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Implications of soluble E-cadherin level of antiviral treatment in patients with chronic hepatitis C virus infection

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ABSTRACT

Background and aims: Soluble E-cadherin (sE-cadherin) has been observed elevated in patients with various diseases, and implicated in the occurrence and development of those diseases. The implications of sE-cadherin in chronic hepatitis C virus (HCV) infection are still unclear. The purpose of this study is to explore the significance of sE-cadherin in chronic hepatitis C infection and the correlation with treatment response.

Methods: 87 chronic HCV infected patients and 60 healthy subjects were enrolled in this study. Blood samples from patients receiving the combined treatment of pegylated interferon-a (Peg-IFN-α) with ribavirin (RBV) were collected before treatment, during 4th, 12th therapy weeks, end of the treatment, and 24 weeks post-therapy. Plasma sE-cadherin level was detected by enzyme-linked immunosorbent assay (ELISA) and the relationship between sE-cadherin and antiviral treatment outcome was analyzed.

Results: Plasma sE-cadherin concentrations of Chronic HCV infected patients were significantly higher than that of healthy controls. A strong correlation between sE-cadherin level and the HCV viral load, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and also glutamyl transpeptidase (GGT) level was detected. Chronic HCV infected patients achieving rapid virological response (RVR) and sustained virological response (SVR) had lower baseline sE-cadherin concentrations compared with the non-RVR and non-SVR groups respectively. Univariate and multivariate regression analyses suggested that baseline plasma sE-cadherin level was predictive of therapeutic effect in patients with chronic HCV infection.

Conclusion: Baseline sE-cadherin level could be considered as an independent predictor of SVR with Peg-IFN-α plus ribavirin therapy in the Chinese Han population chronic HCV infection patients. Effective antiviral therapy might restore sE-cadherin at physiological levels.

1. Introduction

With about 200 million chronically infected patients worldwide, the incidence of hepatitis C virus (HCV) infection is also on the rise in China [1,2]. Due to long-term chronic HCV infection eventually results in severe liver diseases, such as advanced fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC), HCV infection is still a serious health problem throughout the world. HCV eradication is the most effective treatment to halt disease progression. Thanks to the progress in the understanding of HCV viral kinetics, genome and other factors influenced in HCV infection, multiple direct-acting antivirals (DAAs) have revolutionized therapy against HCV infection, with current sustained virological response (SVR) rates over 90% [3,4]. Because of the limitation of source of drugs and the high costs, combination of Pegylated IFN (Peg-IFN) and ribavirin (RBV) treatment plan has still been used as the first-line clinical therapeutic regimen in most of the Chinese HCV patients in the recent years.

Encoded by gene CDH1, E-cadherin is one of the epithelial-mesenchymal transition (EMT) related markers together with N-cadherin, and vimentin. It is a calcium-dependent cell-cell adhesion glycoprotein composed of five extracellular cadherin repeats, a transmembrane region, and a highly conserved cytoplasmic tail. Because the importance

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of this transmembrane glycoprotein in epithelial cell adhesion, loss or decreased expression of membrane E-cadherin has been involved in various human malignancies, including gastric cancer, HCC, colon cancer, breast cancer and ovarian cancer [5][6–9]. Results from several recent studies have also demonstrated that the aberrant expression of E-cadherin was related to tumor stage, early tumor invasion, poor prognosis and eventually leading to reduced survival of these cancers.

Studies also found that the extracellular domain of E-cadherin can be hydrolyzed and released into circulation in the form of sE-cadherin (80 kDa fragment of E-cadherin) [10]. Members of the A Disintegrin and Metalloprotease (ADAM) family (ADAM10 and 15), bacterial proteases, cathepsins (B, L, S), members of matrix metalloprotease (MMP) family (MMP-2, 3, 7, 9, and 14), and plasmin have all been implicated in the generation of sE-cadherin [11–18]. Mounting of studies showed that elevated levels of sE-cadherin reflected the progression of various disease such as tumor and infectious disease. Patients with epithelial carcinoma such as prostate cancer, gastrointestinal cancer and ovarian cancer have increased serum sE-cadherin [19–23]. In addition to tumors, increased sE-cadherin level was also present in some infectious diseases, trauma, organ failure and other diseases [14,24,25,26]. The level of sE-cad in acute pancreatitis was also significantly elevated and could predict the severity of pancreatitis, allowing for the appropriate intervention [24]. In HIV infection, the levels of sE-cadherin correlate with viral load in patients [24], suggesting that sE-cadherin is a marker for severity of infection.

However, to date, the significance and function of sE-cadherin in chronic HCV infection remains unknown. The purpose of this study was to investigate a series of changes in plasma sE-cadherin levels in patients with chronic HCV infection receiving Peg-IFN-α plus ribavirin treatment, and to explore the correlations between plasma sE-cadherin level and antiviral treatment response.

