Identification of Potential Metabolic Biomarkers in Predicting Esophageal Varices Needing Treatment in Patients With Liver Cirrhosis

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Research

Keywords: liver cirrhosis, varices needing treatment, hepatic metabolite, potential biomarker

Posted Date: February 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-217869/v1

Abstract

Background: The goal of this study was to determine the diagnostic performance of *in vivo* quantitative proton magnetic resonance spectroscopy (^{1}H-MRS) to identify the presence of esophageal varices needing treatment (VNT), as well as to investigate its correlation with clinical variables in patients with liver cirrhosis.

Methods: Forty cirrhotic patients without VNT showing negative red color sign, and 40 cirrhotic patients with VNT showing positive red color sign underwent laboratory tests, esophago-gastro-duodenoscopy, and ^{1}H-MRS with single-voxel localization in cirrhotic liver parenchyma.

Results: The levels of lactate+triglyceride (TG) and choline in cirrhotic patients with VNT were significantly higher than those of cirrhotic patients without VNT. Lactate+TG and choline levels were positively correlated with spleen diameter and negatively correlated with platelet count in the combined group of cirrhotic patients with and without VNT. In cirrhotic patients with VNT, older age, longer spleen diameter, lower platelet counts, and lower ratios of platelet count/spleen diameter were independently associated with an increase of lactate+TG and choline metabolites in the presence of esophageal VNT. Additionally, the area under the curve used to distinguish cirrhotic liver with VNT from cirrhotic liver without VNT was 1.00 (95% confidence interval [CI]: 0.95–1.00) for lactate+TG and 0.67 (95% CI 0.55–0.77) for choline.

Conclusions: Our study demonstrated that higher hepatic lactate+TG and choline levels in cirrhotic patients in conjunction with longer spleen diameter, lower platelet counts, and lower ratios of platelet count to spleen diameter were associated with the presence of esophageal VNT and the risk of developing variceal bleeding. Therefore, *in vivo*, ^{1}H-MRS may be an effective tool for diagnosing and predicting esophageal VNT in patients with liver cirrhosis.

Background

Liver cirrhosis is a severe and irreversible disease of the liver that is known to lead to metabolic hepatic failure and portal hypertension [1]. One of the major complications of portal hypertension is the development of esophageal varices (EVs), which have been shown to be correlated with the severity of chronic liver disease [2]. Although screening endoscopy for esophageal varices needing treatment (VNT) has been recommended for cirrhotic patients due to the high mortality rate associated with variceal bleeding [3], this method is invasive and frequently requires sedation, limiting its clinical utility as a screening test. Therefore, there is a clinical demand for noninvasive assessment, particularly in predicting active bleeding from EV.

In this context, there has been an increasing interest in discovering noninvasive parameters that might help detect the presence of EV or determine groups at high risk of developing variceal bleeding among cirrhotic patients, such as the diameter of the spleen or portal vein, platelet count, Child-Pugh score, prothrombin time assay, or a combination of measurements with multiple biomarkers, along with an

ultrasound or magnetic resonance elastography [4]. However, the consistent utility of these methods might not be supported when applied to other independent patient series [5]. As a robust noninvasive imaging technique, proton magnetic resonance spectroscopy (^{1}H-MRS) facilitates the identification of metabolite profiles *in vivo*, providing biochemical characterization of normal and abnormal tissues through quantitative measures of cellular metabolites [6]. The liver is one of the most metabolically diverse organs, as it is involved in many critical metabolic processes [7]. Clinically, metabolic information indicative of liver disease could help clinicians better characterize disease pathophysiology. Although cirrhosis represents a major change in the tissue, conventional approaches for assessing this disease rely on relatively few biomarkers and are not sufficient when providing global biochemical properties and definitive diagnoses [7]. Based on clinical observations, we hypothesized that quantitative ^{1}H-MRS for hepatic metabolite changes *in vivo* might serve as a potential tool when diagnosing and predicting esophageal VNT in cirrhotic patients.

Thus, the purpose of our study was to evaluate the diagnostic performance of ^{1}H-MRS and to investigate its correlation with clinical variables in cirrhotic patients with and without VNT with conventional endoscopy as the reference standard.

Methods

Patients

This prospective study was approved by the institutional review board of Chonnam National University Hospital and conformed to the ethical guidelines of the 2008 Declaration of Helsinki. All subjects provided written informed consent. To quantify significant differences in cellular metabolite levels between the groups with a level of significance of $\alpha = 0.05$ and $\beta = 0.2$ in this study, at least 40 subjects had to be included per group.

