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Metabolic profile of liver damage in non-cirrhotic virus C and autoimmune hepatitis: A proton decoupled ^{31}P -MRS study

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ABSTRACT

Purpose: To study liver ^{31}P MRS, histology, transient elastography, and liver function tests in patients with virus C hepatitis (HCV) or autoimmune hepatitis (AIH) to test the hypothesis that ^{31}P MR metabolic profile of these diseases differ.

Materials and methods: 25 patients with HCV (n = 12) or AIH (n = 13) underwent proton decoupled ^{31}P MRS spectroscopy performed on a 3.0T MR imager. Intensities of phosphomonoesters (PME) of phosphoethanolamine (PE) and phosphocholine (PC), phosphodiester (PDE) of glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC), and γ , α and β resonances of adenosine triphosphate (ATP), and nicotinamide adenine dinucleotide phosphate (NADPH) were determined. Liver stiffness was measured by transient elastography. Inflammation and fibrosis were staged according to METAVIR from biopsy samples. Activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALT) and thromboplastin time (TT) were determined from serum samples.

Results: PME had a stronger correlation with AST (z = 1.73, p = 0.04) and ALT (z = 1.77, p = 0.04) in HCV than in AIH patients. PME, PME/PDE, PE/GPE correlated positively and PDE negatively with inflammatory activity. PE, PC and PME correlated positively with liver function tests.

Conclusion: ^{31}P -MRS suggests a more serious liver damage in HCV than in AIH with similar histopathological findings. ^{31}P -MRS is more sensitive in detecting inflammation than fibrosis in the liver.

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1. Introduction

Histological analysis of biopsy sample is currently considered the golden standard in the assessment of inflammatory activity and

fibrosis in the liver. However, liver biopsy has several drawbacks concerning both patient safety and reliability. Overall complication rates of over 6% have been reported with bleeding risks of 1–2% and even a small mortality [1]. Since a biopsy sample represents only approximately 1/50 000 of the total liver volume, sampling errors are frequent. Regev et al. have reported discordant results in 33% of patients undergoing two simultaneous biopsies from different locations of the liver [2]. Both intra- and interobserver variation have been reported in the interpretation of liver biopsies by histopathologists [2].

Fibrosis has been shown to be associated with restricted diffusion of water measured by diffusion-weighted imaging (DWI) [3]. However, there is some incongruity between studies and Boulanger et al. were unable to demonstrate this on HCV patients [4]. By assessing mechanical properties of the tissue, transient (TE) [5] and magnetic resonance elastography (MRE) [6] can detect fibrosis. MRE performed better than ^{31}P magnetic resonance spectroscopy (MRS) or DWI in detecting liver fibrosis [3,7]. However, in the presence of inflammatory activity, MRE can overestimate fibrosis and the effect of these two components cannot be distinguished [6].

Abbreviations: AIH, autoimmune hepatitis; ALD, alcoholic liver disease; ALP, alkaline phosphatase; ALT, alanine transaminase; AMARES, advanced method for accurate, robust and efficient spectral fitting; AST, aspartate transaminase; ATP, adenosine triphosphate; DAA, direct acting antivirals; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; HCV, virus C hepatitis; IQR, interquartile ratio; ISIS, image selective in vivo spectroscopy; LFT, liver function test; MRE, magnetic resonance elastography; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; NOE, nuclear overhauser enhancement; PBC, primary biliary cirrhosis; PC, phosphocholine; PDE, phosphodiester; PE, phosphoethanolamine; Pi, inorganic phosphates; PME, phosphomonoesters; STEAM, stimulated echo acquisition mode; SVR, sustained virological response; TE, echo time; TR, time of repetition; TT, thromboplastin time; VOI, volume of interest.

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Currently, liver fibrosis is considered treatable or reversible if diagnosed at an early stage [8]. Therefore, it is of great importance not only to detect fibrosis early enough, but also to identify a rapidly progressing disease. The degree of inflammatory activity in the liver has prognostic value in the evaluation of progression of the liver damage [9]. New second generation direct acting antiviral drugs for hepatitis C virus (HCV) are expensive and therefore their use should be limited to patients who are in high risk of progressive liver disease and who benefit from the treatment. At present, histological analysis of a liver biopsy at presentation is recommended to establish autoimmune hepatitis (AIH) diagnosis and to guide the treatment decision, but follow-up biopsies are not recommended [10]. A non-invasive method to, on one hand, recognize HCV and AIH patients who are at high risk of progressive liver disease and, on the other hand, to monitor treatment response would be helpful.

