

MORPHOMETRIC ANALYSIS OF SONIC HEDGEHOG IN NON-ALCOHOLIC FATTY LIVER AND STEATOHEPATITIS PATIENTS BY MORPHOQUANT: A POTENTIAL BIOMARKER OF DISEASE SEVERITY

Authors: Cindy Serdjebi¹, Bastien Lepoivre¹, Florine Chandes¹, Linda Willis², Yvon Julé¹

¹Biocellvia, Marseille, France
²HistologiX, Innovation Building | BioCity Nottingham, Pennyfoot Street, Nottingham, NG1 1GF, United Kingdom

Cindy Serdjebi, R&D Director,
cindy.serdjebi@biocellvia.com

BACKGROUND AND AIMS

Sonic hedgehog (Shh) is an important pathway activated in non-alcoholic fatty liver diseases and its expression is known to increase along with the severity of non-alcoholic steatohepatitis (NASH). In addition, computer-assisted morphometry previously demonstrated that Shh expression was associated with ballooning degeneration score as well as fibrosis grading. Yet, the use of this immune-histo-chemistry (IHC) has long been ignored in the current general practice and/or in clinical trials. In this study, we have developed a fully automated morphometry software to detect and quantify Shh expression and investigated the interest of measuring the area of active injury in patients' liver biopsies.

METHOD

Liver biopsies were scored by a blinded expert pathologist according to the NASH CRN for steatosis, inflammation, ballooning and fibrosis. Shh labeling was developed using SG vector as chromogen. MorphoQuant, a fully automated and deterministic artificial intelligence, based on morphometry and expert system - completely independent from pathologist's annotations, was developed to specifically detect Shh and areas of active liver injury from whole slide images. As an edge effect was seen on a significant number of labeled slides, a 120-µm safety margin was automatically removed from all biopsy fragments before analysis. The quantitative data of Shh expression and areas of active injuries were thus compared to the pathologist's reading and correlations were calculated using the Spearman correlation test for fibrosis, steatosis, lobular and portal inflammation, Mallory-Denk bodies, interface hepatitis, NAS and NASH status.

.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 AUTOMATED DIGITAL QUANTIFICATION