Between November 2017 and December 2020, 517 patients with cirrhosis confirmed by liver biopsy or imaging findings, such as morphologic changes in the liver or sequelae of portal hypertension [8], were referred to the department of radiology, where a hepatic ^{1}H-MRS was performed as part of routine imaging. Among them, patients who met the following criteria were excluded based on our protocol: presence of multiple or infiltrative hepatocellular carcinoma (HCC) with limited parenchymal evaluation (n = 155); fatty deposition (more than 5%) in the liver (n = 182). presence of a lipiodol-uptake lesion in the liver due to previous trans-arterial chemoembolization for HCC (n = 41); patients who did not undergo MRI examination due to poor respiratory condition (n = 25); patients who had not undergone esophago-gastro-duodenoscopy (EGD) within the previous 6 months (n = 19); and patients with a history of treatment for EV (n = 15).

An EGD was performed by gastroenterologists who had more than 5 years of experience in clinical practice. EVs were classified according to location, form, color, and the red color sign as follows [9]: (1) the location of the varices was classified into the upper, middle, or lower third of the esophagus or upper

stomach; (2) variceal forms included no observation of varices (F0), small and straight varices (F1), enlarged and tortuous varices (F2), or large and coil-shaped varices (F3); (3) the color of the varices was graded as white (Cw) or blue (Cb); and (4) the red color sign was considered to be present in cases with dilated, small vessels (red wale sign), and telangiectasias or cherry-red spots on the surface of the varices. Cirrhotic patients were divided into two groups. One group included patients without VNT (F0 or F1) showing a negative red color sign (no history of bleeding) and the other group included patients with VNT (F2 or F3) showing the presence of esophageal variceal bleeding (presence of red color signs, which are venules or red spots on the varices) [2, 10].

Consequently, this prospective study consisted of 40 cirrhotic patients without VNT including negative red color sign and 40 cirrhotic patients with VNT including positive red color sign. The causes of liver cirrhosis were hepatitis B (n = 51), hepatitis C (n = 17), both hepatitis B and C (n = 9), alcohol-related (n = 2), and idiopathic (n = 1).

MR imaging and spectroscopy

MR images and spectra were acquired using a 3-T MR scanner (Magnetom TimTrio, Siemens Healthcare, Erlangen, Germany). The T2-weighted images were acquired using a half-Fourier acquisition single-shot turbo spin-echo (HASTE) sequence with the following parameters: repetition time/echo time (TR/TE) = 2000/167 msec; field of view = 380 × 380 mm; matrix size = 320 × 256; number of excitations = 1; slice thickness = 5 mm; interslice gap = 0.5 mm; number of slices = 32 ~ 50; and scan time = 60 ~ 90 sec. To saturate fat and detect lactate and choline metaboles with a long T2 relaxation time, ^{1}H single-voxel spectra was acquired using a point-resolved spectroscopy sequence with the following parameters: TR/TE = 2000/288 msec, six acquisitions (within a single breath-hold), 2000 Hz spectral width, and 2 × 2 × 2 cm^3 voxel size, which was in the region of interest (ROI) voxel on the cirrhotic liver parenchyma [11–13]. Figure 1 shows representative localized voxel and ^{1}H-MR spectra with a long TE acquired from each group. MRS acquisition began when the level of water suppression was over 90% and the bandwidth was below 10 Hz after auto-shimming. To reduce respiratory motion artifacts, a compression belt was used while the patients' breathing was monitored, and MRS acquisition was stopped earlier or whenever the patient had to breathe again [11].

MR spectra analysis

MR spectra were analyzed using a Java-based MR user interface software (jMRUI version 4.0; developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium) following the procedure described in previous studies [11, 12]. Major hepatic metabolites were assigned from the MR spectra as follows: triglyceride (TG, 0.9 ppm), lactate + TG (1.3 ppm), and choline (3.2 ppm). All spectra were then fitted in the time domain using a non-linear least-squares algorithm in the jMRUI software package (AMARES). The signal intensity of all spectra was normalized to that of the residual water peak at 4.7 ppm as an internal reference.

Clinical measurements

To evaluate blood cell counts and liver function as blood-based biomarkers, blood samples were analyzed using a routine clinical chemistry analyzer. The spleen diameter was measured on MR images and was defined as the greatest longitudinal dimension at the level of the splenic hilum on the PACS monitor using electronic calipers [14]. Spleen diameter and platelet count have been proposed as predictive values that may be directly or indirectly associated with the presence of EV [15, 16]. Additionally, the platelet count/spleen diameter ratio is mostly used as the noninvasive predictor of EV [17].