^{31}P MRS has been shown to correlate with and even to differentiate the degrees of hepatitis of various aetiologies [11,12]. Especially, phosphomonoester/phosphodiester (PME/PDE) -ratio has been shown to be a sensitive but rather unspecific indicator of liver damage [11,13]. PME/PDE -ratio has also shown potential as an indicator of treatment response in HCV patients [14,15]. Van Wassenae-Van Hall et al. [16] found a correlation between aspartate transaminase activity (AST) and portal inflammation with PME. However, mixed results have been reported on the relationship between fibrosis and ^{31}P MRS. Some groups have found a correlation between fibrosis stage and ^{31}P MRS [11,13], while others have not [7]. Dezortova et al. [17] have reported significant differences in ^{31}P MR spectra of functionally compensated cirrhosis compared to decompensated cirrhosis. Commonly, patients with liver disease of different aetiologies have been pooled when studying relationships between histology and ^{31}P MRS [7,13]. There is some evidence that ^{31}P MR spectra may vary according to the aetiology of cirrhosis [18].

The PME signal has contributions from several resonances. Its increase in the presence of liver damage has been linked to elevated levels of cell membrane precursors, phosphocholine (PC) and phosphoethanolamine (PE). A decrease in PDE resonance has been linked to reduction in the levels of cell membrane degradation products, glycerophosphocholine (GPC) and glycerophospho-ethanolamine (GPE). These subcomponents of PME and PDE cannot be distinguished using conventional ^{31}P MRS at clinical field strengths ($\leq 3.0\text{T}$) and therefore the sum of PME and PDE resonances have been reported. However, coupling interactions between protons and ^{31}P nuclei can be cancelled out by applying an additional proton decoupling scheme to the MRS pulse sequence, which allows separation of PME and PDE subcomponents at clinical field strengths. Previously, Sevastianova et al. have reported increased PE/(PME + PDE) and decreased GPC/(PME + PDE) in cirrhosis compared to pre-cirrhotic levels of non-alcoholic fatty liver disease (NAFLD) [17]. Also, Schlemmer et al. found GPE/GPC to be elevated in alcoholic liver disease (ALD) patients, and PE/PC to be elevated in cirrhotic ALD patients compared to healthy controls [19]. As an indication of disturbed liver energy metabolism, reduced ATP levels have been associated to obesity [20], diabetes mellitus type 2 [21] and cirrhosis [17].

The object of this study was to evaluate the relationship between ^{31}P MRS, liver histology, transient elastography and liver function tests (LFT) in patients with histologically proven liver damage due to HCV or autoimmune hepatitis (AIH) and to study whether the ^{31}P MR spectral appearance in these diseases differ.

2. Methods

25 consecutive patients with either AIH (N = 13) or HCV genotype 1 (N = 12) from the Department of Gastroenterology at Helsinki University Hospital underwent liver biopsy on clinical indications

for staging the liver injury. Liver samples were analysed by a single pathologist blinded to clinical and MRS data. Fibrosis and inflammation activity were assessed using METAVIR score [22]; inflammation was scored from 0 to 3 with 0 being no; 1 mild; 2 moderate; and 3 severe activity. Accordingly, the grade of fibrosis was assigned a number from 0 to 4: 0, no scarring; 1 portal fibrosis without septa; 2 portal fibrosis and rare fibrotic septa; 3 portal fibrosis and numerous fibrotic septa; 4 cirrhosis. All but two samples had 10–30 portal areas (Table 1). Activities of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and thromboplastin time (TT) were determined from plasma samples. We also determined serum HCV antibodies and HCV RNA for HCV and immunoglobulin G (IgG) for AIH patients. All AIH patients received immunosuppressive drugs and their treatment was in a steady state. MRS was performed within 5 weeks (median) of the liver biopsy (Table 1).

