Digital pathology using stain-free imaging indices allows direct prediction of all-cause mortality, hepatic decompensation, and hepatocellular carcinoma development in patients with non-alcoholic fatty liver disease

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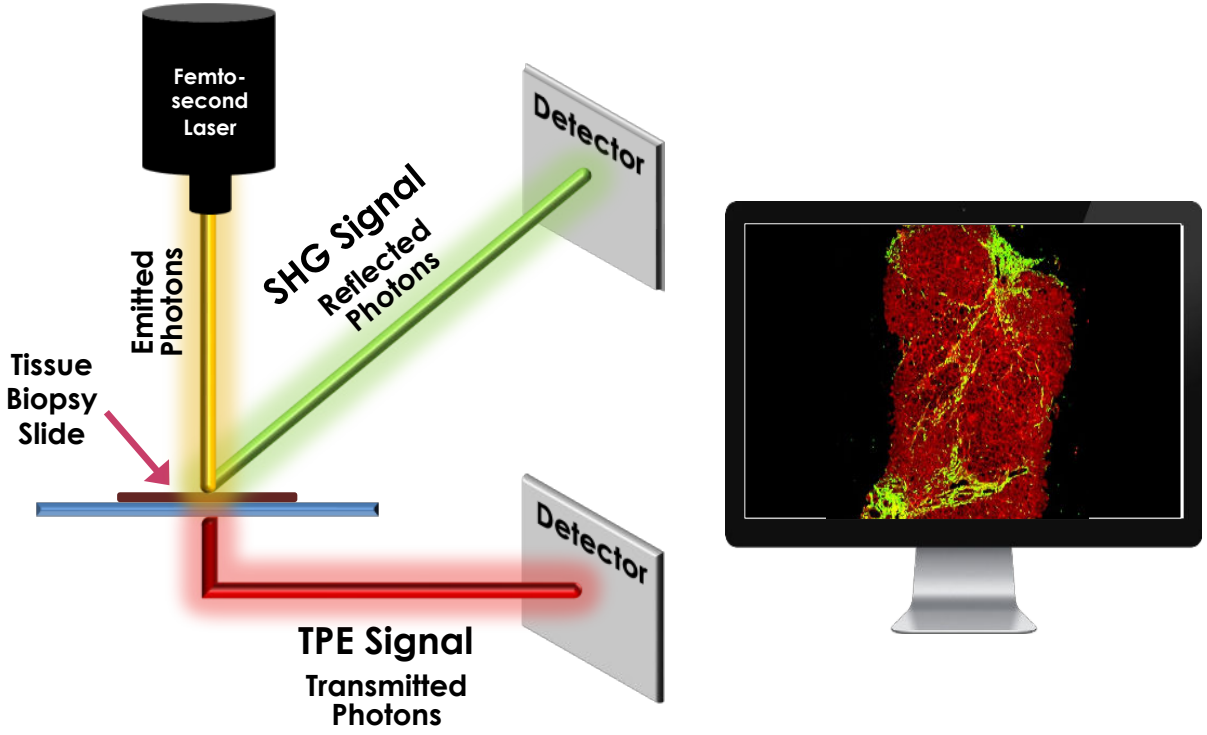
Background

- Digital quantification of scarring from either stained liver sections or stain-free imaging reduces observer-related variability in the histological assessment of NAFLD.
- To date, computational methods have mainly provided ordinal scores analogous to those provided by a pathologist as disease outcomes are strongly correlated with stage.
- Direct prediction of outcomes from tissue, without using fibrosis stage as a surrogate, has not been possible due to the absence of suitable event-rich cohort data.

NAFLD, non-alcoholic fatty liver disease

Stain-free imaging

- Stain-free imaging examines architectural features unapparent to human observers.
- SHG microscopy identifies collagen and TPEF microscopy identifies hepatocytes.



SHG, second harmonic generation; TPEF, two-photon excitation fluorescence

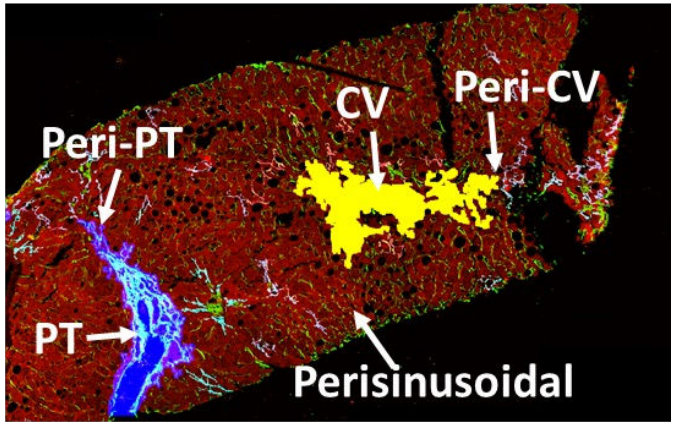
Xu S, et al. J Hepatol. 2014;61(2):260-269.