Statistical analysis

Data were statistically evaluated using independent two-sample t-tests to compare clinical variables and analysis of covariance (ANCOVA) with adjustments for age and sex to compare the metabolic levels using statistical software (SPSS for Windows, version 18; SPSS Inc, Chicago, IL) between the two groups. The associations of metabolite levels with splenic diameter, platelet count, and the ratio of platelet count to splenic diameter were assessed using Pearson's correlation. Additionally, a multiple linear regression analysis was used to analyze the significant factors affecting cellular alterations of hepatic metabolism. To determine the diagnostic accuracy for the prediction of esophageal VNT, receiver operating characteristic (ROC) curves with the corresponding area under the curve (AUC), sensitivity, and specificity were calculated using MedCalc software (MedCalc Software, Mariakerke, Belgium).

Results

Comparison of clinical variables

Table 1 presents the characteristics of the two patient groups. When the study population was stratified according to the spleen diameter and platelet count, the cirrhotic patients with VNT showed a stepwise manner increase of spleen diameter with reduction of platelet count compared to the cirrhotic patients without VNT. Cirrhotic patients with VNT had lower platelet counts, lower levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), higher total bilirubin, lower serum albumin levels, longer spleen diameters, and lower ratios of platelet count to spleen diameter than those without VNT. Except for AST and ALT levels, these clinical variables were all significantly different between the two groups ($P < 0.05$).

Comparison of the hepatic metabolite levels for lactate + TG, choline, and TG

Cellular metabolites showed distinct patterns of hepatic metabolism between the groups (Fig. 2). The levels of lactate + TG and choline in cirrhotic patients with VNT were significantly higher compared to those of cirrhotic patients without VNT ($P < 0.05$), while the level of TG at 0.9 ppm was similar between the two patient groups.

Correlation of the hepatic metabolite levels with spleen diameter and platelet count

Pearson's correlation coefficients were calculated to evaluate the linear relationship between the clinical measurements and quantitative concentrations of ^{1}H-MRS metabolites. Spleen diameter showed a positive significant correlation with lactate + TG and choline in cirrhotic patients without VNT (lactate + TG: $R^2 = 0.908$, $P < 0.001$; choline: $R^2 = 0.842$, $P < 0.001$) and with VNT (lactate + TG: $R^2 = 0.952$, $P < 0.001$; choline: $R^2 = 0.846$, $P < 0.001$), as well as in the combined group of cirrhotic patients without/with VNT (lactate + TG: $R^2 = 0.893$, $P < 0.001$; choline: $R^2 = 0.849$, $P < 0.001$) (Fig. 3a and 3b). On the contrary, platelet count was negatively correlated with lactate + TG and choline in cirrhotic patients without VNT (lactate + TG: $R^2 = -0.181$, $P = 0.263$; choline: $R^2 = -0.230$, $P = 0.154$) and with VNT (lactate + TG: $R^2 = -0.217$, $P = 0.179$; choline: $R^2 = -0.207$, $P = 0.199$), as well as in the combined group of cirrhotic patients without/with VNT (lactate + TG: $R^2 = -0.518$, $P < 0.001$; choline: $R^2 = -0.336$, $P = 0.002$) (Fig. 3b and 3d).

Independent predictive factors for metabolite levels in cirrhotic liver with/without VNT

To evaluate the role of the different independent factors affecting metabolite levels, a multiple linear regression analysis was performed. In cirrhotic patients with VNT, older age, longer spleen diameter, lower platelet counts, and lower ratios of platelet count/spleen diameter were significantly and independently associated with an increase in both lactate + TG and choline metabolites in the presence of esophageal VNT (except age, which was not significantly associated with choline), which were shown in Table 2.

Diagnostic performance of ^{1}H-MRS in identifying the risk of varices needing treatment

The AUC to distinguish cirrhotic liver without VNT from cirrhotic liver with VNT was 1.00 (95% CI 0.95–1.00; $P < 0.001$) for lactate + TG and 0.67 (95% CI 0.55–0.77; $P = 0.005$) for choline. The diagnostic accuracy had a sensitivity and specificity of 98% and 100%, respectively, at a cut-off value of 0.61 for lactate + TG, and a sensitivity and specificity of 58% and 73%, respectively, at a cut-off value of 0.09 for choline (Fig. 4).

Discussion

The current study was the first to identify metabolic biomarkers that could be used to predict esophageal VNT in cirrhotic patients using ^{1}H-MR spectroscopy in conjunction with their relationships with the clinical variables of spleen diameter, platelet count, and the ratio of platelet count to spleen diameter. We found that hepatic lactate + TG and choline content measured using ^{1}H-MRS strongly correlated with esophageal variceal bleeding, which is one of the complications of liver cirrhosis. In multivariate analysis,

the ^{1}H-MR spectroscopic signal intensities of both lactate + TG and choline were associated with the potential risk factors of age, spleen diameter, platelet count, and the ratio of platelet count to spleen diameter in cirrhotic patients with and without VNT. Our findings, therefore, demonstrate the value of ^{1}H-MRS as a diagnostic tool *in vivo* to evaluate possible esophageal variceal bleeding as well as provide valuable information for understanding the cellular alterations in hepatic metabolism related to progressive EV in cirrhotic liver disease. If ^{1}H-MRS is included in routine MR imaging protocols for the screening and surveillance of VNT or management of liver cirrhosis, it could provide a better targeted, more cost-effective strategy of diagnostic and therapeutic endoscopy in cirrhotic patients.

