Comparison of Circulating Endothelial Cell/Platelet Count Ratio to Aspartate Transaminase/Platelet Ratio Index for Identifying Patients with Cirrhosis

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Background/Objectives: **Circulating endothelial cells (CECs) are indicative of vascular injury and correlate with severity of vascular diseases. A pilot study showed that the ratio of CEC to platelet count (CEC/PC) was effective in predicting cirrhosis. Therefore, we evaluated CEC/PC in a larger cohort of patients, correlated it with cirrhosis, and compared its operating characteristics with previously described biomarker for cirrhosis, the AST/platelet ratio index (APRI).** ***Methods:*** **Fifty-three patients with cirrhosis, 20 matched healthy controls, and 9 patients with noncirrhotic liver disease were recruited. Peripheral blood sample was collected and analyzed to enumerate nucleated CEC CD146+, CD105+, CD45− using a commercial assay.** ***Results:*** **Median CEC counts were significantly higher in patients with cirrhosis (62 cells/4 mL, interquartile range [IQR]: 43.5–121) as compared with controls (31 cells/4 mL, IQR: 22.2–40). The CEC/PC was also significantly elevated in cirrhotics (0.69, IQR: 0.39–1.48) compared with controls (0.12, IQR: 0.09–0.20) and noncirrhotics (0.21, IQR: 0.08–0.43). Receiver operator characteristic (ROC) analysis revealed that CEC cutoff value of ≥37 cells/4 mL showed sensitivity of 81% and specificity of 75% for differentiating cirrhosis from controls (area under the curve [AUC]: 0.80; 95% confidence interval [CI] 0.67–0.91). The CEC/PC ratio cutoff value of ≥0.23 showed sensitivity of 91% and specificity of 82% (AUC: 0.92; 95% CI 0.83–0.99). The APRI cutoff value of ≥0.4 showed sensitivity of 94% and specificity of 85% for differentiating cirrhosis from control patients (AUC: 0.96; 95% CI 0.90–1.0). A product of CEC and APRI, termed CAPRI (CEC-APRI), effectively distinguished patients with cirrhosis from controls; with cutoff value of ≥12.7, showing higher sensitivity of 98% and specificity of 85% (AUC: 0.98; 95% CI 0.96–1.0).** ***Conclusion:*** **The CEC/PC ratio is significantly elevated in patients with cirrhosis and demonstrates comparable operating characteristics to previously described APRI. Furthermore, CAPRI, compiled as product of CEC to APRI showed outstanding ability to distinguish patients with cirrhosis from controls, although larger studies are necessary for validation.** **(J Clin Exp Hepatol 2012;2:19–26)**

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Hepatic fibrosis occurs in response to chronic or repetitive liver injury, which can eventually lead to cirrhosis and its complications of portal hypertension, hepatocellular carcinoma, and liver failure.[1] Even though histopathological examination of a liver biopsy specimen is currently the gold standard for the staging of cirrhosis, various serologic and biochemical tests are under evaluation to assess hepatic fibrosis in a less invasive manner.[2–4] This is particularly important for monitoring disease progression and response to treatment for drugs that have the potential to reverse hepatic fibrosis.

Circulating endothelial cells (CECs) represent a cellular marker of endothelial damage. Circulating endothelial cells are present in very low quantities among healthy subjects[5] but can gain access to the peripheral circulation after sloughing from vessel walls following pathological injury such as mechanical stress, change in adhesion molecule expression, or matrix degradation.[6,7] Circulating endothelial cells are characterized by the expression of at least two endothelial markers (i.e., CD146 and UEA-1) with the absence of expression of leukocyte markers (i.e., CD14

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Abbreviations: APRI: AST/platelet ratio index; AST: aspartate aminotransferase; AUC: area under the curve; CAPRI: CEC with APRI; CEC: circulating endothelial cell; CTP: Child–Turcotte–Pugh; EGD: esophagogastroduodenoscopy; ELF: enhanced liver fibrosis; IQR: interquartile range; MELD: model for end-stage liver disease; PC: platelet count; ROC: receiver operator characteristic
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and CD45).[8] Several recent studies suggest that CEC are elevated in different forms of vascular injury, such as acute coronary syndromes.[9–12]

Since cirrhosis and portal hypertension are associated with vascular injury and endothelial damage,[13,14] we hypothesized that CEC count may be elevated in cirrhotic patients as compared with healthy controls. Indeed, an earlier pilot study conducted in a small patient cohort suggested that the ratio of CEC to platelet count (CEC/PC) could accurately predict cirrhosis and hepatic decompensation.[15] Therefore, the aims of this study were to (1) determine if CEC/PC was significantly higher in subjects with cirrhosis and portal hypertension compared with healthy individuals in a larger cohort of patients and in a cohort of patients with elevated liver tests but no cirrhosis, (2) evaluate the sensitivity and specificity of CEC/PC for distinguishing cirrhosis compared with another commonly utilized non-invasive diagnostic marker of cirrhosis, the aspartate aminotransferase (AST) to platelet ratio index (APRI),[16] and (3) evaluate the efficacy of a new index for cirrhosis integrating CEC with APRI (CAPRI).

