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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

The relationship between transient elastography and histological collagen proportionate area for assessing fibrosis in chronic viral hepatitis

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Abstract
Background Collagen proportionate area (CPA) has a better correlation with hepatic venous pressure gradient (HVPG) than with Ishak stage. Liver stiffness measurement (LSM) is proposed as non invasive marker of portal hypertension/disease progression. Our aim was to compare LSM and CPA with Ishak staging in chronic viral hepatitis, and HVPG in HCV hepatitis after transplantation.
Methods One hundred and sixty-nine consecutive patients with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections pre/post liver transplantation (LT), had a liver biopsy combined with LSM (transient elastography), CPA (biopsies stained with Sirius Red and evaluated by digital image analysis and expressed as CPA) and HVPG (measured contemporaneously with transjugular biopsies in LT HCV patients).
Results LSM was dependent on CPA in HBV ($r^2 = 0.61$, $p < 0.0001$), HCV ($r^2 = 0.59$, $p < 0.0001$) and LT groups ($r^2 = 0.64$, $p < 0.0001$). In all three groups, CPA and Ishak were predictors of LSM, but multivariately CPA was better related to LSM (HBV: $r^2 = 0.61$, $p < 0.0001$; HCV: $r^2 = 0.59$, $p < 0.0001$; post-LT: $r^2 = 0.68$, $p < 0.0001$) than Ishak stage. In the LT group, multiple regression analysis including HVPG, LSM, aspartate aminotransferase to platelet ratio index (APRI) and Ishak stage/grade, showed that only CPA was related to HVPG ($r^2 = 0.41$, $p = 0.01$), both for HVPG $\geq$6 mmHg (OR 1.34, 95 % CI 1.14–1.58; $p < 0.0001$) or $\geq$10 mmHg (OR 1.25, 95 % CI 1.06–1.47; $p = 0.007$).
Conclusion CPA was related to LSM in HBV or HCV hepatitis pre/post-LT. CPA was better related to LSM than Ishak stage. In the LT HCV group, CPA was better related to HVPG than Ishak stage/grade, LSM or APRI. CPA may represent a better comparative histological index for LSM, rather than histological stages.

Keywords Collagen proportionate area · Liver fibrosis · Viral hepatitis · Portal hypertension · Transient elastography · Liver stiffness

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Introduction

Histological assessment of liver fibrosis is the "best standard" for diagnosing and assessing fibrosis in chronic liver disease, in particular for chronic viral hepatitis [1]. The different staging systems, METAVIR scale [2], Ishak [3] and Scheuer [4] systems, assess predominantly architectural changes and do not measure of the degree of liver fibrosis per se. Cirrhosis is only classified as a single stage histologically, being the most severe categorical assignment [5], with no histological sub classification [6].

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As scoring systems do not quantify fibrosis [5, 6], morphometry is used for this purpose, which could also be used to validate non-invasive markers of liver fibrosis, and to evaluate effects of therapy on fibrosis.

A reliable morphometric method for measuring liver fibrosis is computer-assisted digital image analysis (DIA). The areas of collagen stained with picroSirius red, and of the liver tissue are measured, and the relative proportion of collagen to tissue is derived as the collagen proportionate area (CPA) [7–9].

Calvaruso et al. [9] evaluated CPA, and its relationship with Ishak stage and hepatic venous pressure gradient (HVPG), in patients with recurrent hepatitis C virus (HCV) after transplantation, finding that CPA was a better histological correlate with HVPG than Ishak stage.

Hepatic venous pressure gradient is used to evaluate portal hypertension in patients with chronic viral hepatitis both pre [10] and post transplantation [11, 12]. Normally HVPG ranges from 1 to 5 mmHg; pressures $\geq$6 mmHg indicate portal hypertension (PHT) [13]. HVPG $\geq$10 mmHg predicts the development of complications of cirrhosis [14]. Patients transplanted for HCV cirrhosis with a HVPG $\geq$6 mmHg at 1 year after LT, are at high risk of developing clinical decompensation [11, 12]. We showed that CPA in 1 year biopsies in patients transplanted for HCV related cirrhosis was highly predictive of clinical outcome with good sensitivity and specificity, and was better than Ishak stage or HVPG [15]. We also recently demonstrated that CPA increases with worsening cirrhosis in HCV patients post liver transplant, suggesting it can be used for prognostic stratification and assessment of disease progression within the category of severe fibrosis/cirrhosis [16].