As an interesting feature of hepatic metabolite differences, the lactate + TG level was significantly higher in cirrhotic patients with VNT than in cirrhotic patients without VNT, indicating that esophageal variceal bleeding induced by cirrhosis could be associated with elevated lactate levels in conjunction with progressive hepatic dysfunction. Although the lactate signal at 1.3 ppm overlapped with large TG resonance in the liver and could not be separated from TG peak in our spectra [18], we assumed that lactate contributed to differential patterns of metabolite changes between cirrhotic liver with and without VNT, similar to previous studies [11, 12] of *in vivo* ^{1}H-MRS with a long TE, which have demonstrated a disease-specific lactate + TG signal intensity. The liver plays an important role in modulating cellular homeostatic pathways such as the metabolism of organic acid anions including lactate and amino acids [19, 20]. However, in chronic liver diseases, lactate clearance is impaired due to a reduction of functional hepatocytes, while healthy liver has a major functional reservoir of metabolizing lactate [19, 20]. Several studies [19, 21, 22] using hyperpolarized ^{13}C-MRS with liver disease models reported that the lactate-to-pyruvate ratio could be used as a metabolic marker indicating an inadequate supply of oxygen and glucose, suggesting that higher lactate levels compared with normal controls are induced by hypoxic and hypermetabolic states under anaerobic glycolysis in pathological states. More importantly, our study demonstrated that hepatic metabolite values for lactate + TG were associated with the presence of esophageal VNT. However, the relationships between the metabolic parameters and the endoscopic findings of EVs have been poorly investigated. A recent study [23] reported that the presence of EVs and the risk of developing variceal bleeding correlated with the severity of cirrhosis. Enomoto et al. [24] reported the association of the branched-chain amino acids to tyrosine ratio (BTR) with the severity of liver fibrosis and EVs in patients with hepatitis C virus-positive chronic liver disease. In that study, a decreased value of BTR was found to be associated with the progression of liver fibrosis and severity of varices.

Given the results of our correlation analysis, spleen diameter was positively correlated with lactate + TG level in both cirrhotic patients without VNT and with VNT, as well as in the combined group of cirrhotic patients without/with VNT. These positive correlations indicate that any increase in spleen diameter was associated with higher levels of hepatic metabolites, and therefore with impaired metabolic status associated with the progression of EV as well as the presence of esophageal VNT. Additionally, in the combined group of cirrhotic patients without/with VNT, platelet count was negatively correlated with lactate + TG level, suggesting that higher levels of hepatic metabolites could be related to the severity of

impaired metabolic status, which might be associated with the development of esophageal VNT in patients with cirrhotic liver. Therefore, the increased lactate + TG level might be a noninvasive metabolic biomarker reflecting disease-specific metabolism in the hepatic pathophysiology to predict the occurrence of esophageal VNT or to help determine an effective follow-up strategy.

Our results also revealed that in cirrhotic patients with VNT, the choline metabolite levels were significantly higher than those in cirrhotic patients without VNT. Choline is an important constituent of the cell membrane in phospholipid metabolism and is an active marker of cellular proliferation, indicating that an elevated choline peak may be associated with increased membrane phospholipid biosynthesis [25]. Given the altered level of choline-containing compounds including choline, phosphocholine, glycerophosphocholine, and taurine in cirrhotic liver, a previous study [7] reported that alterations of choline-containing compounds may suggest abnormal synthesis or degradation of cell membranes, or may just be related to the mobility of the choline-containing compound parts. In our correlation analysis, choline levels were positively correlated with spleen diameter in both cirrhotic patients without VNT and with VNT, as well as in the combined group of cirrhotic patients without/with VNT. Also, these levels were negatively correlated with platelet count in the combined group of cirrhotic patients without/with VNT. These findings may suggest that in cirrhotic liver with VNT and/or the risk of developing variceal bleeding, the increase in choline as a cellular biomarker reflects the continual destruction of functional hepatic tissue and hepatocytes, which could eventually result in hepatic failure.

