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Indocyanine Green Retention Test as a Noninvasive Marker of Portal Hypertension and Esophageal Varices in Compensated Liver Cirrhosis

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Noninvasive markers would be useful for the assessment of portal hypertension (PH) and esophageal varices (EV) in patients with cirrhosis. The aim of our study was to evaluate the performance of the indocyanine green (ICG) retention test as a noninvasive marker of PH and EV, measured against the gold standards (hepatic venous pressure gradient [HVPG] measurement and upper endoscopy). We prospectively enrolled patients with compensated cirrhosis referral to our unit. All patients underwent laboratory tests, abdominal ultrasound, upper gastrointestinal endoscopy, HVPG measurement, and the ICG 15-minute retention (ICG-r15) test. We evaluated the sensitivity and specificity of the ICG retention test and other noninvasive tools for the diagnosis of PH and EV. Ninety-six consecutive Child-Pugh A patients (67 male and 29 female; 60.3 ± 11.8 years of age) were enrolled. Seventy-four patients had clinically significant portal hypertension (CSPH), of whom 59 had severe portal hypertension (SPH). ICG-r15 and Lok index were independently related to the presence of both CSPH and SPH, whereas ICG-r15 and INR were related to EV. ICG-r15 values (<6.7% and <6.9%, respectively) were able to rule out the presence of CSPH and SPH (LR^- 0.15 and 0.14); ICG-r15 <10% provided a 97.8% sensitivity (LR^- 0.042) for the exclusion of EV and a 100% sensitivity (LR^- 0.0) for large EV. *Conclusion:* The ICG-r15 test is an effective tool for assessment of PH in patients with compensated cirrhosis. Although this would not replace endoscopy, the ICG-r15 appears able to identify patients with advanced liver disease in which endoscopy is mandatory as well as rule out the presence of EV in patients with compensated cirrhosis. (HEPATOLOGY 2014;59:643-650)

Histological and hemodynamic alterations occurring in the course of chronic liver disease (CLD) are characterized by progressive deposition of extracellular matrix (ECM), leading to liver tissue fibrogenesis and intra- and extrahepatic vascular changes. Capillarization of hepatic sinusoids as well as development of intrahepatic shunts and neoangiogenesis lead to increased intrahepatic resistance to blood flow.[1,2] Both architectural and dynamic alterations (activation of myofibroblasts and hepatic stellate cells), together with increased portal blood flow resulting from splanchnic vasodilatation, lead to the development of portal hypertension (PH).

The presence of PH is commonly observed in patients with liver cirrhosis. Development of PH significantly increases the risk of complications, such as

Abbreviations: AAR, AST/ALT ratio; AIC, Akaike information criterion; ALT, alanine aminotransferase; APRI, AST/platelet ratio index; AST, aspartate aminotransferase; AUC, area under the curve; AUROC, area under ROC curve; BMI, body mass index; CLD, chronic liver disease; CPT, Child Pugh Turcotte; CSPH, clinically significant portal hypertension; ECM, extracellular matrix; EV, esophageal varices; FHVP, free hepatic venous pressure; GGT, gamma-glutamyl transpeptidase; GI, gastrointestinal; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HE, hepatic encephalopathy; HVPG, hepatic venous pressure gradient; ICG, indocyanine green; (%); ICG-k, ICG disappearance rate; ICG-r15, ICG retention at 15 minutes; ICG-$t_{1/2}$, ICG half-life; INR, international normalized ratio; LEV, large esophageal varices; LR^-, negative likelihood ratio; LR^+, positive likelihood ratio; LSM, liver stiffness measurement; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis; NPV, negative predictive value; OR, odds ratio; PH, portal hypertension; PSDR, platelet count/spleen diameter ratio; PPV, positive predictive value; ROC, receiver operating characteristic; SD, standard deviation; SPH, severe portal hypertension; TE, transient elastography; UGI, upper GI; US, ultrasound; WHVP, wedged hepatic venous pressure.