To overcome the need to perform liver biopsies to assess the degree of liver fibrosis, a number of non-invasive tests have been developed such as transient elastography (TE) [17–30], and serum direct and indirect markers, such as aspartate aminotransferase (AST) to platelet ratio index (APRI) [31, 32], and recently an algorithm combing 3 biomarkers (3-M-ALG) [34].

The APRI score has been extensively investigated, especially in nontransplant patients with chronic hepatitis C [31, 32]. Only one study [33] evaluated post transplant patients, in which APRI had the highest diagnostic value, compared to other non invasive tests.

Transient elastography gives a liver stiffness measurement (LSM) which correlates with liver disease stage in histological scoring systems. Its performance is best for cirrhosis, but is less accurate in pre-cirrhotic stages [17–21]. LSM correlates better with less severe portal hypertension (HVPG $\leq$10 mmHg), than with more severe portal hypertension [22–25].

Few studies have been performed using TE in a HCV transplanted patient [26–30]. These show a good correlation between LSM and the liver disease histological stage; one also showed a positive association between LSM and HVPG [29]. LSM may distinguish between slow and rapid "fibrosers" [30].

Morphometric quantitation of collagen has previously been compared with LSM but different stains and methodologies have been used, the correlation between collagen area and LSM was weak and the viral hepatitis groups were not evaluated separately in any of these studies [35–38].

No study exists comparing LSM and CPA in liver transplant patients, and no published study has compared CPA with both LSM and HVPG.

Our aim was to evaluate the relationships between LSM and CPA in patients with chronic viral hepatitis HCV or HBV, and to establish the relationships of both LSM and CPA with Ishak staging, and with HVPG in patients with recurrent HCV after transplantation.

Methods

Patients

Between February 2007 and April 2010 we evaluated a consecutive cohort of 169 patients with viral hepatitis who had a liver biopsy, TE and CPA performed concomitantly [9]. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. We used the specific Ethical permission to examine liver biopsy specimens for research with clinicpathological correlation of histology with scoring systems, histochemistry and other techniques (when tissue in excess of that needed for diagnostic purposes is available). It was granted by the National Research Ethics Service (REC reference number 07/Q0501/50) with institutional ID number 7558.

All patients gave written informed consent for the procedures and the histological evaluation. The non-transplanted group consisted of 106 consecutive patients with chronic viral hepatitis (52 with HCV, and 54 with HBV). The transplanted cohort comprised 63 patients transplanted for HCV cirrhosis with a cadaveric graft, who had transjugular liver biopsies (TJB) performed with HVPG [10, 11] measured at the same time.

Liver biopsy and hemodynamic study

The standard percutaneous liver biopsy was performed using the Menghini technique [39], with a 16-gauge disposable needle, in nearly all patients in the HBV and HCV non-transplanted groups. In the remainder TJB was performed.

The TJB and HVPG procedures were performed in all transplanted patients at the time of yearly protocol biopsies or when indicated by abnormal liver function tests for which there was no definitive diagnosis, after 6 h fast, under local anesthesia (lignocaine 1 %, 5–10 ml

subcutaneously); 70 % of patients received intravenous sedation with 5–10 mg diazepam. We used a 19-gauge Tru-cut type biopsy needle (Quick core; Cook, William Cook Europe, Denmark) [9] and performed three or four passes through the same hepatic vein wall (right or middle) to ensure that sufficient liver tissue was obtained [40, 41]. Hepatic vein pressures were measured using a 5-Fr balloon catheter using the technique described previously [10, 42].

Measurement of CPA

Liver biopsy samples were formalin fixed, paraffin-embedded and stained with hematoxylin and eosin and Gordon and Sweet staining for reticulin. Another tissue section was stained with picroSirius red for collagen quantification and determination of CPA by DIA. Each biopsy sample was evaluated histologically according to Ishak et al. [2]. For each biopsy, the total length was more than 15 mm [40]. Portal tracts were defined according to Crawford et al. [43] and were not counted in biopsies with severe distortion of the liver architecture, such as in cirrhosis or marked nodularity, because recognition and enumeration of individual portal tracts in these circumstance is impossible. The section of each biopsy stained with picroSirus red was used for DIA, which was performed by G.I. and V.C. The equipment used consisted of a digital camera (Canon Powershot A 640 attached to a close-up copy-stand with backlighting) connected to a compatible personal computer. After whole section digital image capture, CPA was measured with Zeiss KS300 image analysis software. The CPA measurement included editing steps to eliminate image artifacts and structural collagen in large portal tracts and blood vessel walls as previously described [9]. CPA is expressed as the relative proportion (%) of collagen to tissue [9].