In our study, when evaluating the effect of age, spleen diameter, platelet count, and ratio of platelet count/spleen diameter on metabolite alterations in cirrhotic patients with VNT, significant risk factors that were associated with altered hepatic metabolism and indicated the presence of VNT in cirrhotic liver included older age, longer spleen diameter, lower platelet count, and lower ratio of platelet count to spleen diameter. Meanwhile, when a cut-off value of 0.61 for lactate + TG and 0.09 for choline was used, the ROC analysis revealed 100% specificity for lactate + TG and 73% specificity for choline in differentiating cirrhotic patients with esophageal VNT from cirrhotic patients without VNT. This result suggests that ^{1}H-MR spectroscopy could be effectively used to stratify cirrhotic patients in terms of the risk of esophageal variceal bleeding.

Our study has several limitations. First, we did not apply multi-voxel scanning when acquiring *in vivo* quantitative ^{1}H-MRS because such a technique requires much longer scanning and breath-holding times. Second, the number of patients in our study was relatively small, therefore a larger number of patients in a prospective study may be necessary to verify our results. Third, as mentioned in the discussion, the lactate signal at 1.3 ppm could not be separated from the TG peak. Thus, utilizing high-field MRI equipment, spectrally overlapped lactate and TG signals at 1.3 ppm should be separated and quantified in future studies.

Conclusions

In this study, we utilized ^{1}H-MRS to quantify hepatic lactate + TG and choline levels in cirrhotic patients, demonstrating that higher values of these metabolites were associated with the presence of esophageal VNT and the risk of developing variceal bleeding. Further, those metabolic changes were correlated with longer spleen diameters, lower platelet counts, and lower ratios of platelet count to spleen diameter.

Declarations

Acknowledgements

None.

Authors' contributions

CMM and SSS designed the study; CMM performed the majority of experiments; CMM and SSM contributed to the analysis and interpretation of results; CMM wrote the first draft of the manuscript; SSM has approved the final manuscript and completed manuscript; also, all authors agree with the content of the manuscript.

Funding

This work was supported by the fund from the National Research Foundation of Korea (2018R1D1A3B07043473).

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Tables

Table 1. Characteristics of cirrhotic patients with and without esophageal varices needing treatment (VNT)

Variables	Patients without VNT (n = 40)	Patients with VNT (n = 40)	P-value
Mean age (y)	59.00 ± 21.45	63.00 ± 22.90	0.02
No. of men (%)	20 (50.0)	20 (50.0)	-
Aspartate aminotransferase (U/L)	61.89 ± 40.12	40.00 ± 10.96	0.23
Alanine aminotransferase (U/L)	55.00 ± 35.41	21.67 ± 6.25	0.48
Total bilirubin (mg/dL)	1.17 ± 0.43	1.39 ± 0.44	0.00
Albumin (g/dL)	4.13 ± 1.51	3.30 ± 1.01	0.00
Spleen diameter (cm)	11.08 ± 1.24	13.24 ± 1.99	0.00
Platelet count (×1000/μL)	183.43 ± 84.24	103.33 ± 38.58	0.00
Platelet count/spleen diameter ratio	16.98 ± 8.84	8.14 ± 3.83	0.00

Independent two-sample *t*-test was used for statistical analysis.

Table 2. Multiple linear regression analysis for the independent effect of each variable associated with metabolite changes in the two groups

Variables	Lactate+TG			Choline		
	β coefficient	P-value	95% CI	β coefficient	P-value	95% CI
Cirrhotic patients without VNT						
Age	0.003	**0.005**	0.001 – 0.005	0.001	0.384	-0.001 – 0.002
Spleen size (cm)	0.020	**0.034**	0.002 – 0.038	0.012	**0.035**	0.001 – 0.024
Platelet count (×1000/μL)	0.000	0.499	0.000 – 0.001	0.000	0.613	0.000 – 0.001
Platelet count/spleen size ratio	-0.002	0.616	-0.010 – 0.006	-0.001	0.625	-0.006 – 0.004
Cirrhotic patients with VNT						
Age	0.003	**0.013**	0.001 – 0.005	0.002	0.123	0.000 – 0.004
Spleen size (cm)	0.031	**0.000**	0.020 – 0.041	0.015	**0.017**	0.003 – 0.026
Platelet count (×1000/μL)	-0.001	**0.004**	-0.002 – 0.000	-0.001	**0.036**	-0.001 – 0.000
Platelet count/spleen size ratio	0.013	**0.001**	0.006 – 0.021	0.009	**0.027**	0.001 – 0.018

Bolded values are significant at $P < 0.05$.