METHODS

Patients

This study was approved by the Institutional Review Board of Mayo Clinic, Rochester. Patients attending the outpatient hepatobiliary clinics, liver transplant clinics, and hospital services at Mayo Clinic, Rochester, MN, were screened for inclusion in the study. Fifty-three patients with cirrhosis, 20 normal healthy subjects, and 9 patients with noncirrhotic liver disease were enrolled after informed consent. *Inclusion criteria* were age >18 years, a diagnosis of cirrhosis by histology or by compatible imaging, laboratory (thrombocytopenia, elevated international normalized ratio [INR], hypoalbuminemia), and clinical complications of cirrhosis/portal hypertension (one or more of the following: splenomegaly, esophageal varices, ascites, hepatic encephalopathy). *Exclusion criteria* included any systemic or localized diseases associated with vascular injury (e.g., coronary artery disease, peripheral vascular disease, vasculitis, thromboembolic disease, malignancies, or transplantation), critically ill patients, current smoking, or use of statins, warfarin, nonsteroidal anti-inflammatory drugs or immunosuppressive/immunomodulatory agents within 3 months of recruitment. Age and sex frequency-matched volunteers served as controls. Controls did not report any hepatic or vascular disease, which was confirmed by chart review. The noncirrhotic group included patients with elevated liver enzymes but without clinical, laboratory, or radiologic features of cirrhosis.

Clinical Data

The following data were collected by chart review: serum liver function tests, PC, coagulation parameters, clinical, and laboratory parameters required for model for end-stage liver disease (MELD) score and Child–Turcotte–Pugh (CTP) staging. The presence of splenomegaly and ascites was determined by ultrasound examination. The presence of esophageal varices was determined by diagnostic esophagogastroduodenoscopy (EGD). The APRI was calculated by dividing the patient's AST by the upper limit of normal AST and then multiplying that number by 100/PC.[16]

Circulating Endothelial Cell Enumeration

A 4 mL sample of peripheral blood was obtained by nontraumatic venipuncture from patients and controls after discarding the first 2 mL that were drawn. Samples were processed within 72 hours of collection using the CellSearch™ CEC kit and CellTracks® Analyzer II (Veridex, Raritan, NJ). The kits were purchased commercially for this study. The number of CEC was enumerated by using an immuno-magnetic isolation technique and quantifying cells with a CEC phenotype of CD146+, CD105+, DAPI+, and CD45–. The CEC count was expressed as cells/4 mL. Each gallery was reviewed by two independent operators with expertize in cell search gallery interpretation who were blinded to the origin of the samples. The final CEC count was calculated as an average of the two values for each case. Our prior studies showed very low intra- and interobserver variability in CEC counts.[15]

Sample Size Calculation

Based on the prior pilot study,[15] the estimated difference in the mean CEC for cases relative to controls was expected to be about 77 cells/4 mL, with a standard deviation of 102. This predicted that we would need a sample size estimation of 76 patients (57 cases and 19 controls) to have an 80% power (assuming an estimated type I error of 0.05) to detect a statistically significant difference between the two arms. Ultimately, we enrolled 58 cases and 20 controls, but could not analyze five of the samples because of an unanticipated delay in processing time of >72 hours.

Analysis

Statistical analyzes were performed using SPSS version 17.0 for PC. Continuous data were summarized using median values with interquartile range (IQR). Ordinal and categorical data were summarized with ratios or proportions. Between-group differences were assessed using the Mann–Whitney *U* test. For correlation studies, a Spearman's rank correlation coefficient was determined. The optimal diagnostic threshold value of CEC, CEC/PC, APRI, and CAPRI, for differentiating cirrhotics from controls, was assessed using receiver operator characteristic (ROC) curve methodology. Statistical significance was considered with $P < 0.05$.

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RESULTS

Patient Demographics

The study included 53 patients with cirrhosis (Table 1), 9 patients with noncirrhotic liver disease (Table 2), and 20 healthy volunteers. The cirrhotic patients included 31 females and 22 males with median age of 58 years (IQR 50–64). The noncirrhotic group with chronic liver disease included 4 males and 5 females with median age of 59 years (IQR 52–68). The control group included 11 females and 9 males with a median age of 51 years (IQR 42–62). Of 53 cirrhotic patients, 42 had splenomegaly, 36 had esophageal varices, 31 had ascites, and 23 had encephalopathy. Fourteen patients were Child A, 28 were Child B, and 11 were Child C. The median MELD score was 11 (IQR 9–15). Twenty-six patients were on beta-blockers for varices. A diagnosis of cirrhosis was made in patients by abdominal ultrasound (42), computed tomography (CT) (34), magnetic resonance imaging (MRI) (23), and liver biopsy (15).

Table 1 Patient demographic, clinical, and biochemical features of cirrhotic cohort ($n=53$).