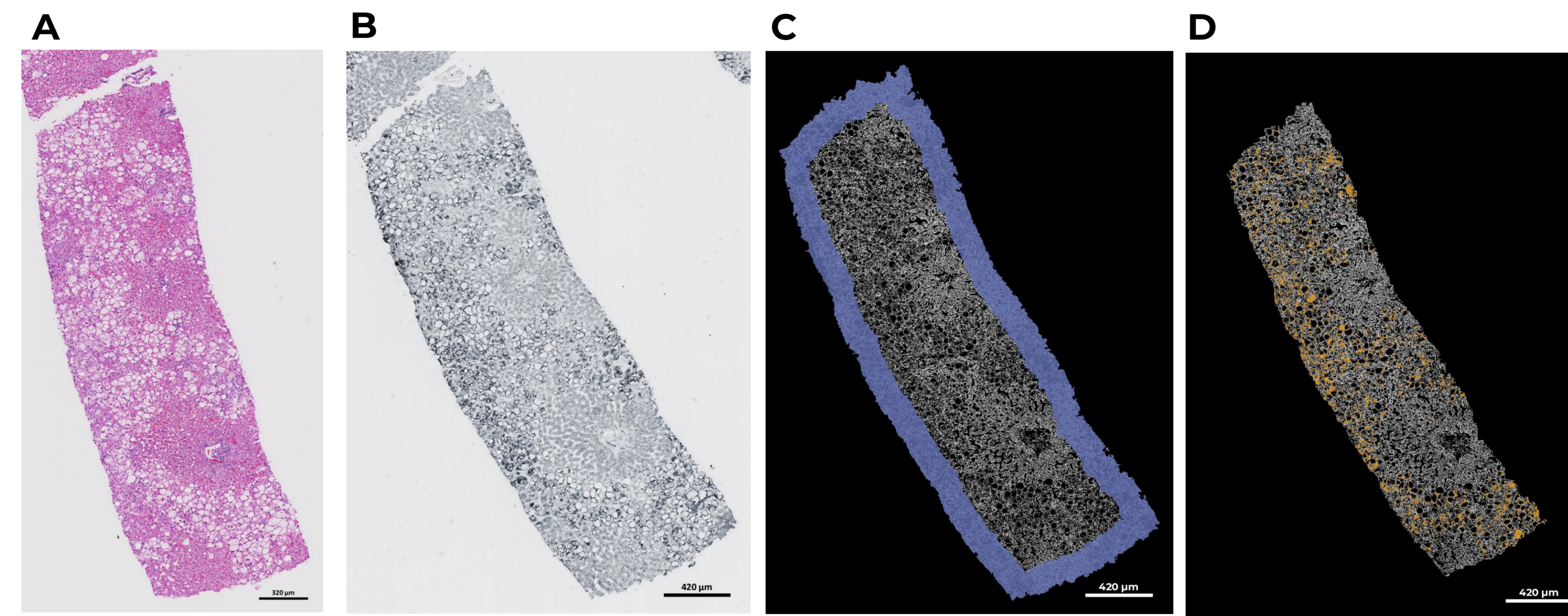


Figure 1. Representative images of computational analysis by MorphoQuant™. A. Native scan of H&E section. B. Native scan of the same biopsy labelled with Sonic Hedgehog. C. Automated newly delineated section to suppress edge effect. D. Shh expression. E. Active injury area.