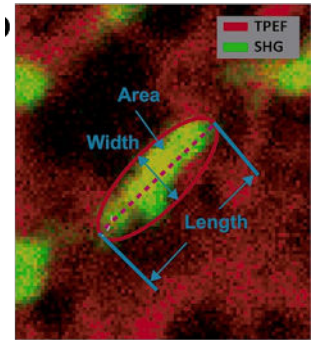
qFibrosis

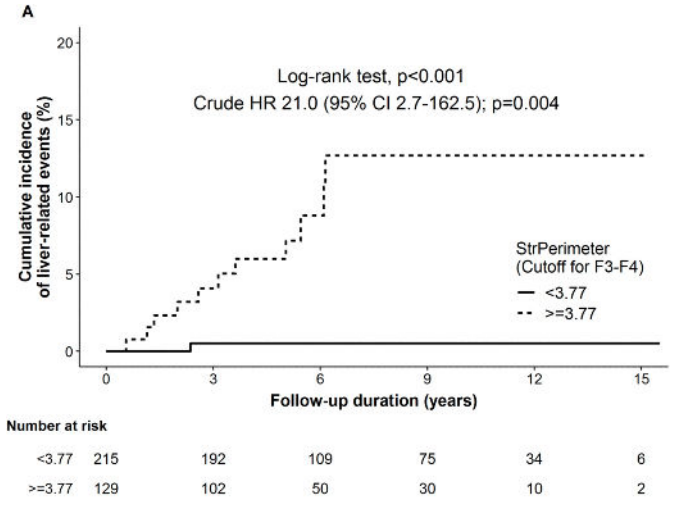
- ML-based algorithm with SHG/TPEF imaging provides a visual mapping of collagen burden/distribution & permits measurement of quantifiable collagen fibrillar properties.

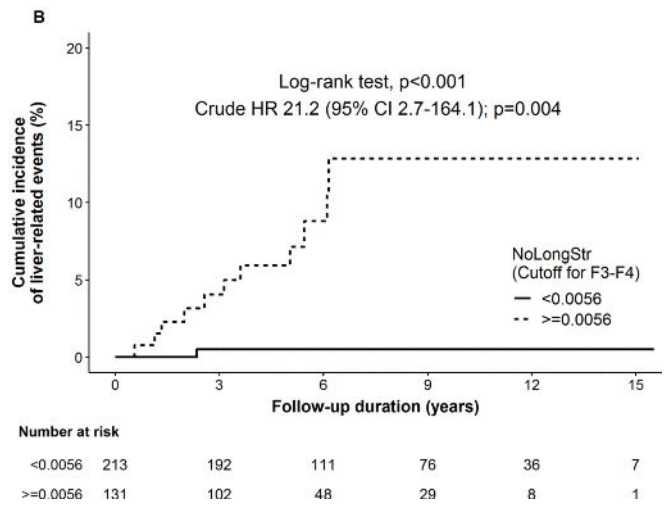
  e.g., length, width and area of fibers according to their distribution in the histopathological regions.

- Previous longitudinal study demonstrated the potential prognostic significance of q-FPs where subjects with high q-FPs had increased incidence of liver-related events.









ML, machine learning; q-FPs, fibrosis-related parameters

StrPerimeter: Perimeter of collagen fibres; NoLongStr: number of long collagen fibres

Liu et al., Hepatology, 2020;71:1953-1966; Wang et al., *Gut* 2019; 69(6):1116-1126.

SteatoSITE

- SteatoSITE (https://steatosite.com/) is a resource containing integrated clinical and pathological data from 940 cases across NAFLD spectrum.
- Includes outcome data from electronic health record and pathologist-assigned scores from H&E and PSR-stained sections including NASH-CRN fibrosis.
- A single unstained section was available.