Four out of twelve HCV patients received antiviral treatment and were asked for a follow-up ^{31}P MRS experiment; two of them agreed. This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics committee of the Helsinki University Hospital and all subjects gave their written informed consent.

2.1. MRS

MRS of liver was performed on a 3T clinical imager (Achieva, Philips Medical Systems, Best, The Netherlands) with subjects lying in a supine position. T1-weighted ultrafast gradient echo MR images were collected in three orthogonal directions with 10 mm slice thickness in the upper abdomen. The number of slices was individually adjusted to cover the liver. Images were collected during end-exhalation breath holds. A $125\text{--}216\text{ cm}^3$ voxel was placed in the center of the right liver lobe using image selective in vivo spectroscopy (ISIS) with TR of 6000 ms and 128 acquisitions, and using proton decoupling and nuclear Overhauser enhancement (NOE) as previously described [12]. ^{31}P MRS data was collected with a circular non-flexible ^{31}P transmit-receive loop coil with a diameter of 14 cm, while a ^1H body coil was used for proton decoupling and NOE during data acquisition. The subjects were instructed to adjust their breathing cycle to the pulse sequence noise, so that the excitation pulse and data acquisition were timed to end exhalation. Breathing was monitored using a standard navigator belt.

For determination of liver fat content, we performed a ^1H MRS measurement during the same session using a standard body coil. A $25 \times 25 \times 25\text{ mm}^3$ voxel was placed in the right liver lobe avoiding vascular structures and contamination from surrounding tissues. 16 acquisitions were collected as a time series using STEAM technique for spatial localization with TE of 10 ms and mixing time of 15 ms. Data collection was triggered to the end-exhalation using a navigator belt so that TR was kept above 4000 ms.

All spectra were analysed with jMRUI v5.0 software (<http://www.jmrui.eu>). In the analysis of ^{31}P MR spectra, intensities of PE, PC, Pi, GPE, GPC, γ -NTP, α -NTP, NADPH, UDPG and β -NTP were determined using AMARES algorithm with prior knowledge [23]. Intensities were expressed as a ratio to the total phosphorus signal. Total PME and PDE intensities were calculated adding individual signal components. In the analysis of ^1H MRS spectra, each phase cycle of the time series were phased and frequency shifted before averaging. Intensities of water at 4.7 ppm and methylene groups of fatty acid chain at 1.3 ppm were determined. Signal intensities were corrected for relaxation effects. Methylene/(water + methylene) -ratios were further converted to mass fractions as previously described [24].

Table 1Patient characteristics. Normally distributed variables are shown as mean $\pm$ SD, non-normally as median (IQR).

	HCV	AIH	TOTAL	P-value [*]
Characteristics				
Subjects	12	13	25	
Age (years)	50.3 $\pm$ 10.2	45.4 $\pm$ 18.7	47.7 $\pm$ 15.3	0.43
Gender	4M	3M	7M	
LFAT (%)	1.5 (0.3–1.2)	1.0 (0.2–1.2)	1.3 (0.3–1.2)	0.45
Biopsy samples				
Portal areas	18 (13–22)	15 (12–18)	15 (12–21)	0.49
Time gap vs. MRS (weeks)	4 (2–6)	5 (3–10)	5 (2–9)	0.22
Histology (METAVIR)				
Fibrosis	2xF0, 5xF1, 2xF2, 3xF3	1xF0, 3xF1, 4xF2, 4xF3		0.44
Inflammation	2xG0, 5xG1, 3xG2, 2xG3	6xG0, 4xG1, 1xG2, 1xG3		0.07
Laboratory parameters				
ALP (IU/L)	91 $\pm$ 39	65 $\pm$ 21	79 $\pm$ 34	0.02
AST (IU/L)	129 $\pm$ 141	33 $\pm$ 20	84 $\pm$ 113	0.05
ALT (IU/L)	97 $\pm$ 58	37 $\pm$ 36	68 $\pm$ 57	0.01
TT (%)	107 $\pm$ 19	96 $\pm$ 22	101 $\pm$ 21	0.29
IgG (g/L)		13.9 $\pm$ 4.1		
HCV RNA (IU/L)	8.6 (1.2–11.5) $\times 10^9$			
Transient Elastography				
Stiffness (kPa)	9.7 $\pm$ 7.7	6.4 $\pm$ 2.0	8.4 $\pm$ 6.2	0.18

HCV, virus C hepatitis; AIH, autoimmune hepatitis; LFAT, liver fat content (by ^1H MRS); ALP, alkaline phosphatase; AST, aspartate transaminase; ALT alanine transaminase; TT, thromboplastin time; IgG, immunoglobulin G.

