

MORPHOQUANT-LIVER, AN AUTOMATED PATHOLOGIST-INDEPENDENT MORPHOMETRIC SOFTWARE, IDENTIFIED SIGNIFICANT DIFFERENCES BETWEEN STEATOTIC AND MASH LIVER BIOPSIES

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OBJECTIVES

- To assess the capability of MorphoQuant, an automated pathologist-independent morphometric software of digital pathology, to identify histological differences between steatotic liver disease (SLD) and metabolic dysfunction-associated steatohepatitis (MASH) liver biopsies through quantification of objective features.
- To compare MorphoQuant findings with the pathologist's visual scoring.
- To assess the interest in quantifying Sonic Hedgehog (Shh) expression with digital pathology.

MATERIALS AND METHODS

- 271 liver biopsies scored by a blinded expert pathologist according to the NASH CRN.
- MorphoQuant, a fully automated and deterministic artificial intelligence was developed to assess MASH features and fibrosis.
- For digital quantification, slides stained with H&E, picosirius red alone or combined with CK19, labeled with CD68 or with Sonic Hedgehog, and digitized.
- MASH (NAS ≥ 4 , with at least 1 point per feature) and non-MASH biopsies were compared.
- Correlations with pathologist were assessed and digital analysis of Shh expression was tested for correlations with histological features.

CONCLUSION

The current study demonstrates that MorphoQuant allows an objective quantification of MASH and fibrosis-related features, and to stratify steatotic and MASH patients. It also shows the relevance of using Shh as a potential marker for disease activity. In addition, this study highlights that reliable digital pathology software can be developed independently from pathologists' annotations.

.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

PSR or PSR-CK19 (n = 271)	<ul style="list-style-type: none"> Biopsy area (mm²) Number of fragments Biopsy adequacy Tissue density (%) Steatosis S and T (%) Mean vesicle area (μm²) Collagen S and T (%) Periductular collagen (%) Perisinusoidal / Perivascular/ Septal collagen (%) CK19 S and T (%)
H&E (n = 262)	<ul style="list-style-type: none"> Inflammation area (%) Inflammatory foci (n/mm²)
CD68 (n = 120)	<ul style="list-style-type: none"> CD68 (%) Hepatic crown-like structures (n/mm²)
Shh (n = 271)	<ul style="list-style-type: none"> Shh (‰) Active injury area (‰)

