Poster WED-342

Artificial intelligence-driven qFibrosis® in alpha-1-antitrypsin deficiency-associated liver disease: correlation with METAVIR stage and other disease specific features

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At the time of the study

Background

- Alpha-1 antitrypsin deficiency-associated liver disease (AATD-LD) is caused by the accumulation of misfolded alpha-1 antitrypsin (Z-AAT) in hepatocytes.[1]
- There are no approved pharmacological therapies available for AATD-LD; fazirsiran is an investigational small interfering RNA therapy undergoing phase 3 development in patients with AATD-LD.[2,3,4]
- Currently, liver biopsy is the reference standard for the assessment of AATD-LD.[5]
- Severity of liver fibrosis is determined by pathologist-reported Meta-analysis of Histological Data in Viral Hepatitis (METAVIR) fibrosis stage, however, histological evaluation provides categorical scores which may be influenced by inter- and intra-reader variability.[6-8]
- Change in liver fibrosis is an important endpoint for the evaluation of efficacy of investigational therapies;[7] reproducible and automated scoring methods that objectively evaluate histological changes in clinical trials are needed.
- Digital quantification by collagen proportionate area (CPA), an area-based method, offers a reproducible and continuous score, capable of capturing dynamic changes in fibrosis[9]; however, there is an unmet need for more advanced artificial intelligence (AI)-driven methods of liver fibrosis quantification in patients with AATD-LD.
- Second harmonic generation/two-photon excitation fluorescence (SHG/TPEF) microscopy and an AI-driven quantification method (qFibrosis®; HistoIndex Pte Ltd, Singapore) incorporate collagen morphology assessments and measurements of zonal distribution of fibrosis to provide a more sensitive approach for the detection of collagen and quantification of liver fibrosis, in addition to pathologist-reported histological evaluation.[8,10]
  - qFibrosis utilizes more than 150 collagen morphological features to facilitate fibrosis scoring.[10]

Aims

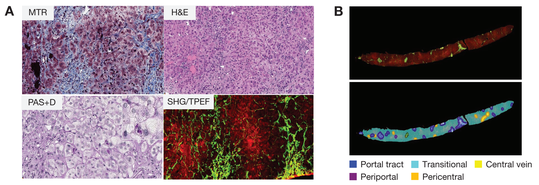
- To evaluate the utility of AI-driven qFibrosis analyses in AATD-LD by comparing qFibrosis (METAVIR) stage and continuous value with pathologist-reported METAVIR fibrosis stage, non-invasive tests (NITs) of fibrosis and Z-AAT polymer burden using data from phase 2 clinical trials of fazirsiran.

Methods

- Patients with AATD-LD who had both baseline and post-treatment liver histological samples and who received fazirsiran/placebo in the AROAAT-2001 (NCT03945292) and AROAAT-2002 (NCT03946449) trials were included.
  - Liver biopsy samples were collected at baseline and at Week 48/72/96 in AROAAT-2001 and at baseline and Week 24/48 in AROAAT-2002.
- Unstained formalin-fixed paraffin-embedded liver biopsy samples were utilized to prepare 4 µm thick sections for HistoIndex Pte Ltd's proprietary SHG/TPEF imaging and qFibrosis analysis platform.
- The performance of SHG/TPEF imaging for the detection of fibrosis and outcomes associated with qFibrosis analysis were evaluated by hepatopathologists during method development.
- Correlation of qFibrosis analyses (METAVIR stage, continuous value, zonal distribution) with pathologist-reported METAVIR fibrosis stage (evaluated by a central pathology reading conducted by two pathologists and one adjudicator), intrahepatic Z-AAT polymer burden (assessed by periodic acid Schiff staining with diastase [PAS+D] and liquid chromatography–mass spectrometry [LC–MS]) and other NITs were evaluated by Spearman correlation analysis.
  - Cross-sectional analysis (baseline and post-treatment) was used to determine correlations.
- Pathologist-reported METAVIR fibrosis staging, liver stiffness measurement (LSM) via FibroScan® or magnetic resonance elastography (MRE) and additional methodological details have been described previously.[3,4]

Figure 1. Overview of SHG/TPEF microscopy and digital pathology analysis method for the quantification of fibrosis in AATD-LD

SHG/TPEF imaging and analysis sensitively detect collagen and enable the quantification of the zonal distribution of fibrosis; presence of PAS+D-positive polymer does not interfere with collagen detection.



(A) Consecutive sections of a liver biopsy sample from a patient with AATD-LD stained (Masson's trichrome stain [MTR]/ haematoxylin and eosin [H&E] stain/PAS+D) and scanned using a Leica AT2 scanner or SHG/TPEF imaging using Genesis 200. (B) Representative SHG images and identification of different zones in a liver biopsy. An illustration of the quantification of septa features is shown in **Figure S1** (accessed via the QR code).