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development of esophageal varices (EV), ascites, hepatic encephalopathy [HE], and gastrointestinal (GI) bleeding; moreover, PH increases the overall risk of liver-related death. Therefore, available guidelines strongly suggest to assess the presence and grade of PH in all patients with liver cirrhosis.[3-5]

The gold standard for the diagnosis and assessment of PH is the measurement of hepatic venous pressure gradient (HVPG); however, HVPG measurement is not routinely available, is costly, invasive, and needs specific expertise. On these bases, the development of noninvasive parameters that are able to identify the presence and grade of PH (absence of PH, pre-clinical, clinically significant and severe) is useful for the routine management of patients with CLD and liver cirrhosis. Several noninvasive parameters have been proposed for the diagnosis and assessment of PH and detection of EV, even though none reached an important role in clinical practice[6-16]; in particular, liver stiffness measurement (LSM) was recently shown to be a valid noninvasive tool, even though it is closely correlated to the etiology of the underlying liver disease and different cut-off values have been proposed for different conditions (i.e., HCV, hepatitis C virus, hepatitis B virus [HBV], nonalcoholic steatohepatitis [NASH], alcoholic and cholestatic).[14-16]

Indocyanine green (ICG) is a tricarbocyanine dye that binds to albumin and alpha-1 lipoproteins; after an active uptake from hepatocytes, it is secreted unchanged into the bile. ICG is removed exclusively from the liver and does not undergo enterohepatic circulation.[17,18] ICG clearance is a quantitative liver function test representing both parenchymal function and hepatic blood flow.[19] Based on its characteristics, the ICG retention test is routinely used, especially in Eastern countries, for assessment of liver function in patients undergoing hepatic surgery (hepatocellular carcinoma [HCC] or biliary cancer resection),[20,21] and it has been recently proposed and clinically evaluated as a prognostic marker in patients with advanced cirrhosis and in those listed for liver transplantation.[22,23] Finally, ICG clearance is modified by acute changes in vascular liver perfusion.[24-26] We hypothesized that among patients with well-preserved liver function, the ICG retention test could directly reflect the alteration of liver blood flow and, indirectly, the presence and grade of PH.

The aim of our study was to evaluate the ICG 15-minute retention (ICG-r15) test as a noninvasive marker of PH and EV in patients with compensated liver cirrhosis, compared to the gold standards, HVPG and upper GI (UGI) endoscopy, respectively.

Patients and Methods

Patients. All patients, referred to our unit for newly diagnosed liver cirrhosis, were prospectively enrolled from January 2010 to January 2011. Patients were diagnosed with liver cirrhosis based on clinical, biochemical, histological, or ultrasound (US) criteria.

Exclusion criteria were age younger than 18 years, obesity (body mass index [BMI]: $\geq$30), Child-Pugh class B or C (Child Pugh Turcotte [CPT] score $\geq$7), previous or ongoing clinical decompensation (ascites, HE or UGI bleeding, jaundice, and spontaneous bacterial peritonitis), presence of HCC, significant alterations of liver function tests (alanine aminotransferase [ALT] >10-fold upper limit of normal, international normalized ratio [INR] >1.5, total bilirubin >2 mg/dL, and Model for End-Stage Liver Disease [MELD] >15), ongoing treatment for PH, recent alcohol intake, presinusoidal or extrahepatic causes of PH, and pregnancy status.

All patients completed the study protocol within 7 days. All patients gave their written informed consent in accord with the principles of the Declaration of Helsinki (revision of Edinburgh, 2000).

Clinical and Biochemical Assessment. Clinical evaluation and routine biochemical screening (including complete liver function tests) were performed at enrollment. Laboratory tests included: hemochrome, total platelet count, transaminase levels (ALT and aspartate aminotransferase [AST]), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, total bilirubin, serum albumin, creatinine, blood urea nitrogen, sodium (Na), and prothrombin time (INR).

Noninvasive Score of PH and EV. We also calculated, for all patients, the AST/ALT ratio (AAR),[6]

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platelet count/spleen diameter ratio (PSDR),[7] AST/platelet ratio index (APRI),[9] Lok index,[10] FIB-4,[11] and Park index,[12] according to published formulas.

US Examination. US examinations were performed by the same operator, using a 3.5-7.0-MHz convex probe provided with color, power, and pulsed Doppler device (Aloka Prosound 5000; Hitachi Aloka Medical, Ltd., Tokyo, Japan). Liver margins, size of left, right, and caudate lobes, spleen bipolar diameter, anteroposterior diameter of the portal trunk, mean portal vein flow, hepatic vein flow, and resistance index were recorded.