H&E, hematoxylin and eosin; PSR, picrosirius red

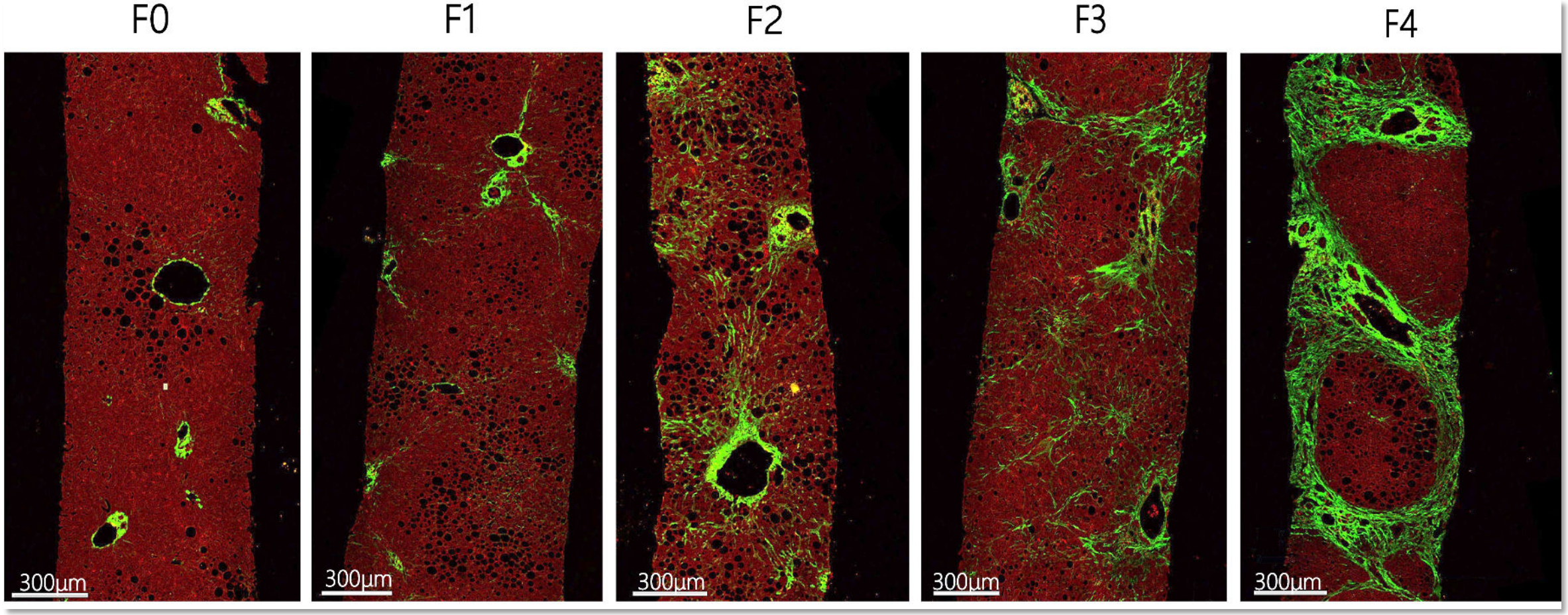
Aims

- To establish individual indices for risk of all-cause mortality, hepatic decompensation and HCC from key fibrotic architectural features identified using stain-free imaging.
- To compare the predictive power of the risk indices established with (i) the stain-free imaging-derived qFibrosis stage (qF0/1/2 v qF3/4) and (ii) with the assigned NASH-CRN fibrosis stage (F0/1/2 v F3/4).

HCC, hepatocellular carcinoma

Methods

- Unstained sections from a training set of 294 core biopsies from SteatoSITE were imaged using SHG/TPEF microscopy.



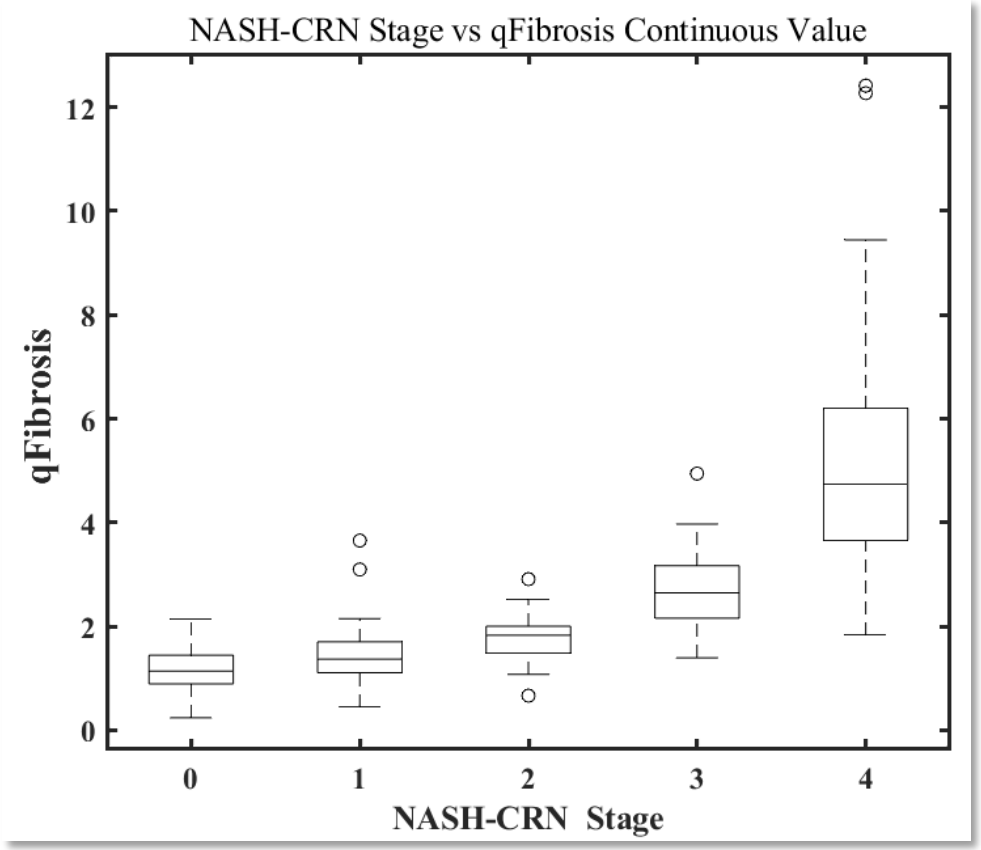
Statistical approach

- Using sequential feature selection, 10, 10 and 5 parameters were chosen out of 184 fibrosis-related parameters and a linear regression method was used to construct individual indices for risk of all-cause mortality, hepatic decompensation and HCC, respectively.
- Time-to-event analysis was performed using the Kaplan–Meier method, with death as a competing risk for decompensation and HCC, and distributions compared using the log-rank test.
- A Cox proportional hazards model was used to estimate hazard ratios.

