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ORIGINAL ARTICLE

^{31}Phosphorus magnetic resonance spectroscopy of the liver for evaluating inflammation and fibrosis in autoimmune hepatitis

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ABSTRACT

Background: Liver biopsy is the gold standard in evaluating inflammation and fibrosis in autoimmune hepatitis.

Aims: In search of non-invasive follow-up tools in autoimmune hepatitis, we evaluated ^{31}phosphorus magnetic resonance spectroscopy (^{31}P MRS).

Methods: Twelve consecutive AIH patients (mean age 42.8 years, 10 women) underwent liver biopsy, routine laboratory liver function tests, which were compared to findings in ^{31}P MRS and transient elastography (TE).

Results: Phosphoenolpuryvate (PEP) correlated with the grade of inflammation ($r = 0.746$, $p = .005$) and thromboplastin time ($r = 0.592$, $p = .043$). It also differentiated patients with active inflammation from patients without ($t = 3.781$, $p = .009$). There was no correlation between PEP and aminotransferase or immunoglobulin G levels.

The phosphoethanolamine (PE)/phosphocholine (PC) ratio, PE/glyserophosphoethanolamine (GPE) ratio and PC/[total phosphomonoester (PME) + phosphodiester (PDE)] ratios correlated with immunoglobulin G ($r = 0.764$, $p = .006$; $r = 0.618$, $p = .043$; and $r = -0.636$, $p = .035$, respectively).

PME/PDE and PE/GPE correlated with fibrosis ($r = 0.668$, $p = .018$ and $r = 0.604$, $p = .037$). PE/GPE also differentiated F3 from F0-2 patients ($t = 3.810$, $p = .003$).

Phosphorus metabolites did not correlate with TE results and TE did not correlate with liver histology or laboratory parameters.

Conclusions: ^{31}P MRS seems to detect active inflammation and advanced fibrosis in AIH patients. TE was ineffective in fibrosis quantification.

Abbreviations: AC: anabolic charge; AIH: autoimmune hepatitis; Alb: albumin; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CI: confidence interval; F: fibrosis; G: grade; GPE: glyserophosphoethanolamine; GPC: glycerophosphocholine; IgG: Immunoglobulin G; MR: magnetic resonance; NADPH: nicotinamide adenine dinucleotide phosphate; NPV: negative predictive value; ; NTP: nucleotide triphosphate; PC: phosphocholine; PC: phosphocholine; PDE: total phosphodiester level; PE: phosphoethanolamine; PEP: phosphoenolpuryvate; PEP/PtdC: phosphoenolpyruvate/phosphatidylcholine; Pi: phosphorus; PME: total phosphomonoester level; ^{31}P MRS: ^{31}phosphorus magnetic resonance spectroscopy; PPV: positive predictive value; PtdC: phosphatidylcholine; TE: transient elastography; TT: thrombolastin time; UDPG: uridine diphosphoglucose

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Autoimmune hepatitis; liver histology; magnetic resonance spectroscopy; transient elastography; fibrosis; inflammation

Introduction

The goal of therapy of autoimmune hepatitis is serological and histological remission in order to prevent fibrosis progression and cirrhosis development and to control inflammation-induced symptoms. With standard therapy (prednisolone or prednisolone in combination with azathioprine), this is achieved in most patients [1] with up to 89% with 10 years survival. Liver biopsy is regarded to be important for diagnosis, but in addition, it provides important information about the disease prognosis, as proposed by the American Association for the Study of Liver Disease and the British Society of Gastroenterology [1,2]. The role of follow-up biopsies is more controversial. Both of these societies recommend follow-up biopsies to confirm remission during maintenance therapy and prior to drug withdrawal. Inflammation activity during therapy can be estimated by alanine aminotransferase (ALT) and immunoglobulin G (IgG) levels, but histological inflammation resolves more slowly than the laboratory tests become normal [3]. Also, a proportion of patients have ongoing active inflammation despite of normal laboratory values, which can render them for progressive fibrosis development. These patients are also likely to relapse after treatment discontinuation [3,4]. It has been shown that histological cirrhosis seems to be partially reversible in some AIH patients. In paired liver biopsies, fibrosis/cirrhosis

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category was demonstrated to improve during therapy in 57% (16/28) of patients including 9/14 patients with resolution of nodular fibrosis/cirrhosis [5].

