microscopy

– Second harmonic generation/two-photon excitation fluorescence laser microscopy (SHG/TPEF)

Artificial intelligence-assisted systems

Benefits

- Good correlation with conventional semiquantitative scoring
- Continuous scale measurements
- Minimal inter- and intra-rater variability
- High sensitivity to detect small changes in histological patterns

Caveats

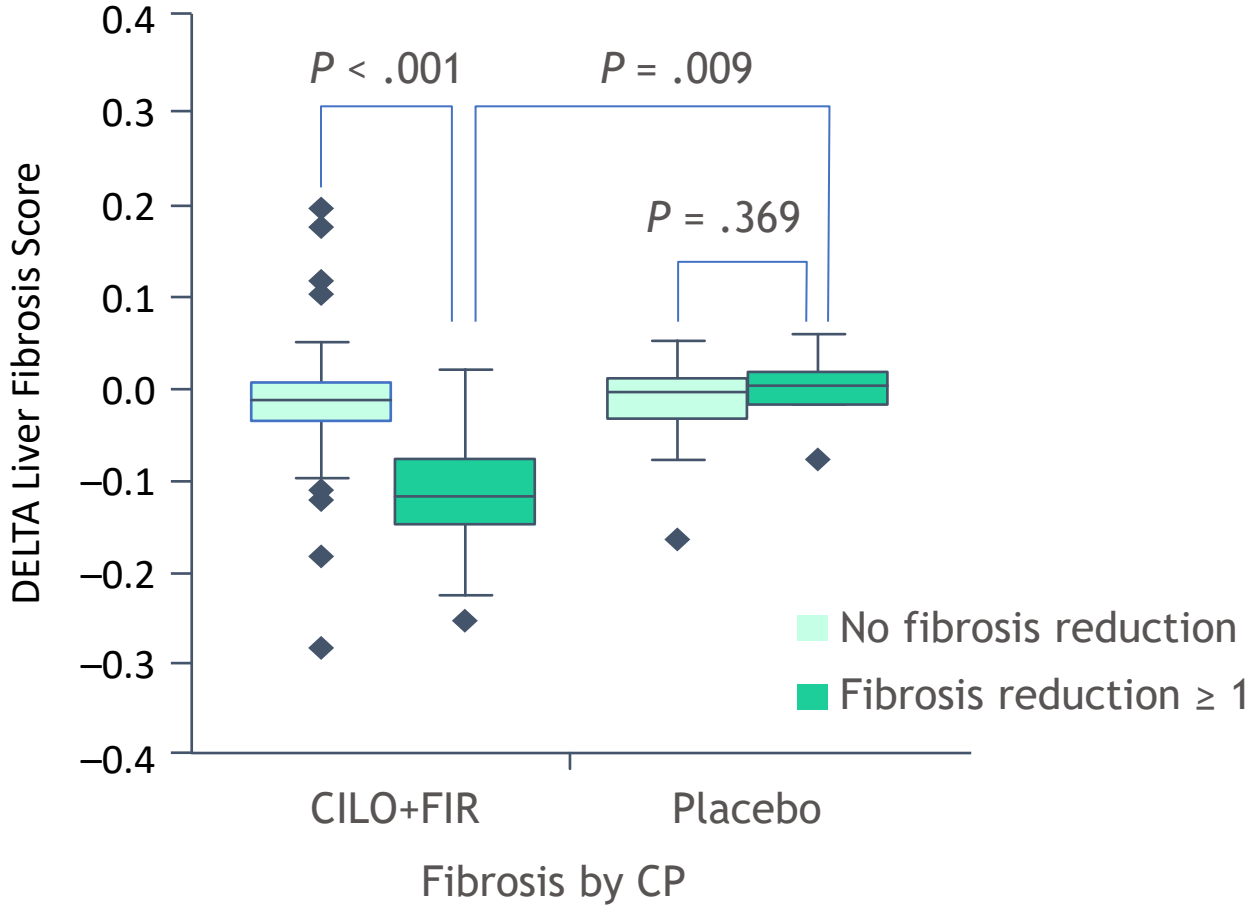
- No detection of non-NAFLD types of liver disease
- Thresholds at which a morphological change is associated with clinical effect is unknown
- Dependent on sample quality

