Corrigendum to 'Level of MFAP4 in ascites independently predicts 1-year transplant-free survival in patients with cirrhosis' [JHEP Reports 3 (2021) 100287]

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Background & Aims: Prognostic models of cirrhosis underestimate disease severity for patients with cirrhosis and ascites. Microfibrillar-associated protein 4 (MFAP4) is an extracellular matrix protein linked to hepatic neoangiogenesis and fibrogenesis. We investigated ascites MFAP4 as a predictor of transplant-free survival in patients with cirrhosis and ascites.
Methods: A dual-centre observational study of patients with cirrhosis and ascites recruited consecutively in relation to a paracentesis was carried out. Patients were followed up for 1 year, until death or liver transplantation (LTx). Ascites MFAP4 was tested with the model for end-stage liver disease (MELD-Na), CLIF Consortium Acute Decompensation (CLIF-C AD), and Child-Pugh score in Cox regression models.
Results: Ninety-three patients requiring paracentesis were included. Median ascites MFAP4 was 29.7 U/ml [22.3–41.3], and MELD-Na was 19 [16–23]. A low MELD-Na score (<20) was observed in 49 patients (53%). During follow-up, 20 patients died (22%), and 6 received LTx (6%). High ascites MFAP4 (>29.7 U/ml) was associated with 1-year transplant-free survival ($p = 0.002$). In Cox regression, ascites MFAP4 and MELD-Na independently predicted 1-year transplant-free survival (hazard ratio [HR] = 0.97, $p = 0.03$, and HR = 1.08, $p = 0.01$, respectively). Ascites MFAP4 and CLIF-C AD also predicted survival independently (HR = 0.96, $p = 0.02$, and HR = 1.05, $p = 0.03$, respectively), whereas only ascites MFAP4 did, controlling for the Child-Pugh score (HR = 0.97, $p = 0.03$, and HR = 1.18, $p = 0.16$, respectively). For patients with MELD-Na <20, ascites MFAP4 but not ascites protein predicted 1-year transplant-free survival (HR 0.91, $p = 0.02$, and HR = 0.94, $p = 0.17$, respectively).
Conclusions: Ascites MFAP4 predicts 1-year transplant-free survival in patients with cirrhosis and ascites. In patients with low MELD-Na scores, ascites MFAP4, but not total ascites protein, significantly predicted 1-year transplant-free survival.
Lay summary: Patients with cirrhosis who have fluid in the abdomen, ascites, are at an increased risk of death and in need for liver transplantation. Our study identified patients with ascites and a poor prognosis by measuring microfibrillar associated protein 4 (MFAP4), a protein present in the abdominal fluid. Patients with low levels of the MFAP4 protein are at particularly increased risk of death or liver transplantation, suggesting that clinical care should be intensified in this group of patients.

Introduction

Ascites is the most frequent type of decompensation in cirrhosis.[1] The transition from compensated to decompensated cirrhosis with ascites marks a substantial deterioration in disease severity with a reduction in 5-year survival from 80% to around 30%.[2] Prognostic models in decompensated cirrhosis are widely used and well validated. They include the model for end-stage liver disease (MELD-Na), the CLIF Consortium Acute Decompensation (CLIF-C AD) score, and the Child-Pugh.[3–5] However, the ability of the models to differentiate between patients with a poor or good prognosis is limited in patients with ascites. For the Child-Pugh, this is because of the important limitation in the combination of objective and subjective criteria, rendering it susceptible to inter-observer variance.[6] For MELD-Na and CLIF-C AD, both are solely based on biochemical parameters and age but make use of serum creatinine as a surrogate of glomerular filtration rate (GFR), which is known to overestimate GFR in patients with ascites.[7,8] Furthermore, patients with low MELD-Na scores continue to be at a high risk of liver-related deaths,[9] in particular those with moderate ascites.[10] Therefore, improved models to assess the prognosis of patients with

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ascites are needed. Aspects of chronic liver disease that could hold additional prognostic information include hepatic neo-angiogenesis and fibrosis formation. Biomarkers reflecting these intrahepatic events may enhance current models, improve patient care, and optimise allocation of healthcare resources.

Microfibrillar-associated protein 4 (MFAP4) is an extracellular matrix (ECM) protein belonging to the fibrinogen-related protein superfamily distributed throughout the human body.[11] It is believed to be involved in integrin signalling where it links tissue repair and remodelling with inflammation and angiogenesis.[12] Human studies confirm this link. Indeed, serum MFAP4 correlates closely with alcohol-related liver fibrosis stage and can be used as an accurate diagnostic marker of fibrosis, as shown by our group in a large biopsy-controlled study.[13] Similarly, a proteome study of hepatic tissue showed that MFAP4 expression correlated with increasing hepatic fibrosis in patients infected with chronic viral hepatitis C,[14] a finding later confirmed in 2 clinical studies.[15,16] A recent single-cell RNA-sequencing study showed that MFAP4 secreted by hepatic stellate cells was an integral part of the transcriptome in murine models of liver fibrosis and confirmed the MFAP4 gene as 1 of 4 hepatic expressed genes that are robust predictors of advanced fibrosis.[17] Observational follow-up studies of early-stage liver disease have shown that fibrosis is the most important prognostic factor in liver disease.[18,19] Biomarkers of liver fibrosis may therefore also provide prognostic information.[20] We have previously shown that serum MFAP4 is a good predictor of advanced fibrosis but unfit to predict time to decompensation in 45 patients with compensated cirrhosis.[13] Early literature showed that total ascites protein could predict the development of spontaneous bacterial peritonitis (SBP)[21–23] and indirectly the poor prognosis associated with SBP development.[24] However, later studies have not been able to replicate the association between total ascites protein, SBP development, and mortality.[25–27] Thus, it has been speculated if specific subtypes of ascites proteins are more accurate prognostic markers.[28]