Results

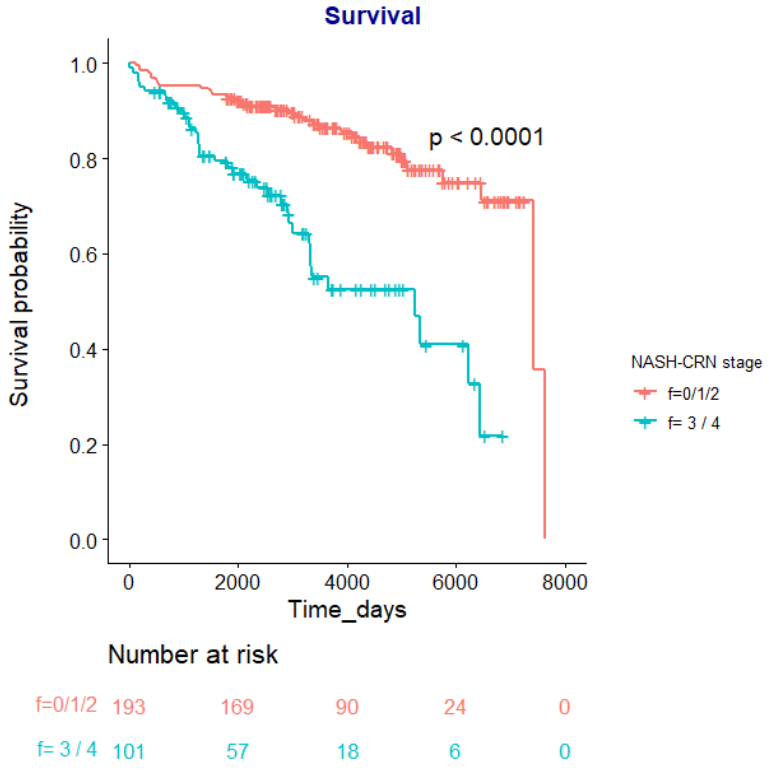
- qFibrosis has been applied in over a dozen non-cirrhotic and cirrhotic NASH retrospective studies, where correlation with central pathologists' (CRN staging) ranges from 0.57 – 0.81.

- The correlation of qFibrosis with CRN staging from the current training set of 294 core biopsies is r = 0.82 ($p<0.001$).



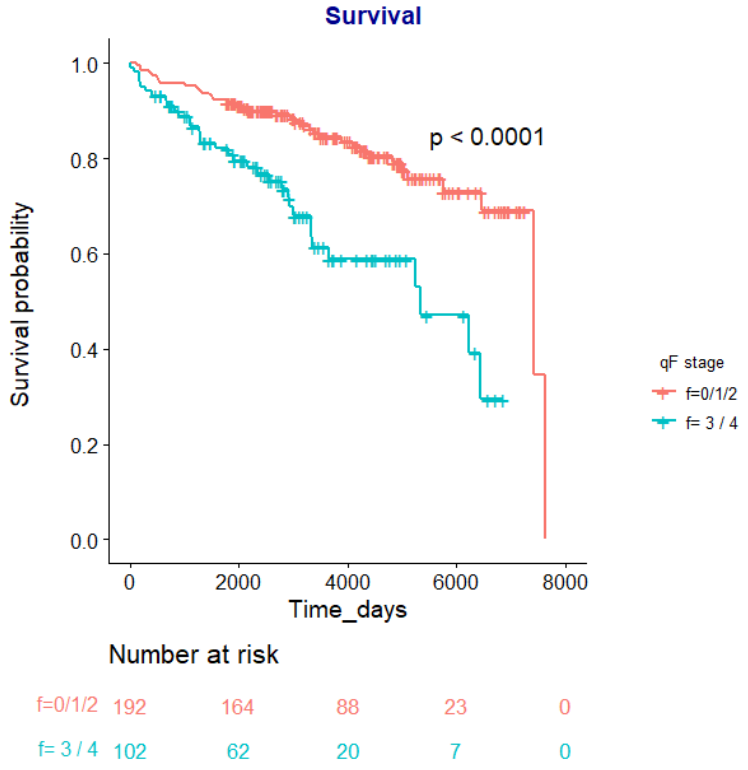
Results: All-cause mortality index

NASH-CRN



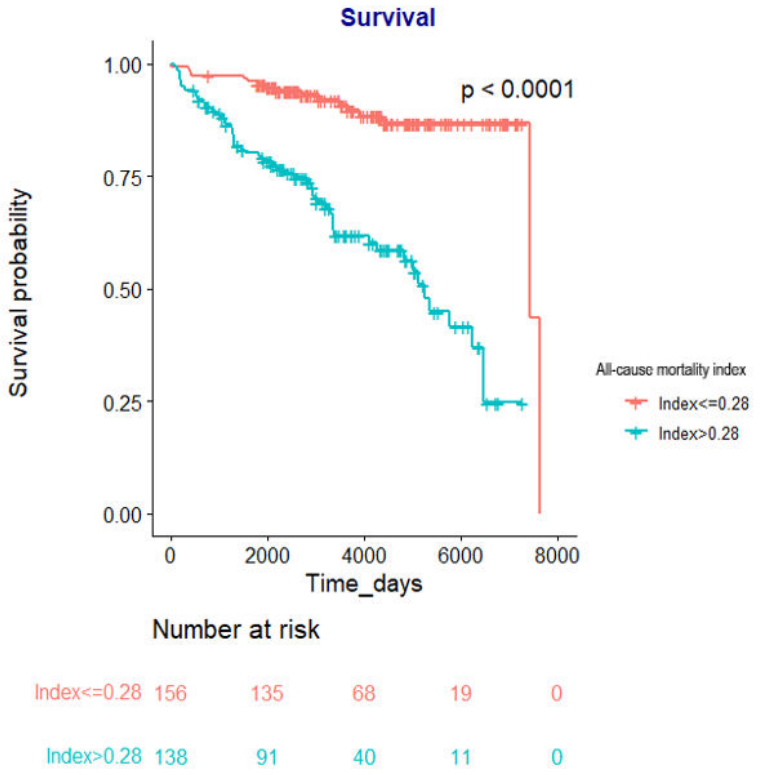
NASH-CRN	p value	Hazard ratio	HR 95.0% CI
F3/4 vs F0/1/2	0.000	**3.54**	2.19-5.73