CI, confidence interval; TG, triglycerides; VNT, varices needing treatment

Figures

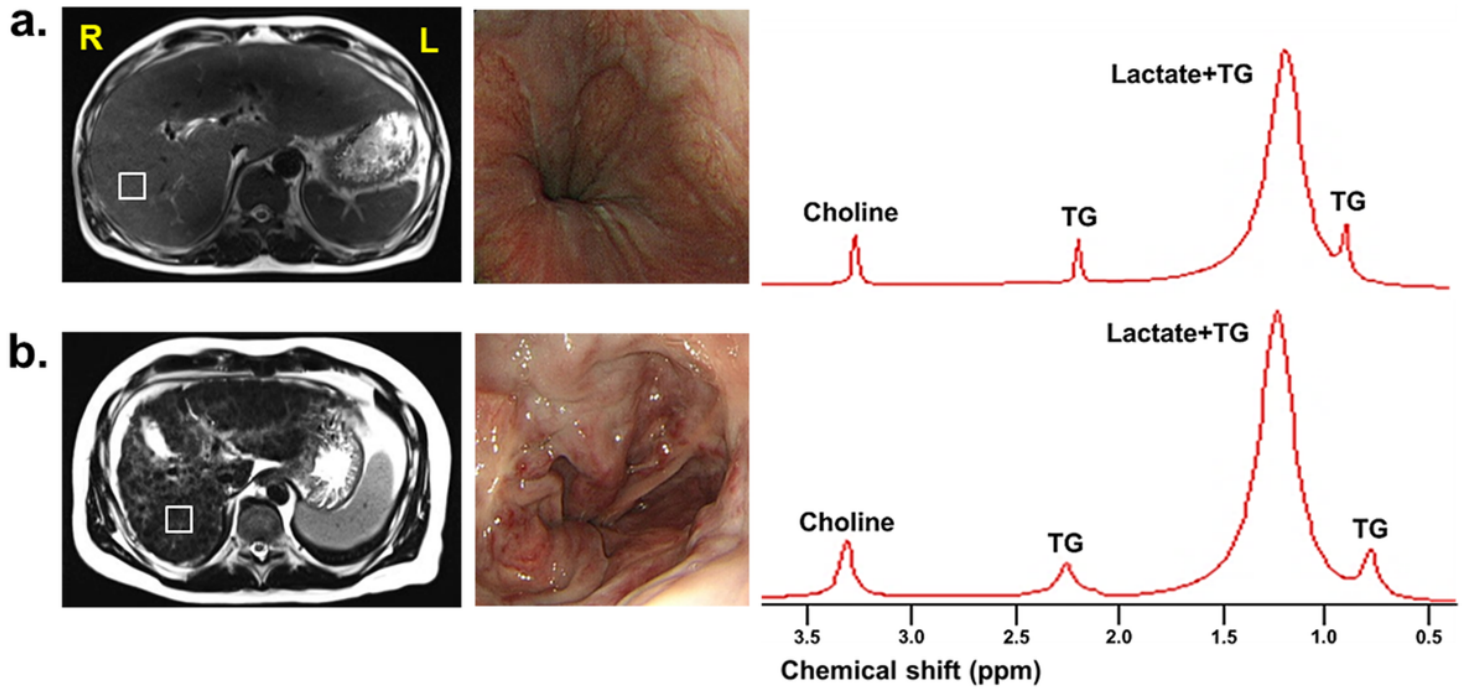

Figure 1

Axial MRI (left) with endoscopic image (middle) and representative MR spectra (right) acquired from a voxel (white square on axial MR image) localized in the right lobe of the liver in a cirrhotic patient without VNT (a), and a cirrhotic patient with VNT (b). L, left; R, right; TG, triglycerides; VNT, varices needing treatment