Demographics (units)/ (normal range)	Median (range) or number (%)
Age (yr)	58 (50–64)
Females	31/53 (58)
Compensated cirrhosis (Child A)	14/53 (26)
Decompensated cirrhosis (Child B and C)	39/53 (74)
Etiology of liver disease	
NASH	12/53 (23)
PSC	3/53 (5.6)
Alcohol	14/53 (26)
HCV	7/53 (13)
PBC	7/53 (13)
Autoimmune hepatitis	1/53 (2)
Alcohol + hepatitis C	5/53 (9.4)
Alcohol + NASH	1/53 (2)
Cryptogenic	3/53 (5.6)
Ascites	31/53 (57)
Splenomegaly	42/53 (80)
Esophageal varices	36/53 (68)
Encephalopathy	23/53 (39)
Platelet count $\times 10^6$/L (150–450 $\times 10^6$/L)	90 (20–348)
Total bilirubin (mg/dL) (0.1–1.0 mg/dL)	1.8 (0–37)
Albumin (g/dL) (3.5–5.0 g/dL)	3.5 (2–5)
Alanine aminotransferase (U/L) (7–55 U/L)	40 (11–591)
Aspartate aminotransferase (U/L) (8–48 U/L)	58 (18–531)
Alkaline phosphatase (U/L) (45–115 U/L)	133 (46–930)
INR (0.9–1.2)	1.2 (1–2)
Liver biopsy	15/53 (28)
Ultrasound abdomen	42/53 (79)
CT abdomen	34/53 (64)
MRI abdomen	23/53 (43)
EGD	38/53 (72)
Beta-blocker	26/53 (49)

CT: computed tomography; EGD: esophagogastroduodenoscopy; HCV: hepatitis C virus; INR: international normalized ratio; MRI: magnetic resonance imaging; NASH: non-alcoholic steatohepatitis; PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis.

Circulating Endothelial Cell, Circulating Endothelial Cell/Platelet Count Ratio, APRI, and CAPRI in Healthy Individuals and Patients

Median CEC values for different etiologies of cirrhosis are depicted in Figure 1 and were not significantly different between the etiologies. Median CEC levels were significantly higher in patients with cirrhosis as compared with controls (median [IQR]); cirrhosis: 62 cells/4 mL (43.5–121); controls: 31 cells/4 mL (22.2–40); $P<0.001$; Mann–Whitney) (Figure 2A). Median CEC levels in patients with noncirrhotic liver disease were in an intermediate range between controls and cirrhosis, however not significantly different from either group (IQR; 35 cells/4 mL [20.2–83.5]; $P=0.06$ and $P=0.63$, respectively; Mann–Whitney) (Figure 2A). The PC was significantly lower in cirrhotic patients compared with controls and noncirrhotic patients (IQR; cirrhosis: 90 [67–141]; controls: 238 [180–247]; noncirrhotic liver disease: 202 [168–226] $P<0.0001$ and $P=0.0006$, respectively; Mann–Whitney) (Figure 2B). The CEC/PC ratio was also significantly different in patients with cirrhosis compared with controls and noncirrhotics (IQR; cirrhosis: 0.69 [0.39–1.48]; controls: 0.12 [0.09–0.20]; noncirrhotic liver disease: 0.21 [0.08–0.43]; $P<0.001$ and $P=0.0012$, respectively; Mann–Whitney) (Figure 2C). The previously established APRI[4] was also significantly higher in patients with cirrhosis as compared with controls (IQR; cirrhosis: 1.26 [0.82–2.32]; controls: 0.22 [0.19–0.30]; $P<0.0001$; Mann–Whitney) (Figure 2D).

Table 2 Patient demographic, clinical, and biochemical features of the noncirrhotic liver disease cohort ($n=9$).

Demographics (units)/ (normal range)	Median (range) or number (%)
Age (yr)	59 (35–80)
Females	5/9 (55)
Etiology of liver disease	
NAFLD	3 (33)
HCV	2 (22)
Autoimmune hepatitis	2 (22)
Idiopathic	2 (22)
Platelet count $\times 10^6$/L (150–450 $\times 10^6$/L)	202 (135–289)
Total bilirubin (mg/dL) (0.1–1.0 mg/dL)	0.9 (0.6–2.6)
Alanine aminotransferase (U/L) (7–55 U/L)	70 (30–196)
Aspartate aminotransferase (U/L) (8–48 U/L)	48 (23–143)

HCV: hepatitis C virus; NAFLD: non-alcoholic fatty liver disease.