Soon and Wee, Clinical & Molecular Hepatology. 2021; Liu F, Hepatology. 2020 ; Forlano R, Clinical Gastroenterology & Hepatology 2020; Rowe IA and Parker R Clinical Gastroenterology & Hepatology. 2021

The deep learning treatment assessment (DELTA) liver fibrosis score: a novel tool to detect treatment response

ATLAS Study

- Adult NASH patients, Stage F3/4
- 48 weeks of treatment
  - Selonsertib
  - Firsocostat (FIR)
  - Cilofexor (CILO)
- Alone or in two-drug combinations

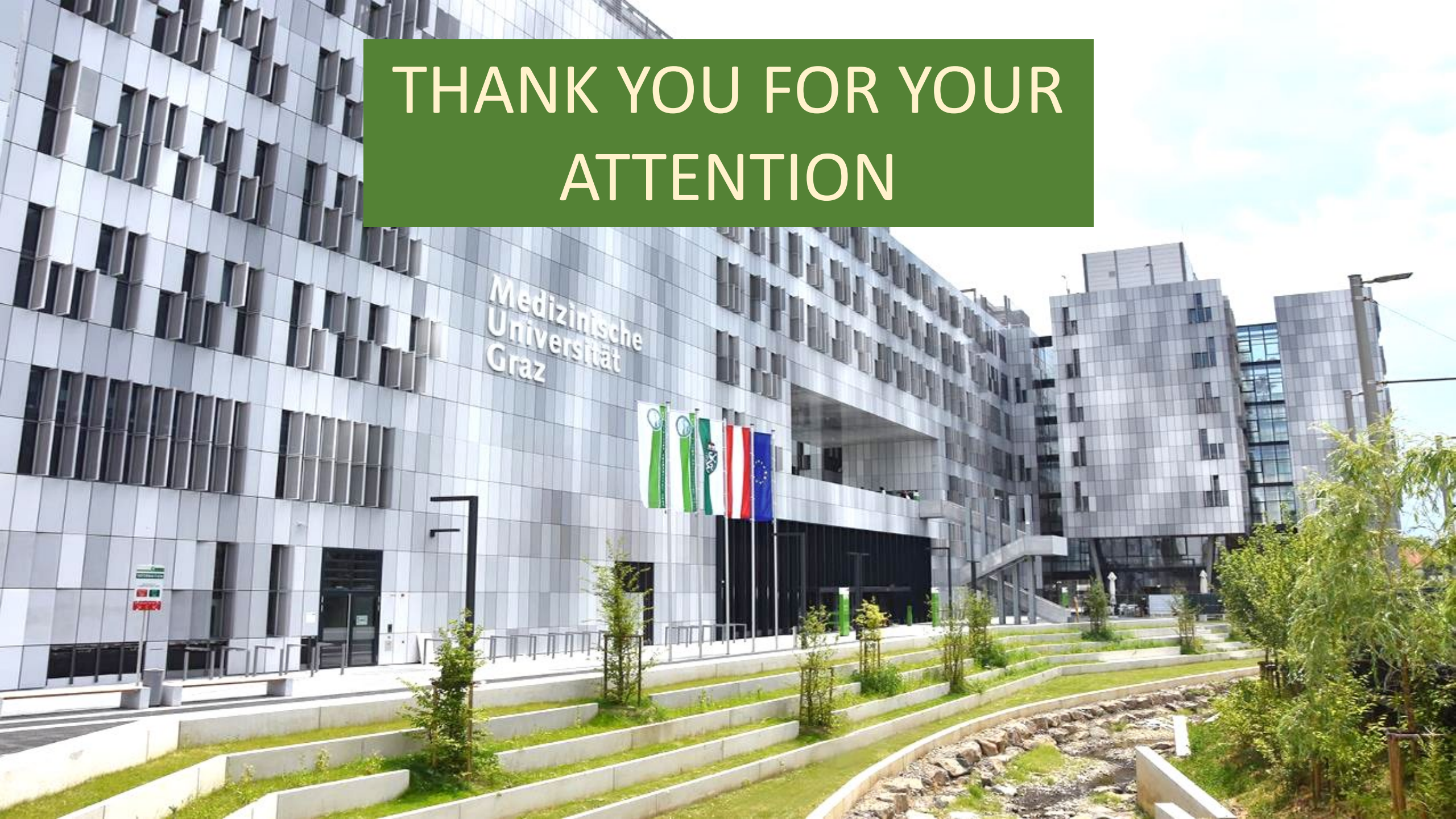


Taylor-Weiner AH, Hepatology. 2021

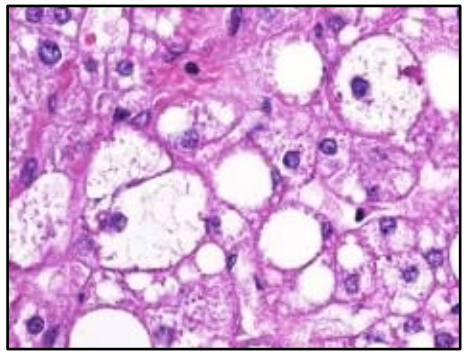
Summary

- The utility of semiquantitative histological scoring systems is impaired by low inter- and intra-observer agreement.
- Inadequate accuracy may be due to variable definitions of histological features and applications of scoring systems.
- The International NAFLD Pathology Group aims to provide guidelines for the standardized semiquantitative histological evaluation of NAFLD.