UGI Endoscopy. Gastroscopies were all performed at our endoscopy unit by the same operator. Presence and degree of EV, gastric varices, and hypertensive gastropathy were recorded and classified according to the Baveno V Consensus Conference criteria; patients were considered as having large esophageal varices (LEV) when grade $\geq$II was found.[3-5]

HVPG Measurement. As previously described,[27] we performed HVPG measurement placing a 7-F venous catheter introduced into the right arm through the basilica vein using the Seldinger technique. A 5-F balloon-tipped catheter (OB-Medi-Tech; Boston Scientific, Cork, Ireland) was advanced into the right hepatic vein to measure wedged (WHVP) and free hepatic venous pressures (FHVP) by connection to an external transducer and polygraph (PowerLab; ADInstruments, Inc., Colorado Springs, CO). After inflating the balloon catheter, adequacy of occlusion was obtained by injection of a small amount of radiological contrast medium. Portal pressure gradient (HVPG) was calculated as the difference between WHVP and FHVP. All HVPG measurements were performed by an experienced radiologist and supervised by a hepatologist. All assessments were performed according to the most recent guidelines.[28]

Clinically significant (CSPH) and severe portal hypertension (SPH) were defined as HVPG $\geq$10 and $\geq$12 mmHg, respectively, according to consensus definition.[3,4]

ICG Retention Test. After a basal blood-sample collection, ICG (ICG-PULSION; Pulsion Medical Systems SE, Munich, Germany), at a dose of 0.5 mg/kg, was administered by the antecubital vein of the opposite arm. Then, venous peripheral blood samples were collected every 5 minutes for 20 minutes. After clotting, samples were centrifuged (2,500$\times g$ for 10 minutes). Then, ICG absorbance in serum was measured at 805 nm using a Cary 50 Scan UV-Vis spectrophotometer (Varian, Palo Alto, CA). The ICG retention test was performed in serum, instead of plasma, to reduce possible interferences resulting from plasma turbidity; ICG concentrations obtained by employing ICG absorbance at 805 nm in serum were comparable to those measured in plasma after correction for blank density by the method of Nielsen.[29] ICG serum disappearance rate (ICG-k), ICG half-life time (ICG-$t_{1/2}$), and ICG-r15 were calculated by fitting the serum disappearance curve by a single-exponential decay equation.[18]

Statistical Analysis. Statistical analysis was performed using MedCalc package v.11.5 for Windows. Categorical variables are reported as numbers (percentage); continuous variables were reported as mean and standard deviation (SD) or in median and range on the basis of their distribution analyzed by the means of Kolmogorov-Smirnov's test. The unpaired Student t test (normally distributed) and Mann-Whitney's test (non-normally distributed) were used for comparison of continuous variables. Fisher's exact test was used for comparison of categorical variables. Receiver operating characteristic (ROC) curves were used to identify sensitivity, specificity, and the best cut-off values for the diagnosis of CSPH, SPH, and EV. The Akaike information criterion (AIC) was used to identify the relative goodness of fit of every statistical variable or model evaluated in this study: AIC = 2k - 2 ln(L), where k is the number of parameters in the statistical model and L is the maximized value of the likelihood function for the estimated logistic regression model.[30] Cut-off values were selected according to the aim of the test (rule in or rule out the presence of any condition), choosing, respectively, the highest positive likelihood ratio (LR^+) and the lowest negative likelihood ratio (LR^-). Positive predictive value (PPV), negative predictive value (NPV), LR^+, and LR^- were calculated for each cut-off point. P values <0.05 were considered statistically significant.

Results

Characteristics of Patients. Ninety-six consecutive patients with compensated liver cirrhosis (67 male and 29 female; mean age: 60.3 $\pm$ 11.8 years) were prospectively enrolled; demographic, clinical, and biochemical characteristics of the entire population are shown in Table 1. All patients (n = 96) presented Child-Pugh class A. Etiologies of the underlying liver diseases are reported in Table 1. All patients had PH; among them, 22 (22.9%) had pre-clinical PH (HVPG between 5 and 9 mmHg), and 74 (77.1%) had CSPH (HVPG $\geq$10 mmHg), of whom 59 (61.5%) had SPH (HVPG $\geq$12 mmHg).