For patients with decompensated cirrhosis as a result of ascites, paracentesis is the primary treatment option to relieve abdominal distension and to identify underlying infections. As the ascitic fluid is sampled and analysed in all patients where a therapeutic or diagnostic paracentesis is performed, an additional analysis of the fluid can be performed without much added effort at the bedside. Analyses into specific constituents of the ascitic fluid such as ascitic MFAP4 could therefore yield new insights into the prognosis of patients and help guide future care for clinicians.

We therefore hypothesised that the level of ascites MFAP4 is associated with transplant-free survival in patients with decompensated cirrhosis and ascites.

Our primary aim was to evaluate ascites MFAP4 ability to prognosticate transplant-free survival in patients with cirrhosis and ascites alone and in combination with established prognostic models. Our secondary aims were to explore the correlation between ascites MFAP4, prognostic models, and ascites and serum proteins.

Patients and methods

Study design
We conducted retrospective analyses based on data collected from 2 prospective studies in tertiary liver centres. The aim of the analysis was to investigate the prognostic role of ascites MFAP4 in patients with cirrhosis and ascites in need of paracentesis. The study centres were located at Copenhagen University Hospital, Hvidovre, Denmark, and Bonn University Medical Center in Germany. The recruitment period lasted from 2010 up until 2013. Study procedures adhered to the Declaration of Helsinki, and ethical approval was granted for the cohort at both Hvidovre Hospital in Denmark (H-C-2009-020 and H-2-2010-126) and Department of Internal Medicine I, University of Bonn in Germany (203/13) (Supplementary CTAT Table). Written informed consent was obtained before participation in the study for all participants. Study methods are previously described elsewhere.[29–31]

Study population
Patients were recruited at the inpatient and outpatient hospital clinics of both study centres. The inclusion criteria were liver cirrhosis with ascites in need of paracentesis, age above 18 years, and signed informed consent. Cirrhosis was verified by typical clinical findings (*e.g.* complications to portal hypertension such as oesophageal varices or splenomegaly), ultrasonic evidence of cirrhosis, or liver biopsy. Ascites was diagnosed either clinically or by ultrasonic evidence of fluid in the peritoneal cavity. The diagnostic and therapeutic paracenteses were performed independently of the study. Clinical indications for paracentesis were suspected SBP and tense ascites. Exclusion criteria were treatment with antibiotics 1 week before inclusion and ascites as a result of malignant disease after analysis of the ascitic fluid sample.

Investigations
Age, sex, and aetiology of cirrhosis were recorded in relation to sampling of ascites. Clinical grading of disease severity was done based on the clinical presentation of the patient together with biochemical parameters at time of ascites sampling. Biochemistry and clinical data were used to calculate the MELD-Na, Child-Pugh, and CLIF-C AD scores for all patients.[3–5]

Ascites fluid was sampled in relation to either a diagnostic or therapeutic paracentesis. Standard measurements of total ascites protein, neutrophil count, and total white blood cell count were performed according to local procedures. Ascites fluid for MFAP4 measurements was centrifuged for 10 min, and supernatants were stored at −20°C until analysis. Blood samples for MFAP4 measurements were collected in EDTA vacuum tubes. Serum was obtained after centrifugation at 3500g for 10 min, and aliquots were also kept at −20°C. Analyses of MFAP4 were performed in all ascites samples (n = 93) and in a subset of serum samples (n = 41) following the scope of the ethical approval.

Outcomes and follow-up period
The primary outcome of transplant-free survival was evaluated through medical records. Patients were followed up for up to 365 days from the day of inclusion and sampling of ascites. The cause of death was denoted from death certificates and labelled if the cause of death was identified. Follow-up for patients receiving liver transplantation (LTx) ended at the date of transplantation. Complications to cirrhosis were not systematically recorded.

Measurements of MFAP4
MFAP4 in both ascites and serum was measured in Spring 2016. Preanalytical handling of MFAP4 has shown that the protein is stable at −20°C.[32] Sample preparation and measurements of MFAP4 have been described in detail elsewhere.[11,32] In brief, levels of MFAP4 were quantified using an AlphaLISA technique.

We conducted duplicate measurements and accepted the sample only if covariance was <10%. Two different monoclonal anti-MFAP4 IgGs (HG-HYB7-14 and HG-HYB 7-18; Supplementary CTAT Table) were used to perform sandwich ELISA reactions in both serum and ascites samples. All measurements of MFAP4 levels were performed at the Institute of Molecular Medicine of the University of Southern Denmark.