^{*} Between HCV and AIH groups (Student's *t*-test for normal and Mann-Whitney for non-normal distributions).

2.2. Transient elastography

Transient elastography examinations were carried out by a single radiologist (RK) with Fibroscan[®] (Echosens, France) guided by anatomical ultrasound to confirm the location of the measurements. The examinations were performed through one intercostal space that provided an optimal unobstructed view to the right lobe of the liver. The results were based on a median of ten validated measurements. Transient elastography results with an interquartile ratio (IQR) below 30% of the median value and a success rate of at least 60% were accepted, according to manufacturer's recommendations.

2.3. Statistical analysis

Differences between AIH and HCV groups were tested using Student's *t*-test for normally distributed continuous variables. Log-normally distributed data was transformed accordingly before the testing. For non-normally distributed variables, a non-parametric Mann-Whitney test was used. Normality was tested using Kolmogorov-Smirnov test. Spearman's and Pearson correlations were used to assess statistical relationships for non-continuous and continuous variables, respectively. Fisher's transformation was used to test differences in parameter correlations between AIH and HCV groups. A *p*-value below 0.05 was considered statistically significant.

3. Results

AIH and HCV groups did not differ in terms of grade of inflammatory activity or stage of fibrosis. There was no difference in liver stiffness measured by transient elastography nor in liver fat content measured by ^1H MRS. Patient characteristics are shown in Table 1 and differences in all ^{31}P MRS metabolites in AIH and HCV groups are shown in Table 2. PC (4.9 $\pm$ 2.0 vs. 8.1 $\pm$ 2.4; *p* = 0.002) and PME (11.9 $\pm$ 3.3 vs. 17.9 $\pm$ 4.9; *p* = 0.002) were higher and γ -ATP (12.2 $\pm$ 1.7 vs. 14.4 $\pm$ 2.5; *p* = 0.019) and α -ATP (12.3 $\pm$ 1.8 vs. 14.5 $\pm$ 2.5; *p* = 0.023) lower in HCV than in AIH (Fig. 1).

Table 2

^{31}P MRS metabolite levels and signal ratios in autoimmune (AIH) and virus C (HCV) hepatitis groups. Metabolite levels are expressed as% (mean $\pm$ SD) of total phosphorus signal.

Metabolites	Autoimmune hepatitis	Virus Hepatitis C	P-value
PE	7.0 $\pm$ 2.4	9.9 $\pm$ 4.4	0.061
PC	4.9 $\pm$ 2.0	8.05 $\pm$ 2.4	0.002
Pi	11.4 $\pm$ 3.4	11.4 $\pm$ 2.8	1.000
GPC	12.7 $\pm$ 2.5	12.4 $\pm$ 2.9	0.766
GPE	11.3 $\pm$ 2.7	10.9 $\pm$ 2.6	0.712
PtdC	4.2 $\pm$ 1.2	4.6 $\pm$ 2.0	0.544
NADPH	8.9 $\pm$ 3.9	6.8 $\pm$ 3.2	0.158
UDPG	3.9 $\pm$ 1.9	4.4 $\pm$ 1.5	0.496
PME	11.9 $\pm$ 3.3	17.9 $\pm$ 4.9	0.002
PDE	24.1 $\pm$ 4.1	23.4 $\pm$ 5.3	0.718
γ -ATP	14.5 $\pm$ 2.5	12.3 $\pm$ 1.8	0.023
α -ATP	14.4 $\pm$ 2.5	12.2 $\pm$ 1.7	0.019
β -ATP	6.6 $\pm$ 3.1	7.1 $\pm$ 5.0	0.772
Ratios			
PME/PDE	0.50 $\pm$ 0.13	0.81 $\pm$ 0.31	0.004
PE/GPE	0.56 $\pm$ 0.18	0.82 $\pm$ 0.38	0.044
PC/GPC	0.44 $\pm$ 0.20	0.79 $\pm$ 0.33	0.004
PE/PC	1.71 $\pm$ 0.82	1.34 $\pm$ 0.79	0.278
GPE/GPC	1.16 $\pm$ 0.26	1.14 $\pm$ 0.19	0.889

