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BRIEF DEFINITIVE REPORT

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Histological sub-classification of cirrhosis using collagen proportionate area in patients with chronic hepatitis C

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Abstract

Collagen proportionate area (CPA, %) is used to quantify liver fibrosis. Here, we assessed CPA performance to sub-classify cirrhosis. CPA was measured in explanted livers from consecutively transplanted patients for hepatitis C virus-related cirrhosis. Model for end-stage liver disease (MELD), Child-Pugh score and decompensating events (ascites, variceal bleeding, non-obstructive jaundice and encephalopathy) were recorded at the time of liver transplant. Of the 154 patients, 24%, 12%, 35%, 24% and 5% had zero, one, two, three and four previous decompensating events. Patients with decompensation had significantly higher CPA than those without (25.1 ± 8.4 vs 15.8 ± 5.5, $P < .001$). Decompensation was independently associated with CPA, bilirubin and albumin or with CPA and MELD score. CPA did not differ between patients with one, two, three or four decompensating events (22.2 ± 6.3 vs 26.6 ± 8.9 vs 24.5 ± 7.7 vs 24.4 ± 10.9, $P = .242$). Overall, CPA correlates with the clinical severity of cirrhosis until the advent of decompensation but not with subsequent decompensating events.

KEYWORDS

ascites, decompensation, fibrosis, liver transplantation, MELD, variceal bleeding

1 | INTRODUCTION

Liver cirrhosis is no longer considered an end-stage condition with an invariably dismal prognosis, but represents a dynamic multistage progressive disease, that can be further sub-classified into clinical stages in order to reflect the changing prognosis over time.[1]

Approximately one-third of patients with chronic liver disease develop cirrhosis as a result of recurring complex angio-architectural changes in the liver.[2] Increasing fibrogenesis and deposition of extracellular matrix from activated hepatic stellate cells, as well as chronic inflammation in response to hepatocyte damage, lead to the formation of regenerative nodules that are surrounded by fibrotic septa. This constitutes a typical presentation of the cirrhotic liver histology.[2,3] In addition to fibrosis, worsening hepatic endothelial dysfunction, mainly due to angiogenesis and formation of intrahepatic shunts as well as

Abbreviations: CPA, collagen proportionate area; DIA, digital image analysis; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

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insufficient release of vasodilatory factors, is another significant contributor to the establishment and progression of portal hypertension, which is regarded as the key cause of the overt complications often seen in cirrhotic patients.[4]

Liver fibrosis is the most studied among cirrhosis mechanisms and is considered of paramount significance for the prognosis of disease severity and mortality among patients with chronic liver disease.[2,3] Consequently, histological diagnosis of cirrhosis has been based on fibrosis assessment and architectural changes, which are therefore present in all common ordinal staging systems (Metavir, Ishak, Brunt) as well as semi-quantitative ones (Laennec, Nagula, Kumar).[5]

It has been now shown that morphometric quantification of liver fibrosis outranks all other histological systems for the subclassification of cirrhosis and prognosis.[6] This method, known as collagen proportionate area (CPA), first introduced by our group,[7] is a fibrosis ratio generated using a computer-assisted digital image analysis (DIA) of Sirius red-stained sections that measures the relative areas of collagen and parenchymal tissue. CPA has been associated with hepatic venous pressure gradient, as well as hepatic decompensation and liver-related mortality.[8,9]

In this study, we evaluated CPA measurements from the explanted livers of patients transplanted for hepatitis C virus (HCV)-related cirrhosis to assess the ability of CPA to sub-classify cirrhosis based on clinical stages. The aim was to explore if CPA (as a surrogate of worsening fibrosis) is increasing in cirrhosis according to the number of decompensating events.

2 | MATERIALS AND METHODS

2.1 | Study patients

We included 154 consecutive adult patients with biopsy-proven HCV-related cirrhosis who underwent liver transplantation at Royal Free Hospital, London, UK, from 2000 to 2011. All patients had active HCV infection at the time of transplantation, as explants were retrieved from the pre-directing-acting antivirals era. We excluded patients with additional aetiologies of liver disease or those who were retransplanted. Basic demographic characteristics were obtained from clinical records, and model for end-stage liver disease (MELD) and Child-Pugh scores were calculated according to the known risk formulas.

2.2 | Study clinical outcomes

All history of clinical outcomes, including hepatic decompensating events and/or hepatocellular carcinoma, at the time of liver transplant were recorded. Hepatic decompensation was defined as the development of ascites, variceal bleeding, clinical non-obstructive jaundice (total bilirubin levels ≥ 50 μmol/L) and/or hepatic encephalopathy.

