Prediction of oesophageal varices in patients with primary biliary cirrhosis by non-invasive markers

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Abstract

Introduction: Preliminary data suggested that non-invasive methods could be useful to assess presence of oesophageal varices (OV) in liver cirrhosis. The primary objectives were to investigate non-invasive markers for diagnosing and grading OV in patients with primary biliary cirrhosis.
Material and methods: This study included a total of 106 consecutive treatment-naive patients with primary biliary cirrhosis (PBC). Results of physical examination, blood tests, and abdominal ultrasound scan (USS) were measured. Performance of non-invasive markers for OV was expressed as sensitivity, specificity, positive, and negative predictive values (PPV, NPV), accuracy, and area under the curve (AUC).
Results: Oesophageal varices were found in 54 (50.9%) and large OV in 28 of the 106 patients. Variables found to differ significantly between patients with any grade or large and without OV included increased spleen length, increased portal vein diameter, low platelet count, and low levels of albumin or low γ-glutamyltranspeptidase (γ-GTP) values. Area under the receiver operating characteristic curve showed that spleen length (cutoff = 156.0) had AUC 0.753 (95% CI: 0.657–0.849), and high NPV (82.1%) to exclude any grade OV. Large OV could be excluded with NPV 70.6% by spleen length.
Conclusions: Predictive risk factors that use readily available laboratory results and ultrasound scan results may reliably identify esophageal varices in patients with PBC.

Key words: primary biliary cirrhosis, oesophageal varices, non-invasive serum markers.

Introduction

Primary biliary cirrhosis (PBC) is a chronic cholestatic disease characterized by the progressive destruction of small septal and interlobular bile ducts [1].

Chronic cholestasis may result in hepatic fibrosis and portal hypertension. Portal hypertension is a frequent complication of cirrhosis, and a major complication of portal hypertension is the development of oesophageal varices (OV), which may occur in up to 90% of patients with liver cirrhosis [2].

Development of OV is an ominous sign that is observed in approximately one-third of patients with PBC during extended follow-up. Almost

40% of these patients experience one or more episodes of variceal bleeding within 3 years of developing OV [3]. The recent Baveno IV Consensus Conference on portal hypertension recommended that all cirrhotic patients should be screened for presence of OV. Endoscopy should be performed at 2–3 years intervals in patients without varices and at 1–2 years intervals in patients with small varices [4].

However, a generalized screening program of periodical upper endoscopy in PBC patients may lead to high costs and low compliance since the procedure is invasive and may be poorly accepted by the patients if required repeatedly. For these reasons, an increasing number of studies have been focused on developing novel non-invasive approaches to assessing oesophageal varices. Several studies [5–10] have addressed the issue of identifying patients with varices by non-invasive or minimally invasive methods, with the aim of avoiding endoscopy in those at low risk of having varices. However, because of poor validation on an extensive scale or the inadequate accuracy of most studied markers, none of them can be recommended in daily clinical practice.

This study therefore aimed to investigate the diagnostic accuracy of a series of non-invasive markers, readily available in clinical practice, in PBC patients for presence of clinically relevant OV.

Material and methods

Patients

We included patients who underwent upper endoscopy in Beijing 302 Hospital between January 2008 and September 2010. In total 106 patients with PBC were included in the retrospective study. The diagnosis of PBC required: (1) the presence of chronic cholestatic liver disease of at least 6 months' duration; (2) serum alkaline phosphatase (ALP) level at least 1.5 times the upper limit of normal; (3) antimitochondrial antibody positivity; (4) absence of biliary obstruction by ultrasound, computed tomography, or cholangiography; and (5) a liver biopsy in the previous 3 months compatible with or diagnostic of PBC.

Exclusion criteria: Patients with a history of use of β-blockers, previous portal-systemic shunt surgery or transjugular-intrahepatic-portal-systemic shunt, endoscopic ligation, or sclerotherapy of varices or upper gastrointestinal bleeding excluded. Patients with malignancy or who had undergone organ transplantation were also excluded. All patients gave their informed consent to be included in the study.

Patients were evaluated by upper endoscopy for the assessment of esophageal varices. Routine biological parameters were recorded for every patient according to the follow-up protocol of each condition. Routine practice at our hospital is to measure spleen length and portal vein diameter during abdominal ultrasound scan (USS).