2. Materials and methods

2.1. Patient selection

A total of 87 patients with HCV infection were included in this study. All patients were outpatients or present for follow-up examinations in the Affiliated Infectious Disease Hospital of Soochow University from Oct 2015 to Dec 2017. All the patients involved in the study were treatment naïve and without cirrhosis, HCV antibody tested positive, with viral RNA exceeding 20 IU/ml. The presence of chronic HCV infection was defined as detectable HCV RNA at initiation of therapy. The patient who co-infected with Hepatitis B Virus (HBV), human immunodeficiency virus (HIV), active tuberculosis, other immune-related diseases and malignancy were excluded.

Of the 87 patients, 67 (77.0%) were HCV genotype 1b, 11 (12.6%) were genotype 2a, 4 (4.6%) were genotype 3a and 5 (5.8%) were genotype 6a. For normal healthy controls (HCs), sixty healthy individuals were included, with a gender ratio and average age matching the patient group. All patients and controls were Chinese Han population and a written informed consent was obtained from each subject. The study was conducted in accordance with the principals of the Declaration of Helsinki and was approved by the ethics committee of Soochow University and the affiliated infectious disease hospital.

2.2. Study design

All patients followed a personalized treatment with Peg-IFN a-2a (Pegasys, Roche, Switzerland, 180 μg/week, subcutaneously) combined with oral ribavirin (Robatrol, Roche, Shanghai, China, weight-adjusted dose, 800 mg/day for < 65 kg, 1000 mg/day for 65–85 kg and 1200 mg/day for ≥ 85 kg) for 24–48 weeks. Treatment duration was 48 weeks for genotypes 1/4/5 or 6, 24 weeks for genotype 2, and 24 or 48 weeks for genotype 3 based on rapid virologic response (RVR). Treatment was discontinued if HCV RNA loads dropped < 2 Log_{10} at week 12 compared with baseline values, or if HCV RNA load still could be detected at week 24. Blood samples were taken before treatment to determine baseline values. Plasma samples were collected during the 4th, 12th therapy weeks, end of the therapy (EOT) and the 24th week post therapy, and stored at −80 °C for later use. Plasma sE-cadherin levels together with other clinical laboratory parameters and clinical outcomes were measured and collected. The final hepatitis C viral RNA test was performed after week 24 of treatment. The gathered data were then statistically analyzed.

2.3. Outcome definition

Treatment responses were defined as follows: rapid virological response (RVR), HCV RNA load < 20 IU/ml after four weeks of treatment; early virological response (EVR) was defined as seronegative or at least a 2 log_{10} decrease from baseline of serum HCV RNA at week 12 of treatment, the former was complete early virological response (cEVR), while the latter was partial early virological response (pEVR). Sustained virological response (SVR) was defined as HCV RNA load < 20 IU/ml at least 24 weeks after end of treatment, and any other outcome was considered as nonsustained virological responses (non-SVR). Non-respond (NR) was defined as after 12 weeks of treatment, the decrease in HCV RNA level was < 2 log_{10} IU/ml compared with the baseline, and HCV RNA could still be detected at the end of standard treatment. Relapse was defined as HCV RNA reappearance during the follow-up period in patients who achieved seronegative at end of treatment (EOT).

2.4. Laboratory measurements

Serum HCV RNA was quantified with COBAS® AmpliPrep/COBAS® Taqman® HCV Quantitative Test v2.0 (CAP/CTM HCV v2, Roche Molecular Systems, Pleasanton, CA, USA) with detection limit of 20 IU/ml. High HCV viral load was defined as ≥ 600, 000 IU/ml and low viral load was defined as < 600, 000 IU/ml. HCV RNA quantification was performed at baseline, W4, W12, end of treatment and 24 weeks after treatment respectively. Anti-HCV antibody was determined by enzyme-linked immunosorbent assay (Abbott HCV EIA 2.0, Abbott Diagnostic, Chicago, IL, USA). The second-generation line probe assay (Inno-Lipa II; Innogenetics, Zwijndre, Belgium) was used for Genotyping of HCV. An automatic analyser (Hitachi 7170A; Hitachi Ltd., Tokyo, Japan) was used to determine serum biochemical indexes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT). The fibrosis index based on four factors (fibrosis-4 index, FIB-4 index) was calculated according to the formula: FIB-4 index = age (years) × AST [U/L]/(platelet counts [10^9/L] × (ALT [U/L])$^{1/2}$).

2.5. ELISA for sE-cadherin measurement

Plasma concentration of sE-cadherin was determined using a commercial ELISA kit (R&D Systems, Abingdon, UK) following the instruction of manufacturer. The sE-cadherin assay employs the quantitative sandwich enzyme immunoassay technique. In brief, the capture monoclonal antibody specific for human *E*-cadherin has been precoated onto a microplate, after the standard solutions and samples incubated in the microtiter plate wells, an enzyme-linked detection antibody specific for human E-Cadherin is added to the wells, a substrate solution is added to the wells and color develops. The reaction was terminated by the addition of 2 N sulfuric acid. sE-cadherin in plasma of patients with chronic HCV were detected at baseline, W4, W12, end of treatment and 24 weeks after treatment. The plasma sE-cadherin was also determined in the healthy age-matched control group. For each plasma sample, diluted at 1:1 with phosphate-buffered saline. Absorbance was determined by microplate spectrophotometer at 450 nm with the correction wavelength set to 540 nm, and plasma

levels of sE-cadherin were quantified. All the samples were measured in duplicates.