Table 1. Baseline Characteristics of the Study Population

Characteristic	Patients (n = 96)
Gender, male/female	67/29
Age mean ± SD, years	60.3 ± 11.8
Median (range)	61 (33-84)
BMI, kg /m^2	25.6 ± 2.6
Ethiolgies	
HCV n (%)	57 (59.4)
HBV n (%)	13 (13.5)
Alcohol n (%)	19 (19.8)
NASH/criptogenetic n (%)	7 (7.3)
EV n (%)	46 (47.9)
LEV n (%)	12 (12.5)
ALT, IU/mL	40.5 (15-303)
AST, IU/mL	42.0 (17-187)
GGT, IU/mL	53.0 (14-383)
Bilirubin, mg/dL	0.98 ± 0.45
Albumin, g/dL	4.0 ± 0.4
INR	1.16 (0.97-1.50)
MELD	8 (6-15)
Platelet count, $\times 10^9$/L	121.50 (40.0-512.0)
Spleen diameter, cm	13.8 ± 2.3
HVPG, mmHg	13.7 ± 5.4
ICG-r15, %	20.0 ± 13.8
ICG-$t_{1/2}$	5.6 (0.21-22.9)
ICG-k	0.127 ± 0.06
APRI	1.00 (0.04-5.56)
AAR	1.09 ± 0.37
FIB-4	3.88 ± 2.26
Lok index	0.81 ± 1.37
Park index	−0.86 ± 1.90
PSDR	880.1 (210.5-2562.9)

Factors Associated With the Presence of CSPH and SPH. Among all variables evaluated (Supporting Table 1), sex (male), bilirubin, albumin, INR, MELD score, platelet count, APRI, AAR, Lok index, FIB-4 index, Park index, PSDR, and ICG-r15 were significantly associated with the presence of both CSPH and SPH.

Among them, at multivariate analysis, ICG-r15 (odds ratio [OR]: 1.100 [1.002-1.209]; $P = 0.045$) and Lok index (OR, 2.119 [1.158-3.878]; $P = 0.015$) were independently related to the presence of CSPH; the diagnostic performance of this model was an area under the curve (AUC) of 0.853 (0.766-0.917; $P < 0.0001$).

ICG-r15 (OR, 1.113 [1.032-1.200]; $P = 0.005$) and Lok index (OR, 1.707 [1.019-2.859]; $P = 0.042$) were independently related also to the presence of SPH (AUC, 0.842 [0.753-0.908]; $P < 0.0001$), whereas sex, bilirubin, albumin, INR platelet count, MELD score, APRI, AAR, FIB-4, Park index, and PSDR were not included in both models.

Performance of Noninvasive Markers for the Diagnosis of CSPH and SPH. Diagnostic performances of noninvasive markers for detection of CSPH are shown in Table 2. Among the tested variables, Lok index showed the highest area under the ROC curve (AUROC; 0.830 [0.740-0.899]; Park index and ICG-r15: AUC = 0.809 and 0.808, respectively) demonstrated also excellent performances, according to ROC curve analysis. Nevertheless, AIC showed slightly higher values for ICG, compared to Lok and Park indices, identifying ICG-r15 as the best tool for the diagnosis of CSPH because of the lower number of parameters used in the model (ICG-r15 AIC = 81.807; Lok index AIC = 88.157; Park index AIC = 87.914). According to both ROC curve analysis and AIC (Table 3), ICG-r15 presented the best diagnostic performance for the diagnosis of SPH (AUROC = 0.821; AIC = 95.371).

Correlation Between ICG-r15 and HVPG. A linear correlation was observed between values of ICG-r15 and HVPG ($R^2 = 0.571$; Fig. 1).