Upper endoscopy

Upper tract endoscopy was performed by experienced operators who were unaware of the results of non-invasive markers, apart from those necessary to safely perform the endoscopy (platelet count and prothrombin time). All operators applied the following classification of OV: (1) grade 0: absent; (2) grade 1: small, straight esophageal varices; (3) grade 2: enlarged, tortuous varices occupying less than one third of the lumen; and (4) grade 3: large, coil-shaped esophageal varices occupying more than one third of the lumen. According to the criteria proposed at the Baveno I Consensus Conference, patients were considered as carrying large OV when grade was ≥ 2 [11].

Non-invasive markers

Blood work and abdominal USS results were obtained from the test performed closest in time to the esophagogastroduodenoscopy (EGD) and always within 2 months. The Mayo risk score was calculated for each patient at the time of his or her EGD. This score was defined as follows: 0.871 × Ln (bilirubin in mg/dl) + (–) 2.53 × Ln (albumin in g/dl) + 0.039 × age in years + 2.83 Ln (prothrombin time in seconds) + 0.859 × edema [12]. In the Child-Pugh score for primary biliary cirrhosis, the bilirubin references are changed to reflect the fact that these diseases feature high conjugated bilirubin levels. The upper limit for 1 point is 68 µmol/l (4 mg/dl) and the upper limit for 2 points is 170 µmol/l (10 mg/dl). Since the non-invasive markers considered were originally generated as a surrogate measurement of OV, new cutoffs for OV and large OV were defined according to area under the curve (AUC) analysis.

Statistical analysis

Database management and all statistical analyses were performed using SPSS for Windows (ver. 17.0, SPSS, Chicago, IL, USA). Descriptive results were expressed as mean ± SD (standard deviation) or number (percentage) of patients with a condition. The *t*-test or non-parametric Mann-Whitney test was used to compare quantitative data, and the χ^2 test was applied for comparison of frequency data. All tests were two-tailed and *p*-values < 0.05 were considered significant. Multivariate analysis was performed by means of a stepwise logistic-regression procedure on parameters which were significantly different in univariate analysis in order to determine the variables independently associated

with presence of OV. To assess associations between OV and tested variables, odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated using simple logistic-regression analysis. Performance of the non-invasive methods considered was expressed as sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), accuracy, and positive and negative likelihood ratio (LR). Sensitivity, specificity, PPV, NPV, and accuracy were expressed as percentages. The diagnostic value of the non-invasive methods was expressed using the AUC and its corresponding 95% CI. The AUC analysis was also used to determine for each non-invasive marker the optimized cutoff to determine presence of any grade OV and of large OV.

Table I. Clinical and demographic characteristics of study population (N = 106)

Characteristics	Value
Age [years]:	
Mean ± SD	53.8 ±9.2
Range	31–74
Male sex, *n* (%)	7 (6.6)
Platelet count [10^9/l]	126.3 ±72.8
White cell count [10^9/l]	4.1 ±1.9
Bilirubin [μmol/l]	44.6 ±43.3
AST [IU/l]	102.7 ±56.7
ALT [IU/l]	69.2 ±44.3
Albumin [g/l]	33.8 ±5.5
INR	1.1 ±0.2
ALP [IU/l]	273.0 ±196.3
γ-GTP [IU/l]	245.3 ±324.6
Cholesterol [mmol/l]	4.9 ±2.7
Mayo risk score	2.9 ±1.7
Child-Pugh class:	
A (scores 5–6)	55 (51.9%)
B (scores 7–9)	47 (44.3%)
C (scores 10–15)	4 (3.8%)
Oesophageal varices, *n* (%):	
Grade 0	52 (49.1)
Grade 1	26 (24.5)
Grade 2	21 (19.8)
Grade 3	7 (6.6)

Values are mean ± standard deviation (SD) or n (%). AST – aspartate aminotransferase, ALT – alanine aminotransferase, INR – international normalized ratio, ALP – alkaline phosphatase, γ-GTP – γ-glutamyltranspeptidase.