Transient elastography

The evaluation was performed before the liver biopsy on the same day, and the HVPG measurement (if performed). Liver stiffness was determined on the right lobe of liver, as previously described [17]. At least ten acquisitions were performed in each patient. The success rate was calculated as the number of validated measurements. The results were expressed in kilopascal (kPa). Only procedures with ten validated measurements and a success rate of at least 60 % and interquartile range (IQR) less than 30 % were considered reliable and used in the analysis.

Statistical analysis

All data were analyzed using the statistical package SPSS (version 15.0; SPSS Inc, Chicago, IL, USA) and MedCalc statistical software (version 9.3.3). Significance testing was two-sided, and the type 1 error rate was set at 0.05. Linear regression analysis was used to assess the relationship between LSM and CPA, Ishak stage/grade and the relationship between APRI and CPA, Ishak stage/grade for all patients; HVPG was included in the analysis for transplanted patients only. In the latter group, multiple linear regression analysis was used to evaluate the dependence of HVPG on CPA, LSM, Ishak grade/stage, and APRI score. Logistic regression analysis was used to assess the dependence of the dichotomized HVPG variable (HVPG cut-offs $\geq$6 and $\geq$10 mmHg representing, respectively, presence of portal hypertension [10], and clinically significant portal hypertension [14]) on CPA, LSM, Ishak stage/grade and APRI. We also evaluated the area under the Receiver Operating Curve (AUROC) for CPA in relation to LSM, in all patients, and also the CPA and LSM in relation to HVPG cut-offs only in transplanted patients. Considering CPA, we divided the range of values in each patient group into quartiles. The cut-offs at 25, 50 and 75 % were compared for the three groups of patients, and the median LSM was calculated for each quartile. The statistical significance of inter-quartile differences for LSM was evaluated by the Mann–Whitney test.

As we have previously published [9] and confirmed in this paper, CPA cut-off more than 7 % was the best for distinguishing a normal HVPG from a raised HVPG, and early from advanced stage liver disease, and for this reason we considered patients with CPA >7 % and CPA $\leq$7 % separately and evaluated LSM medians and cut-offs for predicting advanced liver fibrosis for each group.

The difference of LSM medians for CPA >7 % and CPA $\leq$7 % in each group was evaluated by the Mann–Whitney test. The difference for LSM medians among the groups for CPA >7 % and for CPA $\leq$7 % was evaluated by Kruskal–Wallis test.

Results

One hundred and sixty-nine patient were evaluated in this study: 63 had recurrent HCV hepatitis after liver transplant, 52 chronic HCV hepatitis and 54 chronic HBV hepatitis. Clinical and histological characteristics of the patients are given in Table 1. All patients had a BMI $\leq$30 and a liver biopsy length >15 mm.

Relationship between CPA and LSM

There was a significant dependence of LSM on CPA in all groups: LT group $r^2 = 0.64$ ($\beta = 0.80$, $p < 0.0001$), HCV group $r^2 = 0.59$ ($\beta = 0.77$, $p < 0.0001$) and HBV group $r^2 = 0.61$ ($\beta = 0.78$, $p < 0.0001$) (Fig. 1). The quartile cut-offs for CPA were very similar in the chronic HCV

Table 1 Characteristics of patients with chronic viral hepatitis with respect to evaluation of histological Ishak stage and grade, HVPG [post liver transplant (LT) only], collagen proportionate area (CPA), liver stiffness measurement (LSM), and AST to platelet ratio index (APRI)

	HCV post LT	HCV	HBV
Patients (*n*)	63	52	54
Sex (male)	49	30	32
Age (years)	54 (39–66)	51 (24–70)	42 (24–68)
BMI (kg/m^2)	23.5	24.5	24.0
Ishak grade	4 (0–10)	4 (2–9)	4 (0–10)
Ishak stage (*n*)			
Stage 0–2	40	30	38
Stage 3–4	12	10	10
Stage 5–6	11	12	6
HVPG (mmHg)	4 (0–32)	NP	NP
HVPG ≥6 mmHg	16		
HVPG ≥10 mmHg	7		
CPA (%)	4.3 (0.5–32.0)	4.4 (0.5–28.0)	3.0 (0.2–24.0)
LSM (kPa)	7.2 (3.1–34.8)	6.4 (3.0–75.0)	6.4 (2.8–39.8)
APRI	0.9 (0.2–19.0)	0.6 (0.2–10.5)	0.5 (0.2–5.5)

Results are given in median (ranges)

NP not performed

hepatitis and recurrent HCV hepatitis following liver transplantation groups, whereas these cut-offs were lower in the HBV group.