Variables Related to the Presence of EV. Among the whole study population, 46 patients (47.9%) had EV, of whom 34 had grade 0 or I, whereas 12 presented with LEV (grade II or III). Clinical and biochemical characteristics of the study population, according to the presence of EV, are shown in Supporting Table 2. Bilirubin, albumin, INR, MELD, platelet count, HVPG, APRI, AAR, Lok index, FIB-4, Park index, PSDR, and ICG-r15 were significantly associated with the presence of EV. Variables independently related with the presence of EV were ICG-r15 (OR, 1.094 [1.029-1.162]; $P = 0.0037$) and INR (OR, 890.196 [8.928-88760]; $P = 0.0038$); the diagnostic performance of the model was an AUC of 0.874 (0.791-0.933; $P < 0.0001$).

Performance of Noninvasive Test for the Diagnosis of EV. The diagnostic performance of noninvasive parameters and HVPG for the diagnosis of EV are reported in Table 4; ICG-r15 showed the highest

Table 2. Diagnosis of CSPH (HVPG ≥10 mmHg)

	AUC	95% CI	P	−2 ln(L)	AIC
ICG-r15	0.808	0.715-0.882	<0.001	79.807	81.807
ICG-$t_{1/2}$	0.768	0.671-0.848	<0.001	86.513	88.513
ICG-k	0.783	0.688-0.861	<0.001	86.218	88.218
Platelet count	0.796	0.702-0.871	<0.001	82.493	84.493
Spleen diameter	0.614	0.509-0.712	0.090	100.567	102.567
APRI	0.736	0.637-0.821	<0.001	94.008	98.008
AAR	0.710	0.609-0.798	0.003	94.886	98.886
FIB-4	0.756	0.657-0.838	<0.001	89.134	95.134
Lok index	0.830	0.740-0.899	<0.001	78.157	88.157
Park index	0.809	0.716-0.882	<0.001	81.914	87.914
PSDR	0.794	0.700-0.870	<0.001	84.612	86.612

Abbreviations: 95% CI, 95% confidence interval; *P*, *P* value for the ROC curve analysis; −2 ln(L), the full model −2 ln likelihood.

Table 3. Diagnosis of SPH (HVPG ≥2 mmHg)

	AUC	95% CI	*P*	−2 ln(L)	AIC
ICG-r15	0.821	0.729-0.892	<0.001	93.371	95.371
ICG-$t_{1/2}$	0.829	0.738-0.898	<0.001	90.104	92.104
ICG-k	0.801	0.707-0.875	<0.001	102.517	104.517
Platelet count	0.764	0.667-0.845	<0.001	111.208	113.208
Spleen diameter	0.635	0.530-0.731	0.022	127.994	129.994
APRI	0.670	0.566-0.762	0.004	121.719	125.719
AAR	0.733	0.633-0.818	<0.001	112.898	116.898
FIB-4	0.728	0.628-0.814	<0.001	113.030	119.030
Lok index	0.814	0.722-0.887	<0.001	99.479	109.479
Park index	0.779	0.683-0.857	<0.001	105.492	111.492
PSDR	0.744	0.645-0.828	<0.001	114.028	116.028

Abbreviations: 95% CI, 95% confidence interval; *P*, *P* value for the ROC curve analysis; −2 ln(L), the full model −2 ln likelihood.

accuracy (AUROC = 0.859 and AIC = 99.235) for the diagnosis of EV.

ICG-r15 Cut-Off Values for the Noninvasive Diagnosis of PH and EV. Based on the diagnostic performance observed, we reported (in Table 5) the ICG-r15 cut-off values proposed for the diagnosis of CSPH, SPH, EV, and LEV.

ICG-r15 presents a good sensitivity, both for the detection of CSPH and SPH: ICG-r15 <6.7% and <6.9% cut-off values are able to rule out the presence of CSPH and SPH (sensitivity, 95.9% and 96.6; LR^- = 0.15 and 0.14, respectively).

The ICG-r15 is able to rule in and rule out the presence of EV: ICG-r15 <10% show an optimal sensitivity (97.8%; LR^- = 0.042) for the exclusion of EV, whereas ICG-r15 ≥22.9% reported a good specificity (90.0%; LR^+ = 5.43) for EV diagnosis. Moreover, ICG-r15 <10% presented a 100% sensitivity (LR^- = 0.0) for the detection of LEV.

