MORPHOQUANT™ THE FIRST FULLY-AUTOMATED MORPHOMETRIC SOFTWARE FOR THE ASSESSMENT OF LIVER BIOPSY, REVEALS SIGNIFICANT DIFFERENCES BETWEEN NON-ALCOHOLIC FATTY LIVER AND STEATOHEPATITIS BIOPSIES

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BACKGROUND AND AIMS

Non-alcoholic steatohepatitis (NASH) is the most severe form of fatty liver diseases, and no treatment has been approved so far. Among the reasons for this lack of valid pharmacological treatment, the subjectivity and variability in the pathology reading has been identified as a confounding factor, probably weakening the ability to assess a treatment effect. We have assessed the performances of the first fully-automated user-independent morphometric software (MorphoQuant™) on liver biopsies.

MATERIALS & METHODS

271 liver biopsies were collected and analyzed. Patients were scored by a blinded expert pathologist according to the NASH CRN for steatosis, inflammation, ballooning and fibrosis using hematoxylin-eosin (H&E) and Masson's trichrome stained slides. MorphoQuant™, a fully automated and deterministic artificial intelligence, based on morphometry and expert system - completely independent from pathologist's annotations, was developed to assess NASH features. For digital quantification, slides were stained with H&E (inflammation area, and number of foci), picrosirius red (PSR) alone or combined with CK19 (steatosis, vesicle size, total collagen, periductular, perisinusoidal, perivascular and septal collagens), labelled with CD68 (CD68 and hepatic crown-like structures) or with Sonic hedgehog (Shh and ballooning/injury), and digitized as whole slide images. MorphoQuant™ readouts of NASH (NAS ≥ 4, with at least a score of 1 in each category) and non-NASH (NAS <4) biopsies were compared using a Mann-Whitney test.

.01 PATIENTS' CLINICAL CHARACTERISTICS

	All Patients (n = 271)
Demography	
Age (mean; min-max)	(53.7; 19 - 74)
Sex ratio (F/M)	151/120
TD2M (no/yes/yes and treated)	159 / 20 / 92
Steatosis Score (NAS)	
S0	0
S1	40
S2	155
S3	66
Inflammation Score (NAS)	
I0	16
I1	156
I2	83
I3	8
Ballooning Score (NAS)	
B0	76
B1	133
B2	54
Fibrosis Score (SAF)	
F0	48
F1	89
F2	67
F3	57
F4	4

Table 1. Summary of patients' clinical characteristics. NAS: NAFLD activity score. SAF: Steatosis, Activity, Fibrosis.

.02 MORPHOQUANT™ READOUTS FROM LIVER HISTOLOGY

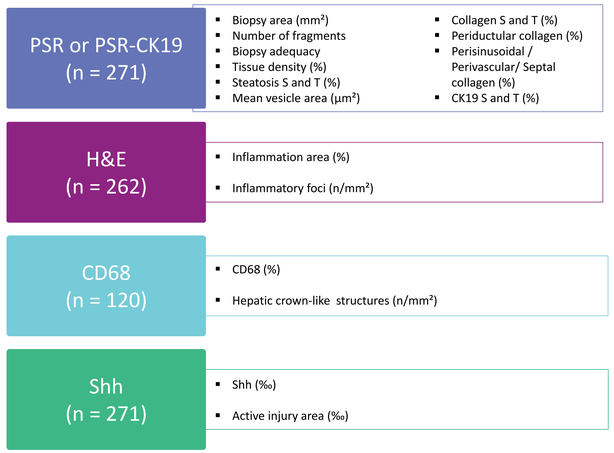

Table 2. Summary of MorphoQuant™ readouts and the respective histology techniques. PSR: picrosirius red; H&E: hematoxylin and eosin; Shh: Sonic Hedgehog.

.03 AUTOMATED DIGITAL QUANTIFICATION

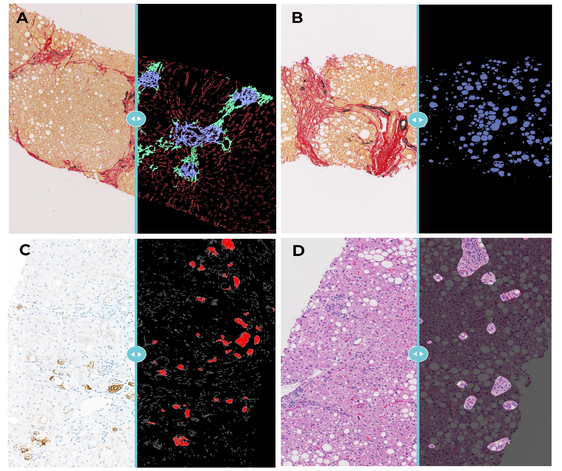

Figure 1. Representative images of computational analysis by MorphoQuant™. **A.** Fibrosis. **B.** Steatosis. **C**. Active injury. **D.** Inflammation.