^{31}Phosphorus magnetic resonance spectroscopy of the liver (^{31}P MRS) has been evaluated in various liver diseases and has shown to be promising [6,7] in evaluating progressive liver disease, while being able to show both fibrosis and inflammation in the liver. Anabolic charge (AC, elevation of PME and decreased PDE) has been shown to rise in a fibrotic liver and also separate advanced from mild fibrosis in diffuse liver disease [8,9]. What makes this method ideal is that it has been reported to predict both fibrosis and inflammation, both being important when making treatment decisions regarding AIH patients. It is also non-invasive.

Transient elastography (TE) [10] has been evaluated in autoimmune hepatitis: In two series, comprising a total of 28 AIH patients, the authors concluded that TE was effective in finding patients with Metavir [11] fibrosis $\geq$ two patients, although these studies reported only the pooled results with other non-viral hepatitis patients [12,13]. Case reports have also demonstrated that in AIH, inflammation results in disproportionally high TE levels, which normalise after treatment initiation [14]. TE finds cirrhosis reliably after six months of treatment with a cut-off of 16 kilopascals but is less reliable in non-cirrhotic patients [15] and prior to suppression of inflammation with treatment.

At present, liver biopsy is the gold standard for detecting inflammation and fibrosis. Liver biopsy is commonly painful and not without risks, although mortality is rare [16]. It would be of great value to find novel non-invasive tools to assess inflammation and fibrosis.

The aim of this prospective study was to show whether ^{31}P MRS and TE detect inflammation and fibrosis in AIH patients, when compared to histology and liver function tests.

Materials and methods

Patients

Twelve consecutive AIH patients, who had a liver biopsy with clinical indications, were recruited into the study at Helsinki University Hospital, Department of gastroenterology. We used Simplified criteria for the diagnosis of autoimmune hepatitis [17], and patients with both a definite or a probable diagnosis were included, since 2008 criteria have been shown to be fairly exclusive, and the disease behaviour in both groups has been shown to be similar [17–19].

Methods

TE with Fibroscan® (Echosens, France) examinations were done by one radiologist, who used a traditional ultrasound probe in association with a TE probe to confirm the location of the measurements in the liver parenchyma. The results were based on a median of ten successful measurements. TE results with an interquartile range below 30% of the median value and a success rate of at least 60% were accepted according to the manufacturer's recommendations.

Liver biopsies were evaluated by the same pathologist (SB), who scored the biopsies of all patients according to the Metavir classification regarding inflammatory activity and fibrosis. The Metavir and Ishak scoring systems have been shown to correlate well with necroinflammatory changes and fibrosis [20].

Biopsy, blood samples, TE and ^{31}P MRS were performed within a one-month period and before treatment was changed.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), immunoglobulin G (IgG), alkaline phosphatase (ALP), thromboplastin time (TT) and albumin (Alb) were measured.

^{31}P MRS of the 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 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 whole liver. Images were collected during end-exhalation breath holds. A 125–216 cm^3 voxel was placed in the centre of the right liver lobe using image selective *in vivo* spectroscopy with TR of 6000 ms and 128 acquisitions and using proton decoupling and nuclear Overhauser enhancement (NOE) as previously described [6]. ^{31}P MRS data were collected with a circular non-flexible ^{31}P transmit-receive loop coil with a diameter of 14 cm, while the ^{1}H 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. The spectra were analysed with jMRUI v5.0 software [21]. Intensities of phosphoetanolamine (PE), phosphocholine (PC), phosphorus (Pi), glyserophosphoethanolamine (GPE), glycerophosphocholine (GPC), phosphoenolpyruvate/phosphatidylcholine (PEP/PtdC), α-, β-, and μ-nucleotide triphosphate (NTP), nicotinamide adenine dinucleotide phosphate (NADPH) and uridine diphosphoglucose (UDPG) were determined using an AMARES algorithm with prior knowledge [22]. Intensities were expressed as a ratio to the total phosphorus signal. Total phosphomonoester level (PME) and total phosphodiester level (PDE) intensities were calculated from individual signal components, while PME comprises PE and PC, while PDE is the sum of GPE and GPC.