The main clinical outcomes that were evaluated in this study were:

1. decompensation among all patients,
2. the number of decompensating events (ie any single decompensating event, any combination of two events, any combination of three events, all four events) among patients with decompensated cirrhosis.

2.3 | Histological assessment

Histological assessment of the explanted liver of all patients were performed at the time of the transplantation by a single histopathologist (APD) who was blinded to the clinical data. Liver biopsy samples were formalin-fixed, paraffin-embedded and routinely stained with haematoxylin and eosin, periodic-acidic schiff stain with diastase digestion, orcein, Victoria blue and Perl's. Another section of tissue was stained with picro-Sirius red for collagen quantification and determination of CPA by DIA.

2.4 | CPA analysis

Collagen proportionate area was measured as described in detail previously[7] in tissue blocks from the right lobe that were at least 2 cm^2 in area. In summary, the picro-Sirius red-stained biopsy samples were used for DIA. Whole biopsy macro-images were captured with a Canon PowerShot A640 digital camera attached to a close-up copy-stand with non-flicker backlighting and connected to a compatible personal computer. After whole-section digital image capture, CPA was measured with Zeiss KS300 image analysis software. Liver collagen was distinguished from the parenchyma with binary segmentation of RGB (red, green, blue) colour channels. Tissue within 5 mm of the capsule and large blood vessels were avoided. A manual editing step was included to eliminate image artefacts.

Collagen proportionate area was calculated as the proportion of the whole parenchyma area occupied by the collagen and expressed as a percentage (%). The measurement was performed by a single experienced operator who was blinded to the clinical data (AH).

2.5 | Statistical analysis

All data were analysed using the statistical package SPSS (version 26.0, IBM) and R Version 3.6.0 (2019-04-26). A two-tailed $P < .05$ was considered statistically significant.

Comparisons of continuous variables between or among groups were performed using *t* test, ANOVA, Mann-Whitney or Kruskal-Wallis tests, while, for comparisons of qualitative data corrected, chi-square method or two-tailed Fisher's exact test were used.

Spearman's *r* coefficient was reported for correlations of quantitative data.

For the evaluation of decompensation among study patients, logistic regression analysis was performed, and odds ratios (OR) with their 95% confidence intervals (CI) and *P*-values were reported. Only if variables were statistically significant at univariate analysis ($P < .05$) were they entered into the multivariate analysis models using backward elimination. Collinearity was assessed with tolerance values, and values < 0.2 were considered for serious collinearity among variables in multivariate regression models. Discriminatory ability of multivariate regression models was assessed using the area under the curve (AUC) with the respective 95% CI, derived from the ROC curve.

In order to identify factors associated with the number of decompensated events, ordinal logistic regression models were used, and *B* estimates, as well as their 95% CI and *P*-values, were reported. Finally, for multivariate ordinal regression models, where again only parameters with significant association were included, chi-square for model fit and R^2 square were assessed.

3 | RESULTS

3.1 | Main patient characteristics

In total, 154 patients (mean age: 51 ± 8 years, 72% males) were included in the study; their basic characteristics are presented in Table S1. As expected, Child-Pugh and MELD scores were significantly higher in decompensated cirrhosis ($P < .001$ in all comparisons). CPA was also significantly higher in patients with decompensated cirrhosis compared with compensated cirrhosis (25.1% ± 8.4% vs 15.8% ± 5.5%, $P < .001$) (Figure 1A). CPA demonstrated a positive correlation with MELD ($r = 0.493$, $P < .001$) (Figure S1A) and Child-Pugh score ($r = 0.477$, $P < .001$) (Figure S1B).

3.2 | Clinical events in patients with decompensated cirrhosis

Among 117 patients with decompensated cirrhosis, 19 (12%), 54 (35%), 37 (24%) and 7 (5%) had one, two, three and four previous decompensating events, respectively. Patients with a single decompensating event had more often ascites (n = 10, 52%) or jaundice (n = 9, 47%). Among patients with two decompensating events, ascites and jaundice was the most common combination (n = 47, 87%), followed by ascites and variceal bleeding (n = 4, 7%) and ascites and encephalopathy (n = 2, 4%). Finally, among patients with three decompensating events, ascites, jaundice and encephalopathy was the most common combination (n = 22, 59%), followed by ascites, jaundice and variceal bleeding (n = 12, 32%).