qFibrosis index



qFibrosis	p value	Hazard ratio	HR 95.0% CI
F3/4 vs F0/1/2	0.000	**2.70**	1.67-4.34

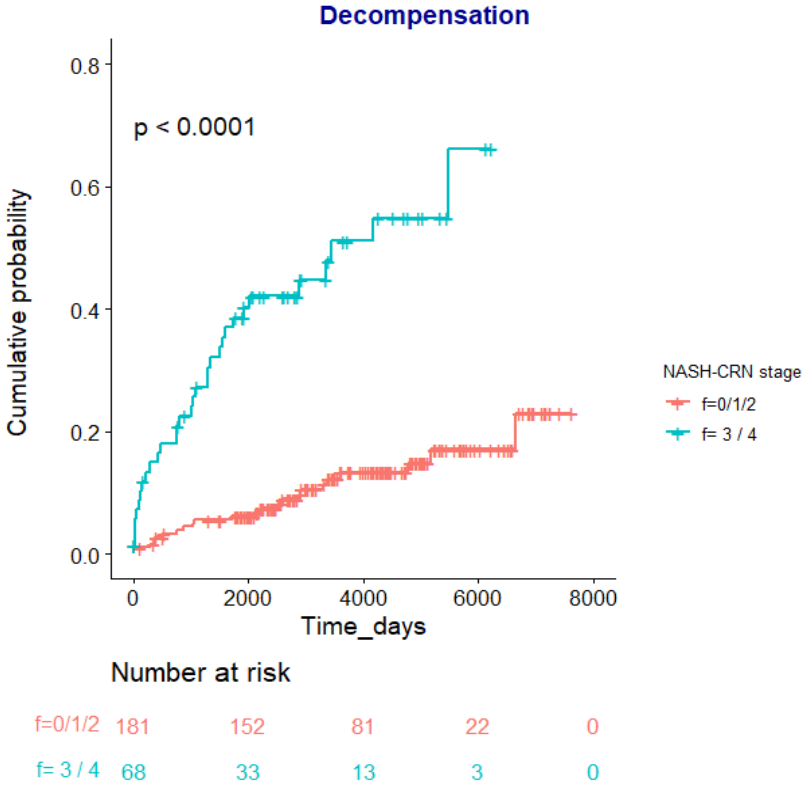
All-cause mortality index



Index	p value	Hazard ratio	HR 95.0% CI
Index>0.28 vs index<=0.28	0.000	**5.33**	3.00-9.47