PE, phosphoethanolamine; PC, phosphocholine; Pi, inorganic phosphates; GPE, glycerophosphoethanolamine; GPC, glycerophosphocholine; PtdC, phosphatidylcholine; NADPH, nicotinamide adenine dinucleotide phosphate; UDPG, uridine diphosphate glucose; PME, phosphomonoesters; PDE, phosphodiester; ATP, adenosine triphosphate.

3.1. MRS and LFT

LFT's did not correlate with phosphodiester, but correlated with phosphomonoester resonances (Table 3). PME had a stronger correlation with AST (*z* = 1.73, *p* = 0.04) and ALT (*z* = 1.77, *p* = 0.04) in HCV than in AIH patients (Fig. 2). In HCV, intensities of α -ATP and γ -ATP correlated negatively with TT (*r* = −0.724, *p* = 0.008) and in all patients with ALP (*r* = −0.411, *p* = 0.04).

3.2. MRS and histology

In a pooled group of AIH and HCV patients, inflammatory activity correlated positively with PME (*r* = 0.45, *p* = 0.024), PME/PDE

Fig. 1. ^{31}P MR liver spectra and a detail of corresponding liver histology (hematoxylin and eosin stain; original magnification, $200\times$) of AIH patients with a) high inflammatory activity (inflammation grade 3, fibrosis stage 3) and b) without inflammation or fibrosis (inflammation grade 0, fibrosis stage 0). Similar spectra and histology of HCV patients with c) high inflammatory activity (inflammation grade 3, fibrosis stage 2) and d) without inflammation or fibrosis (inflammation grade 0, fibrosis stage 0). Histology shows portal inflammation, interface activity and lobular activity in a) and c). Only portal inflammation is seen in b) and d), but no activity. In the spectra, the residual signal is shown above the original spectra and fitted model (red). AIH, autoimmune hepatitis; HCV, virus C hepatitis; PE, phosphoethanolamine; PC, phosphocholine; Pi, inorganic phosphates; GPE, glycerophosphoethanolamine; GPC, glycerophosphocholine; ATP, adenosine triphosphate; NADPH, nicotinamide adenine dinucleotide phosphate.

($r=0.52$, $p=0.007$) and PE/GPE ($r=0.45$, $p=0.024$), and negatively with PDE ($r=-0.615$, $p=0.025$).

In HCV, inflammatory activity correlated positively with PME ($r=0.65$, $p=0.023$), PME/PDE ($r=0.791$, $p=0.002$), PE/GPE ($r=0.708$, $p=0.01$) and negatively with PDE ($r=-0.615$, $p=0.025$). Other metabolites or their ratios did not correlate with inflammation. There were no correlations between MRS metabolites and fibrosis stage.

In AIH, there were no correlations between MRS metabolites and liver histology parameters.

3.3. Transient elastography and histology

Liver stiffness measured by transient elastography correlated with both fibrosis ($r=0.712$, $p=0.0002$) and with inflammation ($r=0.506$, $p=0.016$).

3.4. Follow-up of HCV patients

HCV RNA was 1.47×10^9 UI/L and 4.83×10^9 UI/L for the first and second patient, respectively, before treatment and undetectable in both patients after treatment. Parameters changed from baseline to follow-up for the first and the second patient, respectively, as followed: PME (-48% , -72%), PDE (27% , 49%), PME/PDE (-59% , -44%),

Table 3

Relationships between phosphomonoesters (PE, PC and combined) and liver enzymes and alkaline phosphatase.

