AASLD The Liver Meeting®

CONCORDANCE OF HISTOLOGICAL AND LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY-BASED INTRAHEPATIC Z-ALPHA-1 ANTITRYPSIN (Z-AAT) BURDEN ASSESSMENTS IN PATIENTS WITH ALPHA-1 ANTITRYPSIN DEFICIENCY-ASSOCIATED LIVER DISEASE

Poster 4453

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INTRODUCTION

- Alpha-1 antitrypsin deficiency (AATD) is an autosomal codominant genetic condition characterized by low levels of serum alpha-1 antitrypsin (AAT), a liver-derived serine protease inhibitor (Pi) synthesized mainly in hepatocytes that maintains the protease–antiprotease balance in the lung.[1,2]
- The Pi*ZZ genotype produces misfolded AAT (Z-AAT), resulting in proteotoxic hepatic Z-AAT aggregates that cause AATD-associated liver disease (AATD-LD), and reduced antiprotease activity in the lungs.[1]
- Currently, no approved pharmacological therapies are available for patients with AATD-LD.[3]
  - Liver transplantation is the only treatment option in patients with AATD and advanced liver cirrhosis or failure.[3]
- Fazirsiran is an investigational small interfering RNA therapy undergoing phase 3 development in patients with AATD-LD.
- Periodic acid–Schiff staining with diastase (PAS-D) and liquid chromatography–mass spectrometry (LC–MS) are used to assess intrahepatic Z-AAT burden and response to potential therapies, such as fazirsiran.
  - However, their concordance has not been examined in cross-sectional and longitudinal settings.

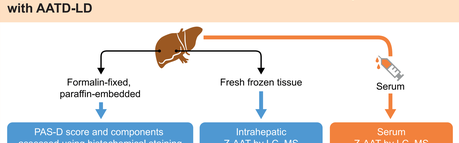
AIM

- To leverage data from two clinical trials of fazirsiran to assess the correlations of PAS-D composite score, intrahepatic Z-AAT and serum Z-AAT.

METHOD

- Baseline and post-baseline serum and liver biopsy samples from patients with baseline fibrosis enrolled in the phase 2 AROAAT-2001 (NCT03945292) and AROAAT-2002 (NCT03946449) trials were used.
  - In AROAAT-2001, patients were randomized to receive fazirsiran or placebo and had a liver biopsy taken at baseline; only those with fibrosis at baseline had a second liver biopsy taken at Weeks 48, 72 or 96.
  - In AROAAT-2002, patients received fazirsiran and had liver biopsies taken at baseline, Week 24 and Week 48.
- Formalin-fixed, paraffin-embedded liver biopsy samples were utilized for PAS-D staining at a central laboratory.
- PAS-D score was read centrally and adjudicated by three histopathologists using a semi-quantitative scale (0–9) that combines scores from three measurements, each scored 0–3: degree of portal tract involvement, zone 1 globule periportal involvement and zonal location.
  - A higher score indicates a higher globule burden.
- Serum Z-AAT and intrahepatic Z-AAT (total, soluble and insoluble) were analyzed by LC–MS (**Figure 1**).
  - Fresh frozen liver tissues were homogenized with lysis buffer and centrifuged.
  - Supernatant or complete homogenate, and serum samples were denatured, reduced and mixed with internal peptide standard before LC–MS analysis.
- Cross-sectional and longitudinal correlations of histology-based PAS-D composite score, and intrahepatic and serum Z-AAT were evaluated by Spearman correlation analysis.
- Further methodological details were described by Clark *et al.* in 2024 and Strnad *et al.* in 2022.[4,5]

Figure 1. Methods for the evaluation of Z-AAT burden in patients with AATD-LD



RESULTS

Table 1. Baseline demographics and characteristics

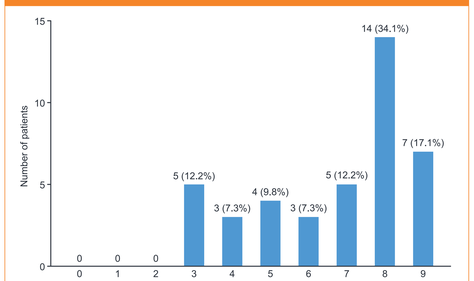
Of 41 patients from the AROAAT-2001 (*n* = 25) and AROAAT-2002 (*n* = 16) trials, median (Q1, Q3) age was 56.0 (47.0, 63.0) years and 28 (68.3%) were male