Statistical analyses

We report numerical data by means and standard deviations or medians and 25th and 75th percentiles depending on distributions. The Wilcoxon rank-sum test was applied for unpaired non-parametric continuous data. A Kaplan–Meier curve was used to visualise transplant-free survival rates during 1-year follow-up using the median value of ascites MFAP4 to split the study cohort equally into low (≤29.7 U/ml) and high ascites MFAP4 (>29.7 U/ml). The log-rank test was performed to test statistical significance between the survival distributions for the low and high ascites MFAP4 groups. Associations between variables was graphically represented by scatterplots, and Spearman's rank correlation coefficient was used to determine significance of correlations. Patients' estimated GFR (eGFR) were computed based on the formula proposed by the Chronic Kidney Disease Epidemiology Collaboration.[33] We applied univariable Cox regression analysis to test the individual prognostic value of ascites MFAP4, MELD-Na, CLIF-C AD, Child-Pugh, serum albumin, and total ascites protein at 3- and 12-month follow-up. The proportional hazard assumption was tested, and martingale residual plots were used to determine the appropriate distribution of the data. Three separate multivariable regression models were built to test if ascites MFAP4 with MELD-Na, Child-Pugh, and CLIF-C AD independently predicted transplant-free survival at the 3- and 12-month follow-up. Incorporating MELD-Na, CLIF-C AD, and Child-Pugh into the same multivariable analyses with ascites MFAP4 was not possible because of multicollinearity between the models and the limited sample size. For subgroup analyses, patients were stratified by MELD-Na scores with a cut-off equal to or above 20. At this threshold the MELD-Na improves the mortality prognostication of patients with hyponatraemia and ascites compared the original MELD score.[3] All calculations were performed in STATA 16.1 (College Station, TX, USA) (Supplementary CTAT Table). Values of p <0.05 were considered significant, and 2-tailed analyses were performed.

Results

Patients

Ninety-three patients with decompensated cirrhosis and ascites in need of paracentesis were included in the study. Baseline characteristics are presented in Table 1. The mean age was 60 years, and 71 (76%) were male. Alcohol-related cirrhosis was the main aetiology (83%), whereas cirrhosis was caused by viral hepatitis in 4 (4%), non-alcoholic steatohepatitis in 1 (1%), and other causes including primary sclerosing cholangitis and autoimmune hepatitis in 11 (12%) patients. Fifty-one (55%) patients were Child-Pugh B, and 42 (45%) were Child-Pugh C; the median Child-Pugh score was 9 [8–11]. The median MELD-Na score was 19 [16–23], and 49 (53%) had a MELD-Na score of <20. For CLIF-C AD, the median score was 55 [50–61], and the median ascites MFAP4 was 29.7 U/ml [22.3–43.1]. In 41 patients with sampling of both serum and ascites MFAP4, the median serum MFAP4 was 73.6 U/ml [56.0–92.5] and ascites MFAP4 37.2 U/ml [28.1–52.0].

Table 1. Patient characteristics.

	Overall (n = 93)
Sex (n, men)	71 (76%)
Age (years)	60.3 ± 9.3
Aetiology	
Alcohol	77 (83%)
Viral	4 (4%)
NASH	1 (1%)
Other	11 (12%)
Clinical scores	
Child-Pugh (B/C)	51 (55%) /42 (45%)
Child-Pugh score	9 [8–11]
MELD-Na	19 [16–23]
CLIF-C AD	55 [51–60]
Biochemical data	
Serum MFAP4 (U/ml)*	73.6 [56.0–92.5]
Leucocytes (10^9/L)	7.3 [5.9–10.4]
Platelets (10^9/L)	164 [112–245]
Creatinine (μmol/L)	96.8 [64.0–158.4]
INR	1.4 [1.2–1.6]
Bilirubin (μmol/L)	33.3 [19.0–53.0]
Albumin (g/L)	26.5 [22.9–29.7]
Sodium (mmol/L)	135 [132–138]
CRP (mg/L)	18 [10–31]
Ascites data	
Ascites MFAP4 (U/ml)	29.7 [22.3–43.1]
Total ascites protein (g/L)	10 [7–19]
Ascites neutrophils (cells/mm³)	20 [8–42]
Ascites leucocytes (cells/mm³)	131 [77–236]

Counts are presented as n (%), continuous data are presented as mean ± SD or median [p25–p75].
CLIF-C AD, CLIF Consortium Acute Decompensation; CRP, C-reactive protein; INR, internationalised normal ratio; MELD-Na, model for end-stage liver disease; MFAP4, microfibrillar-associated protein 4; NASH, non-alcoholic steatohepatitis.
* Measured in 41 patients.

Twenty-eight (30%) had refractory ascites at inclusion. Eight patients with malignant ascites were excluded (pancreatobiliary cancer, n = 6; hepatocellular carcinoma, n = 1; renal cell carcinoma, n = 1).