- The evolution of novel histology-based quantitative methods of liver tissue analysis is presumed to enhance the utility of morphological liver tissue analysis for the assessment of treatment effects in clinical trials of NASH.

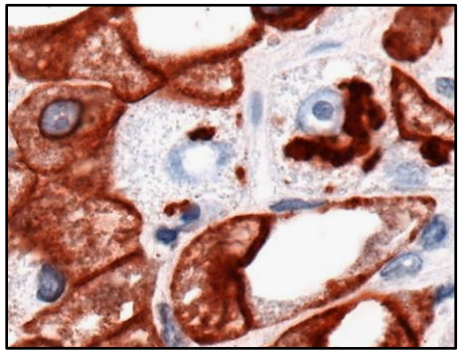
THANK YOU FOR YOUR ATTENTION



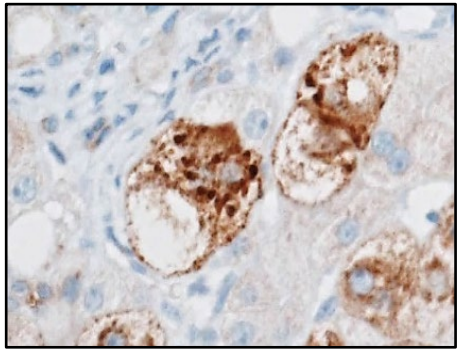
Challenges with the histological diagnosis of NASH: Classification of hepatocellular ballooning



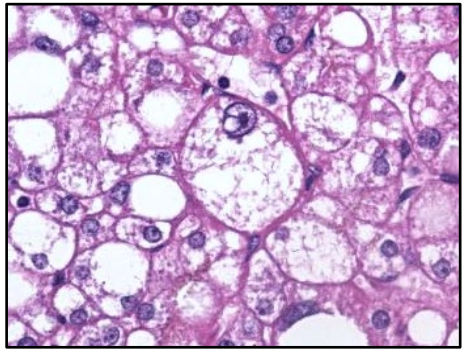
Ballooned hepatocyte
Grade 2 ballooning
2-3x regular hepatocyte
Rounded shape
Cytoplasmic clarification



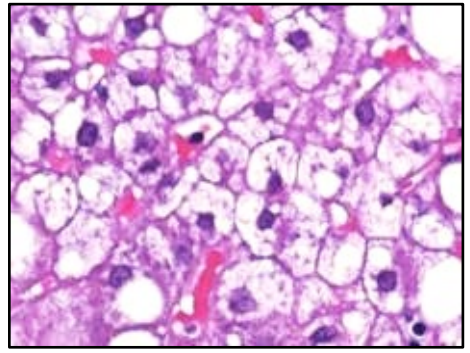
Loss of reactivity with antibodies against K8/18



Reactivity with antibodies against sonic hedgehog



Ballooned hepatocytes contain small droplet fat



Grade 1 ballooning
Regular sized hepatocytes
Rounded shape
Cytoplasmic clarification

„Classical ballooning“

„Non-classical ballooning“