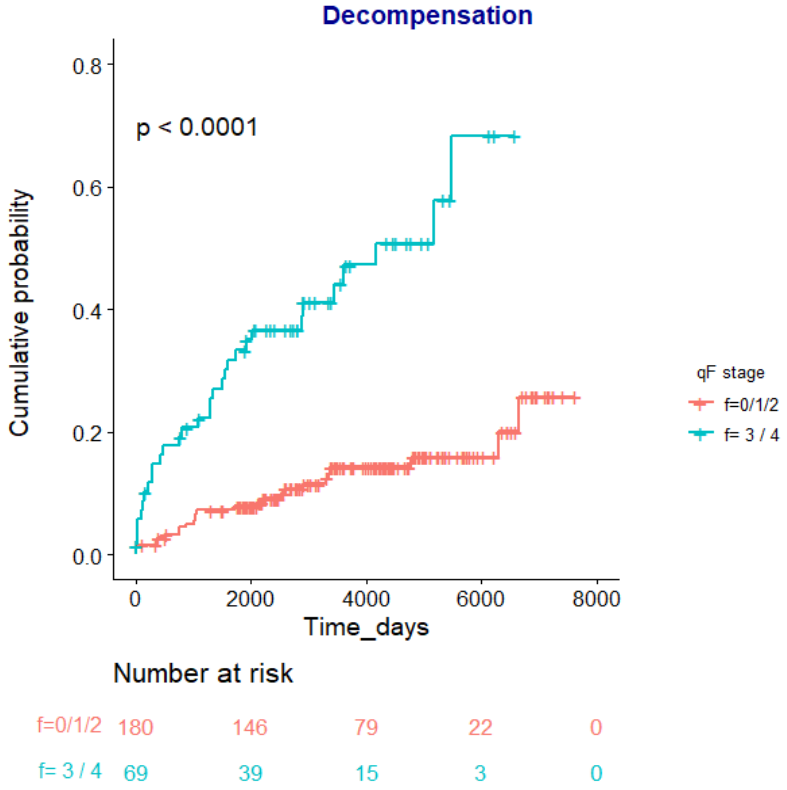
Results: Hepatic decompensation index

NASH-CRN



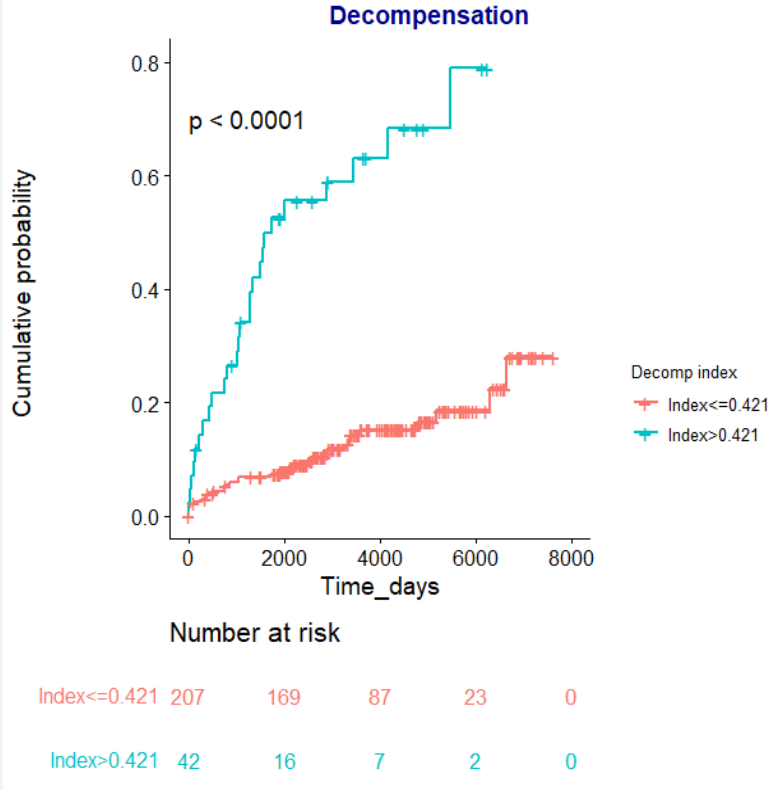
NASH-CRN	p value	Hazard ratio	HR 95.0% CI
F3/4 vs F0/1/2	0.000	**6.02**	3.48-10.41

qFibrosis index



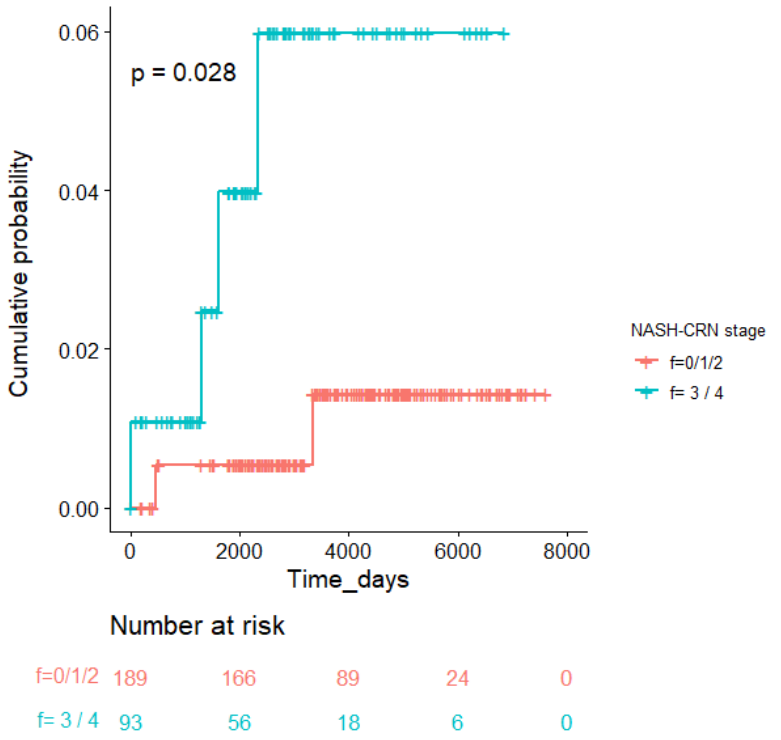
qFibrosis	p value	Hazard ratio	HR 95.0% CI
F3/4 vs F0/1/2	0.000	**4.63**	2.70-7.93