2.6. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics software (version 23.0, SPSS, Inc., Chicago, IL, USA) or GraphPad Prism (version 7.0, GraphPad Softioare Inc., San Diego, CA, USA). Continuous variables are expressed as means ± standard deviations (mean ± SD), while categorical variables are expressed as actual numbers where appropriate. The comparisons between groups were performed via Student's *t*-test, χ^2 test or Fisher's exact test. The Pearson correlation analysis method was performed to analyze the correlation. Univariate logistic regression analysis was applied to identify factors associated with baseline plasma sE-cadherin or to predict the therapeutic outcome. Multiple logistic regression analysis was applied to identify the independent risk factors predictive of non-SVR. Receiver operating characteristic (ROC) was to determine the optimum cut-off values for indicators associated with the treatment outcome of chronic HCV infection patients. All tests were two-tailed, and *P*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Treatment responses of Peg-IFN/RBV therapy

Of the 87 patients receiving antiviral therapy, 58 (66.7%) achieved RVR, 73 (83.9%) were cEVR, and 74 (85.1%) achieved SVR. There were 9 relapsed after cessation to therapy and 4 were NR, the patients did not achieve SVR (non-SVR) were 13 in all. The baseline clinical characteristics of all chronic HCV infection patients of different virological response were compared and summarized in Table 1. Patients with different treatment outcomes and virological response (SVR vs non-SVR, RVR vs non-RVR and cEVR vs non-cEVR) were similar in terms of the demographic and HCV genotype. The baseline HCV RNA loads of patients from non-RVR, non-cEVR and non-SVR groups were significantly higher than that of patients from RVR, cEVR and SVR group respectively. The same was true of pre-treatment AST, which were significantly higher in non-RVR, non-cEVR and non-SVR groups than that of RVR, cEVR and SVR groups. Meanwhile, significant difference was also observed in the baseline values of ALT and GGT between SVR and non-SVR, RVR and non-RVR groups. The FIB-4 index which used to evaluate liver fibrosis was significantly higher both in non-cEVR and non-SVR groups compared to their counterparts (Table 1).

3.2. Increased plasma level of sE-cadherin in HCV-infected patients

In order to understand the role of sE-cadherin in patients with chronic HCV infection, the plasma sE-cadherin of 87 pre-treatment patients and 60 age-matched healthy subjects were determined. Results showed that plasma sE-cadherin levels in chronic hepatitis C infection patients were significantly higher than that of healthy donor group (HD group) (71.84 ± 24.31 vs. 35.37 ± 12.20 ng/ml; Student's *t*-test, $P < 0.001$, Fig. 1). Furthermore, there was a significant correlation between sE-cadherin level and HCV viral load in chronic HCV patients before treatment (Pearson's $r = 0.5557$, $P < 0.0001$, Fig. 2A). Moreover, sE-cadherin levels were also positively correlated with ALT, AST and GGT levels (Fig. 2B–D). We used univariate and multivariate linear regression analyses to identify factors associated with plasma baseline sE-cadherin levels in the patients with chronic HCV infection. In univariate analysis, younger age, lower baseline HCV RNA levels, lower AST and FIB-4 index were significantly associated with a lower baseline sE-cadherin level (Table 2). No significant differences in baseline plasma sE-cadherin could be observed between genders, ages and HCV genotypes. Logistic regression analysis revealed that pre-treatment GGT level above normal (OR/CI: 3.299/1.154–9.434, $P = 0.026$) and baseline HCV RNA loads ≥600,000 IU/ml (OR/CI: 3.091/1.024–9.038, $P = 0.045$) were independent predictors of higher plasma sE-cadherin (Table 2).

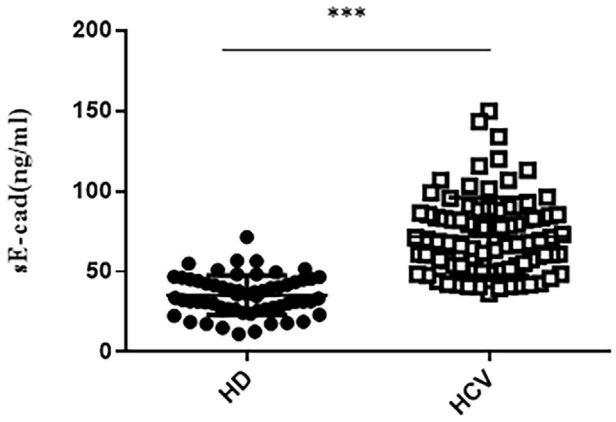

Fig. 1. Plasma sE-cadherin concentrations in patients with chronic HCV infection were significantly higher than those in healthy donors (HD). The scatter dot plot shows the plasma sE-cadherin value of each subject. Data was presented as means ± SD. ***$P < 0.001$.