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

.03 AUTOMATED DIGITAL QUANTIFICATION

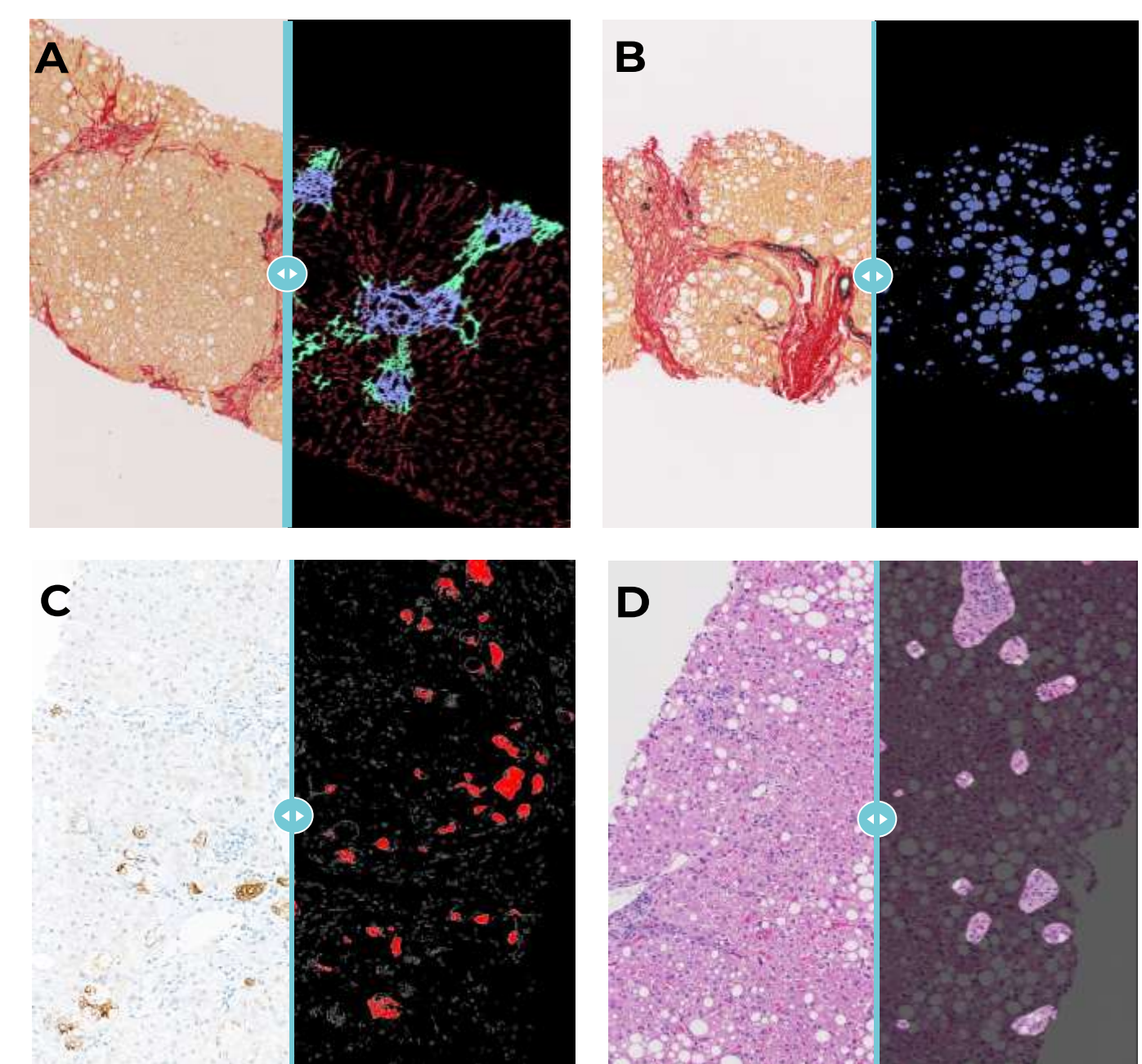


Figure 1. Representative images of computational analysis by MorphoQuant™. A. Fibrosis. B. Steatosis. C. Active injury. D. Inflammation.