The study was approved by the Ethics Committee, Department of Medicine, Helsinki University Hospital (diary number 150/13/03/01/2012), and all subjects gave their written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in *a priori* approval by the institution's human research committee.

Statistics

The Pearson correlation coefficient for parametric tests and the Spearman rank correlation coefficient for nonparametric tests correlation were used in comparing between the biopsy, ^{31}P MRS, TE and laboratory tests. A two-tailed *t*-test

for unpaired samples was used to compare the means between two groups. $p < .05$ was considered statistically significant. Statistical analyses were performed with SPSS v 22 (IBM corp, NY), SAS (SAS institute, NC).

Results

Patient characteristics and liver histology are described in Tables 1 and 2. Examples of normal and abnormal (grade 2, stage 2). AIH liver spectra and histology are shown in Figure 1. The mean age of patients was 42.8 years. Most were women (10/12), and seven out of 12 were in serological remission. All were non-cirrhotic.

PEP correlated with histological inflammation grade ($r = 0.746$, $p = .005$), TT ($r = 0.592$, $p = .043$ and ALP ($r = 0.598$, $p = .040$). PEP was able to differentiate patients with active histological inflammation (G1-3) from patients with none (G0) ($t = 3.781$, $p = .009$) (Figure 2). PEP levels >0.35 had a sensitivity of 100% (95%CI: 54.1–100%), specificity of 66.7% (95%CI: 22.3–96.7%), positive predictive value (PPV) of 75% (95%CI: 34.9–96.8%) and negative predictive value (NPV) of 100% (95%CI: 39.8–100%) to differentiate active inflammation (G1-3) from patients with none.

PE/PC (Figure 3), PE/GPE and PC/(PME + PDE) ratios correlated with inflammation activity as measured with IgG ($r = 0.764$, $p = .006$; $r = 0.618$, $p = .043$; and $r = -0.636$, $p = .035$, respectively). PE/PC and PE/GPE also separated patients with elevated IgG (>15.0) from patients with normal IgG ($t = 2.233$, $p = .0496$ and $t = 2.672$, $p = .023$, respectively). PE/PC had sensitivity of 80% (95%CI: 28.4–99.5%), specificity of 85.7 (95%CI: 42.1–99.6), PPV of 80% (95%CI: 28.4–99.5%) and NPV of 85.7% (95%CI: 42.1–99.6%) with ratios of >2 and PE/GPE 75% (95%CI: 19.4–99.4%), 87.5% (95%CI: 44.7–99.7%), 75% (95%CI: 19.4–99.4%) and 75% (95%CI: 19.4–99.4%) with a ratio of >6, respectively, for detecting patients with elevated IgG.

PME/PDE and PE/GPE correlated with fibrosis score ($r = 0.668$, $p = .018$ and $r = 0.604$, $p = .037$, respectively (Figures 4,5.). PE/GPE also differentiated patients with fibrosis F3 from patients with no or mild fibrosis (F0-2) ($t = 3.810$, $p = .003$) but could not differentiate patients with milder fibrosis (between stages 1–2) from patients with no fibrosis (F0). Sensitivity of PE/GPE for differentiating F3 from F0-2 was 100% (95%CI: 39.8–100%), specificity 50% (95%CI: 15.7–84.3%), PPV 50% (95%CI: 15.7–84.3) and NPV 100% (95%CI: 39.8–100%) with 0.5 cut-off. With a cut-off of 0.7, the values were 50% (95%CI: 6.8–93.2%), 100% (95%CI: 63.1–100%), 100% (95%CI: 15.8–100%) and 80% (95%CI: 44.4–97.5%).

The histological inflammation correlated with ALT and TT ($r = 0.767$, $p = .004$ and $r = 0.725$, $p = .008$, respectively), but not with IgG.