3.3. The normalization of plasma level of sE-cad was associated with successful antiviral treatment

To investigate the serial changes of sE-cadherin level associated the antiviral treatment and the correlation between the pre-treatment baseline sE-cadherin and treatment response, plasma sE-cadherin levels in patients with chronic hepatitis C were detected at baseline, W4, W12, end of treatment and 24 weeks after treatment, respectively. Compared to the patients who achieved SVR, patients with non-SVR had

Table 1
The baseline characteristics of chronic HCV infection patients stratified by response to Peg-IFN-α plus ribavirin therapy.

Variables	RVR (n)		*P* value	cEVR (n)		*P* value	SVR (n)		*P* value
	Yes (58)	Non (29)		Yes (73)	Non (14)		Yes (74)	Non (13)	
Gender (male/female)	30/28	19/10	0.257	40/33	9/5	0.769	40/34	9/4	0.375
Age (years)	41.28 ± 13.17	44.72 ± 13.56	0.257	41.99 ± 13.31	45.17 ± 13.64	0.446	41.62 ± 13.33	47.00 ± 12.81	0.181
HCV RNA (Log_{10}IU/ml)	5.69 ± 0.91	6.94 ± 0.86	0.000	5.96 ± 1.03	7.07 ± 0.79	0.001	5.92 ± 1.02	7.19 ± 0.64	0.000
HCV genotype (1b/non-1b)	47/11	20/9	0.207	58/17	9/3	1.000	58/16	9/4	0.715
ALT (IU/L)	54.59 ± 29.49	89.17 ± 67.51	0.013	63.16 ± 47.94	84.58 ± 48.16	0.155	61.81 ± 47.20	90.62 ± 48.71	0.047
AST (IU/L)	41.03 ± 18.20	59.00 ± 36.98	0.019	41.99 ± 23.86	66.25 ± 38.31	0.007	43.05 ± 23.11	69.62 ± 37.21	0.026
GGT (IU/L)	36.53 ± 23.14	57.31 ± 40.47	0.015	39.52 ± 25.70	68.08 ± 49.92	0.076	38.54 ± 24.83	71.46 ± 48.07	0.031
FIB-4 index	1.23 ± 0.66	1.55 ± 0.85	0.058	1.23 ± 0.67	1.77 ± 0.81	0.014	1.20 ± 0.66	1.88 ± 0.74	0.001

Abbreviations: RVR, rapid virological response; SVR, sustained virological response; cEVR, Complete early virological response; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, glutamyl transpeptidase; FIB-4 index, Fibrosis-4 index.