Characteristic	AROAAT-2001 (*n* = 25)	AROAAT-2002 (*n* = 16)	Overall (*N* = 41)
Age, years, median (Q1, Q3)	57.0 (47.0, 64.0)	56.0 (49.0, 62.2)	56.0 (47.0, 63.0)
Male, *n* (%)	14 (56.0)	14 (87.5)	28 (68.3)
BMI, kg/m², median (Q1, Q3)	28.9 (24.8, 36.1)	25.1 (21.8, 27.9)	26.6 (24.4, 31.2)
Liver test, median (Q1, Q3)			
ALT (U/L)	35.5 (22.8, 46.0)	60.5 (49.5, 74.2)	45.5 (27.0, 60.2)
AST (U/L)	31.5 (24.8, 39.2)	41.5 (32.0, 54.5)	33.5 (28.5, 46.8)
GGT (U/L)	32.0 (22.0, 45.2)	68.5 (34.8, 86.5)	38.0 (24.5, 69.0)
Z-AAT, median (Q1, Q3)			
Total intrahepatic Z-AAT (nmol/g)	32.7 (24.9, 68.2)	56.8 (33.4, 83.8)	39.4 (26.4, 83.8)
Soluble intrahepatic Z-AAT (nmol/g)	20.0 (15.3, 25.3)	24.0 (19.0, 31.9)	20.8 (15.9, 28.6)
Insoluble intrahepatic Z-AAT (nmol/g)	16.2 (6.7, 45.9)	31.9 (9.0, 53.1)	18.4 (8.2, 53.1)
Serum Z-AAT (μg/mL)	21.7 (18.6, 27.6)	26.5 (21.8, 27.8)	23.5 (19.1, 27.7)
PAS-D composite score, median (Q1, Q3)	7.0 (5.0, 8.0)	8.0 (6.8, 8.2)	8.0 (5.0, 8.0)

- Baseline values for liver tests and Z-AAT (intrahepatic and serum) were higher in patients from the AROAAT-2002 trial than from the AROAAT-2001 trial.

Figure 2. Distribution of PAS-D composite scores at baseline

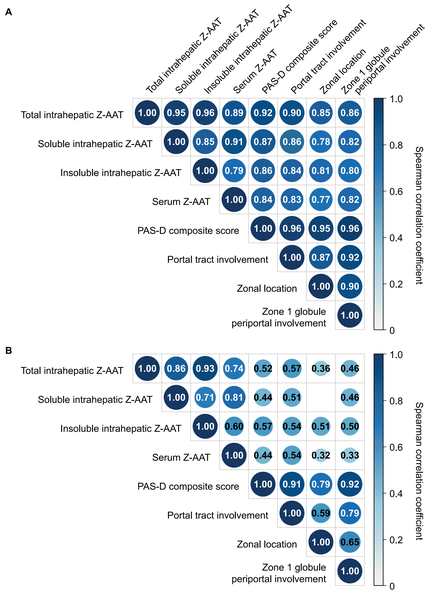
The most common PAS-D composite score at baseline was 8 (*n* = 14 [34.1%])



PAS-D score comprises three components: portal tract involvement, zone 1 globule periportal involvement and zonal location.

Figure 3. Correlations between PAS-D composite score, PAS-D components, intrahepatic Z-AAT and serum Z-AAT when analyzed (A) cross-sectionally and (B) longitudinally

PAS-D composite score and intrahepatic Z-AAT significantly correlated with each other when analyzed cross-sectionally ($p < 0.0001$) and longitudinally ($p < 0.01$). The strongest serum Z-AAT correlation was with soluble intrahepatic Z-AAT



Spearman correlation coefficients (r) range from −1 (strong negative correlation) to +1 (strong positive correlation). In panels A and B, circles indicate $p < 0.0001$ and $p < 0.05$, respectively. Empty cells indicate not statistically significant. The sizes of the circles align with the correlation coefficients.