Clinical outcomes

In 12-month analyses, the median follow-up time was 298 [30–365] days. Twenty (22%) patients died, and 6 patients (6%) received a LTx (Supplementary Materials and Methods; Tables S1 and S2). In total, 16 deaths were related to liver disease, 1 died of non-primary liver cancer, and the cause of death was unknown for 3 patients. When segregated according to hospitalisation status, 51 were inpatients and 42 outpatients. In the inpatient group, 13 deaths and 6 LTx were observed during follow-up, whereas 7 died in the outpatient group. Transjugular intrahepatic portosystemic shunts were implanted in 10 patients (11%) during follow-up.

Ascites MFAP4 independently predicts 1-year transplant-free survival

Higher ascites MFAP4 showed to be associated with higher 1-year transplant-free survival (HR = 0.96, 95% CI 0.93–0.99, p = 0.01) (Table 2). Child-Pugh score (HR = 1.29, 95% CI 1.03–1.62, p = 0.03), MELD-Na scores (HR = 1.10, 95% CI 1.04–1.17, p <0.01), and higher CLIF-C AD scores (HR = 1.06, 95% CI 1.01–1.10, p = 0.01) correlated with significantly lower 1-year transplant-free survival. Serum MFAP4, serum albumin, and total ascites protein were not associated with transplant-free survival at 1-year follow-up.

In patients with low ascites MFAP4 (<29.7 U/ml), 14 died and 5 received LTx during the 1-year follow-up. In the same follow-

Table 2. Univariable prognostic models.

	3-month follow-up		12-month follow-up	
	HR [95% CI]	*p* value	HR [95% CI]	*p* value
Ascites MFAP4 (U/ml)	0.96 [0.93–1.00]	0.05	**0.96 [0.93–0.99]**	**0.01**
Serum MFAP4 (U/ml)	1.00 [0.97–1.03]	0.77	0.99 [0.97–1.01]	0.26
Child-Pugh score	**1.34 [1.01–1.78]**	**0.04**	**1.29 [1.03–1.62]**	**0.03**
MELD-Na	**1.13 [1.06–1.22]**	**<0.01**	**1.10 [1.04–1.17]**	**<0.01**
CLIF-C AD	**1.08 [1.03–1.14]**	**<0.01**	**1.06 [1.01–1.10]**	**0.01**
Albumin (g/L)	1.00 [0.93–1.08]	0.93	1.01 [0.95–1.07]	0.80
Total ascites protein (g/L)	0.96 [0.90–1.02]	0.18	0.94 [0.89–1.00]	0.05

Values in bold denote statistical significance. Univariable Cox regression of transplant-free survival. CLIF-C AD, CLIF Consortium Acute Decompensation; HR, hazard ratio; MFAP4, microfibrillar-associated protein 4; MELD-Na, model for end-stage liver disease.

up period, 6 died, and 1 received LTx in the group of patients with high ascites MFAP4 (>29.7 U/ml). Patients with a low ascites MFAP4 level (≤29.7 U/ml) had a significantly decreased 1-year transplant-free survival (*p* = 0.002 by the log-rank test) (Fig. 1).

In multivariable Cox regression with ascites MFAP4 and MELD-Na score, both were independently associated with 1-year transplant-free survival (HR = 0.97, 95% CI 0.94–1.00, *p* = 0.03, and HR = 1.08, 95% CI 1.02–1.15, *p* = 0.01) (Table 3). Likewise, ascites MFAP4 (HR = 0.96, 95% CI 0.93–0.99, *p* = 0.02) and CLIF-C AD (HR = 1.05, 95% CI 1.00–1.09, *p* = 0.03) were independently associated with the outcome. In the final regression model, controlling for ascites MFAP4 and Child-Pugh score, only ascites MFAP4 was associated with the outcome of transplant-free survival (HR = 0.97, 95% CI 0.94–1.00, *p* = 0.03), whereas Child-Pugh score was not (HR = 1.18, 95% CI 0.94–1.49, *p* = 0.16). Separate models were computed, combining ascites MFAP4 with MELD-Na, CLIF-C AD, and Child-Pugh score where the area under the receiver operating characteristic curve was calculated. A non-significant tendency towards an improved prognostication was observed in all 3 models (Supplementary Materials and Methods; Fig. S1).
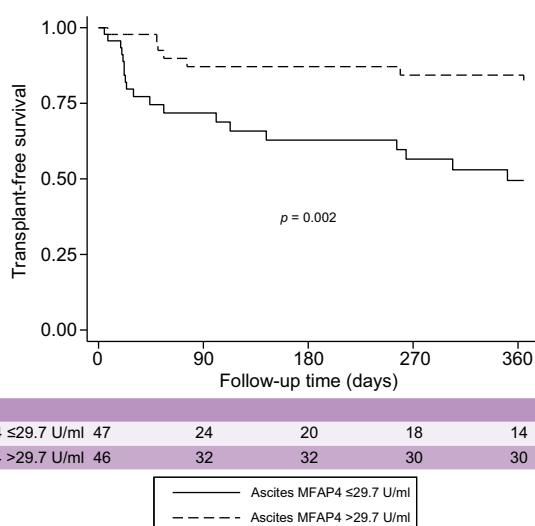
Fig. 1. One-year transplant-free survival function of ascites MFAP4. Kaplan–Meier survival function of ascites MFAP4 with death and liver transplantation as counting events. The solid line represents patients with ascites MFAP4 ≤29.7 U/ml at baseline. The dashed line depicts patients with ascites MFAP4 >29.7 U/ml at baseline. A log-rank test showed a significant association between ascites MFAP4 levels at baseline and 1-year transplant-free survival (*p* = 0.002). MFAP4, microfibrillar associated protein 4.