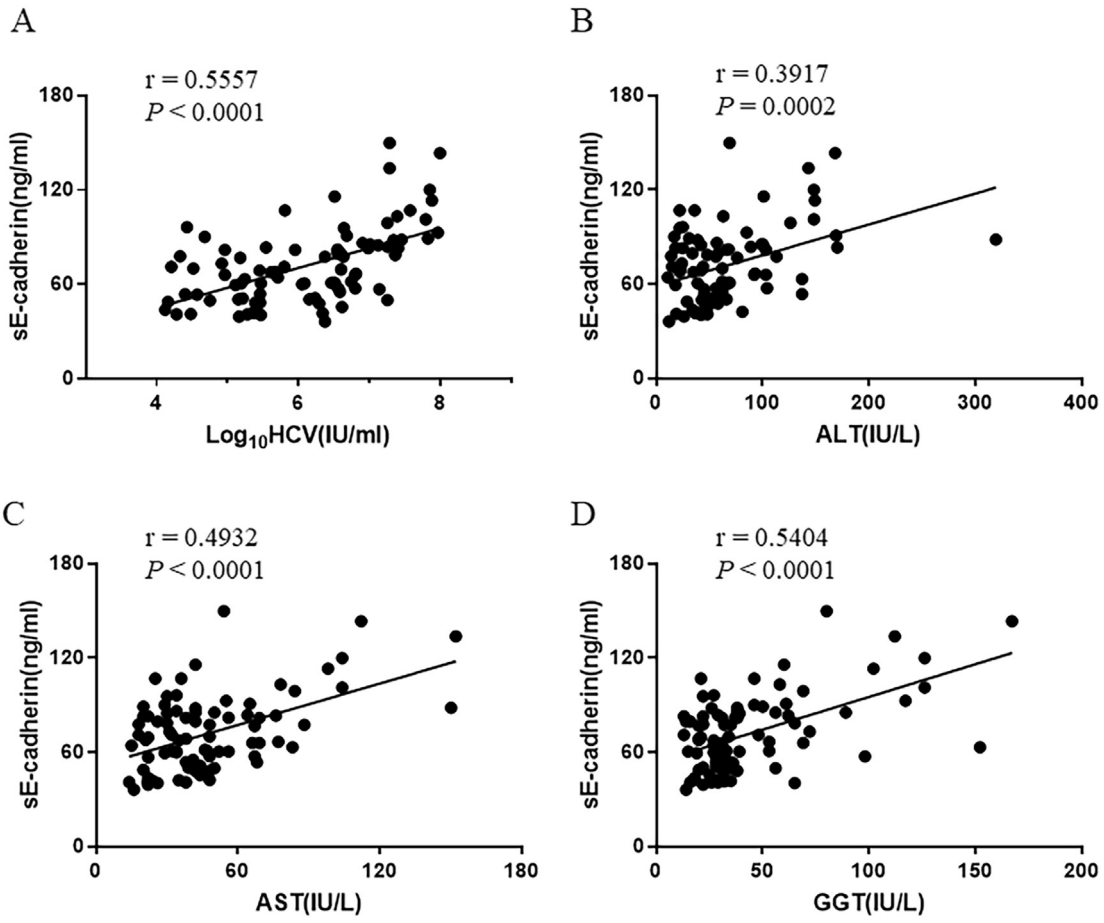

Fig. 2. Correlation between plasma sE-cadherin and clinical assessments in patients with chronic HCV infection. (A) Correlation between sE-cadherin and HCV viral load (Log_{10}HCV RNA). (B) Correlation between sE-cadherin and ALT level. (C) Correlation between sE-cadherin and AST level. (D) Correlation between sE-cadherin and GGT level.

Table 2
Factors associated with baseline sE-cadherin in chronic HCV patients.

Clinical parameters	Cases (n)	Baseline sE-cadherin		Univariate analysis	Multivariate analysis	
		< 69.81 ng/ml n (%)	≥69.81 ng/ml n (%)	0R (95% CI) *P value*	0R (95% CI)	*P value*
Gender						
Male	49	23 (50.0)	26 (63.4)			
Female	38	23 (50.0)	15 (36.6)	0.750 (0.317–1.777) 0.513		
Age, years						
< 40	63	38 (82.6)	25 (59.5)			
≥40	24	8 (17.4)	16 (40.5)	3.040 (1.132–8.160) 0.027	2.431 (0.726–8.140)	0.785
HCV genotype(1b/non-1b)						
1b	67	37 (80.4)	30 (73.2)			
Non-1b	20	9 (19.6)	11 (26.8)	1.507 (0.552–4.114) 0.423		
HCV RNA(Log10 IU/ml)						
< 6	39	27 (58.7)	12 (29.3)			
≥6	48	19 (41.3)	29 (70.7)	3.434 (1.406–8.386) 0.007	3.091 (1.024–9.038)	0.045
AST (IU/L)						
< 37	40	23 (50.0)	17 (41.5)			
≥37	47	23 (50.0)	24 (58.5)	1.412 (0.604–3.298) 0.426		
ALT (IU/L)						
< 40	26	13 (28.3)	13 (31.7)			
≥40	61	33 (71.7)	28 (68.3)	0.848 (0.338–2.127) 0.726		
GGT (IU/L)						
< 45	60	39 (84.8)	21 (51.2)			
≥45	27	7 (15.2)	20 (48.8)	4.006 (1.566–10.248) 0.004	3.299 (1.154–9.434)	0.026
FIB-4 index						
< 1.25	51	33 (71.7)	18 (43.9)			
≥1.25	26	13 (28.3)	23 (56.1)	2.338 (1.059–5.160) 0.036	1.649 (0.582–4.672)	0.347

Abbreviations: OR, Odds ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, glutamyl transpeptidase; FIB-4 index, Fibrosis-4 index.