Among patients with decompensated cirrhosis, CPA values did not differ significantly between patients with one, two, three or four decompensating events (22.2 ± 6.3 vs 26.6 ± 8.9 vs 24.5 ± 7.7 vs 24.4 ± 10.9, $P = .242$) (Figure 1B).

3.3 | Factors associated with presence of decompensation and the number of decompensating events

In the whole cohort of 154 patients (Table 1), CPA was independently associated with decompensation in multivariate regression models. In particular, when CPA, bilirubin, albumin and INR values were included in the model, all but INR were independently associated with decompensation (adjusted OR for CPA per 1% increase: 1.14, 95% CI: 1.04-1.26, $P = .006$; adjusted OR for bilirubin per mg/L: 1.07, 95% CI: 1.01-1.13, $P = .014$; adjusted OR for albumin per mg/L: 0.82, 95% CI: 0.73-0.93, $P = .001$). When MELD score and CPA values were included in the multivariate regression analysis, they were both significantly associated with decompensation (adjusted OR for CPA per 1%

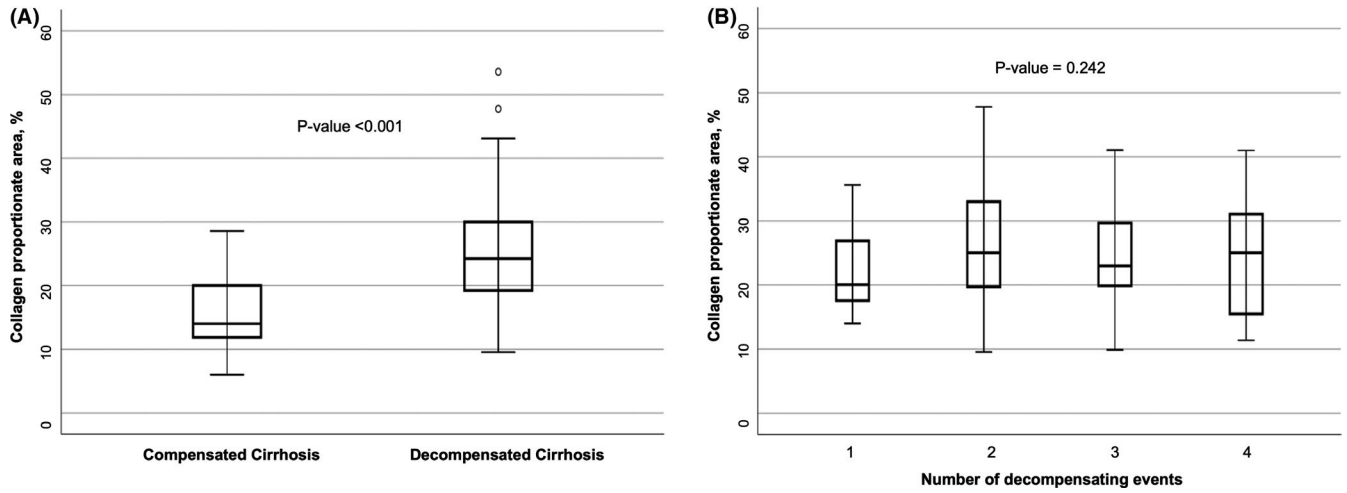

FIGURE 1 Collagen proportionate area (CPA, %) values in patients with compensated versus decompensated cirrhosis (A) and in relation to the number of decompensating events (B). All pairwise comparisons using the *t* test were non-significant ($P > .05$) as was the ANOVA test for comparing CPA values among the different groups. The *P*-value presented in the figure corresponds to the ANOVA test

TABLE 1 Factors associated with decompensation among 154 HCV-cirrhotic study patients. Results from logistic regression analysis. Statistically significant results are marked in bold font

Variables	Univariate regression OR (95% CI) *P*-value	Multivariate regression 1[a] OR (95% CI) *P*-value	Multivariate regression 2[b] OR (95% CI) *P*-value
Age, per year	0.97 (0.92-1.02) *.200*		
Sex, female vs male	0.65 (0.27-1.55) *.329*		
CPA, per 1% increase	**1.24 (1.14-1.34)** ***<.001***	**1.14 (1.04-1.26)** ***.006***	**1.16 (1.06-1.27)** ***.001***
MELD score, per point	**1.51 (1.29-1.77)** ***<.001***		**1.36 (1.16-1.59)** ***<.001***
Bilirubin, per mg/L	**1.13 (1.08-1.19)** ***<.001***	**1.07 (1.01-1.13)** ***.014***	
Albumin, per g/L	**0.78 (0.71-0.85)** ***<.001***	**0.82 (0.73-0.93)** ***.001***	
INR, per unit	**51.35 (8.15-323.22)** ***<.001***	3.57 (0.63-20.27) .151	

Abbreviations: CI, confidence interval; CPA, collagen proportionate area; HCC, hepatocellular carcinoma; INR, international normalized ratio; MELD, model for end-stage liver disease; OR, odds ratio.