Figure 2 represents ICG-r15 individual data points according to the presence of EV. Twenty-seven patients of the entire study population have ICG-r15 <10%; of them, 26 do not present EV on upper endoscopy, whereas 1 patient presents small varices (F1) without red signs. No high-risk varices were misdiagnosed, and 26 (of 50) upper endoscopies could be saved with the proposed cut off.

Discussion

The results of our study suggest that the ICG retention test is a valid tool for assessment of PH and EV in patients with compensated liver cirrhosis. This is the first study evaluating the performance of ICG-r15, compared to the gold standards, for assessment of PH and EV (HVPG and upper endoscopy, respectively) in this setting; thus far, the ICG retention test was considered a prognostic marker in decompensated patients and in patients undergoing liver surgery.[20-23]

The ICG retention test is a quantitative test reflecting total liver blood flow and functional hepatic reserve. We evaluated the accuracy of ICG-r15 in patients with well-preserved liver function to avoid possible interferences resulting from reduced functional hepatic reserve; clearly, when hepatic function is impaired, both liver blood flow and anion excretion are compromised, altering the ICG retention test. The ICG-r15, compared to the gold standards, is less expensive and invasive; furthermore, it is highly

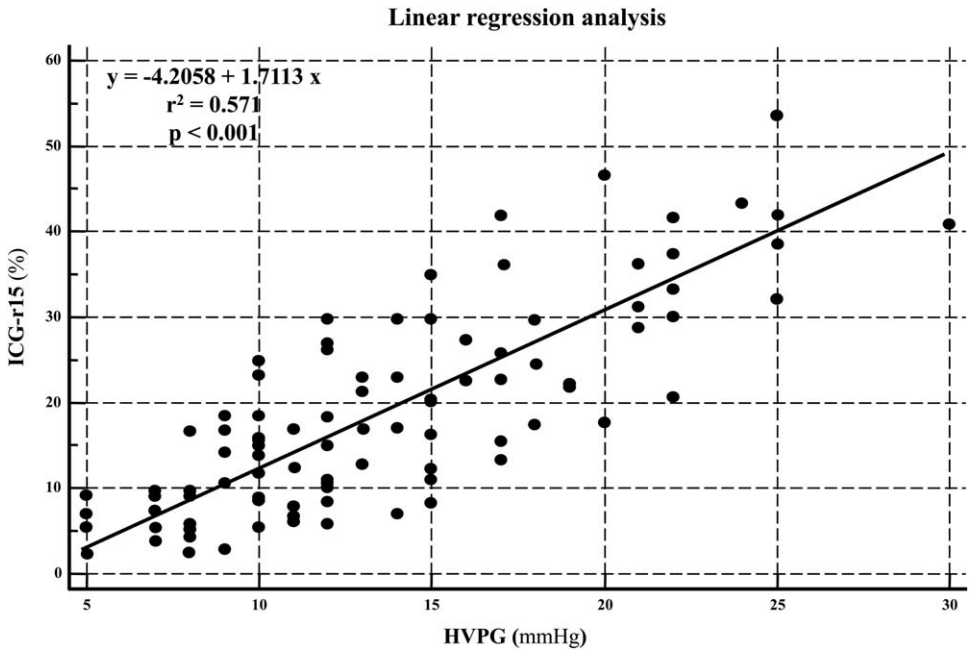

Fig. 1. Linear regression analysis between ICG-r15 (%) and HVPG (mmHg) among the whole study population.

Table 4. Diagnosis of Esophageal Varices

	AUC	95% CI	*P*	−2 ln (L)	AIC
ICG-r15	0.859	0.785-0.933	<0.001	97.235	99.235
ICG-$t_{1/2}$	0.828	0.737-0.897	<0.001	108.224	110.224
ICG-k	0.848	0.760-0.913	<0.001	97.639	99.639
Platelet count	0.780	0.685-0.875	<0.001	121.438	123.438
Spleen diameter	0.722	0.619-0.826	<0.001	131.672	133.672
APRI	0.703	0.597-0.809	<0.001	121.455	125.455
AAR	0.706	0.600-0.813	<0.001	122.104	126.104
FIB-4	0.763	0.666-0.860	<0.001	112.044	118.004
Lok index	0.824	0.742-0.906	<0.001	97.418	107.418
Park index	0.818	0.731-0.905	<0.001	104.112	110.112
PSDR	0.773	0.676-0.870	<0.001	112.233	114.233
INR	0.827	0.746-0.908	<0.001	99.099	101.099
HVPG	0.816	0.731-0.901	<0.001	102.248	104.248

Abbreviations: 95% CI, 95% confidence interval; *P*, *P* value for the ROC curve analysis; −2 ln(L), the full model −2 ln likelihood.

reproducible and does not expose the patient to ionizing radiation.