Considering each quartile of CPA for each group we calculated the median value of LSM, finding a statistically significant difference between the third and the fourth quartile in each group (Table 2).

In the post transplant group, for CPA >7 % the AUROC for LSM was 0.97 (95 % CI 0.94–1.0) and the best cut-off was 9.0 kPa (91 % sensitivity and 99 % specificity). The median value of LSM for CPA ≤7 % was 6.1 kPa (3.1–19.4), whereas for CPA >7 % it was 15.2 kPa (7.4–34.8) ($p < 0.0001$). In the HCV group, for CPA >7 % the AUROC for LSM was 0.91 (95 % CI 0.81–1.0) and the best cut-off was 9.3 kPa (94 % sensitivity and 84 % specificity). The median value of LSM for CPA ≤7 % was 6.1 kPa (3.0–14.3), whereas for CPA >7 % it was 14.0 kPa (4.8–75.0) ($p < 0.0001$). Thus the relationships between CPA and LSM are similar for hepatitis C before and after transplantation, despite different widths of biopsy needles used for the standard biopsy and transjugular ones.

In patients with chronic hepatitis B, for CPA >7 %, AUROC for LSM was 0.99 (95 % CI 0.99–1.0), and the best cut-off of 11.0 kPa (100 % sensitivity and 98 %

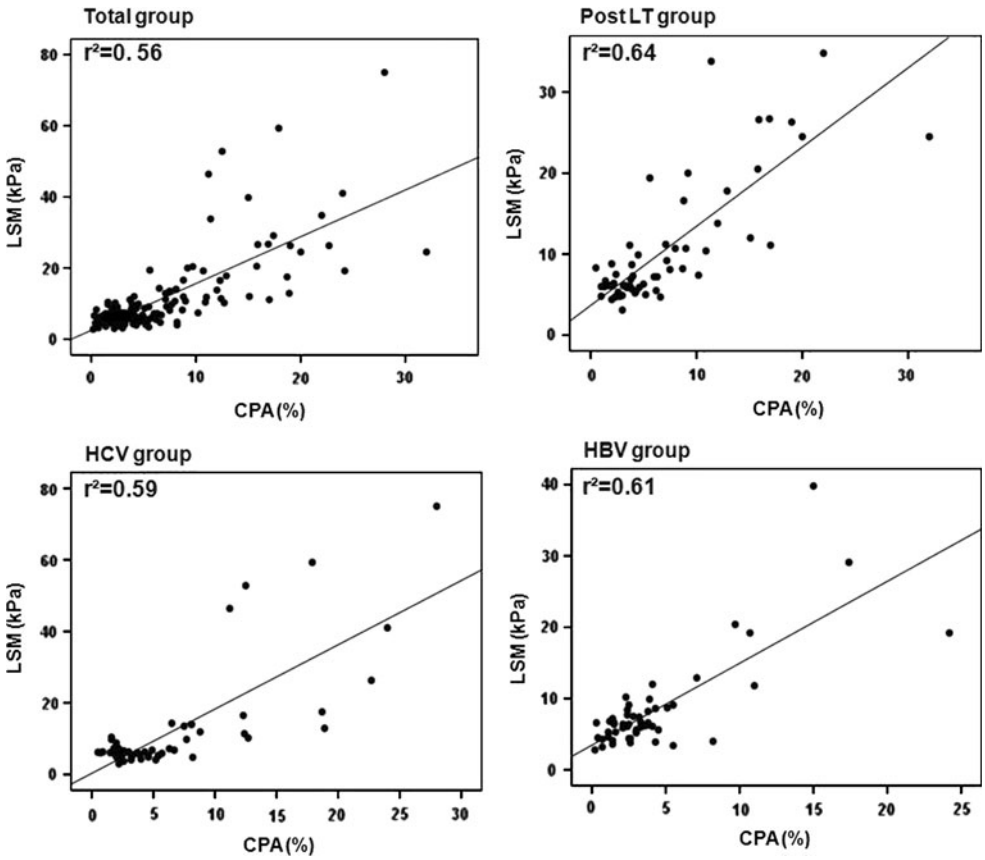

Fig. 1 Correlation between CPA and LSM in total group and subgroups: post-LT group, HCV group and HBV group

Table 2 Quartiles of CPA values in the 3 groups of patients with chronic viral hepatitis (post transplant HCV, HCV and HBV), and corresponding median LSM values, and Ishak stage scores