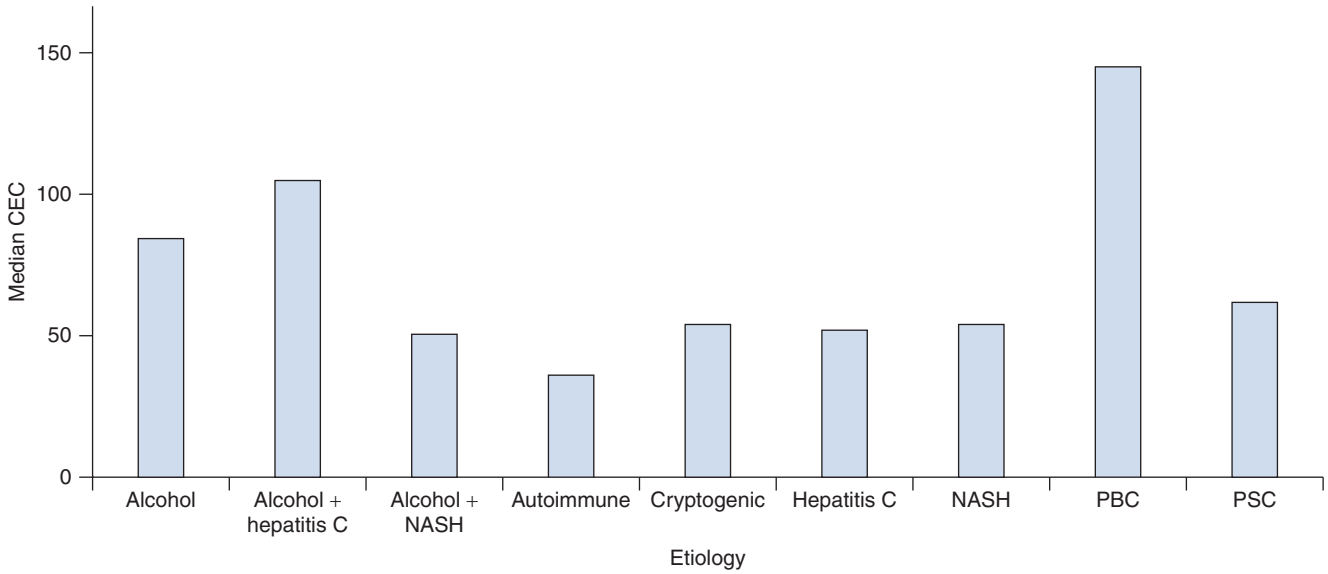

Figure 1 Median circulating endothelial cell counts for different etiologies of cirrhosis ($n=53$).
CEC: circulating endothelial cell; NASH: non-alcoholic steatohepatitis; PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis.

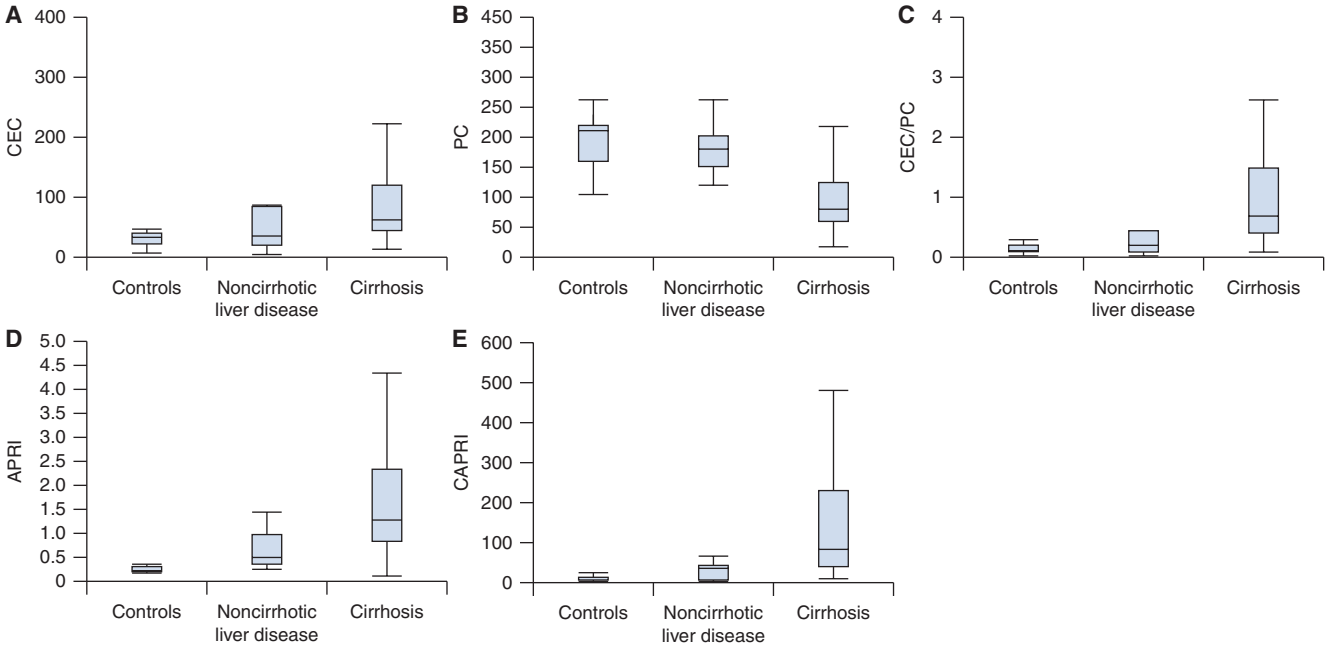

Figure 2 Box and whisker plots comparing circulating endothelial cells (CEC), platelet count (PC), CEC/PC ratio, aspartate aminotransferase to platelet ratio index (APRI), and CAPRI (a product of CEC and APRI) for diagnosis of cirrhosis. (A) CEC were significantly increased in cirrhotic patients compared with controls ($P<0.001$; Mann–Whitney); CEC in noncirrhotic patients were not significantly different from controls or cirrhosis ($P=0.06$ and 0.63, respectively; Mann–Whitney); (B) PC was significantly lower in cirrhotic patients compared with controls ($P<0.0001$; Mann–Whitney) and noncirrhotic liver disease patients ($P=0.0006$; Mann–Whitney); (C) CEC/PC ratio was significantly increased in cirrhotic patients compared with controls ($P<0.001$; Mann–Whitney) and noncirrhotic liver disease patients ($P=0.0012$; Mann–Whitney); (D) APRI was significantly increased in cirrhotic patients compared with controls ($P<0.0001$; Mann–Whitney) and noncirrhotic liver disease patients ($P=0.0056$; Mann–Whitney); (E) CAPRI was significantly increased in cirrhotic patients compared with controls ($P<0.0001$; Mann–Whitney) and noncirrhotic liver disease patients ($P=0.0019$; Mann–Whitney).