We hypothesized that among patients with well-preserved liver function, the ICG-r15 could directly reflect the alteration of liver blood flow and, indirectly, the presence and grade of PH.

Previous studies showed that the ICG retention test directly reflects total liver blood flow and is modified by acute changes in vascular liver perfusion.[24-26] However, when liver function deteriorates, other factors come into place and may influence ICG clearance (i.e., anion excretion reserve); this is the reason why we focused our evaluation on patients with well-preserved liver function and excluded all those with significant alteration.

Table 5. ROC Cut-Off Values of ICG-r15 for the Diagnosis of CSPH, SPH, EV, and LEV

		ICG-r15 (%)	Sensitivity (%)	Specificity (%)	PPV	LR^+	NPV	LR^-
CSPH	Rule out	<6.7	95.9	27.3	66.7	1.32	81.6	0.150
	Rule in	≥16.7	60.8	90.9	95.7	6.69	40.8	0.430
SPH	Rule out	<6.9	96.6	27.0	67.1	1.28	81.8	0.140
	Rule in	≥18.4	62.7	91.9	92.5	7.73	60.7	0.410
EV	Rule out	<10	97.8	52.0	65.2	2.04	96.3	0.042
	Rule in	≥22.9	54.3	90.0	83.8	5.43	68.2	0.510
LEV	Rule out	<13.3	100.0	41.7	19.7	1.57	100	0.000
	Rule in	≥22.9	58.3	72.6	23.3	2.13	92.4	0.570

Our study suggests that the ICG-r15 presents a good performance for the diagnosis of both PH and EV; although quite scattered, on regression analysis, the ICG-r15 appears directly related to HVPG measurement (Fig. 1). Moreover, among noninvasive serum markers evaluated in our study, the ICG retention test presents the best diagnostic performance for assessment of PH, CSPH, and SPH (Tables 2 and 3). According to ROC curves, we identified two cut-off values (<6.7% and <6.9%) able to rule out the presence of CSPH and SPH (Table 5) with a very good sensitivity (95.9% and 96.6%; $LR^- = 0.15$ and 0.14, respectively).

Another finding of our study is the correlation between the ICG-r15 and the presence of EV: The ICG-r15 positively correlated with the presence of EV; ROC curve (Supporing Fig. 3) identified 16.7% as the single cut-off value with the best accuracy (Youden

