AASLD The Liver Meeting®

STAIN-FREE DIGITAL PATHOLOGY IMAGING PROVIDES MICROARCHITECTURALLY-RESOLVED INSIGHTS INTO SCAR EVOLUTION AND HISTOLOGICAL INJURY ALLOWING DIRECT CLINICAL OUTCOME PREDICTION IN METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE

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INTRODUCTION

- Cardinal histological features reflecting activity are central to the diagnosis of metabolic dysfunction-associated steatotic liver disease (MASLD) and fibrosis stage is associated with increased mortality and liver-related events.
- Recent data indicates significant correlation between ballooning and inflammation with long-term clinical outcomes, though this correlation is less significant when combined with steatosis.[1]
- Liver biopsies of the SteatoSITE cohort[2] allowed stain-free quantification of histological features that are predictive of clinical outcomes but unapparent to human observers.

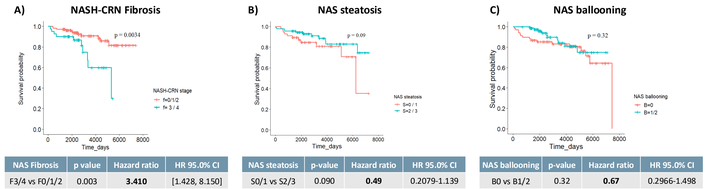
AIM

- Previous research has demonstrated that the linkage between fibrosis morphological features quantified by qFibrosis is more robust than conventional ordinal fibrosis staging systems.[3]
- Building on this approach, we aim to evaluate whether a combined analysis of fibrosis and ballooning/steatosis features can more effectively predict long-term clinical outcomes.

METHOD

- Sections from n=452 biopsies were randomized into training (300) or test (152) sets and imaged for fibrosis, steatosis, and ballooning parameters using second harmonic generation/two-photon excitation fluorescence (SHG/TPE) microscopy.
- 5 of 184 fibrosis parameters have previously been used to derive separate fibrosis-based **Clinical Outcome Mortality Index (COMI-F) and Clinical Outcome Decompensation Index (CODI-F).**
- By feature traversing, the effect of adding 65 steatosis and 21 ballooning parameters to COMI and CODI was tested, and **improved composite outcome indices (-FS and -FB)** generated.
- In the testing set, the predictive power of the new composite indices was compared with assigned NASH-CRN fibrosis stage (F0/1/2 v F3/4), stain-free imaging derived qFibrosis stage (qF0/1/2 v qF3/4), and previously derived fibrosis-only indices using Kaplan–Meier analysis and Cox proportional hazards modelling.

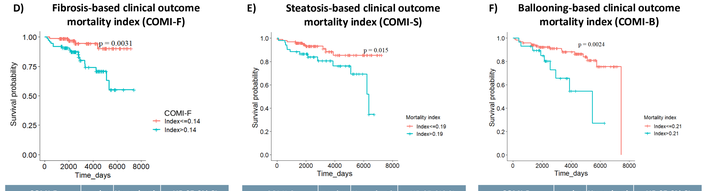
Figure 1: *Kaplan–Meier time-to-event analysis in n = 152 validation cohort with log-rank test p-value for* ***all-cause mortality*** *with high/low risk dichotomisation based on* ***(Top row, L to R):*** *(A) NASH-CRN Fibrosis, (B) NAS Steatosis and (C) NAS Ballooning;* ***(Bottom row, L to R):*** *(D) COMI-F, (E) COMI-S and (F) COMI-B*



NAS Fibrosis	p value	Hazard ratio	HR 95.0% CI
F3/4 vs F0/1/2	0.003	**3.410**	[1.428, 8.150]

NAS steatosis	p-value	Hazard ratio	HR 95.0% CI
S0/1 vs S2/3	0.090	**0.49**	0.2079-1.139

NAS ballooning	p-value	Hazard ratio	HR 95.0% CI
B0 vs B1/2	0.32	**0.67**	0.2966-1.498

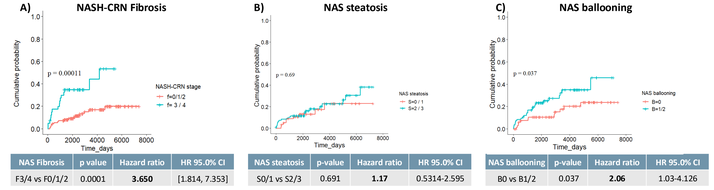


COMI-F	p value	Hazard ratio	HR 95.0% CI
Index >0.14 vs ≤0.14	0.003	**4.490**	[1.500, 13.380]

COMI-S	p value	Hazard ratio	HR 95.0% CI
Index >0.19 vs ≤0.19	0.015	**2.74**	1.172-6.431

COMI-B	p value	Hazard ratio	HR 95.0% CI
Index >0.21 vs ≤0.21	0.0024	**3.39**	1.472-7.81

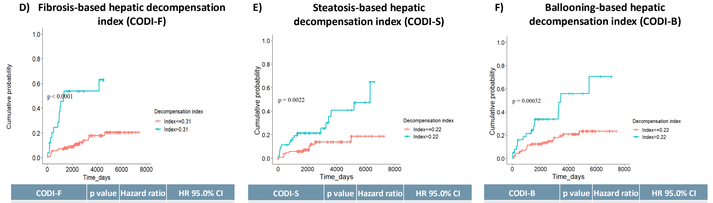
Figure 2: *Kaplan–Meier time-to-event analysis in n = 152 validation cohort with log-rank test p-value for* ***hepatic decompensation*** *(with death as a competing risk) with high/low risk dichotomisation based on* ***(Top row, L to R):*** *(A) NASH-CRN Fibrosis, (B) NAS Steatosis and (C) NAS Ballooning;* ***(Bottom row, L to R):*** *(D) CODI-F, (E) CODI-S and (F) CODI-B*