	PME	PE	PC
Total (n = 25)			
ALP	$r = 0.79^{***}$	$r = 0.64^{**}$	$r = 0.59^{**}$
AST	$r = 0.65^{**}$	$r = 0.43^{*}$	$r = 0.63^{**}$
ALT	$r = 0.59^{**}$	ns	$r = 0.66^{**}$
HCV (n = 12)			
ALP	$r = 0.83^{**}$	$r = 0.68^{*}$	ns
AST	$r = 0.69^{*}$	ns	$r = 0.68^{*}$
ALT	$r = 0.65^{*}$	ns	$r = 0.68^{*}$
AIH (n = 13)			
ALP	ns	ns	$r = 0.55$
AST	ns	ns	ns
ALT	ns	ns	ns

PME, phosphomonoesters; PE, phosphoethanolamine; PC, phosphocholine; ALP, alkaline phosphatase; AST, aspartate transaminase; ALT alanine transaminase.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

Fig. 2. Correlations between phosphomonoesters (PME) and serum a) alanine (ALT) and b) aspartate (AST) transaminases in patients with virus C hepatitis (blue circles) and autoimmune hepatitis (red circles). PME correlates with AST and ALT in HCV, but not in AIH patients.

PE (−3.6%, −75.6%), PC (−75%, −68%), plasma ALT (−54%, −90%).
Fig. 3 shows MR spectra before and after the antiviral treatment.

4. Discussion

In our study, ^{31}P MRS was more sensitive to liver inflammation than liver fibrosis in both HCV and in AIH patients. Patients with HCV showed a more prominent elevation of phosphomonoesters

Fig. 3. Effect of treatment on ^{31}P MR spectra. Averaged spectra from two patients before (black) and after successful (red) treatment.

as a function of disease severity, measured by liver histology, compared to AIH.

Patients with aggressive hepatitis will develop fibrosis, which can further proceed to cirrhosis and hepatocellular carcinoma. MR and US elastographies have proven to be useful for non-invasive detection of liver fibrosis, but at present there are no non-invasive methods in clinical use to score liver inflammation. Currently, liver fibrosis is considered reversible, but some angio-architectural changes, such as vascularized septa that accompany cirrhosis, probably are irreversible and therefore could represent a point-of-no-return for disease progression [25]. Inflammation and fibrosis are tightly connected and regulated; thus, controlling the inflammatory pathway could be an attractive therapeutic strategy for liver fibrosis.

The European Association for the study of the liver (EASL) guidelines recommend that besides HCV patients with advanced liver disease (METAVIR score F3 and F4), treatment is justified also for patients with moderate fibrosis (F2). Should we have a non-invasive method to show inflammation, it could become an additional indication to start HCV treatment even with the expensive direct acting antivirals (DAA) in patients with F2.

^{31}P MRS can easily be repeated for follow-up of treatment response. In both, our HCV patients with follow-up ^{31}P MRS, both blood parameters and MRS showed improvement. HCV RNA was undetectable, plasma ALT was decreased by more than 50% and PME/PDE -ratio had decreased approximately 50% after treatment. This is in line with previous studies, where 41% and 30% decreases in PME/PDE were found in a group of virologic responders [14,15]. In our study, the decrease in PME/PDE arised dominantly from the decrease in PME – especially PC which decreased by approximately 70% in both patients. Treatment response on PC or other subcomponents of PME or PDE have not been reported in HCV previously. Whether PC could serve as a sensitive and disease specific marker of treatment response merits further study on larger study populations.

PME and PDE are thought to represent cell membrane precursors and degradation products, respectively, and PME/PDE ratio serves as an index of cell membrane turnover [26]. In our patients, PME/PDE marked inflammatory activity best. Contrary to our results, changes in PME/PDE ratio have occasionally been linked to fibrosis rather than to inflammation [11,13]. In a study on HCV patients, PME/PDE correlated better with the degree of fibrosis than with inflammation [11]. Lim et al. have also found that PME/PDE ratio was normalised in responding cirrhotic patients receiving antiviral treatment for HCV [14]. Considering that cirrhosis represents a “point-of-no-return” for fibrosis, the normalization of PME/PDE ratio in their study may rather have represented changes in inflammation than in fibrosis. Also, in a mix of non-alcoholic fatty liver disease (NAFLD), AIH, HCV, PBC, alcoholic liver disease (ALD) and autoimmune cholangitis patients, Noren et al. found

PME/(PME/PDE) to correlate with fibrosis, but not with inflammation [13]. Possible changes in the degree of fibrosis can be expected to be relatively slow compared to changes in inflammation. Thus, the smaller time gap between biopsy and MR experiment in our study (median 1 month) compared to study by Lim et al. (3 months) and Noren et al. (median 31 and 5 months) may play a key role in explaining the differences between these observations.