Results

Patients' characteristics

There were 7 male and 99 female patients with a mean age of 53.8 ±9.2 years. The main demographic, laboratory, and endoscopic features of the patients are summarized in Table I. Overall, any grade OV were present in 54 (50.9%) patients, of whom 26 (24.5%) had grade I OV, 21 (19.8%) had grade II OV, and 7 (6.6%) had grade III OV. Large OV were present in 28 (26.4%) patients.

Factors associated with the presence of OV in univariate analysis

Among all the variables listed in Table II, low levels of albumin, low white cell count, low platelet count, low γ-glutamyltranspeptidase (γ-GTP) values, high levels of bilirubin, high spleen length values, and high portal vein diameter values showed a significant association with presence of any grade OV. Factors associated with large OV were: low levels of albumin, low levels of cholesterol, low white cell count, low platelet count, low γ-GGT values, high spleen length values, and high portal vein diameter values.

Factors associated with the presence of OV in multivariate analysis

Tables III and IV show the results of multivariate analysis; stepwise logistic regression analysis was performed to identify factors associated with any grade oesophageal varices and large oesophageal varices in PBC patients.

High spleen length values, and high portal vein diameter values and low levels of albumin were significantly associated with any grade oesophageal varices. High spleen length values, and high portal vein diameter values and low γ-GTP values were significantly associated with large oesophageal varices. The following model was generated when continuous values of prediction factors were used to create a prediction formula: Y = –3.425 + 0.453 * portal vein diameter (PVD) + 0.029 * SL – 0.164 * albumin (ALB) with any grade OV and Y = –8.322 + 0.603 * PVT + 0.034 * SL – 0.014 * γ-GTP with large OV in PBC patients.

Performance of factors for detection of any grade OV

The diagnostic performance of simple non-invasive markers for detection of any grade OV is shown in Table V. The optimized cutoffs for each non-invasive marker, as resulted from AUC analysis, are also shown.

Table II. Univariate analysis of factors associated with the presence of oesophageal varices

Factors	No OV (*N* = 52)	Any grade OV (*N* = 54)	Large OV (*N* = 28)	*P*-value	*P*-value
Age [years]	52.5 ±9.7	55.0 ±8.6	54.6 ±7.2	0.177	0.201
Male, *n* (%)	3 (5.8)	4 (7.4)	0 (0.0)	0.651	0.272
ALT [IU/l]	87.0 ±146.1	51.8 ±34.5	48.1 ±26.8	0.107	0.193
AST [IU/l]	123.7 ±148.6	82.1 ±48.2	78.1 ±51.3	0.069	0.143
ALP [IU/l]	262.2 ±202.4	283.7 ±191.8	238.7 ±185.2	0.597	0.631
γ-GTP [IU/l]	335.5 ±420.9	157.1 ±146.0	97.6 ±70.3	0.007	0.000
Albumin [g/l]	36.1 ±6.0	30.8 ±4.6	31.3 ±4.8	< 0.001	0.000
Bilirubin [µmol/l]	30.2 ±27.4	68.0 ±87.6	52.1 ±70.2	0.006	0.062
INR	1.1 ±0.2	1.2 ±0.7	1.1 ±0.1	0.187	0.435
Cholesterol [mmol/l]	5.3 ±2.0	4.6 ±3.3	4.0 ±1.2	0.205	0.004
PLT [10^9/l]	149.2 ±75.7	106.2 ±64.9	101.1 ±53.4	0.004	0.006
WBC [10^9/l]	4.8 ±1.9	3.5 ±1.7	3.2 ±1.4	0.001	0.000
PVD [cm]	11.4 ±1.1	12.5 ±1.5	12.7 ±1.3	< 0.001	0.000
SL [cm]	126.1 ±31.4	154.0 ±23.1	151.1 ±23.0	< 0.001	0.001
Mayo risk score	2.7 ±0.8	2.8 ±1.0	3.0 ±0.8	0.180	0.142

Results given as mean ± SD (range) or n (%). WBC – white blood cell, PVD – portal vein diameter, SL – spleen length, ALT – alanine aminotransferase, AST – aspartate aminotransferase, INR – international normalized ratio, ALP – alkaline phosphatase, γ-GTP – γ-glutamyl-transpeptidase. P-value between "No OV" and "Any grade OV" in the first column, between "No OV" and "Large grade OV" in the second column.