Ascites MFAP4 predicts 1-year transplant-free survival in patients with low MELD-Na

At 1-year follow-up, 7 deaths and 2 LTx were observed in patients with low MELD-Na scores (<20), and 13 deaths and 4 LTx in patients with high MELD-Na scores (≥20). Ascites MFAP4 predicted transplant-free survival in patients with a low MELD-Na score (HR = 0.91, 95% CI 0.85–0.98, *p* = 0.02), but not in patients with a high MELD-Na score (HR = 0.99, 95% CI 0.95–1.02, *p* = 0.46) (Table 4). Total ascites protein was not a significant predictor of transplant-free survival in patients with a low or high MELD-Na score (HR = 0.94, 95% CI 0.86–1.03, *p* = 0.17, and HR = 0.98, 95% CI 0.89–1.08, *p* = 0.72). Analyses of outcomes were also performed according to hospitalisation status at inclusion. The inpatient group had significantly lower levels of ascites MFAP4 compared with outpatient (*p* <0.01) (Supplementary Materials and Methods; Table S3). As expected, the transplant-free survival was lower in the inpatient group than in the outpatient group (*p* = 0.0001 by the log-rank test) (Supplementary Materials and Methods; Fig. S2). When controlling for hospitalisation status, a trend was observed for ascites MFAP4 as a predictor of transplant-free survival (Supplementary Materials and Methods; Table S4).

Ascites MFAP4 does not predict 3-month transplant-free survival

At 3-month follow-up, 15 (16%) had died and 2 (2%) received LTx (Table 2). In this period, 4 deaths and 1 LTx were observed in the high ascites MFAP4 group (>29.7 U/ml), whereas 11 deaths and 1 LTx were seen in the low ascites MFAP4 group (≤29.7 U/ml). In univariable Cox regression, ascites MFAP4 showed a trend but was not significantly associated with 3-month transplant-free survival (HR = 0.96, 95% CI 0.93–1.00, *p* = 0.05) (Table 2). Higher Child-Pugh, MELD-Na, and CLIF-C AD scores were all associated with lowered 3-month transplant-free survival (HR = 1.34, 95% CI 1.01–1.78, *p* = 0.04; HR = 1.13, 95% CI 1.06–1.22, *p* <0.01; and HR = 1.08, 95% CI 1.03–1.14, *p* <0.01, respectively). Serum MFAP4, serum albumin, and total ascites protein were not associated with death or transplantation at 3-month follow-up. In multivariable Cox regression analyses, ascites MFAP4 was not independently associated with 3-month transplant-free survival (Table 3). Divided by patient MELD-Na scores, neither ascites MFAP4 nor total ascites protein were predictors at 3-month follow-up (Table 4).

Low ascites MFAP4 correlates with increased liver disease severity

Low ascites MFAP4 correlated significantly with increased MELD-Na (r = −0.23, *p* = 0.03) (Fig. 2A), whereas Child-Pugh correlated inversely with ascites MFAP4 (r = −0.23, *p* = 0.03) (Fig. 2B). No

Table 3. Multivariable prognostic models.

	3-month follow-up		12-month follow-up	
	HR [95% CI]	*p* value	HR [95% CI]	*p* value
Model 1				
Ascites MFAP4 (U/ml)	0.97 [0.93–1.01]	0.14	**0.97 [0.94–1.00]**	**0.03**
MELD-Na	**1.12 [1.04–1.20]**	**<0.01**	**1.08 [1.02–1.15]**	**0.01**
Model 2				
Ascites MFAP4 (U/ml)	0.97 [0.93–1.00]	0.11	**0.96 [0.93–0.99]**	**0.02**
CLIF-C AD	**1.07 [1.02–1.13]**	**0.01**	**1.05 [1.00–1.09]**	**0.03**
Model 3				
Ascites MFAP4 (U/ml)	0.97 [0.93–1.01]	0.11	**0.97 [0.94–1.00]**	**0.03**
Child-Pugh score	1.24 [0.93–1.66]	0.14	1.18 [0.94–1.49]	0.16

Values in bold denote statistical significance. Multivariable Cox regression models of transplant-free survival. Three distinct multivariable models were computed. CLIF-C AD, CLIF Consortium Acute Decompensation; HR, hazard ratio; MELD-Na, model for end-stage liver disease; MFAP4, microfibrillar-associated protein 4.

significant correlation was observed between ascites MFAP4 and CLIF-C AD (r = −0.18, *p* = 0.08) (Fig. 2C).