The median TE result was 6.1 kilopascals, range 3.0–10.8. Compared to histology, TE overestimated fibrosis in two patients and underestimated fibrosis in four patients. There was no correlation between phosphorus metabolites and TE or aminotransferase levels. TE did not correlate with phosphorus metabolites, liver histology or laboratory parameters.

Table 1. Patient characteristics ($n = 12$).

Age mean years (range)	42.8 (19.6–69.1)
Time from diagnosis years mean (SD)	6.2 (3.1)
Women *n*	10
ALT median U/L (IQR)	28.5 (17.0–44.3)
Patients with elevated ALT (>45 U/L) *n*	3
ALP median U/L (IQR)	58.0 (45–89.3)
Patients with elevated ALP (>105 U/L) *n*	0
AST median U/L (IQR)	29.0 (24–37)
Patients with elevated AST (>35 U/L) n	2
IgG median g/L (IQR)	11.9 (11.0–18.5)
Patients with elevated IgG (>15 g/L) *n*	5
TT mean percent (SD)	98.0 (20.9)
Patients with decreased TT (<70%) *n*	0
Overlapping primary biliary cirrhosis *n*	2
Patients in serological remission	7

ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; g: gram; IgG: immunoglobulin G; *n*: number; IQR: interquartile range; L: litre; SD: standard deviation; U: unit; TT: Thromboplastin time.

Table 2. Inflammation and fibrosis evaluated by Metavir score [11] and macrovesicular steatosis was evaluated at a medium power from haematoxylin and eosin slides ($n = 12$).

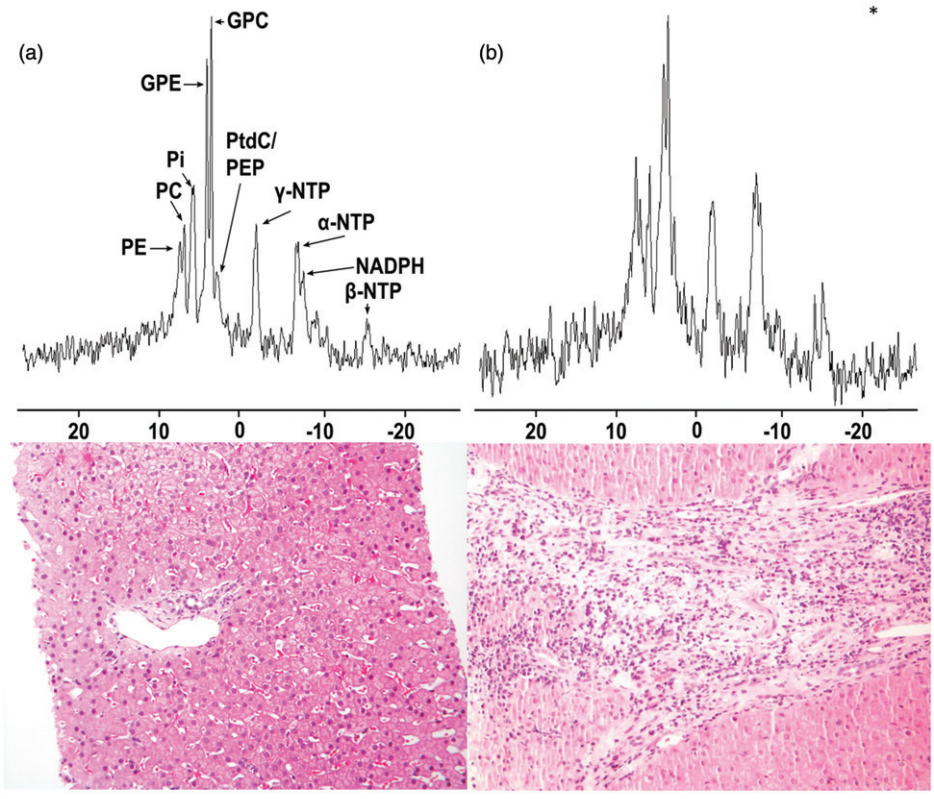
	n
Inflammation grade	
G0	5
G1	3
G2	3
G3	1
Fibrosis stage	
F0	3
F1	1
F2	5
F3	3
F4	0
Steatosis (%)	
≤5	9
6–33	3
≥34	0