Table III. Multivariate analysis of factors associated with any grade oesophageal varices

Factors	Odds ratio (range)	*P*-value
PVD	1.573 (1.052–2.352)	0.027
SL	1.030 (1.007–1.052)	0.009
Albumin	0.849 (0.754–0.955)	0.007

PVD – portal vein diameter, SL – spleen length.

Table IV. Multivariate analysis of factors associated with large oesophageal varices

Factors	Odds ratio (range)	*P*-value
PVD	1.827 (0.977–3.420)	0.049
SL	1.035 (1.004–1.067)	0.027
γ-GTP	0.986 (0.976–0.997)	0.010

PVD – portal vein diameter, SL – spleen length, γ-GTP – γ-glutamyl-transpeptidase.

Table V. Performance of factors for prediction of any grade oesophageal varices

Variable	PVD	SL	Albumin
Cut-off	11.8	156.0	34.5
Sensitivity (%)	79.2	47.9	83.3
Specificity (%)	61.7	89.4	66.0
PPV (%)	72.5	62.7	28.6
NPV (%)	67.3	82.1	20.5
LR+	2.07	4.50	2.45
LR–	0.33	0.58	0.25
AUC	0.731	0.753	0.789
95% CI	0.629–0.833	0.657–0.849	0.696–0.882

PPV – positive predictive value, NPV – negative predictive value, LR – likelihood ratio, AUC – area under the curve, CI – confidence interval, PVD – portal vein diameter, SL – spleen length.

Table VI. Performance of factors for prediction of large oesophageal varices

Variable	PVD	SL	γ-GTP
Cut-off	11.9	156.0	163.5
Sensitivity (%)	84.2	48.0	84.0
Specificity (%)	61.7	89.4	61.7
PPV (%)	88.2	76.4	46.2
NPV (%)	53.8	70.6	12.1
LR+	2.19	4.51	2.19
LR–	0.26	0.58	0.26
AUC	0.775	0.736	0.753
95% CI	0.666–0.884	0.620–0.852	0.642–0.863

PPV – positive predictive value, NPV – negative predictive value, LR – likelihood ratio, AUC – area under the curve, CI – confidence interval, PVD – portal vein diameter, SL – spleen length, γ-GTP – γ-glutamyltranspeptidase.

Albumin and spleen length showed the best performance, as indicated by an AUC of 0.789 and 0.753, respectively. Spleen length presented with the highest positive LR (4.50) and albumin with the lowest negative LR (0.25). None of the non-invasive factors investigated was able to reliably rule in or rule out any grade OV due to an only modest NPV (< 82.1%) and to a low PPV (< 28.6%).

Performance of factors for detection of large OV

The diagnostic performance of simple non-invasive markers for detection of large OV is shown in Table VI. The optimized cutoffs for each non-invasive marker, as resulted from AUC analysis, are also shown. Portal vein diameter and γ-GTP showed the best performance, as indicated by an AUC of 0.775 and 0.753, respectively. Spleen length presented with the highest positive LR (4.51), and both portal vein diameter and γ-GTP had the lowest negative LR (0.26). None of the non-invasive factors investigated was able to reliably rule in or rule out any grade OV due to an only modest NPV (< 88.2%) and to a low PPV (< 12.1%).

Discussion

In patients with PBC, the identification of those with OV is of special interest, because the presence of OV is an important parameter for the subsequent development of variceal hemorrhage and of bleeding-related death. Portal hypertension developing in the advanced stages of PBC is histopathologically related to the regenerative "cirrhotic" nodules, as is the case with most of the causes of hepatic cirrhosis, where resistance to blood flow increases in the sinusoids within the cirrhotic nodules, thereby creating portal hypertension. As for portal hypertension occurring in the early histological stages of PBC, several studies have demonstrated the relationship between histologically significant changes occurring during the course of PBC and the presence of EV, including inflammation confined to the portal tract and periportal areas [13], portal tract venopathy [14], and nodular regenerative hyperplasia [15]. Current guidelines recommend screening all cirrhotic patients by endoscopy, to identify patients at risk of bleeding who should undergo prophylactic treatment. Considering that endoscopy is an invasive procedure and recent evidence suggests that adherence of practicing gastroenterologists to guidelines is unsatisfactory [16], a non-invasive test with high diagnostic accuracy for the determination of significant OV is of great value. The present study was undertaken to develop some markers to predict OV in a consecutive series of treatment-naive patients with PBC.