The APRI in patients with noncirrhotic liver disease was in an intermediate range between controls and cirrhosis and significantly different from both cohorts (IQR; 0.50 [0.5–0.98]; $P=0.0009$ and $P=0.0056$, respectively; Mann–Whitney) (Figure 2D). Because both APRI and CEC showed good discrimination characteristics, we examined a product of the two variables, which we termed the CAPRI which could allow the integration of AST from the APRI

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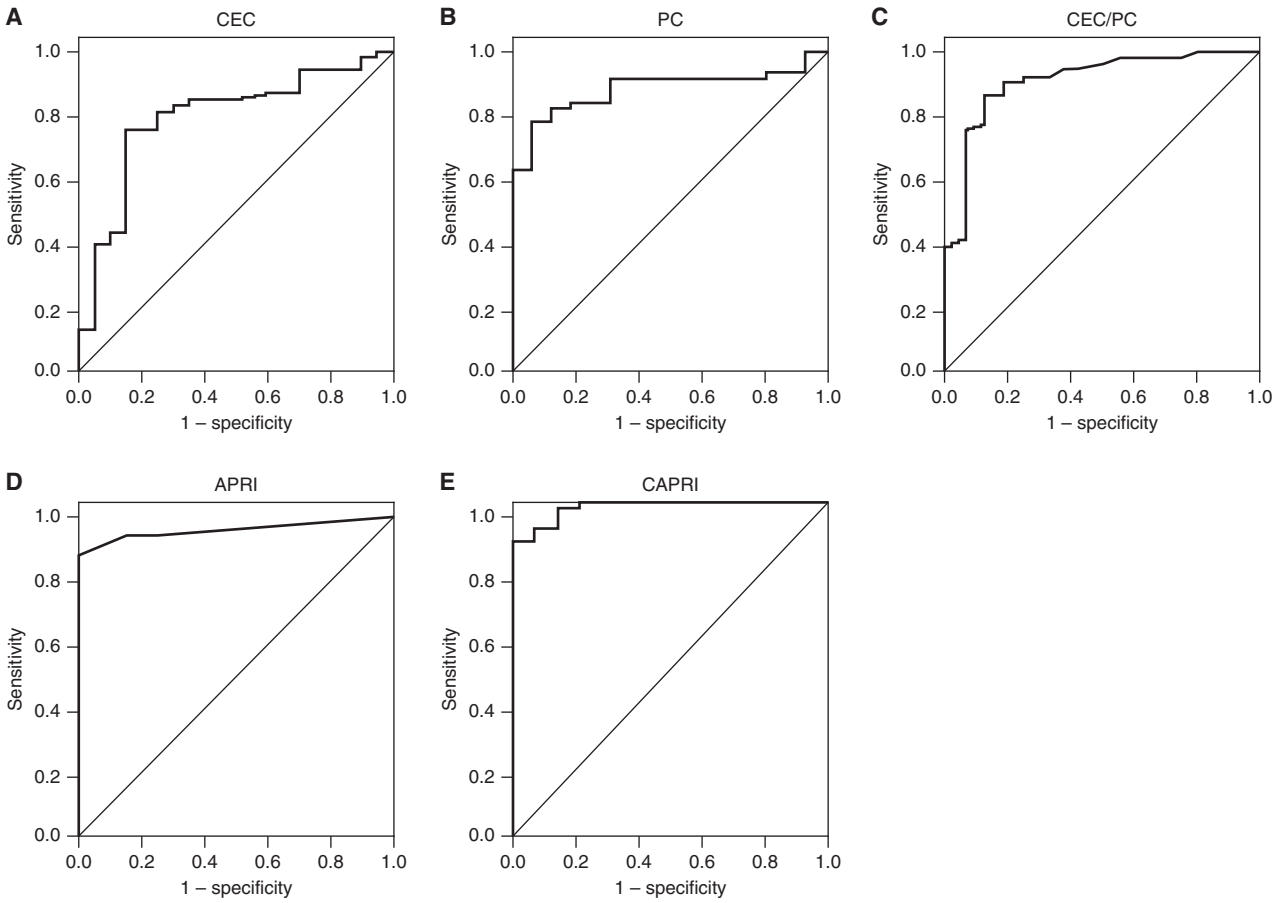