Discussion

In the present study, we demonstrated that ^{31}phosphorus magnetic resonance spectroscopy of the liver is sensitive to inflammatory changes and fibrosis in autoimmune hepatitis patients. While it has previously been shown that ^{31}P MRS operates well in fatty liver disease, alcoholic liver disease and hepatitis C [6,7,23], this has been demonstrated in autoimmune liver diseases.

PEP was shown to be a sensitive marker for hepatitis activity already at grade 1. PEP starts to rise early with histological inflammatory activity and can separate active hepatitis from inactive state. Since PtdC behaves similarly to PEP in the spectrum, there is a concern that we might have been measuring PtdC instead of PEP. We reanalysed the biopsies after this finding to look for bile duct damage in histology but found only occasional mild cholangitis. ALP levels were also normal in all patients.

PME (cell membrane precursors) to PDE (cell membrane degradation products) ratio has previously been shown to correlate with the presence of fibrosis and also fibrosis progression (anabolic charge) [8,9]. As a new finding, PE (subgroup of PME) to GPE (subgroup of PDE) ratio correlated with fibrosis. The PE/PGE ratio was also able to identify pre-



1a) Normal histology and corresponding ^{31}P MRS spectrum
1b) G3F1 liver histology with interphase hepatitis and corresponding ^{31}P MRS spectrum

*Amplitudes (peak heights) have been normalised using GPC as a reference amplitude.

PE, Phosphoetanolamine ; PC, Phosphocholine; Pi, Phosphorus; GPE, Glyserophosphoethanolamine; GPC, Glycerophosphocholine; PtdC/PEP, Phosphatidylcholine/phosphoenolpyruvate; NTP, Nucleotide triphosphate, NADPH, Nicotinamide adenine dinucleotide phosphate; G2P2, Grade 2 and stage 2

Figure 1. Illustrative spectra and histology of two AIH patients with different stages of liver disease.

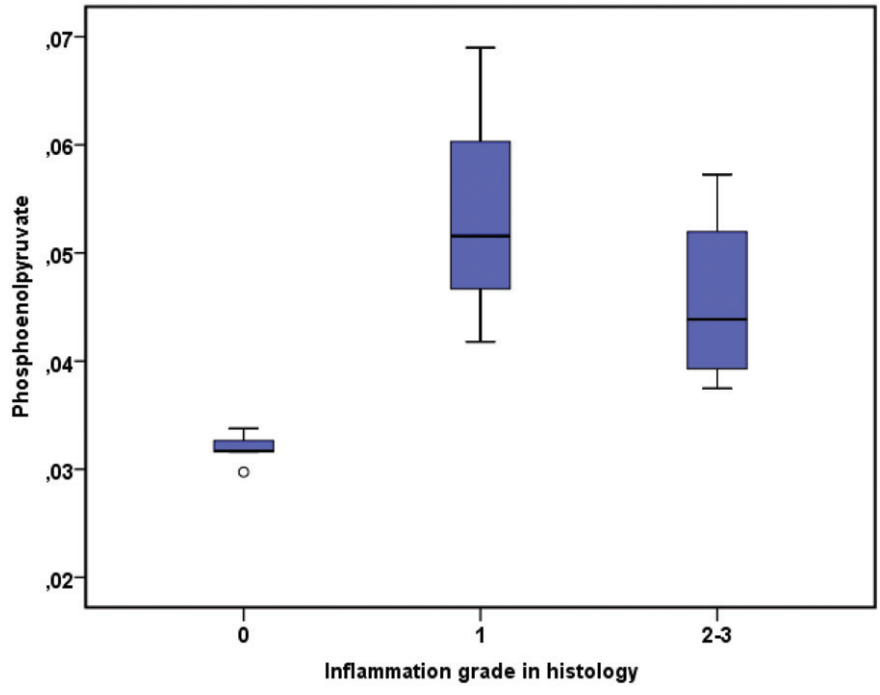


Figure 2. Phosphoenolpyruvate in three groups of inflammation.