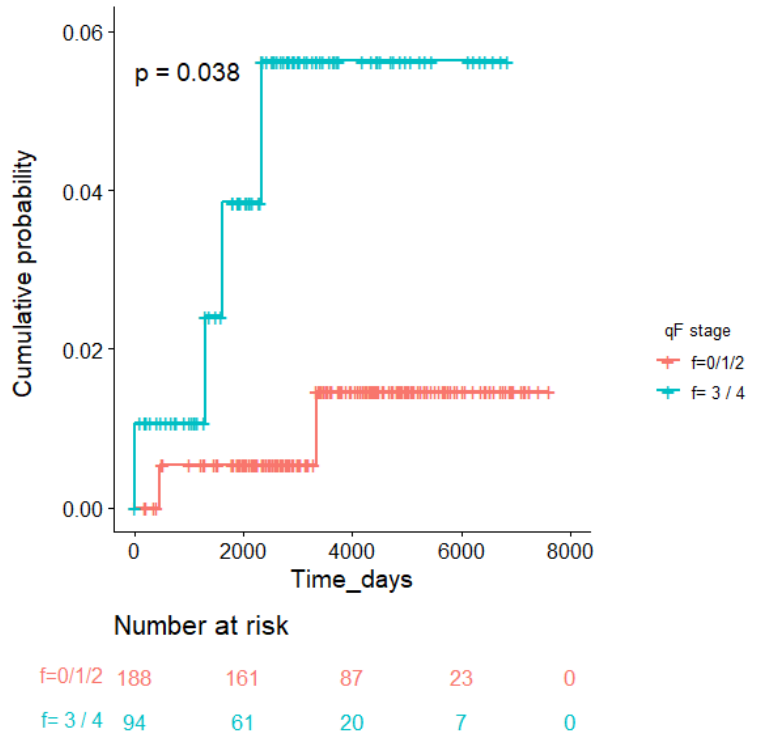
Decompensation index

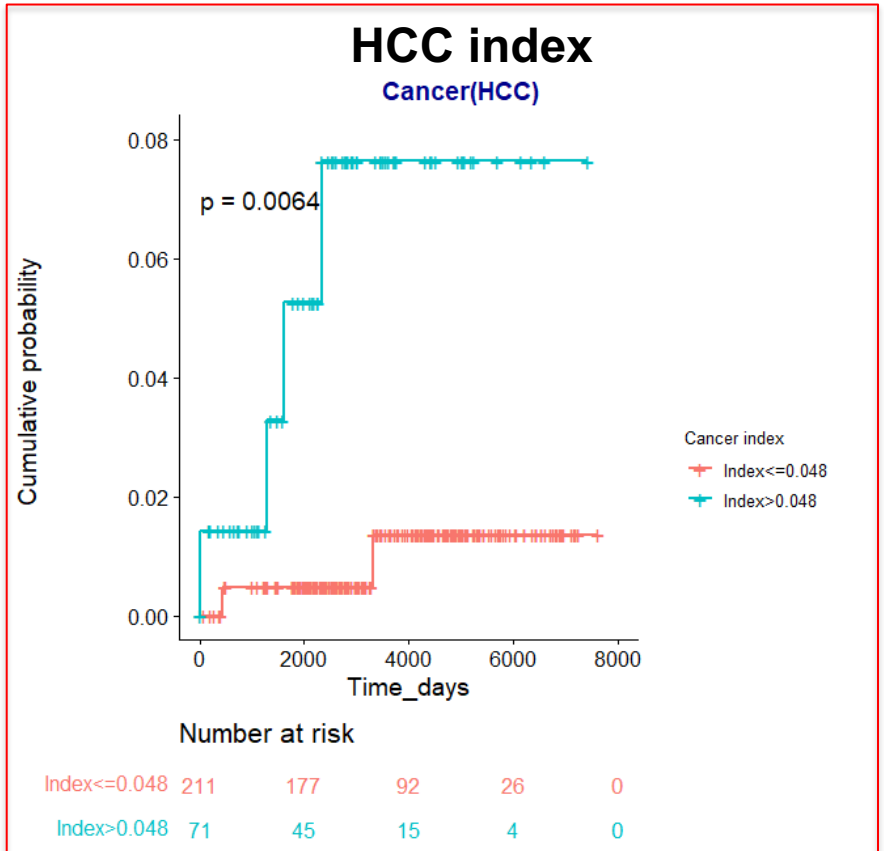


Index	p value	Hazard ratio	HR 95.0% CI
index>0.421 vs index<=0.421	0.000	**6.97**	4.06-11.95

Results: HCC index







NASH-CRN	p value	Hazard ratio	HR95.0% CI
F3/4 vs F0/1/2	0.050	**5.52**	1.00-30.50

qFibrosis	p value	Hazard ratio	HR95.0% CI
F3/4 vs F0/1/2	0.062	**5.08**	0.92-27.91

Index	p value	Hazard ratio	HR95.0% CI
index>0.048 vs index<=0.048	0.020	**7.50**	1.37-41.13

Summary

- Using liver biopsy material with linked long-term clinical outcome data, we developed tools that directly predict hard endpoints in patients with NAFLD and do not rely on ordinal fibrosis scores as a surrogate.
- These tools have greater predictive value than pathologist-assigned NASH-CRN fibrosis stage or computationally-assigned qFibrosis stage.
- These indices will be validated in an additional NAFLD cohort but may provide direct tissue-to-outcome predictions that aid clinical decision-making, offer more nuanced participant stratification and meaningful endpoints in clinical trials.