[a]Including CPA, bilirubin, albumin and INR levels.

[b]Including CPA and MELD score.

increase: 1.16, 95% CI: 1.06-1.27, $P = .001$; adjusted OR for MELD score per point: 1.36, 95% CI: 1.16-1.59, $P < .001$) (Table 1). When the discriminatory performance of those two regression models was examined, it was found that regression model 1 including CPA, bilirubin, albumin and INR levels had the higher AUC compared with CPA and MELD score, MELD score only or CPA only (AUC 0.93, 95% CI: 0.89-0.97, $P < .001$ vs AUC: 0.89, 95% CI: 0.83-0.94, $P < .001$ vs AUC: 0.85, 95% CI: 0.78-0.93, $P < .001$ vs AUC: 0.83, 95% CI: 0.75-0.90, $P < .001$, respectively) (Figure S2).

The increasing number of decompensating events was independently associated only with only baseline MELD score with (estimate: 0.084, 95% CI: 0.021-0.147, $P = .009$) (Table S2).

4 | DISCUSSION

In this well-characterized cohort of HCV-related cirrhosis, we have shown that CPA values were significantly and positively correlated with MELD and Child-Pugh score values and were significantly higher in patients with decompensated than compensated cirrhosis. CPA was independently associated with the presence of decompensation, alongside the MELD score in the multivariate regression model. The regression model with CPA and the MELD components (bilirubin, albumin and INR) was better in classifying patients with decompensated cirrhosis in comparison with the regression model with CPA and MELD score or MELD score alone. Finally, we showed that CPA values did not differ among patients with increasing number of decompensating events. In this patient subgroup, only MELD score was associated with a higher number of decompensating events.

Previous studies have shown that CPA is an independent predictor of hepatic decompensation in patients with chronic liver disease due to a variety of aetiologies, including HCV infection,[7,8,10,11] NAFLD[9] and ALD.[12] We have also previously demonstrated that CPA can sub-classify compensated cirrhosis better than the other semi-quantitative sub-classification systems[6] independently of the MELD score. However, no study to date has explored whether CPA increases alongside disease progression in patients with cirrhosis and therefore whether progressive fibrosis is the driving factor for complications in cirrhosis.

Although fibrosis staging as measured by CPA was associated with cirrhosis progression until the advent of decompensation, there was no relationship with worsening clinical stages after that, potentially implying that fibrosis is no longer the key driving force towards more advanced disease stages. It should be noted, however, that our analysis has explored the role of fibrosis quantified by CPA in the accumulating number of decompensating events in cirrhotic patients, rather than the events sequence in this setting. Our data are in line with the hypothesis that beyond a certain point, worsening fibrosis and portal hypertension are probably driven by increasing microcirculatory dysfunction at the hepatic microvascular network.[13] Along these lines, it has been shown that in patients with hepatic vein pressure gradient (HPVG) > 12 mmHg, the linear relationship between liver stiffness and HPVG values is lost, implying endothelial dysfunction rather than fibrosis as the major contributor in severe portal hypertension.[14]

Model for end-stage liver disease score values were significantly associated with increased number of decompensating events in patients who have already had hepatic decompensation. This finding is consistent with numerous previous studies, suggesting that MELD score is associated with mortality in decompensated liver disease patients.[15] Accordingly, it is considered that MELD score reflects the synthetic dysfunction of a cirrhotic liver and how this worsens as decompensating events accumulate.

We included patients with a single disease aetiology to ensure consistent CPA values, as we have previously shown that alcohol-related and cholestatic liver disease have higher ranges of CPA at the time of transplantation than HCV and HBV,[16] most likely due to the pattern of fibrosis development (porto-central in viral hepatitis versus pericellular/perisinusoidal in alcohol-related liver disease). Although we can only speculate, we believe that these findings most likely apply to all aetiologies of cirrhosis.