.04 CORRELATION WITH PATHOLOGIST VISUAL ASSESSMENT

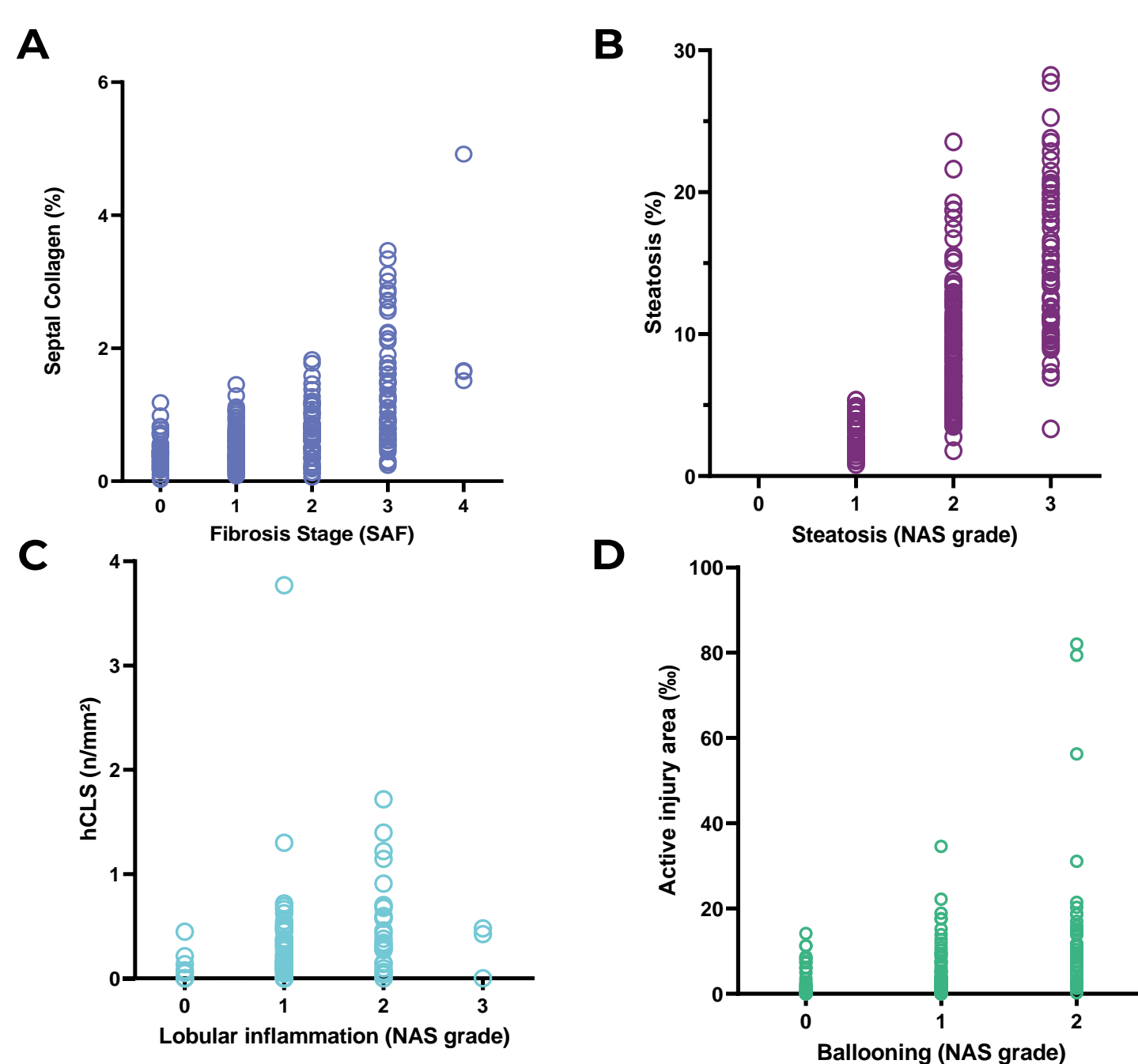


Figure 2. Correlations between quantitative digital assessment of MASH features and NASH CRN score systems for all assessed patients. A. Fibrosis vs septal collagen. B. Steatosis vs steatosis. C. Lobular inflammation vs hepatic crown-like structures. D. Ballooning vs active injury area.

	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.

.05 COMPARATIVE ANALYSIS OF SLD vs MASH PATIENTS

Readouts	SLD (n = 74)	MASH (n = 174)	p-value
	Mean (min - max)	Mean (min-max)	
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 (μm ²)	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/mm ²)	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/mm ²)	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 SLD versus MASH patients (SLD as defined with a NAS score < 4). hCLS: hepatic crown-like structures. Bold means significant.