Ascites MFAP4 is associated with total ascites protein but not serum MFAP4 or albumin in serum and ascites

Ascites MFAP4 and total ascites protein showed a positive correlation (r = 0.52, *p* = <0.01), whereas this was not the case for ascites MFAP4 and serum MFAP4 measurements (r = 0.25, *p* = 0.11) (Fig. 3A and B). No correlation was found between ascites MFAP4 and serum albumin levels in patients (r = 0.04, *p* = 0.73) (Fig. 3C). In a subset of patients with both ascites albumin and ascites MFAP4 measurements, the two did not correlate (r = 0.19, *p* = 0.21), in contrast to ascites albumin and total ascites protein (r = 0.50, *p* <0.01) (Supplementary Materials and Methods; Fig. S3). Ascites MFAP4 was positively associated with platelets as a surrogate of portal hypertension severity (r = 0.37, *p* <0.01) (Supplementary Materials and Methods; Fig. S4). Markers of inflammation in the ascitic fluid, namely leucocytes and neutrophils, were not associated with levels of ascites MFAP4 (r = 0.02, *p* = 0.84 and r = −0.07, *p* = 0.55) (Supplementary Materials and Methods; Fig. S5). Plasma leucocytes and C-reactive protein as markers of systemic inflammation were not associated with ascites MFAP4 (r = −0.17, *p* = 0.10, and r = 0.05, *p* = 0.62, respectively) (Supplementary Materials and Methods; Fig. S6). Ascites MFAP4 correlated weakly with eGFR, but not with plasma creatinine (Supplementary Materials and Methods; Fig. S7). Finally, serum sodium was not correlated with ascites MFAP4 levels (Supplementary Materials and Methods; Fig. S8).

Discussion

In this first study assessing MFAP4 in ascitic fluid, we showed that a high level of ascites MFAP4 predicts 1-year transplant-free survival in patients with cirrhosis and ascites, independent of MELD-Na, CLIF-C AD, and Child-Pugh score. Furthermore, we demonstrated that ascites MFAP4 has important prognostic value in patients with low MELD-Na scores. Against our expectations, we found no strong correlation between ascites and serum MFAP4.

Because the development of ascites is associated with a poor prognosis in patients with cirrhosis, sodium was incorporated into the original MELD score.[2,3] However, some patients with moderate ascites presented with a low MELD-Na score despite a high mortality risk on the LTx waiting list.[10] Continuous refinement of current models in cirrhosis is therefore needed to improve prognostication in this group of patients. In our study, decreasing levels of ascites MFAP4 showed to be a significant predictor of 1-year transplant-free survival, independent of MELD-Na, CLIF-C AD, and Child-Pugh score, in patients with cirrhosis and ascites. We believe this is particularly interesting in patients with a low MELD-Na score, when the current models tend underestimate the actual mortality risk. Especially considering that only ascites MFAP4 but not total ascites protein predicted 1-year transplant-free survival in the subset of patients with a MELD-Na score of <20. This suggest that ascites MFAP4 is a reflection of other pathophysiological processes in decompensated cirrhosis, than what is captured by the MELD-Na. Most likely, ascites MFAP4 identifies patients with severe portal hypertension and a high risk of developing portal hypertension-related complications, a risk not readily predicted by the MELD-Na score. Our study did not have complete data on the risk of developing portal hypertension-related complications during the follow-up. However, we observed an association between ascites MFAP4 and platelets as an indication of the correlation with portal hypertension severity. Psoas muscle thickness measured on computed tomography (CT) scans, in addition to the MELD score, has shown to improve prognostication of LTx waiting-list mortality for patients with cirrhosis.[34] Future studies with both CT scans of the psoas muscle and ascites MFAP4 measurements should seek to compare the combined models.

In early studies of total ascites protein, it was shown to be a predictor of SBP development,[21,23] but more recent studies were unable to replicate these findings or link total ascites protein with SBP-related mortality.[25,27] In the current study, total ascites protein was not associated with overall transplant-free survival. This finding was in contrast to that for ascites

Table 4. Ascites MFAP4 and total ascites protein by MELD-Na score.

	3-month follow-up		12-month follow-up	
	HR [95% CI]	*p* value	HR [95% CI]	*p* value
Ascites MFAP4				
MELD-Na <20	0.96 [0.89–1.04]	0.30	**0.91 [0.85–0.98]**	**0.02**
MELD-Na ≥20	0.98 [0.94–1.02]	0.29	0.99 [0.95–1.02]	0.46
Total ascites protein				
MELD-Na <20	0.97 [0.87–1.07]	0.51	0.94 [0.86–1.03]	0.17
MELD-Na ≥20	1.01 [0.92–1.11]	0.81	0.98 [0.89–1.08]	0.72