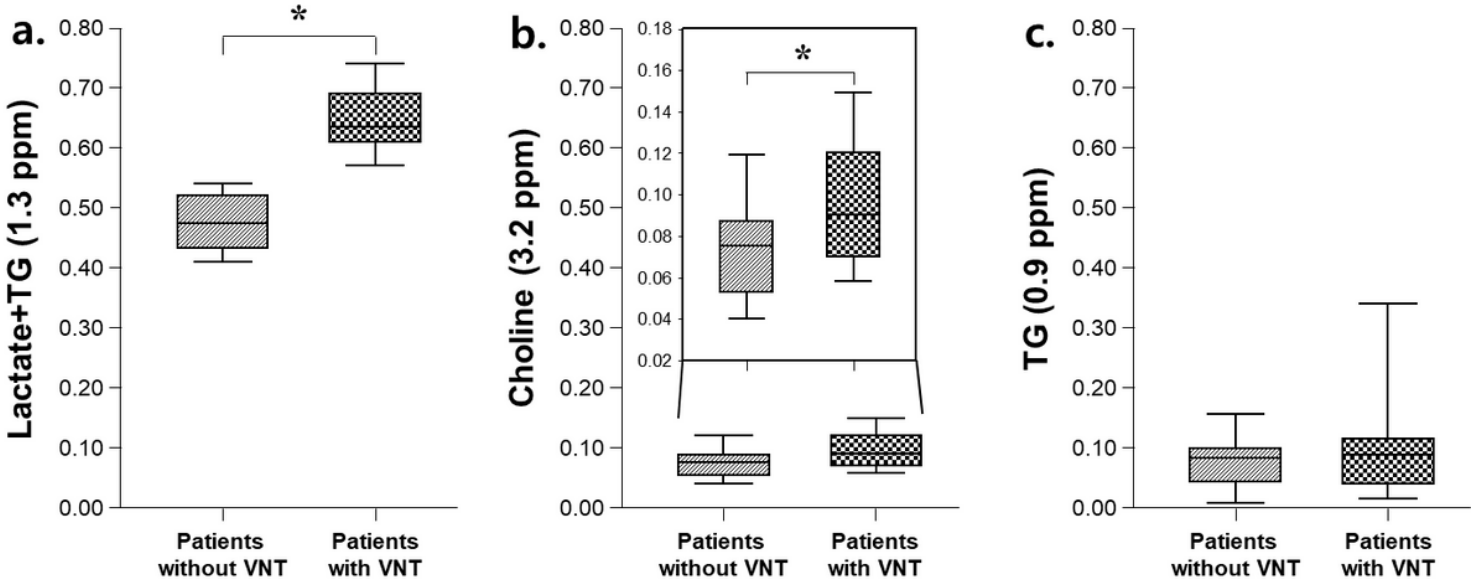

Figure 2

Quantitative comparison of the cellular metabolites for (a) lactate+TG (1.3 ppm), (b) choline (3.2 ppm), and (c) TG (0.9 ppm) in cirrhotic patients with and without VNT. The differential metabolite levels

between the two groups were analyzed using an analysis of covariance (ANCOVA) with adjustments for age and sex at P < 0.05. *significant difference between cirrhotic patients with and without VNT TG, triglyceride; VNT, varices needing treatment