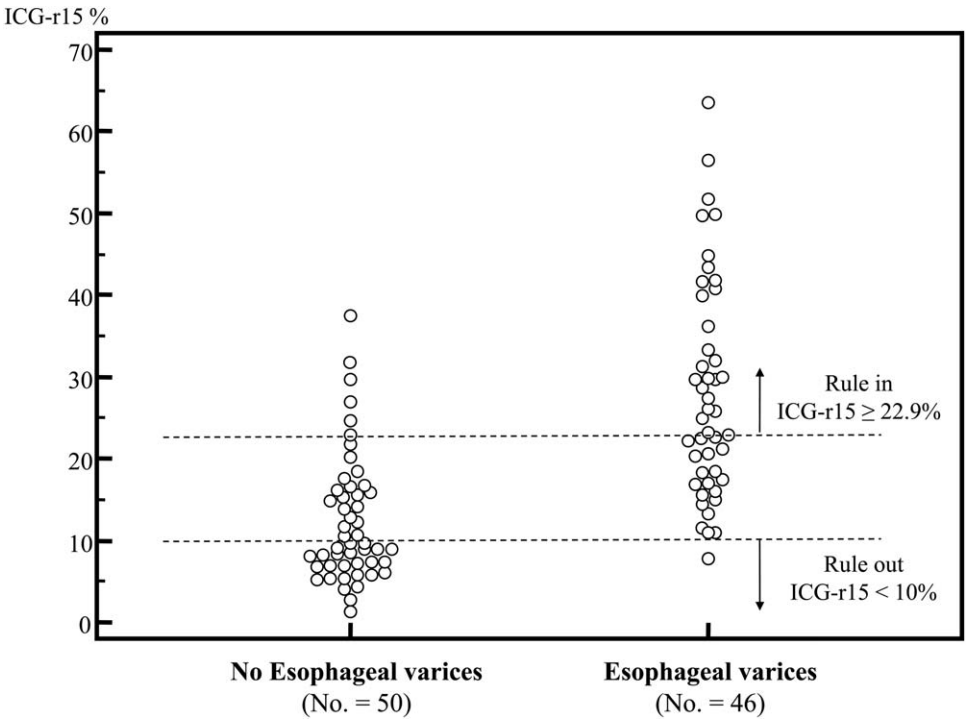

Fig. 2. ICG-r15 individual data points among patients with and without EV.

index: sensitivity, 80.4%; specificity, 80.0%; LR^+ = 4.02; LR^- = 0.24). According to the aim of ruling out or ruling in the presence of EV, we identified two cut-off values, ICG-r15 <10% (sensitivity, 97.8%; NPV, 96.3%; LR^- = 0.042) and ICG-r15 ≥22.9% (specificity, 90.0%; PPV, 83.8%; LR^+ = 5.43), which significantly improve the diagnostic performance of the ICG retention test for detection of EV. Moreover, the cut off proposed to rule out EV (ICG-r15 <10%) is able to exclude the presence of LEV with a sensitivity of 100% and a LR^- of 0.0 (Table 5).

Transient elastography (TE) is a noninvasive technique conceived for the evaluation of liver stiffness; a robust correlation between LSM, PH, and presence of EV has been observed.[14,15] Furthermore, our recent experience reported on the significant ability of LSM and spleen stiffness measurement as noninvasive markers of PH and EV among patients with HCV-related liver cirrhosis.[33]

In this study, we did not consider TE because we enrolled patients with various causes of liver cirrhosis; in fact, it is known that TE cut offs for prediction of EV, CSPH, and SPH are quite different among different ethiologies of underlying liver disease.

Interestingly, we observed that both a noninvasive marker of liver fibrosis, namely, Lok index,[10] and ICG-r15 were identified as variables independently related to the presence of CSPH and SPH; the development of PH is related to excessive production and deposition of ECM by activated myofibroblasts and increased intrahepatic resistance to blood flow resulting from capillarization of hepatic sinusoids, development of intrahepatic shunts, and neoangiogenesis. The observation that Lok index and ICG-r15 were independently related to degree of PH reflects both of these liver architecture alterations. Probably, the incorporation in a single model of a noninvasive marker of liver blood flow to a marker of hepatic fibrosis (i.e., serum marker, LSM, or others) could add more accuracy to noninvasive tools for the diagnosis of PH and for prediction of liver decompensation in longitudinal studies.

Our study has some limitations. First, the sample size of 96 participants is relatively small; however, the study was designed to exclude all extrasinusoidal causes of PH, which resulted in increased selectivity and homogeneity of the study population. However, the distribution of PH and EV seems homogeneous and representative of the population with cirrhosis observed in clinical practice: The etiologies reflect those observed in southern Europe (>70% viral, approximately 20% alcoholic, and increasing amount of NASH); the prevalence of EV and large EV (47.9% and 12.5%, respectively) reflects the trend observed in compensated patients.[5]

In conclusion, the ICG retention test allows an accurate, noninvasive identification of CSPH and SPH in patients with compensated liver cirrhosis. Moreover, the ICG-r15 could help clinicians in identifying patients with high risk of EV development and disease progression (i.e., decompensation); these first findings, based on a single cross-sectional study, have to be validated by larger, multicentric studies and from a longitudinal analysis. This simple, reproducible, and noninvasive test will enable physicians to stratify patients so as to perform a better discrimination for further invasive evaluations, HVPG measurement, and esophagogastroduodenoscopy screening.

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