CPA values	1st quartile ≤25 %	2nd quartile ≤50 %	3rd quartile ≤75 %	4th quartile >75 %
Post LT group				
Patients (*n*)	17	15	16	15
CPA (%)[§]	≤2.6	≤4.3	≤9.0	>9.0
LSM (kPa)	6.1 (4.4–8.8)	5.9 (3.1–11.1)*	8.1 (4.7–19.4)°	20.5 (7.4–34.8)$^{+}$
Ishak 0–2/3–4/5/6	17/0/0/0	12/3/0/0	10/5/1/0	1/4/5/5
HCV group				
Patients (*n*)	13	13	13	13
CPA (%)	≤2.3	≤4.4	≤8.1	>8.1
LSM (kPa)	6.2 (3.0–10.4)	5.5 (3.7–6.8)*	6.8 (4.1–14.3)*	17.5 (4.8–75.0)$^{++}$
Ishak 0–2/3–4/5/6	13/0/0/0	9/3/1/0	8/4/0/1	0/3/1/9
HBV group				
Patients (*n*)	14	16	12	12
CPA (%)	≤1.5	≤3.0	≤4.3	>4.3
LSM (kPa)	4.9 (2.8–10.0)	6.0 (3.8–10.2)*	6.6 (3.4–12.0)*	12.3 (3.4–39.8)$^{+++}$
Ishak 0–2/3–4/5/6	12/2/0/0	15/1/0/0	8/4/0/0	3/3/3/3

LSM results are given in median (range)

Differences for LSM between 1st and 2nd, 2nd and 3rd, 3rd and 4th quartiles of CPA values: * $p > 0.05$, ° $p = 0.02$, $^{+}$ $p < 0.0001$, $^{++}$ $p = 0.003$, $^{+++}$ $p = 0.05$

[§] CPA (%) is the relative proportion of collagen to tissue (stained by picroSirius red), called collagen proportionate area

specificity). The median value of LSM for CPA ≤7 % was 6.2 kPa (2.8–12), whereas for CPA >7 % was 19.2 kPa (4–39.8) ($p < 0.0001$).

No significant difference in LSM was found between the three groups for CPA ≤7 % and CPA >7 %.

Relationship between CPA, LSM and Ishak stage/grade

In all 3 groups, linear regression analysis was used to evaluate the dependence of LSM on CPA, Ishak stage and grade. Univariately LSM was dependent on CPA (post LT: $r^2 = 0.64$, $\beta = 0.80$, $p < 0.0001$, HCV: $r^2 = 0.59$, $\beta = 0.80$, $p < 0.0001$, HBV: $r^2 = 0.61$, $\beta = 0.80$, $p < 0.0001$), and Ishak stage (post LT group: $r^2 = 0.60$, $\beta = 0.80$, $p < 0.0001$, HCV: $r^2 = 0.37$, $\beta = 0.60$, $p < 0.0001$, HBV: $r^2 = 0.52$, $\beta = 0.70$, $p < 0.0001$). As regards Ishak grade, this was only significant in the post LT group ($r^2 = 0.34$, $\beta = 0.56$, $p < 0.0001$). Multivariately CPA was the only independent variable associated with LSM in HCV ($r^2 = 0.61$, $\beta = 0.80$, $p < 0.0001$) and HBV ($r^2 = 0.69$, $\beta = 0.95$, $p < 0.0001$) groups and it ($r^2 = 0.68$, $\beta = 0.47$, $p = 0.001$) was related to LSM better than Ishak stage ($\beta = 0.34$, $p = 0.03$) in the post LT group. The results confirm the strong relationship of LSM with CPA, which was better than with Ishak stage/grade in each patient group. The scatter plots for the relation between CPA and LSM, and Ishak are shown in Figs. 1 and 2, respectively; for LSM and Ishak in Fig. 3. It should be noted that Ishak stages described as numbers do not have a quantitative relationship between them as we have discussed previously [3–7], and this may account for the better relationship seen between LSM and CPA, compared to relationships with the Ishak stage.

Relationship between CPA and APRI score

Collagen proportionate area was significantly related to APRI in all three groups. The evaluation of APRI and CPA, Ishak stage and grade showed that univariately APRI was significantly dependent on CPA (post LT: $r^2 = 0.11$, $\beta = 0.37$, $p = 0.01$; HCV: $r^2 = 0.38$, $\beta = 0.62$, $p < 0.0001$, HBV: $r^2 = 0.19$, $\beta = 0.43$, $p = 0.002$) and Ishak stage (post LT: $r^2 = 0.14$, $\beta = 0.37$, $p = 0.004$; HCV: $r^2 = 0.23$, $\beta = 0.47$, $p < 0.0001$; HBV: $r^2 = 0.23$, $\beta = 0.48$, $p = 0.001$), but it was dependent on Ishak grade in the HBV group only ($r^2 = 0.14$, $\beta = 0.37$, $p = 0.016$). In the multiple regression analysis, CPA was independently associated with APRI score only in the HCV group ($r^2 = 0.40$, $\beta = 0.68$, $p = 0.007$).