Values in bold denote statistical significance. Univariable Cox regression models of transplant-free survival. HR, hazard ratio; MELD-Na, model for end-stage liver disease; MFAP4, microfibrillar-associated protein 4
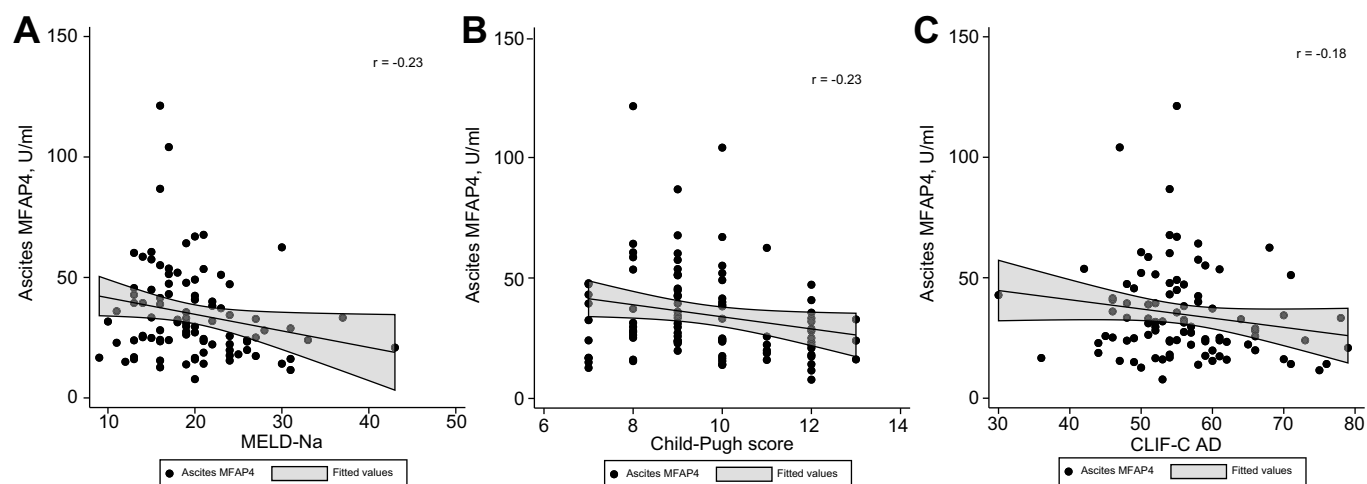
Fig. 2. Ascites MFAP4 and severity of liver disease. (A) Scatterplot of ascites MFAP4 and MELD-Na. The correlation was significant by Spearman's test (r = −0.23, p = 0.03). (B) Scatterplot of ascites MFAP4 levels according to CPS. Ascites MFAP4 and CPS were negatively associated by Spearman's test (r = −0.23, p = 0.03) (C) Scatterplot of ascites MFAP4 and CLIF-C AD score. Ascites MFAP4 did not correlate with the CLIF-C AD score by Spearman's test (r = −0.18, p = 0.08). CLIF-C AD, CLIF Consortium Acute Decompensation; CPS, Child-Pugh score; MELD-Na, model for end-stage liver disease; MFAP4, microfibrillar associated protein 4.

MFAP4, despite the positive correlation between the 2 variables. However, one should take into account the relatively low total ascites protein level observed in the study. This reflects the severe disease state, and future validation across a wider spectrum is relevant.

Our data indicate that the abundance of MFAP4 in the ascitic fluid decreases with increasing severity of cirrhosis. Based on the previous studies of MFAP4 in liver disease, this was contrary to what we expected. Experimental studies have shown that MFAP4 is secreted from hepatic stellate cells and deposited in the hepatic ECM during fibrogenesis.[17] In clinical studies, serum MFAP4 has shown to be positively correlated with liver fibrosis stage in patients with early liver fibrosis and compensated cirrhosis.[13,14] Hence, we expected an increase in serum MFAP4 in the more advanced stage of liver disease, with a reflection of this in ascites MFAP4 level. Surprisingly, our correlation analyses showed that ascites MFAP4 did not reflect serum MFAP4. If MFAP4 in the ascitic fluid originates from the liver, we speculate on whether low levels of MFAP4 were

associated with the more advanced stage of disease, as decompensated cirrhosis is characterised by fewer hepatic stellate cells to drive ECM turnover and irreversible cross-linkage of the fibrotic septae.[35] A decreased MFAP4 synthesis as a result of the fewer stellate cells and a slower release because of irreversible cross-linkage could explain why low MFAP4 was associated with increased disease severity and hereby a poor prognosis. As an alternative explanation, ascites MFAP4 could be a result of local synthesis and degradation in the peritoneal cavity, as a consequence of a pro-inflammation milieu. In the analyses of ascites fluid samples with MFAP4, leucocytes, and neutrophils, we found no correlation between MFAP4 from the ascitic fluid and the local inflammatory cells. Therefore, we believe it is unlikely that ascites MFAP4 is a result of a local inflammatory reaction.