In recent studies, Ali *et al.* [9] reported that male sex, low albumin, elevated bilirubin, and/or prolonged prothrombin time are the predictors for the development of oesophageal varices in early PBC. This result is partly consistent with our data from univariate analysis, as shown in Table II. We found that parameters linked to portal hypertension (platelet count, white blood cells, spleen length and portal vein diameter) as well as low levels of albumin, low γ-GTP values, high level of bilirubin, and low level of cholesterol, were independently associated with presence of any grade or large OV. In our study, those linked to liver dysfunction or advanced disease (ALT, AST, ALP, INR, Mayo risk score and Child score) were not associated with presence of OV. This finding might be different from some previous studies. In our study we found that low γ-GTP values and low level of cholesterol were independently associated with presence of any grade or large OV. Serum γ-GTP reflects release of enzyme caused by biliary tract damage, and its level has been considered as a marker for biliary cell damage in patients with PBC. In our study we found that the levels of serum γ-GTP increased in all PBC patients, but it was lower in the high grade of OV than other groups. This is consistent with a previous study [17], which showed that the levels of γ-GTP increased at the early stage but remarkably decreased at the middle and late stage. The elevated γ-GTP at the middle and late stage may be because of the decrease of functional liver and biliary cells. Ikeda *et al.* [10] reported that high ALP ratios and low platelet counts were useful predictors of esophageal varices in patients with early PBC. Levy *et al.* [8] reported that a platelet count of less than 140,000 and/or a Mayo risk score of 4.5 or greater appeared to identify those patients more likely to benefit from a screening endoscopy. The different performance of the above factors between previous data and ours may be due to a different population and stages of PBC. Most studies [18–20] revealed that total cholesterol level was high in patients with PBC, while in our study we found that low level of cholesterol was associated with large OV, which may be because of the limited population in our study. So additional large-scale, prospective studies are needed to further investigate those predictors for OV in PBC. Furthermore, our multivariate analysis showed that high spleen length values, high portal vein diameter values and low level of albumin were significantly associated with the presence of oesophageal varices in patients with PBC. In our study, we revealed that the markers could identify PBC patients with OV with high accuracy. In addition, albumin had a good AUC in predicting the presence of esophageal varices. Portal vein

diameter was the most powerful independent predictor for large OV.

So far, different non-invasive tools have been recently proposed in the literature for non-invasive screening of OV. However, no valid surrogate for EGD has been developed yet. Several reasons account for this: one of them is that, while endoscopy has been used as the gold standard in all studies, it is by no means a perfect gold standard. Several studies have shown a lack of good agreement between endoscopists in assessing the size of varices [21, 22], and the level of experience of endoscopists may play a role. This is really not surprising, since several variables may affect the endoscopic diagnosis and grading of varices, such as the degree of insufflation of the esophagus, the occurrence of retching during the examination, the duration of oesophageal intubation, etc. Nevertheless, when the result of an alternative method differs from the EGD result, it is automatically labeled as a false-negative (or false-positive) result of the alternative method, even though in reality it might represent a false-positive (or false-negative) result of EGD, and this puts any new method at a disadvantage. All other models based on clinical, biochemical and ultrasound parameters are not accurate enough to avoid endoscopy in PBC patients.