Relationships between LSM, CPA, APRI score and Ishak stage/grade with HVPG in the transplanted patients with recurrent HCV

In the 63 patients post LT, univariately HVPG was dependent on CPA ($r^2 = 0.4$, $\beta = 0.47$, $p = 0.000$), Ishak

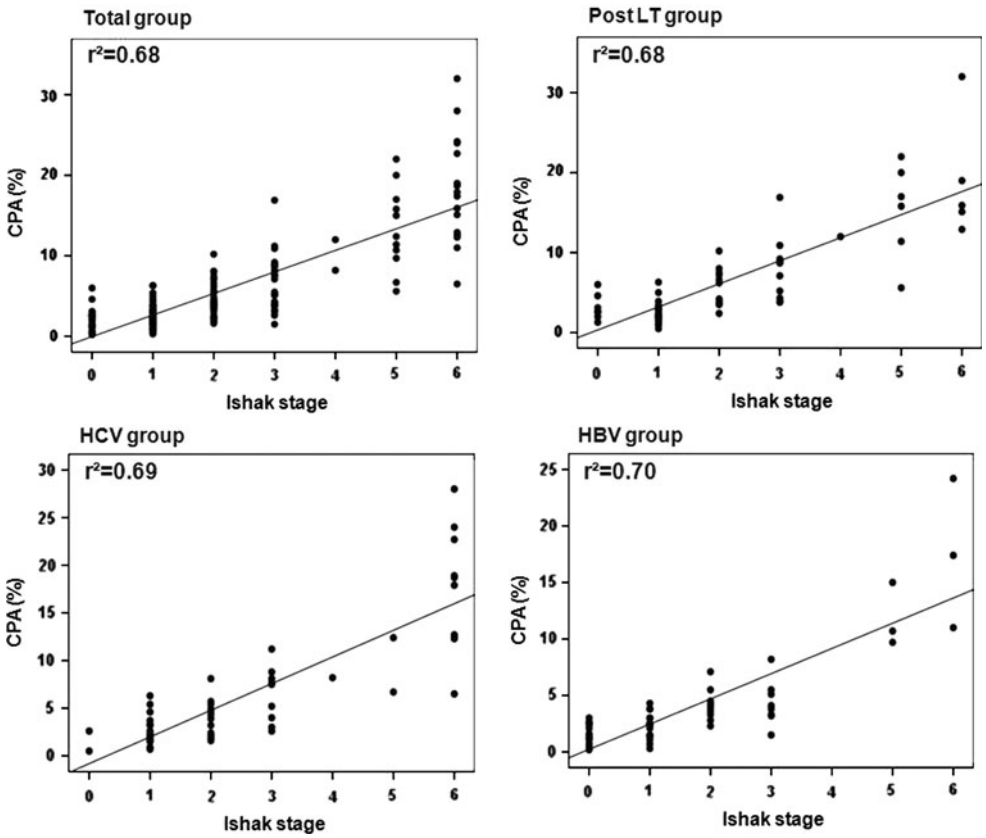

Fig. 2 Correlation between CPA and Ishak stage in total group and subgroups: post-LT group, HCV group and HBV group