PE/GPE correlated with histological inflammation, and PE and PC correlated with LFTs. Previously, Schlemmer et al. [19] have reported elevated PE/PC in ALD patients with cirrhosis compared to non-cirrhotic patients and healthy controls. This suggests that distinguishing subcomponents of PME and PDE may increase the specificity of ^{31}P MRS in characterising liver damage – especially in detecting inflammation. However, PE and PC overlap partly with each other and also with contributions from adenosine monophosphate and glycolytic intermediates, hampering the reliability of quantification. Recently, the first clinical ultrahigh field ($\geq 7\text{ T}$) MR imagers have become available. Chmelik et al. reported good spectral separation of individual PME and PDE components at 7 T without proton decoupling and also observed that T1 of both PME and PDE resonances, unlike Pi and ATP, increased significantly at 7 T compared to 3 T [27]. Based on this observation, they concluded that dipolar interactions prevail as the dominant relaxation mechanism over chemical shift anisotropy for PME and PDE. Thus, NOE and proton decoupling could further improve spectral resolution at 7 T. This subject merits further study.

Due to the inhomogeneous distribution of disease and relatively small sample size, liver histology is prone to sampling errors, which may lead to underestimation of the true degree of fibrosis. In our study, however, liver fibrosis correlated well with transient elastography, but neither correlated with ^{31}P MRS. This makes it unlikely that the lack of correlations between phosphorus metabolite levels and histological fibrosis scores would have arisen from sampling errors.

In our study, patient groups differed not only in terms of aetiology of liver disease, but they also had different treatment protocols. AIH, but not HCV patients received immunosuppressive treatment. Recently, treatment duration has been shown to affect the reliability of transient elastography in staging fibrosis [28]. In AIH, abnormally high liver stiffness values have been reported in patients with very high ALT-levels [29], and transient elastography has been shown to be reliable only after 6 months of immunosuppressive treatment [28]. Also, mean ALT activities in a group with more than 6 months after initiation of the treatment were half of the group with less than 3 months of treatment [28]. It is possible that in our study, differences in ^{31}P MRS between HCV and AIH patients with similar histological liver injury are due to the immunosuppressive treatment of AIH patients. Immunosuppressive treatment may stop the progression of fibrosis or inflammatory cells present in histology may become metabolically less active. If this is true, this would strengthen the hypothesis that ^{31}P MRS is more sensitive to detect inflammation or ongoing fibrogenesis than the presence of fibrosis itself in the liver. Also, this would make ^{31}P MRS an appealing tool to investigate immunosuppressive treatment response in AIH patients.

Patients with HCV showed lower ATP levels compared to AIH. ATP correlated negatively with TT in HCV and with ALP in all patients. Reduced ATP levels have been interpreted as an energy deficit or impaired ATP homeostasis. Our observations are in line with those of previous studies [20,26] and support the interpretation of a more serious metabolic liver damage in patients with HCV than in AIH with similar histology.

Our study was conducted with a relatively small number of patients. Another limitation of our study is the lack of absolute quantification of phosphorus metabolites. In the presence of liver damage, hepatocyte density inside a VOI may decrease. A total

phosphorus signal that we used as a reference would be blind to such general decrease of all metabolite levels.

5. Conclusion

The metabolic profile of liver injury, as measured by ^{31}P MRS, suggested a more serious disturbance in HCV than in AIH with similar histopathological findings, in accordance with blood chemistry results. Our data suggests that increased PME levels in ^{31}P MRS are linked more strongly to the inflammatory process or ongoing fibrogenesis than the presence of fibrosis itself

Conflicts of interest

None.

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