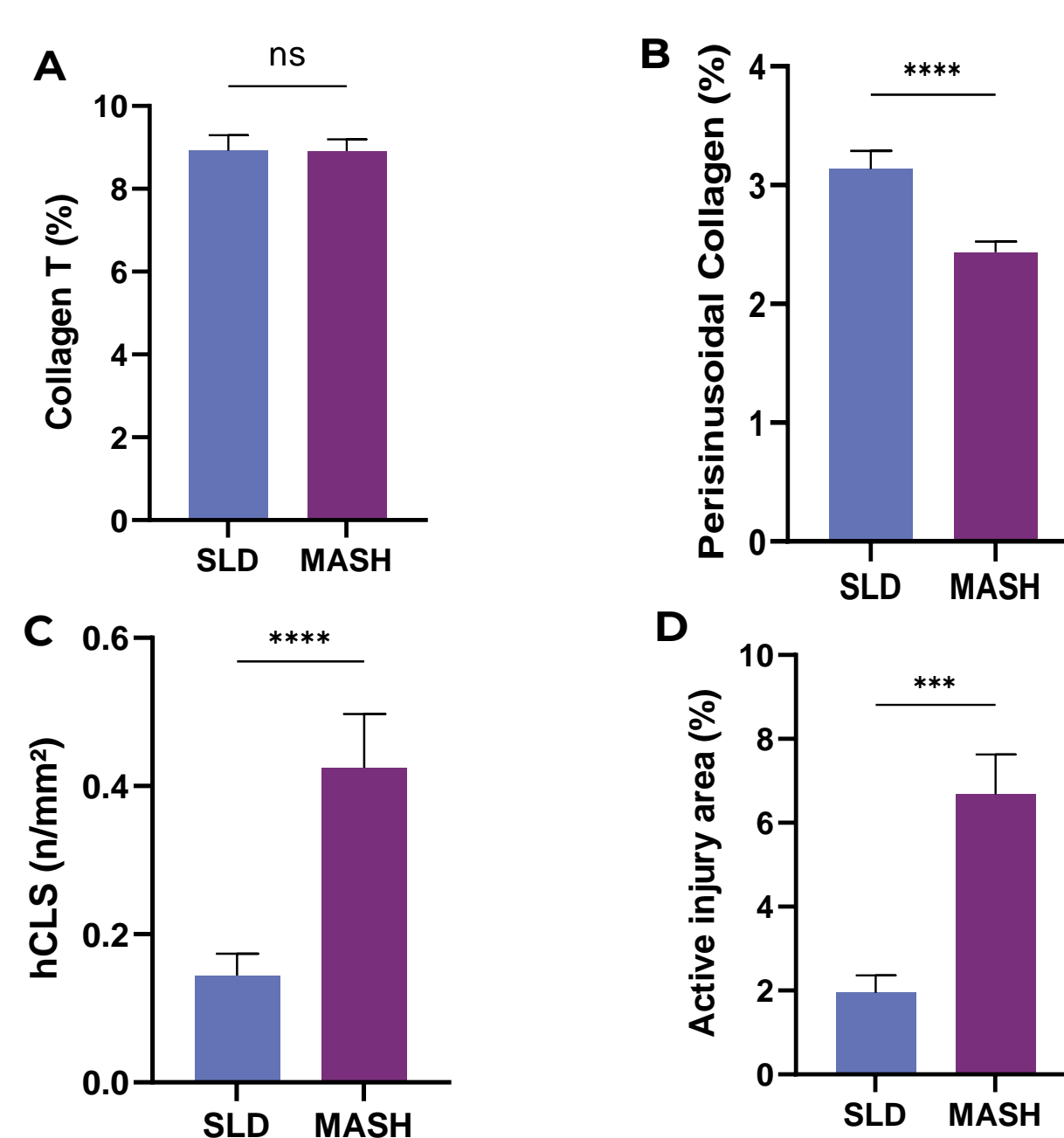


Figure 3. Comparative analysis of SLD versus MASH patients for various readouts. A. Collagen. B. Perisinusoidal collagen. C. Hepatic crown-like structures. D. Active injury area.

AT A GLANCE

- Original combinations of classical stains with immunohistochemistry were used as objective means for more reliable identification of MASH features from 271 liver biopsies.
- Correlations with visual assessment were calculated for MASH features.
- Overall, MASH patients had significant quantitative changes compared to SLD patients
- The current study demonstrates that MorphoQuant is a powerful image analysis tool, using current and original histological methods.
- This study highlights that a reliable digital pathology software can be developed independently from pathologist's annotations.