In conclusion, esophageal varices were observed in 5.5% of the patients with early PBC at the time of diagnosis, and some of them suffered life-threatening complications of variceal bleeding. Our results suggest that the parameters linked to portal hypertension (spleen length and portal vein diameter) and other factors such as albumin or γ-GTP may be used as an initial screening tool for PBC patients to exclude those with very low risk of carrying clinically relevant OV. Available data do not allow for the replacement of endoscopy in OV screening, but may help in stratifying patients with PBC for risk classes and possibly reducing the number of endoscopies needed. Additional large-scale, prospective studies are needed to further define the role of these and other non-invasive markers for diagnosis and grading of OV.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Lee YM, Kaplan MM. The natural history of PBC: has it changed. Semin Liver Dis 2005; 25: 321-6.
2. Jensen DM. Endoscopic screening for varices in cirrhosis: findings, implications, and outcomes. Gastroenterology 2002; 122: 1620-30.
3. Gores GJ, Wiesner RH, Dickson ER, et al. Prospective evaluation of esophageal varices in primary biliary cirrhosis: development, natural history, and influence on survival. Gastroenterology 1989; 96: 1552-9.
4. de Franchis R. Evolving consensus in portal hypertension. Report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. J Hepatol 2005; 43: 167-76.
5. Takeshita E, Kumagi T, Matsui H, et al. Esophagogastric varices as a prognostic factor for the determination of clinical stage in patients with primary biliary cirrhosis. J Gastroenterol 2003; 38: 1060-5.
6. Bressler B, Pinto R, El-Ashry D, et al. Which patients with primary biliary cirrhosis or primary sclerosing cholangitis should undergo endoscopic screening for oesophageal varices detection. Gut 2005; 54: 407-10.
7. Murata Y, Abe M, Furukawa S, et al. Clinical features of symptomatic primary biliary cirrhosis initially complicated with esophageal varices. J Gastroenterol 2006; 41: 1220-6.
8. Levy C, Zein CO, Gomez J, et al. Prevalence and predictors of esophageal varices in patients with primary biliary cirrhosis. Clin Gastroenterol Hepatol 2007; 5: 803-8.
9. Ali AH, Sinakos E, Silveira MG, et al. Varices in early histological stage primary biliary cirrhosis. J Clin Gastroenterol 2011; 45: e66-71.
10. Ikeda F, Okamoto R, Baba N, et al. Prevalence and associated factors with esophageal varices in early primary biliary cirrhosis. J Gastroenterol Hepatol 2012; 27: 1320-8.
11. de Franchis R, Pascal JP, Ancona E, et al. Definitions, methodology and therapeutic strategies in portal hypertension. A Consensus Development Workshop, Baveno, Lake Maggiore, Italy, April 5 and 6, 1990. J Hepatol 1992; 15: 256-61.
12. Dickson ER, Grambsch PM, Fleming TR, et al. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology (Baltimore, Md.) 1989; 10: 1-7.
13. Navasa M, Pares A, Bruguera M, et al. Portal hypertension in primary biliary cirrhosis. Relationship with histological features. J Hepatol 1987; 5: 292-8.
14. Nakanuma Y, Ohta G, Kobayashi K, et al. Histological and histometric examination of the intrahepatic portal vein branches in primary biliary cirrhosis without regenerative nodules. Am J Gastroenterol 1982; 77: 405-13.
15. Colina F, Pinedo F, Solis JA, et al. Nodular regenerative hyperplasia of the liver in early histological stages of primary biliary cirrhosis. Gastroenterology 1992; 102: 1319-24.
16. Zaman A, Hapke RJ, Flora K, et al. Changing compliance to the American College of Gastroenterology guidelines for the management of variceal hemorrhage: a regional survey. Am J Gastroenterol 2004; 99: 645-9.
17. Chen G, Li L, Gao J, Li F, Li B. Primary biliary cirrhosis: an analysis of 153 cases. Zhonghua yi xue za zhi 2002; 82: 1625-8.
18. Wang C, Zhao P, Liu W. Risk of incident coronary artery disease in patients with primary biliary cirrhosis. Int J Clin Exp Med 2014; 7: 2921-4.
19. Kohjima M, Enjoji M, Yada R, et al. Pathophysiological analysis of primary biliary cirrhosis focusing on choline/phospholipid metabolism. Liver Int 2015; 35: 1095-102.

20. Floreani A, Cazzagon N, Franceschet I, Canesso F, Salmaso L, Baldo V. Metabolic syndrome associated with primary biliary cirrhosis. J Clin Gastroenterol 2015; 49: 57-60.
21. Bendtsen F, Skovgaard LT, Sorensen TI, et al. Agreement among multiple observers on endoscopic diagnosis of esophageal varices before bleeding. Hepatology (Baltimore, Md.) 1990; 11: 341-7.
22. Winkfield B, Aube C, Burtin P, et al. Inter-observer and intra-observer variability in hepatology. Eur J Gastroenterol Hepatol 2003; 15: 959-66.