Acknowledgements

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Digital pathology using stain-free imaging indices allows direct prediction of all-cause mortality, hepatic decompensation and hepatocellular carcinoma development in patients with non-alcoholic fatty liver disease

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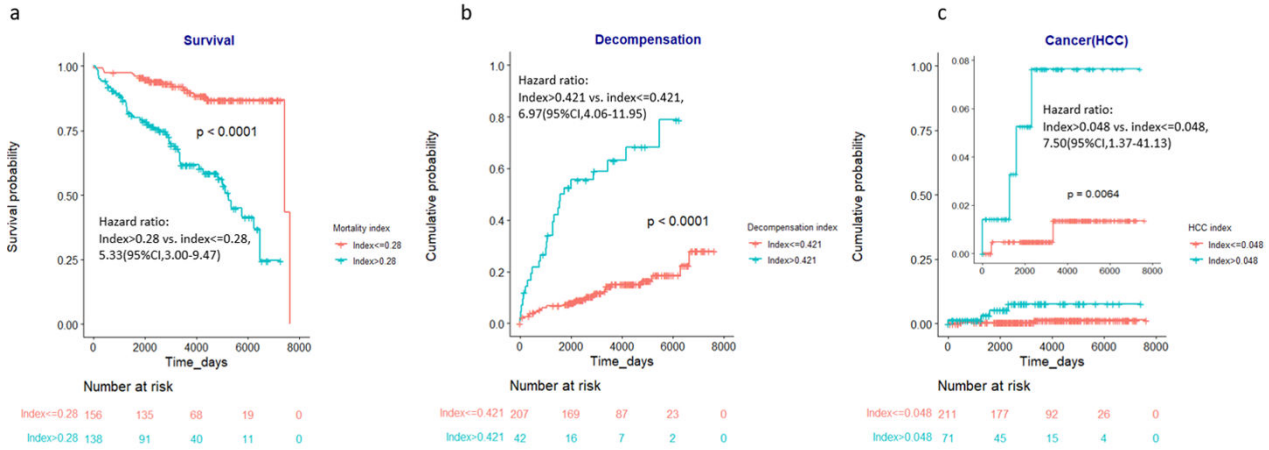
Background and Aims: Digital quantification of scarring from either stained liver sections or stain-free imaging reduces observer-related variability in the histological assessment of non-alcoholic fatty liver disease (NAFLD). To date, computational methods have mainly provided ordinal scores analogous to those provided by a pathologist as disease outcomes are strongly correlated with stage. Direct prediction of outcomes from tissue, without using fibrosis stage as a surrogate, has not been possible due to the absence of suitable event-rich cohort data. Using SteatoSITE (https://steatosite.com/), a resource containing integrated clinical and pathological data, we undertook stain-free imaging to generate tools predictive of patient outcomes using architectural features unapparent to human observers.

Method: Unstained sections from a training set of 294 biopsies were imaged using second harmonic generation/two-photon excitation fluorescence microscopy. Using sequential feature selection, 10, 10 and 5 parameters were chosen out of 184 fibrosis parameters and a linear regression method was used to construct individual indices for risk of all-cause mortality, hepatic decompensation and hepatocellular carcinoma (HCC), respectively. Time-to-event analysis was performed using the Kaplan–Meier method, with death as a competing risk for decompensation and HCC, and distributions compared using the log-rank test (Fig. 1). A Cox proportional hazards model was used to estimate hazard ratios (HRs). The predictive power of the risk indices was compared with the assigned NASH-CRN fibrosis stage (F0/1/2 v F3/4) and the stain-free imaging derived qFibrosis stage (qF0/1/2 v qF3/4).

Results: The newly defined “Mortality Index” had greater predictive power for all-cause mortality (index >0.28 vs. </= 0.28, HR 5.33, 95% confidence intervals (CI) 3.00-9.47, p = 0.000) than either NASH-CRN or qFibrosis stage. The “Decompensation Index” had greater predictive power for decompensation events (index >0.421 vs. </=0.421, HR 6.97, 95% CI 4.06-11.95, p = 0.000) than either NASH-CRN stage or qFibrosis stage. Finally, the “HCC Index” had greater predictive power for HCC development (index >0.048 vs. <=0.048, HR 7.50, 95% CI 1.37-41.13, p = 0.020) than either NASH-CRN stage or qFibrosis stage.

Conclusion: Using liver biopsy material with linked long-term clinical outcome data, we developed tools that directly predict hard endpoints in patients with NAFLD and do not rely on ordinal fibrosis scores as a surrogate. These tools have greater predictive value than pathologist-assigned NASH-CRN fibrosis stage or computationally-assigned qFibrosis stage. These indices will be validated in an additional NAFLD cohort but may provide direct tissue-to-outcome predictions that aid clinical decision-making, offer more nuanced participant stratification and meaningful endpoints in clinical trials.

Figure:


a
Survival
Survival probability
p < 0.0001
Hazard ratio:
Index>0.28 vs. index<=0.28,
5.33(95%CI,3.00-9.47)
Mortality index
Index<=0.28
Index>0.28
Time_days
Number at risk
Index<=0.28 156 135 68 19 0
Index>0.28 138 91 40 11 0
b
Decompensation
Cumulative probability
Hazard ratio:
Index>0.421 vs. index<=0.421,
6.97(95%CI,4.06-11.95)
p < 0.0001
Decompensation index
Index<=0.421
Index>0.421
Time_days
Number at risk
Index<=0.421 207 169 87 23 0
Index>0.421 42 16 7 2 0
c
Cancer(HCC)
Cumulative probability
Hazard ratio:
Index>0.048 vs. index<=0.048,
7.50(95%CI,1.37-41.13)
p = 0.0064
HCC index
Index<=0.048
Index>0.048
Time_days
Number at risk
Index<=0.048 211 177 92 26 0
Index>0.048 71 45 15 4 0