Previous studies identified fibrinolytic activity in the ascites and demonstrated mixed fibrinolytic plasma phenotypes as prognostic markers in decompensated cirrhosis.[36,37] As MFAP4 is part of the
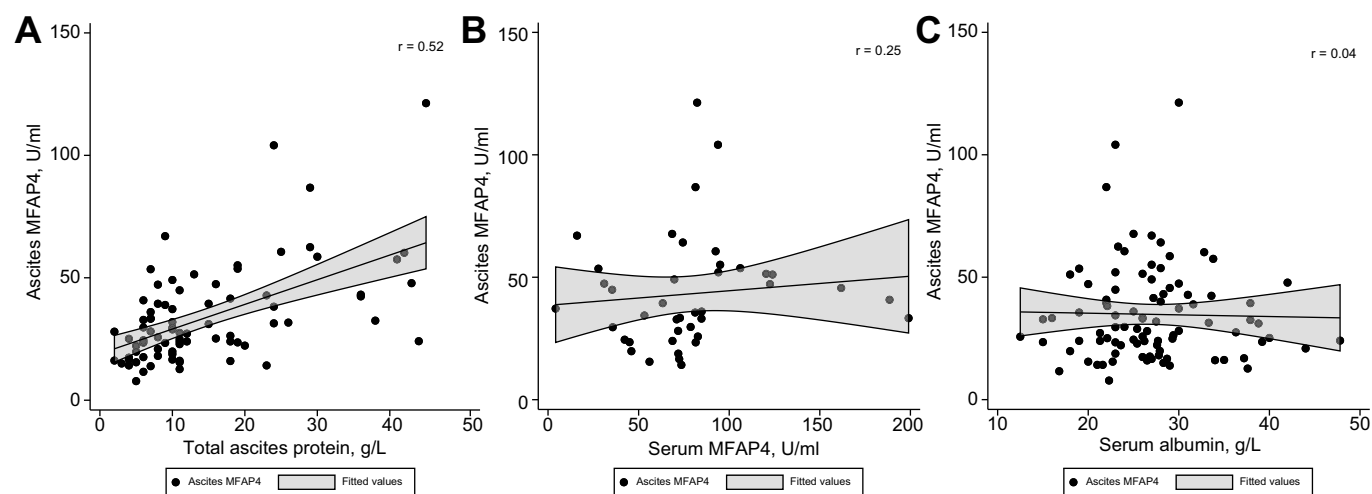
Fig. 3. Ascites MFAP4 correlation with serum MFAP4, total ascites protein, and albumin. (A) Scatterplot of ascites MFAP4 and total ascites protein. The 2 variables were moderately correlated (r = 0.52, p <0.01). (B) Scatterplot of ascites MFAP4 and serum MFAP4 in 41 patients with both measured at sampling. The correlation between ascites MFAP4 and serum MFAP4 was 0.25 (p = 0.11). (C) Scatterplot of ascites MFAP4 and serum albumin. MFAP4 in the ascitic fluid was not associated with levels of albumin in serum (r = 0.04, p = 0.73). All correlations were tested by Spearman's correlation test. MFAP4, microfibrillar associated protein 4.

fibrinogen superfamily and a possible target of fibrinolytic breakdown, future research should aim to clarify the role of fibrinolytic-related degradation of ascites MFAP4.

Our study has limitations to consider. First, no predefined criteria were applied for the paracenteses, which is why clinical practice at each study centre determined eligibility of patients. With diagnostic and therapeutic paracentesis as inclusion criteria, the procedure happened in both inpatient and outpatient settings, making the study population rather heterogeneous. Second, because of the limited size of the study, it was not possible to split our cohort into a test cohort and a validation cohort. Our findings therefore need external validation. Third, only 41 patients had ascites and serum MFAP4 measured at the time of sampling, and we cannot exclude these patients were selected in some way. Fourth, the clinical application of ascites MFAP4 is limited to patients with ascites available for paracentesis, and therefore, ascites MFAP4 cannot be applied in patients with ascites controlled by diuretics, with no indication for paracentesis. Lastly, although ascites MFAP4 was significantly associated with transplant-free survival at 1-year follow-up, we only observed a non-significant trend in the same direction at 3-month follow-up. We believe this may be as result of the limited event rate during the first 3 months of patient follow-up. However, notably in this context, the Child-Pugh score, MELD-Na, and CLIF-C AD performed well at 3 months follow-up. Larger study groups are therefore needed to clarify the role of ascites MFAP4 in short-term mortality prediction.

Ascites MFAP4 was shown to be an independent predictor of 1-year transplant-free survival in patients with decompensated cirrhosis and ascites. In patients with low MELD-Na scores, ascites MFAP4, but not total ascites protein, significantly predicted 1-year transplant-free survival. Ascites MFAP4 seems to be a promising marker of transplant-free survival, with an interesting potential in patients with ascites and low MELD-Na scores.

Abbreviations

CLIF-C AD, CLIF Consortium Acute Decompensation; CPS, Child-Pugh score; CT, computed tomography; CRP, C-reactive protein; ECM, extracellular matrix; eGFR, estimated GFR; GFR, glomerular filtration rate; HR, hazard ratio; INR, internationalised normal ratio; LTx, liver transplantation; MELD-Na, model for end-stage liver disease; MFAP4, microfibrillar associated protein 4; NASH, non-alcoholic steatohepatitis; SBP, spontaneous bacterial peritonitis.

Conflicts of interest
GLS is the inventor of U.S. Patent No. 9,988,442 and EP14743707.3 owned by the University of Southern Denmark. MT received personal fees from Echosens outside the submitted work. The remaining authors have nothing to declare.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
Conceptualised and designed the study: NT, MI, BM, AK. Performed the formal analyses of the data: NT. Conducted the investigations and data collection: BM, PL, CJ, CM, AWK, GLS, UH, JT. Wrote the original draft of the manuscript: NT, MI, AK. Contributed to the manuscript with important intellectual content and approved the final version: All authors

Data availability statement
Owing to the sensitive nature of the data, they will not be shared.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2021.100287.

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Author names in bold designate shared co-first authorship

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