Results

Table 1. Baseline patient characteristics

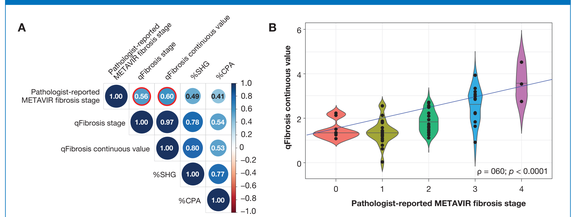
Overall, 41 patients from the AROAAT-2001 (n = 25) and AROAAT-2002 (n = 16) trials had evaluable paired baseline and post-treatment liver histological data.

	AROAAT-2001 (n = 25)	AROAAT-2002 (n = 16)	Overall (N = 41)
Age, years, median (IQR)	57.0 (47.0, 64.0)	56.0 (49.0, 62.2)	56.0 (47.0, 63.0)
BMI, kg/m², median (IQR)	28.9 (24.8, 36.1)	25.1 (21.8, 27.9)	26.6 (24.4, 31.2)
Sex, male, n (%)	14 (56.0)	14 (87.5)	28 (68.3)
Pathologist-reported METAVIR fibrosis stage, n (%)			
0	3 (12.0)	1 (6.3)	4 (9.8)
1	9 (36.0)	2 (12.5)	11 (26.8)
2	11 (44.0)	6 (37.5)	17 (41.5)
3	2 (8.0)	4 (25.0)	6 (14.6)
4	0	2 (12.5)	2 (4.9)
Missing	0	1 (6.3)[a]	1 (2.4)
LSM via FibroScan, kPa, median (IQR)	7.6 (4.4, 11.0)	9.2 (7.1, 11.8)	7.9 (5.6, 11.4)
PAS+D polymer burden, median (IQR)	7.0 (5.0, 8.0)	8.0 (6.8, 8.2)	8.0 (5.0, 8.0)

[a]The baseline pathologist-reported METAVIR fibrosis stage was recorded as 'not evaluable' but a post-treatment METAVIR fibrosis stage was reported.

Figure 2. Correlation of digital pathology-based qFibrosis (METAVIR) stage and continuous value with pathologist-reported METAVIR fibrosis stage

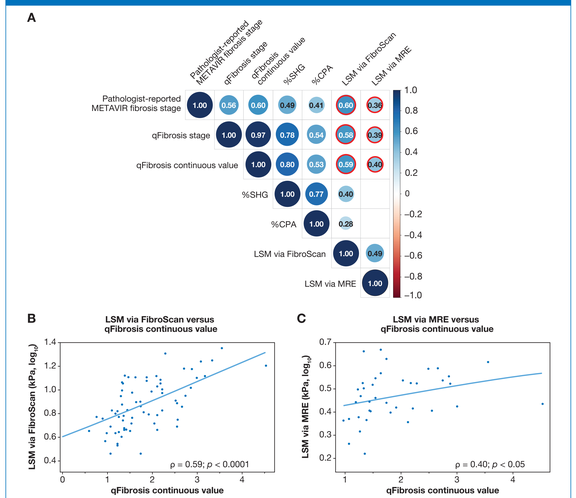
qFibrosis stage and continuous value correlated positively with pathologist-reported METAVIR fibrosis stage (78 pre-/post-treatment visit data points; ρ = 0.56 and ρ = 0.60, respectively; both $p < 0.0001$); correlations were stronger than with area-based methods (%CPA and %SHG).



(A) A correlation heatmap demonstrating the relationship between qFibrosis stage and continuous value and methods of assessment of liver fibrosis. Spearman correlation coefficients range from –1.0 (strong negative correlation) to 1.0 (strong positive correlation). Only correlation coefficients with $p < 0.05$ are shown. (B) Correlation of qFibrosis continuous value with pathologist-reported METAVIR fibrosis stages (F0–F4).

Figure 3. Correlation of digital pathology-based qFibrosis (METAVIR) stage and continuous value with LSM via imaging

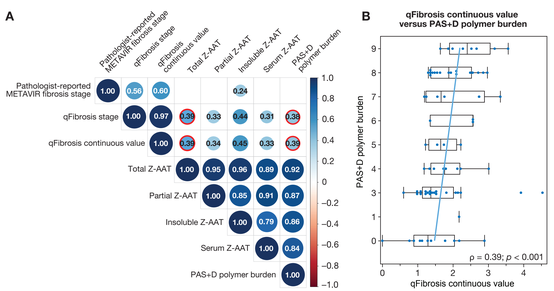
qFibrosis stage and continuous value correlated positively with LSM via FibroScan (70 pre-/post-treatment visit data points; ρ = 0.58 and ρ = 0.59, respectively; both $p < 0.0001$) and via MRE (39 data points; ρ = 0.39 and ρ = 0.40, respectively; both $p < 0.05$); area-based methods (%CPA and %SHG) correlated weakly with LSM via FibroScan.