- Of the five PAS-D components, portal tract involvement, zone 1 globule periportal involvement and zonal location significantly correlated with total intrahepatic Z-AAT ($r = 0.90$, 0.86 and 0.85, respectively; $p < 0.0001$) when analyzed cross-sectionally (**Figure 3A**).
- When analyzed cross-sectionally and longitudinally, serum Z-AAT significantly correlated with PAS-D composite score and intrahepatic Z-AAT ($p < 0.05$) (**Figure 3A and 3B**).

Figure 4. Representative correlations between PAS-D and Z-AAT (serum and intrahepatic) when analyzed (A) cross-sectionally and (B) longitudinally

Intrahepatic Z-AAT (total, soluble and insoluble) and serum Z-AAT measured by LC–MS were significantly ($p < 0.01$) correlated with histology-based PAS-D composite score, both cross-sectionally and longitudinally

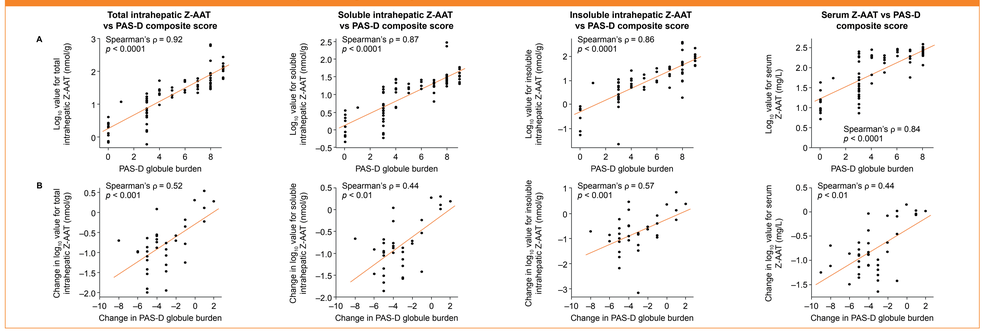
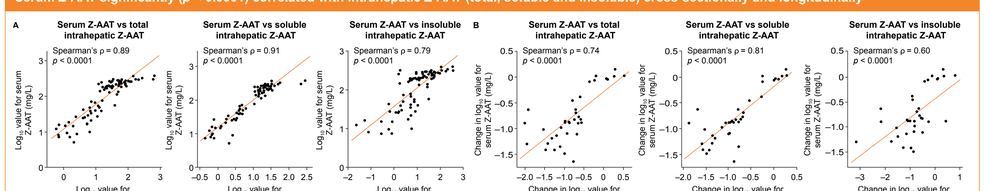


Figure 5. Representative correlations between serum and intrahepatic Z-AAT when analyzed (A) cross-sectionally and (B) longitudinally

Serum Z-AAT significantly ($p < 0.0001$) correlated with intrahepatic Z-AAT (total, soluble and insoluble) cross-sectionally and longitudinally



CONCLUSIONS

- Histology (PAS-D) and LC–MS, two valuable approaches for the measurement of intrahepatic Z-AAT in patients with AATD-LD, demonstrated good concordance cross-sectionally and longitudinally, despite differences in pre-analytical procedures, analytical characteristics and inherent biases.
- These data support the use of portal tract involvement, zone 1 globule periportal involvement and zonal location as the three components of the PAS-D composite score.
- These biopsy-based Z-AAT biomarkers are valuable tools to support clinical development of emerging therapies for AATD-LD.
- Serum Z-AAT significantly correlated with PAS-D composite score and LC–MS-based intrahepatic Z-AAT.
  - Serum Z-AAT demonstrated considerable promise as a non-invasive test to reflect intrahepatic Z-AAT burden, but requires further investigation.
- These study findings are based on a small sample size ($N = 41$) and require further validation.
- Immunohistochemistry-based assays will also be evaluated and correlated with PAS-D and LC–MS-based Z-AAT measurements in future studies.

ABBREVIATIONS

AAT, alpha-1 antitrypsin; AATD, alpha-1 antitrypsin deficiency; AATD-LD, alpha-1 antitrypsin deficiency-associated liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; LC–MS, liquid chromatography–mass spectrometry; PAS-D, periodic acid–Schiff staining with diastase; Pi, protease inhibitor; Q, quartile; Z-AAT, misfolded alpha-1 antitrypsin.

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DISCLOSURES

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