.04 CORRELATION WITH PATHOLOGIST VISUAL ASSESSMENT

	Count (n)	Spearman r	95% confidence interval	p-value
Fibrosis vs				
Collagen S	247	0.1436	0.01567 to 0.2669	**0.0237**
Collagen T	247	0.1498	0.02205 to 0.2728	**0.0182**
Periductular collagen	92	0.5904	0.4387 to 0.7119	**<0.0001**
Perisinusoidal collagen	248	-0.3292	-0.4387 to -0.2101	**<0.0001**
Perivascular collagen	248	0.2753	0.1528 to 0.3896	**<0.0001**
Septal collagen	248	0.5416	0.4444 to 0.6261	**<0.0001**
CK19 S	91	0.3998	0.2065 to 0.5631	**<0.0001**
CK19 T	91	0.3925	0.1982 to 0.5571	**0.0001**
Shh	211	0.4330	0.3125 to 0.5398	**<0.0001**
Active injury area	211	0.5140	0.4035 to 0.6097	**<0.0001**
Steatosis vs				
Steatosis S	248	0.7225	0.6550 to 0.7786	**<0.0001**
Steatosis T	248	0.7212	0.6534 to 0.7775	**<0.0001**
Mean vesicle area	247	0.5258	0.4261 to 0.6129	**<0.0001**
Lobular inflammation vs				
Inflammatory area	231	0.2671	0.1391 to 0.3862	**<0.0001**
Inflammatory foci	231	0.2334	0.1037 to 0.3552	**0.0003**
CD68	101	0.2026	0.001610 to 0.3879	**0.0422**
hCLS	101	0.3604	0.1718 to 0.5236	**0.0002**
Ballooning vs				
Shh	211	0.4257	0.3047 to 0.5332	**<0.0001**
Active injury area	211	0.5329	0.4254 to 0.6256	**<0.0001**

Table 3. Correlations of MorphoQuant™ readouts with visual assessment.

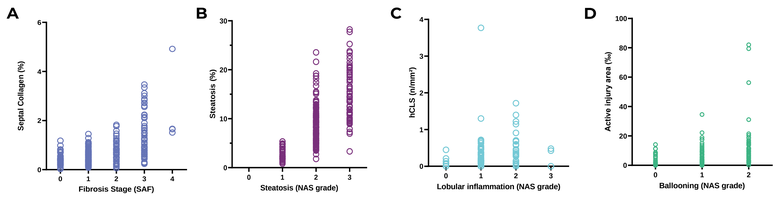

Figure 2. Correlations between quantitative digital assessment of NASH features and NASH CRN score systems for all assessed patients. A. Fibrosis vs Septal collagen. **B.** Steatosis vs Steatosis. **C**. Lobular inflammation vs hepatic crown-like structures. **D.** Ballooning vs Active injury area.

.05 COMPARATIVE ANALYSIS OF NAFL vs NASH PATIENTS

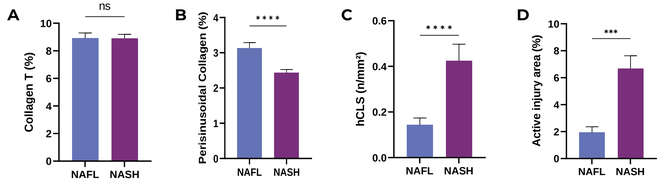

Figure 3. Comparative analysis of NAFL versus NASH patients for various readouts. A. Collagen. **B.** Perisinusoidal collagen. **C.** Hepatic crown-like structures. **D.** Active injury area.

Readouts	NAFL* (n = 74) Mean (min - max)	NASH (n = 174) Mean (min-max)	p-value
Tissue Density (%)	83.09 (64.62 - 94.32)	79.75 (57.09 -95.24)	**0.0013**
Steatosis S (%)	7.83 (0.82 - 23.55)	10.20 (1.19 - 28.23)	**0.0001**
Steatosis T (%)	8.61 (1.04 - 25.90)	11.23 (1.36 - 30.13)	**0.0002**
Mean vesicle area (µm²)	119.9 (34.93 - 273.5)	136.6 (42.23 - 422.2)	**0.0024**
Collagen S (%)	7.74 (1.92 - 20.22)	7.20 (1.25 - 19.96)	0.2241
Collagen T (%)	8.92 (2.67 - 21.44)	8.91 (1.58 - 22.34)	0.4902
Periductular Collagen (%)	3.10 (0.51 - 8.44)	4.86 (0.95-13.84)	**0.0016**
Perisinusoidal Collagen (%)	3.13 (0.89 - 8.03)	2.44 (0.48 - 5.86)	**<0.0001**
Perivascular Collagen (%)	2.97 (0.47 - 12.86)	3.19 (0.18 - 11.60)	0.2207
Septal Collagen (%)	0.61 (0.08 - 4.92)	0.88 (0.03 - 3.47)	**<0.0001**
CK19 S (%)	0.14 (0.002 - 0.49)	0.21 (0.003 - 0.97)	**0.0133**
CK19 T (%)	0.17 (0.002 - 0.60)	0.25 (0.004 - 1.11)	**0.0209**
Inflammatory area (%)	4.78 (0.00 - 13.96)	8.14 (0.04 - 25.70)	**<0.0001**
Inflammatory foci (n/mm²)	29.15 (0.45 - 78.35)	19.81 (0.00 - 58.34)	**<0.0001**
CD68 (%)	2.94 (1.89 - 5.03)	2.94 (1.59 - 5.12)	0.9312
hCLS (n/mm²)	0.14 (0.00 - 0.91)	0.43 (0.00 - 3.77)	**<0.0001**
Shh (‰)	3.27 (0.05 - 18.35)	6.46 (0.06 - 44.88)	**<0.0001**
Active injury area (‰)	1.94 (0.00- 14.15)	6.68 (0.00 - 82.06)	**0.0008**

Table 4. Comparison NAFL versus NASH patients (NAFL as defined with a NAS score < 4). hCLS: hepatic crown-like structures. Bold means significant.

CONCLUSION & DISCUSSION

Original combinations of classical stains with immunohistochemistry were used as objective means for more reliable identification of NASH features from liver biopsies.

Interestingly, correlations with visual assessment were observed for many features, including ballooning, and NASH patients had significant quantitative changes compared to NAFL patients, except for total and perivascular collagen, and CD68 expression.

The current study demonstrates that MorphoQuant is a powerful image analysis tool, using current and original histological methods. In addition, this study highlights that a reliable digital pathology software can be developed independently from pathologist's annotations.

Future work consists of evaluating its usefulness as a support for a more reliable reading of liver pathology.