This study has certain limitations. First of all, it is a cross-sectional study with retrospective collection of data relying on medical records. However, we included consecutive patients that underwent transplantation from the same hospital, which precludes non-uniformity across selection criteria. Also, the number of patients included in the study may be limited, and therefore, larger studies, ideally with prospective study design, are warranted to validate the robustness of our data. Since this was a cross-sectional study, we cannot provide cut-offs to predict decompensation in patients with cirrhosis. Finally, since the inclusion of patients with a single disease aetiology (chronic HCV infection) in our study may not allow us to draw safe conclusions for patient groups with other liver disease aetiologies, such as cholestatic disease and alcoholic or non-fatty liver disease, separate evaluation of these patient populations may be required.

In conclusion, our study has shown that CPA values are associated with the clinical severity of cirrhosis until the advent of the first decompensating event in patients with chronic hepatitis C. After decompensation occurs, worsening fibrosis does not appear to be associated with subsequent decompensating events. Therefore, other mechanisms, such as endothelial dysfunction or systemic inflammation, which may be potentially implicated, require evaluation in future studies in order to assess if they can prove beneficial as biomarkers of risk stratification of patients with decompensated cirrhosis.

ETHICS APPROVAL STATEMENT

The study was approved by the ethical review board of the participating institution (REC reference number 07/Q0501/50).

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None.

CONFLICT OF INTEREST

The authors do not have any discloures to report.

PATIENT CONSENT STATEMENT

The ethics committee waived the requirement for patient consent.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Permission will be considered by the authors upon reasonable request.

DATA AVAILABILITY STATEMENT

Data will be available from the corresponding author upon reasonable request.

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REFERENCES

1. D'Amico G, Morabito A, D'Amico M, et al. Clinical states of cirrhosis and competing risks. *J Hepatol*. 2018;68:563-576.
2. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet*. 2014;383:1749-1761.
3. Garcia-Tsao G, Friedman S, Iredale J, et al. Now there are many (stages) where before there was one: in search of a pathophysiological classification of cirrhosis. *Hepatology*. 2010;51:1445-1449.
4. Fernández M, Semela D, Bruix J, et al. Angiogenesis in liver disease. *J Hepatol*. 2009;50:604-620.
5. Nagula S, Jain D, Groszmann RJ, et al. Histological-hemodynamic correlation in cirrhosis—a histological classification of the severity of cirrhosis. *J Hepatol*. 2006;44:111-117.
6. Tsochatzis E, Bruno S, Isgro G, et al. Collagen proportionate area is superior to other histological methods for sub-classifying cirrhosis and determining prognosis. *J Hepatol*. 2014;60:948-954.
7. Calvaruso V, Burroughs AK, Standish R, et al. Computer-assisted image analysis of liver collagen: relationship to Ishak scoring and hepatic venous pressure gradient. *Hepatology*. 2009;49(4):1236-1244.
8. Calvaruso V, Dhillon AP, Tsochatzis E, et al. Liver collagen proportionate area predicts decompensation in patients with recurrent hepatitis C virus cirrhosis after liver transplantation. *J Gastroenterol Hepatol*. 2012;27:1227-1232.
9. Buzzetti E, Hall A, Ekstedt M, et al. Collagen proportionate area is an independent predictor of long-term outcome in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2019;49:1214-1222.
10. Manousou P, Dhillon AP, Isgro G, et al. Digital image analysis of liver collagen predicts clinical outcome of recurrent hepatitis C virus 1 year after liver transplantation. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2011;17:178-188.
11. Manousou P, Burroughs AK, Sochatzis ET, et al. Digital image analysis of collagen assessment of progression of fibrosis in recurrent HCV after liver transplantation. *J Hepatol*. 2013;58:962-968.
12. Israelsen M, Guerrero Misas M, Koutsoumourakis A, et al. Collagen proportionate area predicts clinical outcomes in patients with alcohol-related liver disease. *Aliment Pharmacol Ther*. 2020. https://doi.org/10.1111/apt.16111
13. Bosch J, Groszmann RJ, Shah VH, et al. Evolution in the understanding of the pathophysiological basis of portal hypertension: how changes in paradigm are leading to successful new treatments. *J Hepatol*. 2015;62:S121-S130.
14. Vizzutti F, Arena U, Romanelli RG, et al. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology*. 2007;45:1290-1297.

15. Zipprich A, Garcia-Tsao G, Rogowski S, et al. Prognostic indicators of survival in patients with compensated and decompensated cirrhosis. *Liver Int*. 2012;32:1407-1414.
16. Hall A, Germani G, Isgrò G, et al. Fibrosis distribution in explanted cirrhotic livers. *Histopathology*. 2012;60:270-277.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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