(A) A correlation heatmap demonstrating the relationship between qFibrosis stage and continuous value and imaging methods of liver fibrosis quantification. Spearman correlation coefficients range from –1.0 (strong negative correlation) to 1.0 (strong positive correlation). Only correlation coefficients with $p < 0.05$ are shown. (B) Correlation of LSM via FibroScan (70 data points) with qFibrosis continuous values (F0–F4). (C) Correlation of LSM via MRE (39 data points) with qFibrosis continuous values (F1–F4).

Figure 4. Correlation of digital pathology-based qFibrosis (METAVIR) stage and continuous value with intrahepatic Z-AAT polymer burden assessed by PAS+D and LC–MS

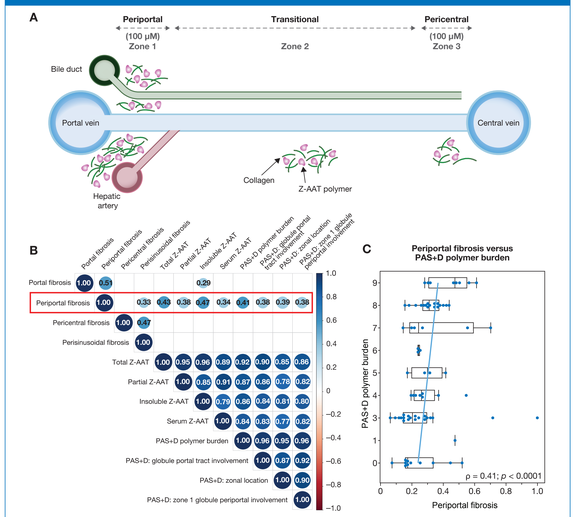
qFibrosis stage and continuous value correlated positively with intrahepatic Z-AAT polymer burden assessed by PAS+D (78 pre-/post-treatment visit data points; ρ = 0.38 and ρ = 0.39, respectively; both $p < 0.001$) and by LC–MS (73 data points; both ρ = 0.39; $p < 0.001$).



(A) A correlation heatmap demonstrating the relationship between qFibrosis stage and continuous value and Z-AAT polymer burden. Spearman correlation coefficients range from –1.0 (strong negative correlation) to 1.0 (strong positive correlation). Only correlation coefficients with $p < 0.05$ are shown. (B) Correlation of PAS+D polymer burden with qFibrosis continuous values (F0–F4).

Figure 5. Digital pathology insights into the spatial correlation between intrahepatic Z-AAT polymer burden and zonal distribution of fibrosis in AATD-LD

Zonal distribution of fibrosis measured by SHG/TPEF imaging and qFibrosis analysis demonstrated that periportal fibrosis specifically correlated with Z-AAT polymer burden assessed by pathologists in the zone 1 globule periportal involvement, which suggests a spatial correlation with Z-AAT polymer burden and collagen deposition.



(A) Illustration of the zonal locations of fibrosis. (B) A correlation heatmap demonstrating the relationship between intrahepatic Z-AAT polymer burden and zonal distribution of fibrosis. Spearman correlation coefficients range from –1.0 (strong negative correlation) to 1.0 (strong positive correlation). Only correlation coefficients with $p < 0.05$ are shown. (C) Correlation between PAS+D polymer burden and periportal fibrosis.

Conclusions

- This is the first study to use SHG/TPEF imaging and qFibrosis analysis for the assessment of liver fibrosis quantification and distribution in patients with AATD-LD.
- qFibrosis stage and continuous value correlated with pathologist-reported METAVIR fibrosis stage.
- qFibrosis stage and continuous value correlated with LSM via FibroScan and LSM via MRE, suggesting its utility as a measure of fibrosis in AATD-LD biopsy and to complement pathologist-reported histological evaluation.
- Periportal fibrosis specifically correlated with Z-AAT polymer burden assessed by different modalities, suggesting a spatial correlation with Z-AAT polymer burden and collagen deposition.
  - These findings suggest that qFibrosis, an AI-driven fully quantitative measure of fibrosis with insights into zonal distribution and potential spatial correlations, has value in the assessment of AATD-LD.
- Although there was a limited sample size in this study (N = 41), the cross-sectional design enabled assessment of multiple data points.
- Novel AI-driven digital pathology approaches may support therapeutic development in AATD-LD but require validation in larger cohorts.

Abbreviations

AATD, alpha-1 antitrypsin deficiency; AATD-LD, alpha-1 antitrypsin deficiency-associated liver disease; AI, artificial intelligence; BMI, body mass index; CPA, collagen proportionate area; H&E, haematoxylin and eosin; IQR, interquartile range; LC–MS, liquid chromatography–mass spectrometry; LSM, liver stiffness measurement; METAVIR, Meta-analysis of Histological Data in Viral Hepatitis; MRE, magnetic resonance elastography; MTR, Masson's trichrome stain; PAS+D, periodic acid-Schiff staining with diastase; Pi, protease inhibitor; SHG, second harmonic generation; SHG/TPEF, second harmonic generation/two-photon excitation fluorescence; Z-AAT, misfolded alpha-1 antitrypsin.

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Disclosures

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