NAS Fibrosis	p value	Hazard ratio	HR 95.0% CI
F3/4 vs F0/1/2	0.0001	**3.650**	[1.814, 7.353]

NAS steatosis	p-value	Hazard ratio	HR 95.0% CI
S0/1 vs S2/3	0.691	**1.17**	0.5314-2.595

NAS ballooning	p-value	Hazard ratio	HR 95.0% CI
B0 vs B1/2	0.037	**2.06**	1.03-4.126



CODI-F	p value	Hazard ratio	HR 95.0% CI
Index >0.31 vs ≤0.31	0.0001	**5.960**	[2.924, 12.14]

CODI-S	p value	Hazard ratio	HR 95.0% CI
Index >0.22 vs ≤0.22	0.002	**3.00**	1.431-6.288

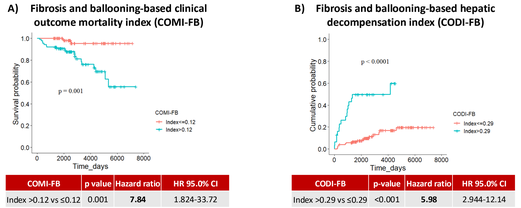
CODI-B	p value	Hazard ratio	HR 95.0% CI
Index >0.22 vs ≤0.22	0.00032	**3.14**	1.628-6.068

RESULTS

Table 1: *Hazard ratios and p-values for prediction of all-cause mortality and hepatic compensation using composite stain-free fibrosis, ballooning and steatosis parameter-derived indices compared with NASH-CRN fibrosis scores, qFibrosis scores, and stain-free fibrosis parameter-only indices.*

	All-cause mortality (COMI)			Hepatic decompensation (CODI)
	Hazard ratio (HR)	*p*-value	95% HR confidence intervals	Hazard ratio
NASH-CRN fibrosis (F0/1/2 v F3/4)	3.41	0.003	1.428-8.15	3.65
qFibrosis (qF0/1/2 v qF3/4)	3.07	0.007	1.295-7.257	3.59
Fibrosis parameter-only indices COMI-F & CODI-F (low v high risk)	4.49	0.003	1.5-13.38	5.96
Composite ballooning and fibrosis parameter indices COMI-FB & CODI-FB (low v high risk)	**7.84**	**<0.001**	**1.824-33.72**	**5.98**
Composite steatosis and fibrosis parameter indices COMI-FS & CODI-FS (low v high risk)	**5.00**	**<0.001**	**1.681-14.88**	**5.87**

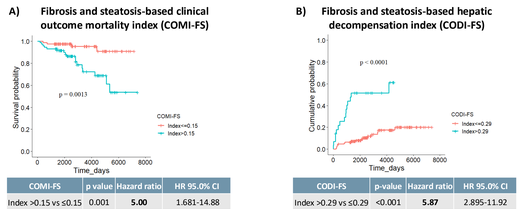
Figure 3: *Kaplan–Meier time-to-event analysis in n = 152 validation cohort with log-rank test p-value for for (A) all-cause mortality (COMI-FB) and (B) hepatic decompensation (CODI-FB) with high/low risk dichotomisation based on composite indices generated by including* ***fibrosis and ballooning*** *parameters.*



COMI-FB	p value	Hazard ratio	HR 95.0% CI
Index >0.12 vs ≤0.12	0.001	**7.84**	1.824-33.72

CODI-FB	p-value	Hazard ratio	HR 95.0% CI
Index >0.29 vs ≤0.29	<0.001	**5.98**	2.944-12.14

Figure 4: *Kaplan–Meier time-to-event analysis in n = 152 validation cohort with log-rank test p-value for for (A) all-cause mortality (COMI-FS) and (B) hepatic decompensation (CODI-FS) with high/low risk dichotomisation based on composite indices generated by including* ***fibrosis and steatosis*** *parameters.*



COMI-FS	p value	Hazard ratio	HR 95.0% CI
Index >0.15 vs ≤0.15	0.001	**5.00**	1.681-14.88

CODI-FS	p-value	Hazard ratio	HR 95.0% CI
Index >0.29 vs ≤0.29	<0.001	**5.87**	2.895-11.92

- The **composite indices for all-cause mortality (COMI-FB) and hepatic decompensation (CODI-FB) generated by including ballooning parameters had greater predictive power** than the COMI-F and CODI-F, respectively, as well as qFibrosis-derived stage and NASH-CRN fibrosis score
- The composite indices for all-cause mortality (COMI-FS) and hepatic decompensation (CODI-FS) generated by including steatosis parameters also had greater predictive power than the COMI-F, as well as qFibrosis-derived stage and NASH-CRN fibrosis score.

CONCLUSIONS

- Advances in AI based imaging analysis have introduced quantitative measures for fibrosis, steatosis, and ballooning, using methodologies now widely validated in MASH clinical trials.
- We show that microarchitectural features of injury and fibrosis quantified by stain-free SHG/TPE imaging can generate indices with greater predictive value for all-cause mortality and liver-related events than indices leveraging fibrosis parameters alone or ordinal fibrosis scores.
- Combining fibrosis and ballooning parameters enhances the hazard ratio, underscoring the critical role of ballooning in developing predictive models for MASH clinical outcomes.
- Additional validation of these findings is required through prospective studies to confirm their applicability and reliability.

REFERENCES

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2. Kendall TJ, Jimenez-Ramos M, Turner F et al. Nat Med 2023;29:2939-2953
3. Kendall TJ, et. al., Liver Int. 2024 Oct;44(10):2511-2516

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