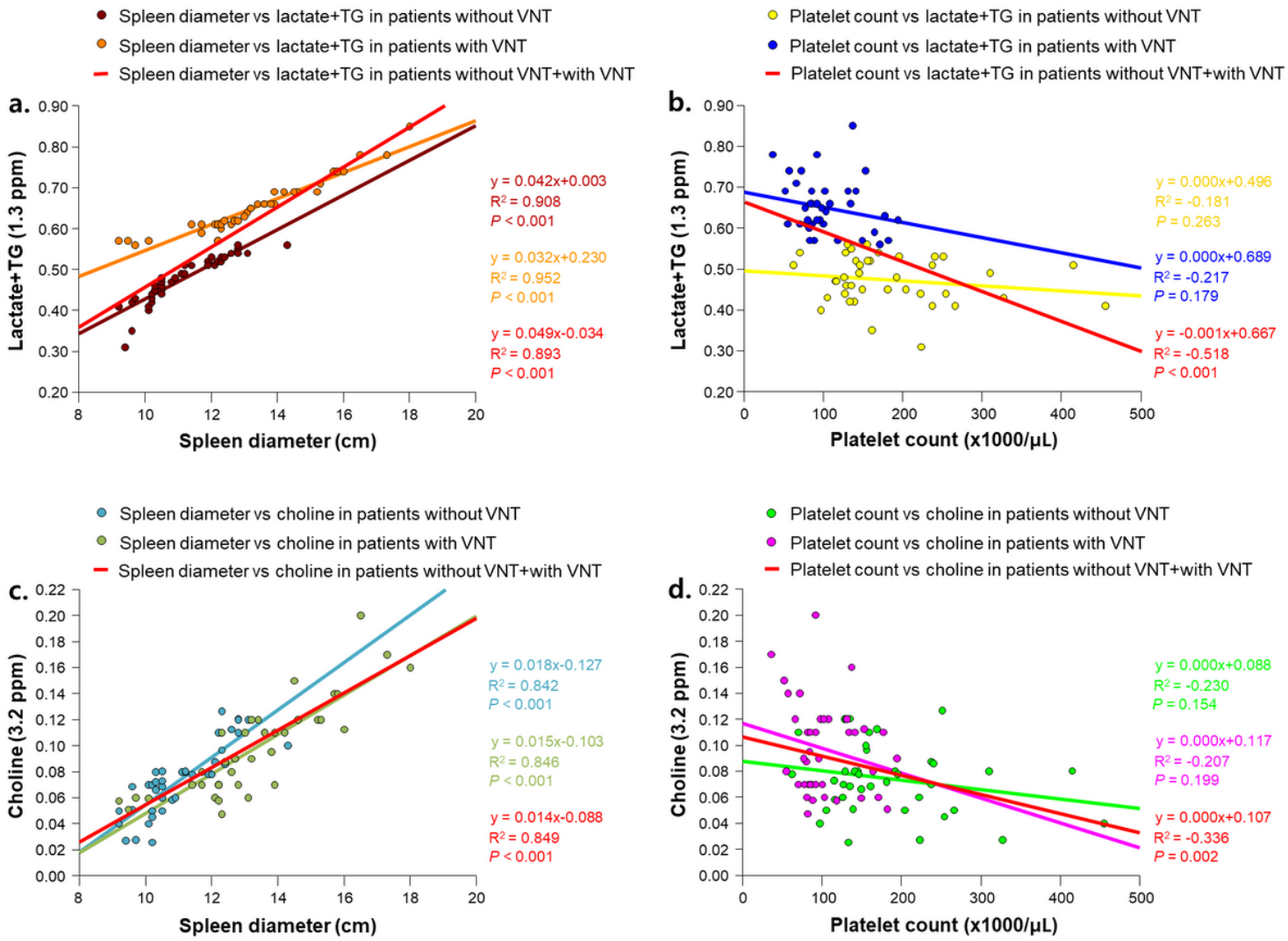

Figure 3

Correlations of lactate+TG and choline metabolites with spleen diameter and platelet count in cirrhotic patients with and without VNT, and in the combined group of cirrhotic patients without/with VNT.

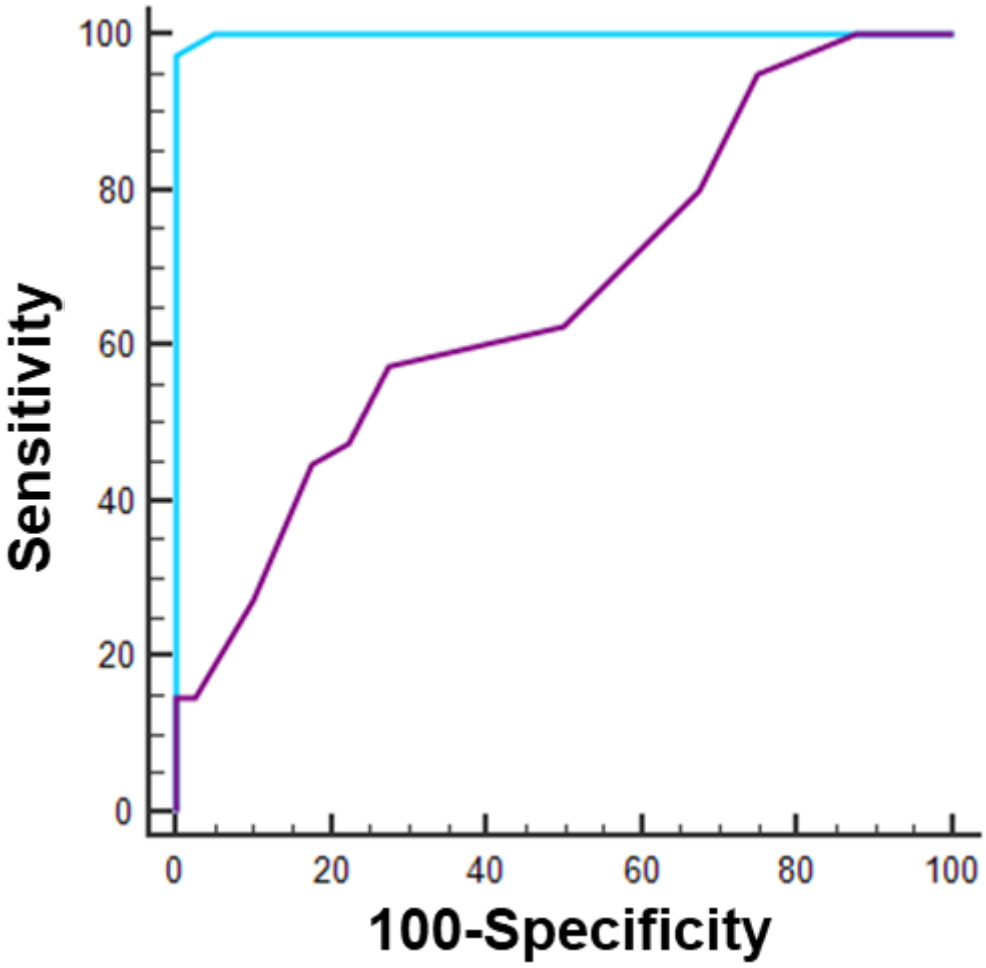

Figure 4

Diagnostic performance of in vivo 1H-MRS in assessing the presence of esophageal VNT in patients with cirrhotic liver. ROC curves of lactate+TG (light blue) and choline (violet) metabolites for the differentiation between cirrhotic liver with and without VNT.