Figure 3 Receiver operator characteristic (ROC) curve for circulating endothelial cells (CEC), platelet count (PC), CEC/PC ratio, aspartate aminotransferase to platelet ratio index (APRI), and CEC with APRI (CAPRI) for diagnosis of cirrhosis. (A) ROC curve for circulating endothelial cells (CEC) for diagnosis of cirrhosis. A CEC cutoff value of 37 cells/4 mL showed sensitivity of 81% and specificity of 75% for differentiating cirrhosis from controls. Area under the curve was 0.80. (B) ROC curve for PC for diagnosis of cirrhosis. The ROC analysis revealed that a PC cutoff value of 179×103 cells/mL had a sensitivity of 85% and specificity of 81% for differentiating cirrhosis from controls. Area under the curve was 0.89. (C) ROC curve for CEC/PC ratio for diagnosis of cirrhosis. A CEC/PC ratio at cutoff value of 0.23 showed a sensitivity of 91% and specificity of 82%. Area under the curve was 0.92. (D) The ROC curve for AST/platelet ratio index (APRI) for diagnosis of cirrhosis. An APRI cutoff value of 0.4 showed a sensitivity of 94% and specificity of 85%. Area under the curve was 0.96. (E) The ROC curve for CAPRI for diagnosis of cirrhosis. A CAPRI cutoff value of 0.34 showed a sensitivity of 98% and specificity of 85%. Area under the curve was 0.98.

with CEC and PC variables. The CAPRI was obtained by multiplying patient's CEC with their APRI (CEC/PC was not used in CAPRI since the PC is already included in the APRI). Median CAPRI values were significantly higher in cirrhotic patients as compared with normal individuals and patients with chronic liver disease without cirrhosis (median [IQR]; cirrhosis: 82 [41.3–231.5]; controls: 6 [4–11.5]; noncirrhotic liver disease: 35 [5–44]; $P<0.0001$ and $P=0.0019$, respectively; Mann–Whitney) (Figure 2E).

Sensitivity and Specificity of Circulating Endothelial Cell, Platelet Count, Circulating Endothelial Cell/Platelet Count Ratio, APRI, and CAPRI for Detecting Cirrhosis

Figures 3A–D depicts ROC analyzes for the ability of different laboratory parameters to accurately identify cirrhotic patients from control patients. Receiver operator characteristic analyzes were not performed with the cohort of patients with liver test elevations in the absence of cirrhosis due to the limited sample size of this cohort. We found that a CEC cutoff value of ≥37 cells/4 mL showed sensitivity of 81% and specificity of 75% for differentiating cirrhosis from controls (area under the curve [AUC]: 0.80; 95% confidence interval [CI] 0.67–0.91] (Figure 3A). A PC cutoff value of $\geq 179 \times 10^3$ cells/μL showed sensitivity of 85% and specificity of 81% (AUC: 0.89; 95% CI 0.81–0.97) (Figure 3B). A CEC/PC ratio cutoff value of ≥0.23 showed sensitivity of 91% and specificity of 82% (AUC: 0.92; 95% CI 0.83–0.99) (Figure 3C). An APRI cutoff value of ≥0.4 showed a sensitivity of 94% and specificity of 85% (AUC: 0.96; 95% CI 0.90–1.0) (Figure 3D). A CAPRI cutoff value of ≥12.7 showed an even higher sensitivity of 98% and maintained a specificity of 85% (AUC: 0.98; 95% CI 0.96–1.0) (Figure 3E).

DISCUSSION

Circulating endothelial cells have been evaluated in several diseases associated with vascular damage.[10,12,17–20]

Since cirrhosis and its associated portal hypertension is also viewed as a vascular disease,[21] this study assessed CEC levels in cirrhosis. The results suggest that CEC levels are significantly higher in patients with cirrhosis as compared with healthy controls. Furthermore, the ratio of CEC/PC is able to discriminate many of the patients with cirrhosis from normal controls. This analysis was pursued to expand on preliminary results obtained in a small preliminary pilot study.[15] The results of this study corroborate the pilot study in several ways. Median CEC levels for cirrhotics in this sample were similar, albeit slightly lower (median of 62) than that observed in the pilot study (median of 74) while median CEC levels for controls were very similar between the two studies (31 in this study and 28 in the prior pilot study). The ROC analyzes also reveal similar results to the pilot study, in which we found that the CEC count had higher specificity for patients with cirrhosis than the PC alone. Here, we find that CEC count has better sensitivity and specificity than the PC. However, unlike the pilot study, we could not confirm a significant correlation of CEC/PC ratio with MELD or CTP scores (data not shown) although the *P* value for the association between CEC/PC count and MELD score did approach statistical significance ($P=0.09$). Overall, the results do support the concept that markers of vascular injury are increased in liver cirrhosis.[22]

Conventional biochemical and serological tests such as PC, when examined alone, are of limited value for the assessment of fibrosis.[23] As a result, percutaneous liver biopsy is the gold standard for the diagnosis and staging of cirrhosis.[24] However, liver biopsy has limitations for assessing cirrhosis because of its invasive nature and associated complications.[25] In addition, since only a minute fraction of the liver is analyzed, it is susceptible to sampling variation.[25] These issues provided the rationale for the development of non-invasive blood assays to estimate the level of hepatic fibrosis to aid in treatment decisions and monitor either progression or resolution of fibrosis.[26] These include Fibrotest, Actitest, and another non-invasive marker, coined enhanced liver fibrosis (ELF) panel.[27,28] Fibrotest involves assessment of alfa 2 macroglobulin, alfa 2 globulin (haptoglobin), gamma globulin, apolipoprotein A1, gamma glutamyl transferase, and total bilirubin, and uses a proprietary calculation that is determined from each of the variables. Actitest is a modification of the Fibrotest that incorporates alanine aminotransferase (ALT) thereby reflecting both liver fibrosis and necroinflammatory activity.[27,29] The ELF comprises hyaluronic acid, amino-terminal propeptide of type III collagen, and tissue inhibitor of metalloproteinase 1, combined in an algorithm.[30,31] While these tests may be effective, some are also expensive and not yet fully validated, which has limited their widespread integration into the clinic, and led some investigators to focus on potential utility of simpler, alternative nomograms such as APRI.