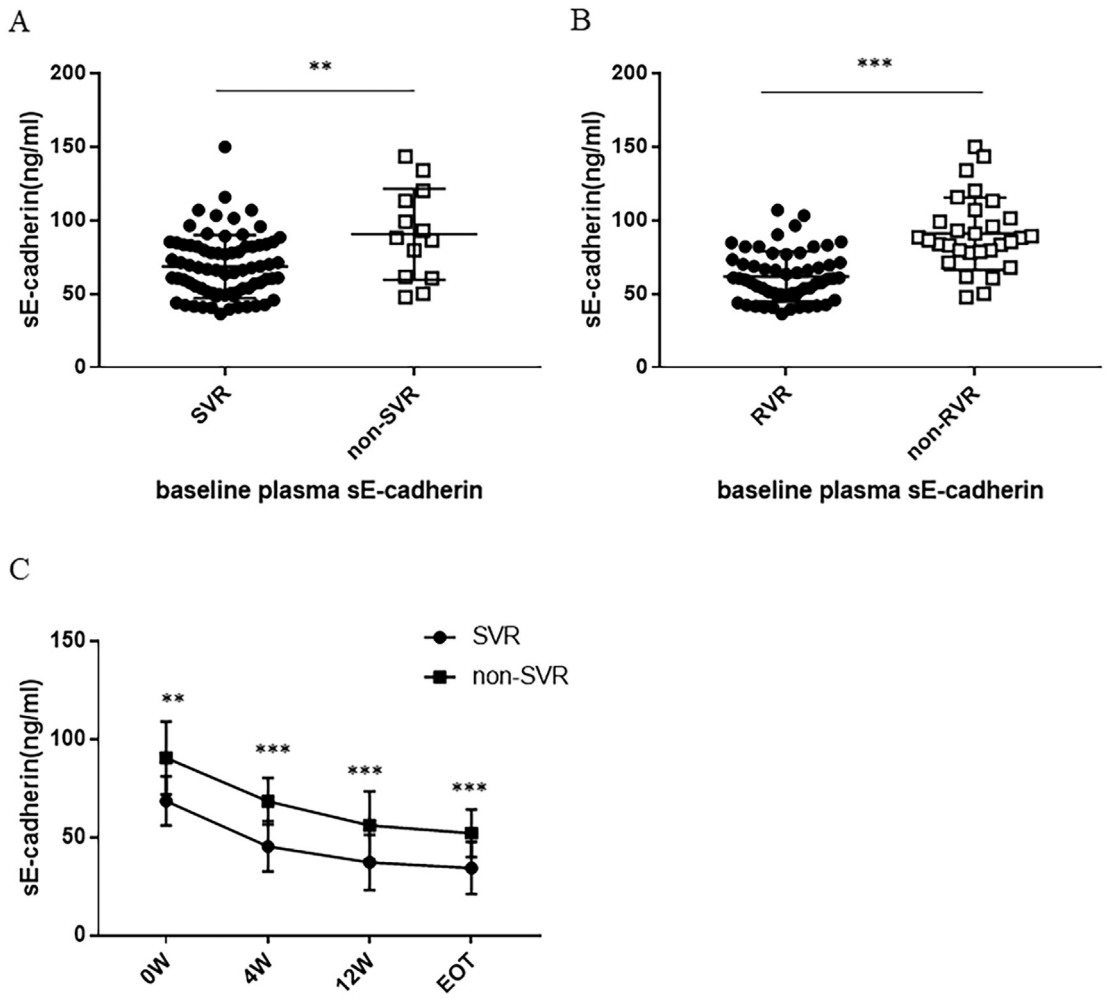

Fig. 3. Correlation between baseline plasma sE-cadherin levels and therapeutic efficacy in patients with chronic hepatitis c virus infection. (A) Baseline plasma sE-cadherin between SVR and non-SVR. (B) Baseline plasma sE-cadherin between RVR and non-RVR. (C) Time course changes of plasma sE-cadherin levels in SVR and non-SVR patients received Peg-IFN/VRVB treatment. EOT, end of treatment. Data was presented as means ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$.

significantly higher baseline sE-cadherin level (mean ± SD: 68.56 ± 23.43 vs. 90.53 ± 30.94 ng/ml; Student's *t*-test, $P = 0.0021$, Fig. 3A). Besides sE-cadherin level, baseline HCV RNA load, AST, ALT, GGT level and FIB-4 index were all higher in non-SVR patients than those in SVR patients (Table 1). There was also a significant difference in plasma baseline sE-cadherin level between patients with RVR and those without RVR (mean ± SD: 61.83 ± 17.03 vs. 90.88 ± 20.40 ng/ml; Student's *t*-test, $P < 0.0001$, Fig. 3B). When further comparing the dynamic changes of sE-cadherin from patients during the process of antiviral therapy with Peg-IFN-a and ribavirin, a similar on-treatment downward trend of plasma sE-cadherin was observed both in SVR and non-SVR patients. The decline of sE-cadherin was sharply at W4 of treatment and then slowed down. Furthermore, the concentration of sE-cadherin in plasma of the SVR group at W4, W12 and EOT were all significantly lower than that of the non-SVR group respectively (Fig. 3C). At the EOT, sE-cadherin levels in the SVR group had decreased to no significant difference from the normal healthy control group (mean ± SD: 34.43 ± 12.53 vs. 35.37 ± 12.20 ng/ml; Student's *t*-test, $P < 0.001$, while although the level of sE-cadherin in non-SVR group also decreased, it was still significantly higher compared with the control group (mean ± SD: 52.17 ± 14.37 vs. 35.37 ± 12.20 ng/ml; Student's *t*-test, $P < 0.001$, Fig. 3C). Normalization of plasma sE-cadherin level in chronic HCV patients achieved SVR demonstrated that the effective antiviral therapy might restore sE-cadherin at low physiological levels.

3.4. Baseline factors associated with the treatment outcome of patients with chronic HCV infection

To further explore the predictive factors associated with the treatment outcome of chronic HCV infection, ROC analysis was to determine the optimum cut-off values for sE-cadherin, Log_{10}HCV RNA, FIB-4 index and indices of liver function ALT, AST and GGT. The optimal cut-off value to predict SVR in our patients were 69.81 ng/ml for sE-cadherin, 6.26 for Log_{10} HCV RNA and 1.19 for FIB-4 index respectively (Supplementary Table 1). The AUCs were illustrated in the ROC curves in Fig. 4. Baseline sE-cadherin showed the AUC (0.72) when used alone for distinguishing between SVR and non-SVR. Log_{10}HCV RNA showed the greatest AUC (0.84) followed by FIB-4 index with an AUC of 0.77. When combining baseline sE-cadherin, Log_{10}HCV RNA and FIB-4 index, all indicators together had a higher AUC of 0.87. By Univariate and Multivariate analysis, the baseline variables associated with an SVR were low Log_{10}HCV-RNA load, sE-cadherin and FIB-4 index. Lower baseline Log_{10}HCV-RNA load (OR 5.702, 95% CI 1.093–29.750, $P = 0.039$), sE-cadherin (OR 5.153, 95% CI 1.149–23.112, $P = 0.032$) and FIB-4 index (OR 4.871, 95% CI 1.193–19.899, $P = 0.027$) were independent factors for achieving SVR to chronic hepatitis C infection (Table 3).