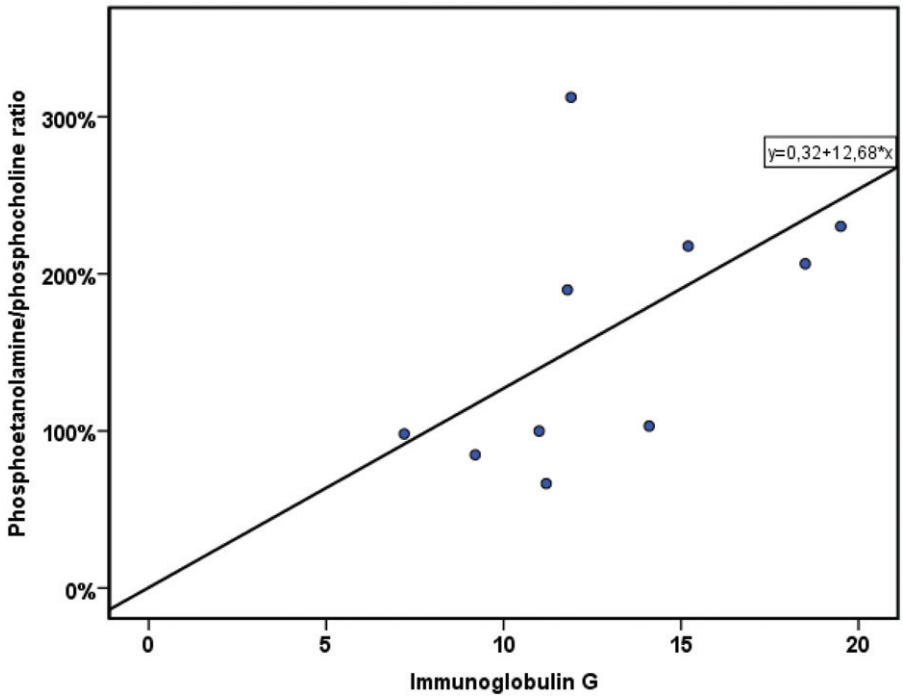


Figure 3. Phosphoetanolamine/phosphocholine ratio as a function of immunoglobulin G.

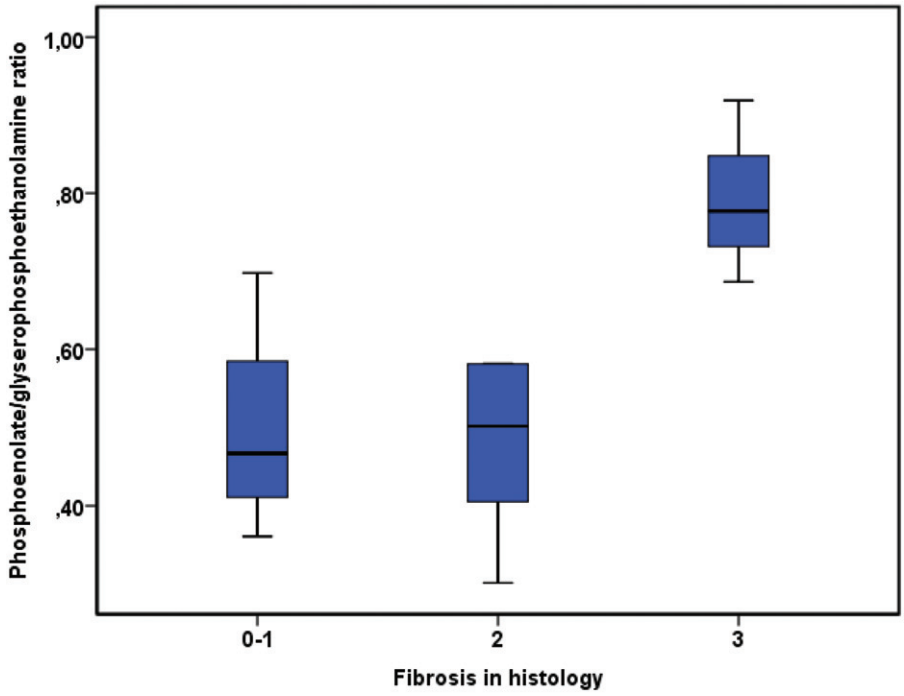


Figure 4. Phosphoenolate/glyserophosphoethanolamine ratio elevation is a sign of advanced fibrosis.