The APRI is calculated by generating the ratio of AST to the upper limit of normal AST and multiplying the value by 100/PC.[29] This index is composed of easily available laboratory tests. Many analyzes of APRI have focused on patients with hepatitis C virus (HCV), HCV/human immunodeficiency virus (HIV) co-infection, and alcoholic liver disease.[32–35] Variable test characteristics have been reported with APRI depending in part upon specific cut-point values and differing patient populations. A recent meta-analysis of 22 studies, predominantly involving patients with chronic HCV, concluded that APRI appears most useful for excluding significant fibrosis in HCV.[36] However, some studies have concluded that APRI may not have adequate clinical accuracy for detection of cirrhosis.[16,25] This has led to the advent of new algorithms that include modifications of APRI or its integration with additional tests.[37–39] For example, a group has proposed an index composed of the AST/ALT ratio, platelets, and INR. This model had an AUC of 0.81 in a validation set.[39] In our study, since CEC/PC performance was similar to APRI, we pursued a conceptually comparable approach by examining whether APRI with its AST variable may improve CEC/PC test performance characteristics. This combination of AST, PC, and CEC, which we termed CAPRI, had greater sensitivity in predicting cirrhosis, when compared with APRI or CEC/PC alone. Furthermore, there was no overlap between normals and those with cirrhosis, an important feature for an initial test of diagnostic accuracy. Although, the results of CAPRI are promising, further validation studies in larger populations will be required especially as the CAPRI emanated from a post hoc analysis.

In this study, we also analyzed a third group of patients with hepatitis but no cirrhosis based on clinical, radiographic, and/or histological criteria. These patients showed significantly lower CEC/PC and CAPRI scores compared with the cirrhosis group suggesting that these tests could be useful for distinguishing cirrhosis not only from normal patients but also in patients with liver test elevations. However, larger studies will be required in the future to validate this concept. Currently, the CEC cost for a given patient would be about $200. Although, this compares favorably to tests such as magnetic resonance elastography or liver biopsy, it would certainly be more than the APRI. However, the cost of CEC would likely decrease with more widespread use owing to increased vendor availability and marketplace competition. Finally, similarly to serum transaminases,[40] CEC may be altered in critically ill patients and therefore its use may be limited to the ambulatory setting when evaluating for cirrhosis.

In summary, this study in a relatively large and well-characterized patient cohort validates the findings of an

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earlier pilot study by showing that CEC levels are elevated in patients with cirrhosis, and that the ratio of CEC/PC is a useful index for differentiating cirrhotic from control patients. Additionally, we have developed a novel score termed CAPRI, which demonstrates greater sensitivity in predicting cirrhosis compared with the more established APRI score. Larger studies are necessary for further validation of CEC/PC and CAPRI in comparison to APRI and other non-invasive tests. Monitoring of CEC and CAPRI at different stages of disease may also help to determine their diagnostic role in the earlier stages of fibrosis.

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CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES