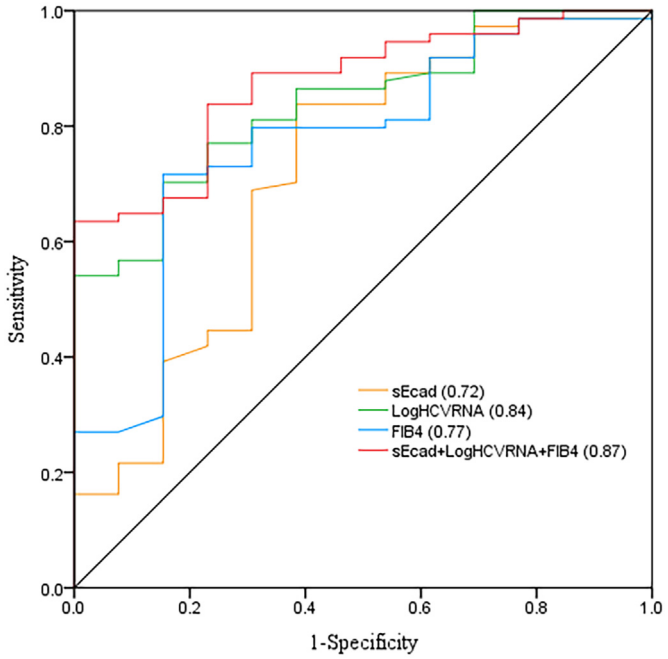

Fig. 4. Receiver operating characteristic (ROC) curves of baseline Log_{10}HCV RNA, plasma sE-cadherin, FIB-4 index and all indicators together for the prediction of SVR vs non-SVR. AUC, area under the curve.

4. Discussion

sE-cadherin is present in a myriad of conditions including caner, infection, organ failure and benign diseases. It could be used as biomarker to contribute insights into disease severity, progression, recurrence, therapeutic response, and prognosis in various cancer. Wang and colleagues' study demonstrated that the sE-cadherin values of patients with chronic hepatitis B, cirrhosis and liver cancer were significantly increased, suggesting that sE-cadherin may be an important indicator of the progression mechanism of HBV-related diseases [27]. Studies have shown that sE-cadherin is not only a biomarker of disease severity, but also a key regulator of the occurrence and development of a variety of diseases. Streeck's study on HIV infection showed that elevated sE-cadherin was induced in HIV infected patients, which in turn impaired antiviral cytokine expression through interact with one of its receptor-Killer cell lectin-like receptor G1 (KLRG1) expressed on HIV-1 specific $CD8^+$ T cells [24]. In addition, Patil has shown that sE-cadherin could mediate tumor progression and metastasis by activating insulin-like growth factor (IGF) [28]. However, the implication of sE-cadherin in chronic HCV infection remains unclear. Therefore, our aim in the present study was to evaluate possible associations of baseline sE-cadherin to severity of chronic HCV infection and the correlation between sE-cadherin and outcome of antiviral therapy in HCV patients treated with Peg-IFN-a and RBV.

In the present study, we observed elevated plasma levels of sE-cadherin in patients with chronic HCV infection compared to healthy individuals. Significant positive correlations between plasma sE-cadherin and major liver function parameters (including ALT, AST, and GGT) and also HCV viral load were observed, suggesting that sE-cadherin could be an appropriate marker for progressive liver damage and HCV replication. Univariate analysis showed that age ≥ 40, Log_{10} HCV RNA ≥ 6, GGT higher than normal range and FIB-4 index were associated with higher sE-cadherin. Further Multivariate analysis demonstrated that only higher Log_{10}HCV RNA and GGT were independent risk factors for high sE-cadherin in chronic HCV infection patients. These results suggested that increased plasma sE-cadherin in chronic hepatitis C may at least partially reflect HCV virus replication and the severity of liver damage.

Another finding of the present study was that treatment naïve patients with chronic hepatitis C achieved SVR after treatment with Peg-IFN-a/RBV demonstrated significant lower baseline plasma sE-cadherin concentrations than the patients failed to attain SVR. In this study, subjects with a lower baseline plasma sE-cadherin level (< 69.81 ng/ml, from the ROC) were significantly associated with favourable therapeutic response both in SVR and RVR. Study also demonstrated that the on-treatment decline degree of sE-cadherin levels in SVR group were significant greater than that of non-SVR group. At the end of treatment, normalization of sE-cadherin level was only observed in the SVR group. Our study suggested that lower pre-treatment plasma sE-cadherin concentration might be a predictor for sustained viral response following a course of Peg-IFN-a/RBV treatment in chronic HCV patients. And effective antiviral therapy might restore sE-cadherin at physiological levels. Univariate and multivariate analysis demonstrated that baseline plasma sE-cadherin level was independent risk factor for antiviral response to chronic hepatitis C infection together with HCV viral load and FIB-4 index. Although many previous studies have shown that HCV viral load and FIB-4 index were effective predictors of SVR, there is still some controversy [29–31]. A combination of pre-treatment sE-cadherin with baseline HCV viral load and Fib-4 index was expected to predict antiviral efficacy more effectively and accurately.