.03 COMPARATIVE ANALYSIS OF NAFL vs NASH PATIENTS

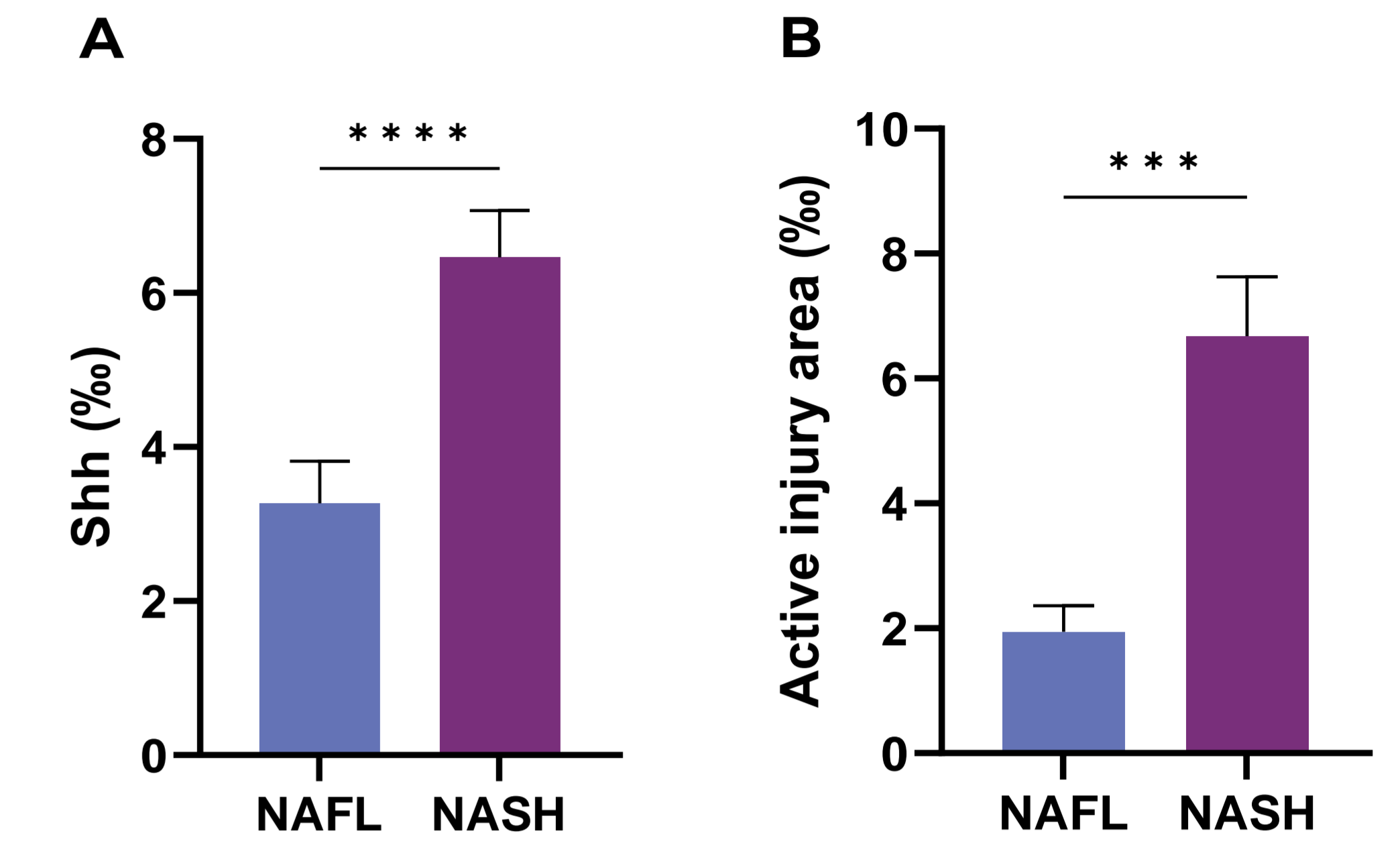


Figure 2. Comparison of Shh and active injury area for NAFL and NASH patients. Shh: Sonic hedgehog. NASH (defined as NAS ≥ 4 with at least 1 in each category)

.04 COMPARATIVE CORRELATION OF SHH vs ACTIVE INJURY AREA

NASH and Fibrosis Scores	Spearman correlation rate r (CI95 interval)	p-value
Fibrosis (SAF)		
Shh	0.4192 (0.2975 - 0.5975)	<0.0001
Active Injury Area	0.4941 (0.3812 - 0.5924)	<0.0001
Steatosis (NAS)		
Shh	0.1193 (-0.02005 - 0.2541)	0.0839
Active Injury Area	0.1222 (-0.01706 - 0.2569)	0.0764
Ballooning (NAS)		
Shh	0.4270 (0.3047 - 0.5332)	<0.0001
Active Injury Area	0.5329 (0.4254 - 0.6256)	<0.0001
Lobular inflammation (NAS)		
Shh	0.2997 (0.1676 - 0.4211)	<0.0001
Active Injury Area	0.4130 (0.2906 - 0.5220)	<0.0001
Portal inflammation		
Shh	0.0669 (-0.07278 - 0.2040)	0.335
Active Injury Area	0.0878 (-0.05186 - 0.2241)	0.2041
Interface hepatitis		
Shh	0.1526 (0.01384 - 0.2855)	0.0267
Active Injury Area	0.1782 (0.04018 - 0.3095)	0.0095
Mallory-Denk bodies		
Shh	0.2826 (0.1495 - 0.4057)	<0.0001
Active Injury Area	0.0343 (0.2142 - 0.4600)	<0.0001
NAFLD Activity Score		
Shh	0.3964 (0.2724 - 0.5075)	<0.0001
Active Injury Area	0.4953 (0.3826 - 0.5934)	<0.0001
NASH status		
Shh	0.3160 (0.1851 - 0.4359)	<0.0001
Active Injury Area	0.3996 (0.02759 - 0.5102)	<0.0001

Table 2. Comparison of correlations between Shh and active injury area with pathologist scoring