1. Schuppan D, Afdhal NH. Liver cirrhosis. *Lancet* 2008;371:838–51.
2. Luxon BA. Noninvasive tests for liver fibrosis. *Postgrad Med* 2006; 119:8–13.
3. Patel K. Noninvasive tools to assess liver disease. *Curr Opin Gastroenterol* 2010;26:227–33.
4. Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; 357:1069–75.
5. Woywodt A, Blann AD, Kirsch T, et al. Isolation and enumeration of circulating endothelial cells by immunomagnetic isolation: proposal of a definition and a consensus protocol. *J Thromb Haemost* 2006;4:671–7.
6. Goon PK, Boos CJ, Lip GY. Circulating endothelial cells: markers of vascular dysfunction. *Clin Lab* 2005;51:531–8.
7. Widemann A, Sabatier F, Arnaud L, et al. CD146-based immunomagnetic enrichment followed by multiparameter flow cytometry: a new approach to counting circulating endothelial cells. *J Thromb Haemost* 2008;6:869–76.
8. Ruegg C, Yilmaz A, Bieler G, Bamat J, Chaubert P, Lejeune FJ. Evidence for the involvement of endothelial cell integrin alphaV-beta3 in the disruption of the tumor vasculature induced by TNF and IFN-gamma. *Nat Med* 1998;4:408–14.
9. Bonello L, Basire A, Sabatier F, Paganelli F, Dignat-George F. Endothelial injury induced by coronary angioplasty triggers mobilization of endothelial progenitor cells in patients with stable coronary artery disease. *J Thromb Haemost* 2006;4:979–81.
10. Bull TM, Golpon H, Hebbel RP, et al. Circulating endothelial cells in pulmonary hypertension. *Thromb Haemost* 2003;90:698–703.
11. Lee KW, Lip GY, Tayebjee M, Foster W, Blann AD. Circulating endothelial cells, von Willebrand factor, interleukin-6, and prognosis in patients with acute coronary syndromes. *Blood* 2005;105: 526–32.
12. Woywodt A, Schroeder M, Gwinner W, et al. Elevated numbers of circulating endothelial cells in renal transplant recipients. *Transplantation* 2003;76:1–4.
13. Fernandez M, Semela D, Bruix J, Colle I, Pinzani M, Bosch J. Angiogenesis in liver disease. *J Hepatol* 2009;50:604–20.
14. Iwakiri Y, Groszmann RJ. Vascular endothelial dysfunction in cirrhosis. *J Hepatol* 2007;46:927—34.
15. Abdelmoneim SS, Talwalkar J, Sethi S, et al. A prospective pilot study of circulating endothelial cells as a potential new biomarker in portal hypertension. *Liver Int* 2010;30:191–7.
16. Snyder N, Gajula L, Xiao SY, et al. APRI: an easy and validated predictor of hepatic fibrosis in chronic hepatitis C. *J Clin Gastroenterol* 2006;40:535–42.
17. Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F. Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. *Blood* 2001;97:3658–61.
18. Janssens D, Michiels C, Guillaume G, Cuisinier B, Louagie Y, Remacle J. Increase in circulating endothelial cells in patients with primary chronic venous insufficiency: protective effect of Ginkor Fort in a randomized double-blind, placebo-controlled clinical trial. *J Cardiovasc Pharmacol* 1999;33:7–11.
19. Mutin M, Canavy I, Blann A, Bory M, Sampol J, Dignat-George F. Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. *Blood* 1999;93:2951–8.
20. Nadar SK, Lip GY, Lee KW, Blann AD. Circulating endothelial cells in acute ischaemic stroke. *Thromb Haemost* 2005;94:707–12.
21. Thabut D, Shah V. Intrahepatic angiogenesis and sinusoidal remodeling in chronic liver disease: new targets for the treatment of portal hypertension? *J Hepatol* 2010;53:976–80.
22. Remkova A, Remko M. Homocysteine and endothelial markers are increased in patients with chronic liver diseases. *Eur J Intern Med* 2009;20:482–6.
23. Sebastiani G, Alberti A. Non invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy. *World J Gastroenterol* 2006;12:3682–94.
24. Manning DS, Afdhal NH. Diagnosis and quantitation of fibrosis. *Gastroenterology* 2008;134:1670–81.
25. Carey E, Carey WD. Noninvasive tests for liver disease, fibrosis, and cirrhosis: Is liver biopsy obsolete? *Cleve Clin J Med* 2010;77: 519–27.
26. Poynard T, Imbert-Bismut F, Munteanu M, et al. Overview of the diagnostic value of biochemical markers of liver fibrosis (FibroTest, HCV FibroSure) and necrosis (ActiTest) in patients with chronic hepatitis C. *Comp Hepatol* 2004;3:8.
27. Halfon P, Bourliere M, Deydier R, et al. Independent prospective multicenter validation of biochemical markers (fibrotest-actitest) for the prediction of liver fibrosis and activity in patients with chronic hepatitis C: the fibropaca study. *Am J Gastroenterol* 2006;101:547–55.
28. Rossi E, Adams L, Prins A, et al. Validation of the FibroTest biochemical markers score in assessing liver fibrosis in hepatitis C patients. *Clin Chem* 2003;49:450–4.
29. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003;38:518–26.
30. Rosenberg WM, Voelker M, Thiel R, et al. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004;127:1704–13.
31. Nobili V, Parkes J, Bottazzo G, et al. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. *Gastroenterology* 2009;136:160–7.
32. Kelleher TB, Mehta SH, Bhaskar R, et al. Prediction of hepatic fibrosis in HIV/HCV co-infected patients using serum fibrosis markers: the SHASTA index. *J Hepatol* 2005;43:78–84.
33. Lackner C, Struber G, Liegl B, et al. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. *Hepatology* 2005;41:1376–82.
34. Lieber CS, Weiss DG, Morgan TR, Paronetto F. Aspartate aminotransferase to platelet ratio index in patients with alcoholic liver fibrosis. *Am J Gastroenterol* 2006;101:1500–8.
35. Schiavon LL, Schiavon JL, Filho RJ, et al. Simple blood tests as noninvasive markers of liver fibrosis in hemodialysis patients with chronic hepatitis C virus infection. *Hepatology* 2007;46:307–14.

36. Shaheen AA, Myers RP. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis C-related fibrosis: a systematic review. *Hepatology* 2007;46: 912–21.
37. Adams LA, Bulsara M, Rossi E, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005;51:1867–73.
38. Becker L, Salameh W, Sferruzza A, et al. Validation of hepascore, compared with simple indices of fibrosis, in patients with chronic hepatitis C virus infection in United States. *Clin Gastroenterol Hepatol* 2009;7:696–701.
39. Lok AS, Ghany MG, Goodman ZD, et al. Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology* 2005;42:282–92.
40. Thomson SJ, Cowan ML, Johnston I, Musa S, Grounds M, Rahman TM. 'Liver function tests' on the intensive care unit: a prospective, observational study. *Intensive Care Med* [Research Support, Non-US Gov't] 2009;35:1406–11.

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