cirrhotic patients (F3) from patients with only mild or moderate fibrosis. MRS cannot separate these subcomponents at 1.5 T strength without proton decoupling. However, proton decoupling with a ^{31}P nucleus with at 3T, is now able to do so. Similar to a previous study [9], in our patients, changes in the phosphorus spectrum seem to develop rather late during fibrosis progression.

In our patients, transient elastography was not effective in the evaluation of fibrosis. It is possible that the study material was too small to find any correlations. A more probable explanation is that TE does not show fibrosis in AIH patients correctly. TE has not been validated in published studies in AIH patients. Also, inflammation has been shown to elevate measured TE values [14,15].

Patients in the study had variable degrees of inflammation in the liver categorised by histology, but laboratory values, especially ALT and AST levels, were normal or only mildly increased, and there was little variance throughout the study population to find any statistical correlation with the MRI spectrum. However, ALT levels correlated with histological

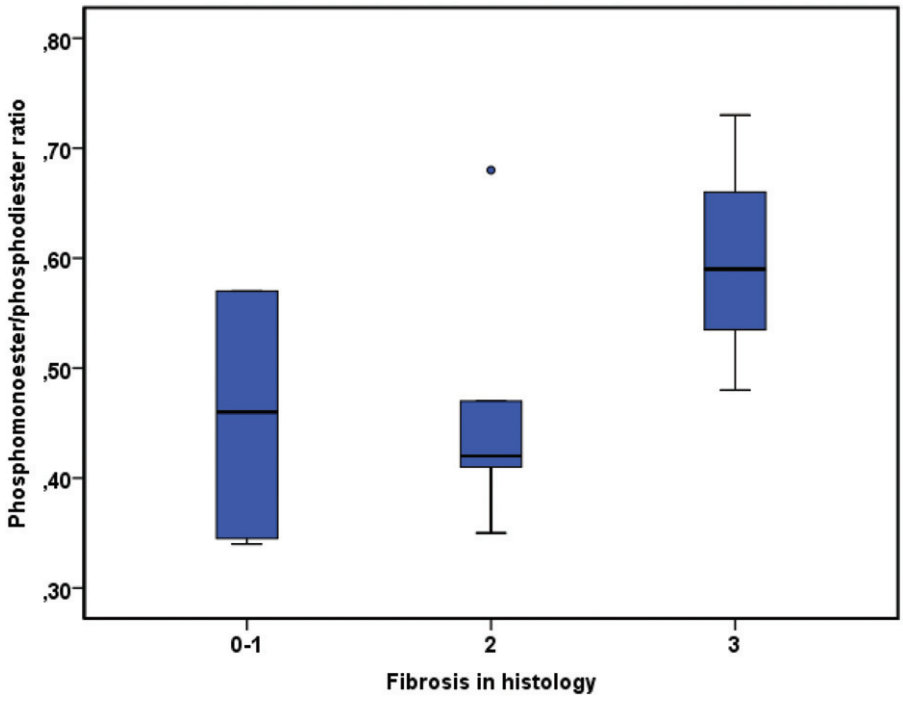


Figure 5. Total phosphomonoester/phosphodiester ratio rises along with fibrosis.

inflammation. IgG, as a marker of inflammation, correlated with ^{31}P MRS.

Liver biopsy is still needed for the diagnosis of AIH. ^{31}P MRS could be used in the follow-up of patients who refuse liver biopsy or in whom biopsy is contraindicated. ^{31}P MRS provides information of both inflammation and fibrosis, with different markers for either parameter.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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