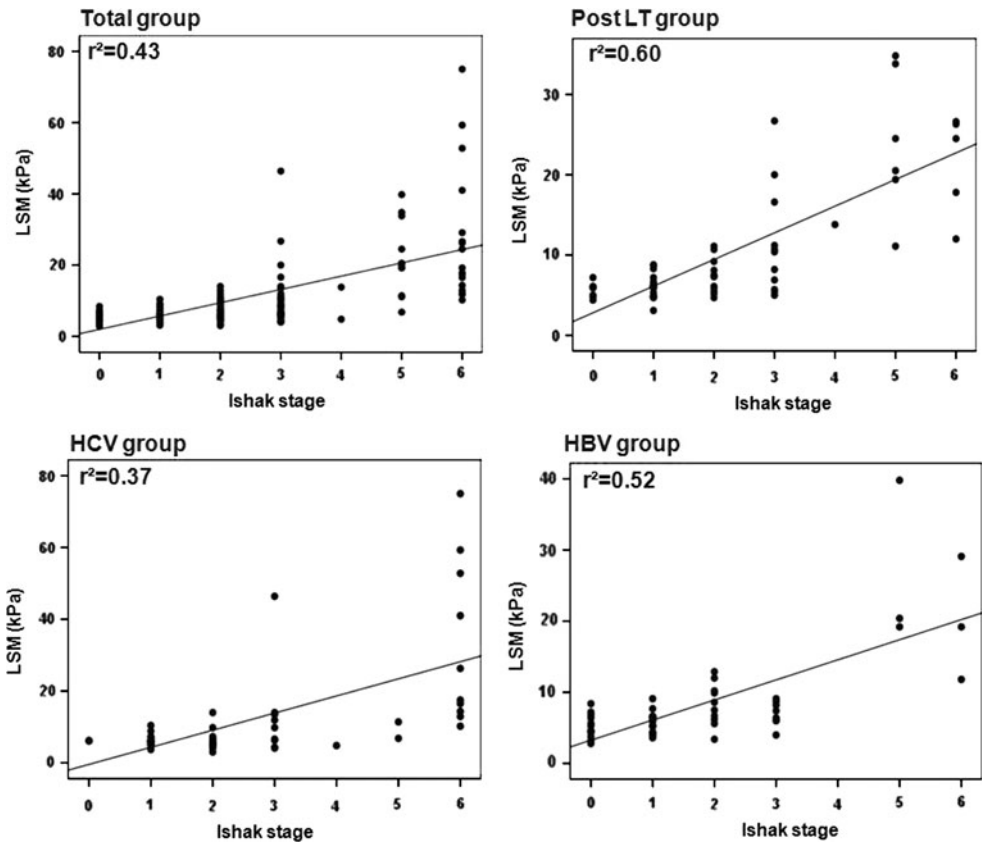

Fig. 3 Correlation between LSM and Ishak stage in total group and subgroups: post-LT group, HCV group and HBV group

stage ($r^2 = 0.35$, $\beta = 0.60$, $p = 0.0001$), Ishak grade ($r^2 = 0.11$, $\beta = 0.33$, $p = 0.012$), LSM ($r^2 = 0.22$, $\beta = 0.50$, $p = 0.000$), and APRI ($r^2 = 0.08$, $\beta = 0.29$, $p = 0.029$). However, in a multivariate analysis, CPA remained the only feature influencing HVPG ($r^2 = 0.41$, $\beta = 0.56$, $p = 0.01$).

Patients with portal hypertension (HVPG ≥6 mmHg)

There were 16 patients. Portal hypertension was related to CPA ($p < 0.0001$), LSM ($p < 0.0001$), Ishak stage ($p < 0.0001$), Ishak grade ($p = 0.023$) and APRI score ($p = 0.04$). Multivariately only CPA (OR 1.34, 95 % CI 1.14–1.6, $p < 0.0001$) remained significantly associated with HVPG ≥6 mmHg.

For HVPG ≥6 mmHg, the AUROC for CPA was 0.91 (95 % CI 0.83–0.98). The best cut-off was 7.7 % (75 % sensitivity and 89 % specificity); AUROC for LSM was 0.83 (95 % CI 0.70–0.95), and the cut-off of 10.9 kPa had 75 % sensitivity and 89 % specificity. Thus, for the presence of portal hypertension, CPA had a better association than LSM (Fig. 4).

Patients with significant portal hypertension (HVPG ≥10 mmHg)

There were seven patients. In the univariate analysis we found a significant dependence on CPA ($p < 0.0001$), LSM ($p = 0.001$) Ishak stage ($p = 0.005$), Ishak grade ($p = 0.013$) APRI score ($p = 0.002$). Multivariately only CPA (OR 1.25, 95 % CI 1.06–1.47, $p = 0.007$) was significantly associated with HVPG ≥10 mmHg.