.05 CORRELATION WITH CENTRAL PATHOLOGY READING

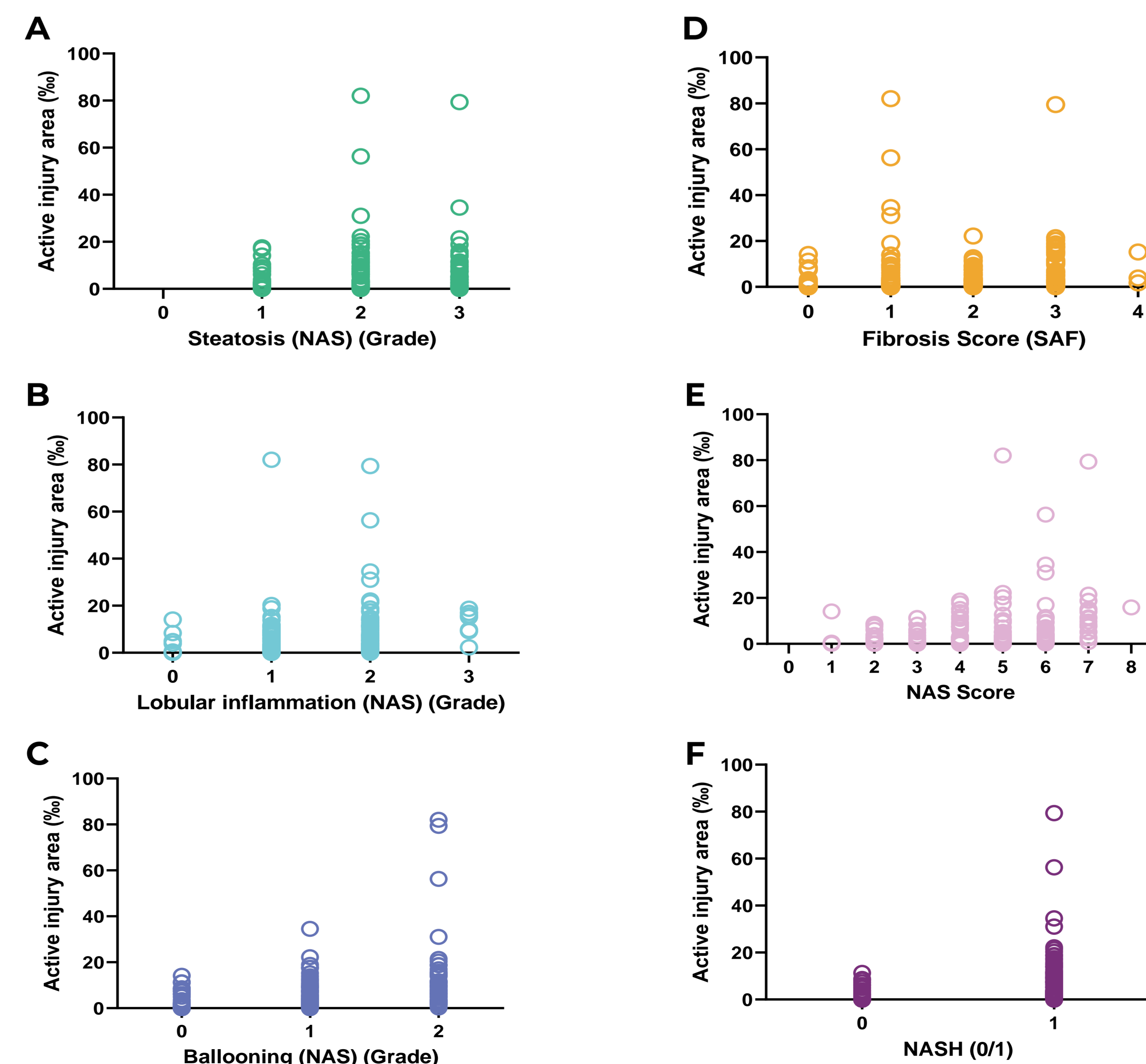


Figure 3. Correlations between quantitative digital assessment of active injury area and pathologist scoring. A. Steatosis. B. Lobular Inflammation. C. Ballooning. D. Fibrosis. E. NAS. F. NASH (defined as NAS ≥ 4 with at least 1 in each category)

CONCLUSION & DISCUSSION

Sonic Hedgehog (Shh) is an important pathway activated in non-alcoholic fatty liver diseases.

Its expression was shown to correlate with fibrosis grade and ballooning.

In the current study, we demonstrate the utility of digital quantification of Shh to discriminate NAFLD from NASH patients. Active injury area, a newly developed readout, is consistently more correlated with the histopathological reading than Shh. Both Shh and active injury area moderately correlated with fibrosis, ballooning, and NAS, and active injury was strongly associated with ballooning, identifying this new readout as a potential biomarker for disease activity.

Future work will investigate Shh and active injury area in NASH cohort with initial and follow-up biopsies.