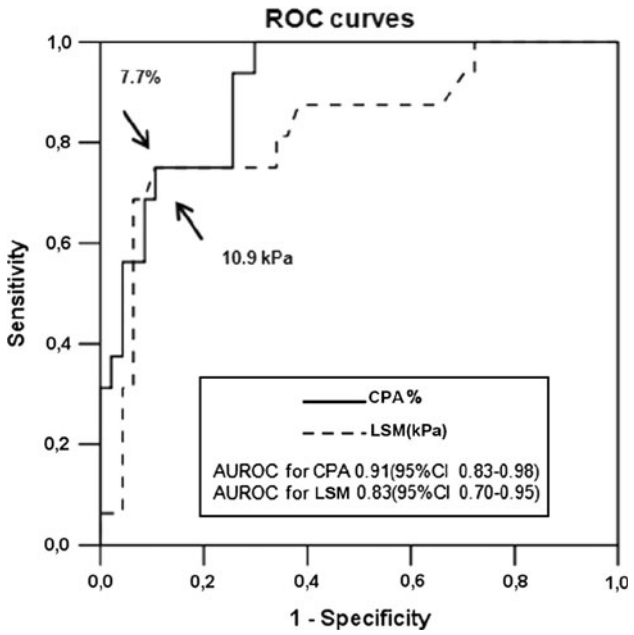

Fig. 4 ROC curves for LSM and CPA for 16 patients with portal hypertension (HVPG ≥6 mmHg) post-LT

Discussion

We assessed CPA and compared this with LSM in three groups of patients with chronic viral hepatitis: 54 with chronic hepatitis B, 52 with chronic hepatitis C and 63 transplanted patients with recurrent HCV. In transplanted patients we also obtained HVPG. The LSM and APRI score calculations were performed at the same time as obtaining a three or four core TJB or percutaneous biopsy [40, 41]. Liver histology was scored according to Ishak et al. [3], and we used our previously published methods for assessing hepatic collagen content using DIA and evaluating CPA [9]. In each patient group, CPA correlated well with LSM. Moreover, in each group the relationship between CPA and LSM was better than the relationship between Ishak stage and LSM. Most previous publications have used the histological stage to compare with LSM [17–21]. Our data on CPA, which is a continuous quantitative variable, suggests this is a better histological index than the categorical stage scores, to compare with LSM and it is likely that this could be the case for all non-invasive tests.

The results support our view that using categorical descriptive variables (grade and stage scores) as a quantitative measure of fibrosis is not only incorrect methodologically [5, 6], but is also a poor "best" standard to compare to LSM [44].

Dividing the CPA values in each group into quartiles, the transplanted HCV and non-transplanted HCV groups had similar values, whereas the CPA values for HBV group were lower, probably related to fewer patients with cirrhosis in this group. However, in this study was not possible to confirm this. The LSM medians between the adjacent quartiles of CPA in each patient group showed a statistically significant difference between the third and fourth quartile. This suggests that LSM has a better association with higher degrees of fibrosis. Indeed LSM is better at identifying higher disease stages (cirrhosis) rather than earlier stages of disease [17–21]. Using the AUROC cut-off of CPA >7 % associated with HVPG ≥6 mmHg (portal hypertension), the LSM cut-offs for HCV with or without transplantation were also similar (9.0 and 9.3 kPa). In the post transplant group, we found as before [9] that HVPG was related to both stage and LSM, as in other studies [22–24]. However when CPA was considered with LSM, Ishak stage, grade APRI score and HVPG all together, then multivariately only CPA was independently related to HVPG. As HVPG is a robust prognostic marker [13, 45], this suggests CPA is likely to be a robust prognostic histological marker [15]. The CPA was also the only

variable independently related to HVPG ≥6 mmHg and HVPG ≥10 mmHg. Thus, CPA has a good relationship with HVPG across a wide range of values, better than LSM. It is important to note that there were only 7 of 63 patients with HVPG ≥10 mmHg, so that LSM was assessed in the HVPG range where LSM is known to have a better correlation with HVPG [22].

Our results are not the only ones assessing collagen morphometrically, but due to different methodologies used in other publications [35–38], a direct comparison is not possible. However, our data are similar to others who have evaluated chronic hepatitis, but in several papers, different etiologies are bunched together. For example Ziol et al. [38] evaluated HCV and HBV patients together. This also makes direct comparisons with our study difficult.

In conclusion we have shown that firstly, CPA is a good measure to quantify fibrosis in liver biopsies of non-transplanted patients with viral hepatitis C and B and recurrent HCV similar to our previous finding in transplanted patients with recurrent HCV [9]. Secondly, CPA in recurrent chronic viral hepatitis C after liver transplantation [9], has a similar relationship to LSM, as in non-transplant chronic hepatitis C patients; moreover, in recurrent HCV hepatitis, CPA has a better relationship with HVPG than Ishak stage, LSM or APRI. Thirdly, the relationship between LSM and CPA was better than the relationship between LSM and Ishak stages in all chronic viral hepatitis groups. Our data suggest that CPA should be the histological parameter with which to compare LSM and other non-invasive markers as we have suggested previously [5, 6, 9]. It should also be used to sub-classify cirrhosis histologically [9, 46].

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Conflict of interest The authors declare that they have no conflict of interest.

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