This study has some limitations. First, although this was the first study to investigate the association between sE-cadherin level and antiviral treatment response to chronic HCV infection, the limited number of patients included in the study limits the validity of the conclusions. Second, genetic background that might be associated with the responses to Peg-IFN-a/RBV treatment was not determined such as the IL28B genetic variation, which may be important predicator for therapeutic effect of chronic HCV infection. Third, the patents enrolled in the study all mild and without liver cirrhosis, the treatment response mechanisms, however, are complex, so more studies with large sample and

Table 3
Univariate and multivariate logistic regression analyses for predictor of SVR to antiviral therapy in 87 chronic HCV infection patients.

Variables	Univariate analysis		Multivariate analysis	
	0R (95% CI)	*P value*	0R (95% CI)	*P value*
Gender (male/female)	1.913 (0.513–5.975)	0.375		
Age (years) ≥ 42.50 vs < 42.50	0.971 (0.929–1.014)	0.183		
HCV RNA (Log10 IU/ml) ≥ 6.26 vs < 6.26	3.696 (1.040–13.137)	0.022	5.702 (1.093–29.750)	0.039
HCV genotype(1b/non-1b)	1.006 (0.248–4.076)	0.993		
ALT (IU/L) ≥ 56.60 vs < 56.50	0.623 (0.212–1.830)	0.390		
AST (IU/L) ≥ 47.50 vs < 47.50	1.582 (0.481–5.203)	0.450		
GGT (IU/L) ≥ 42.50 vs < 42.50	3.150 (0.944–10.512)	0.062		
FIB-4 ≥ 1.19 vs < 1.19	3.696 (1.040–13.137)	0.043	4.871 (1.193–19.899)	0.027
sE-cadherin(ng/ml) ≥ 69.81 vs < 69.81	4.375 (1.112–17.212)	0.035	5.153 (1.149–23.112)	0.032

Abbreviations: OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, glutamyl transpeptidase; FIB-4 index, Fibrosis-4 index.

multi-factors are warranted to validate the results and the underlying mechanisms need to be explored. The molecular mechanisms of sE-cadherin in chronic HCV infection also deserve further investigation.

5. Conclusions

Plasma sE-cadherin levels in chronic HCV patients significantly higher than normal control individuals, baseline sE-cadherin level was an independent risk factor for antiviral response to chronic hepatitis C infection. Clinical application of pre-treatment sE-cadherin might be helpful in predicting early treatment efficacy and thus enable treatment regimens to be optimized and individually tailored.

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Author contributions

Study design: JMW, ZLS; Data collection: ZRW, HC; Performed the experiments: SSG, HQ, HWL; Data analysis: JMW, SSG; Drafting the manuscript: JMW, ZLS. All authors approved the final version of the manuscript.

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Declaration of Competing Interest

The authors declared no conflict of interest with this work.

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References

[1] T.M. Welzel, N. Bhardwaj, C. Hedskog, et al., Global epidemiology of HCV subtypes and resistance-associated substitutions evaluated by sequencing-based subtype analyses, J. Hepatol. 67 (2) (2017) 224–236.
[2] A.P. hrift, H.B. El-Serag, F. Kanwal, Global epidemiology and burden of HCV infection and HCV-related disease, Nat Rev Gastroenterol Hepatol 14 (2) (2017) 122–132.
[3] O. Falade-Nwulia, C. Suarez-Cuervo, D.R. Nelson, M.W. Fried, J.B. Segal, M.S. Sulkowski, Oral direct-acting agent therapy for hepatitis C virus infection: a systematic review, Ann. Intern. Med. 166 (2017) 637–648.
[4] K. Kozbial, S. Moser, R. Al-Zoairy, R. Schwarzer, C. Datz, R. Stauber, et al., Follow-up of sustained virological responders with hepatitis C and advanced liver disease after interferon/ribavirin-free treatment, Liver Int. 38 (2018) 1028–1035.
[5] J. Ding, Z.M. Zhang, G.Q. Liao, et al., Positive expression of LSD1 and negative expression of E-cadherin correlate with metastasis and poor prognosis of colon cancer, Dig. Dis. Sci. 58 (6) (2013) 1581–1589.
[6] A. Kasprzak, K. Rogacki, A. Adamek, et al., Tissue expression of β-catenin and E-and N-cadherins in chronic hepatitis C and hepatocellularcarcinoma, Arch. Med. Sci. 13 (6) (2017) 1269–1280.
[7] H.N. Horne, M.E. Sherman, M. Garcia-Closas, et al., Breast cancer susceptibility risk associations and heterogeneity by E-cadherin tumor tissue expression, Breast Cancer Res. Treat. 143 (1) (2014) 181–187.
[8] X. Xing, Y.B. Tang, G. Yuan, et al., The prognostic value of E-cadherin in gastric cancer: a meta-analysis, Int. J. Cancer 132 (11) (2013) 2589–2596.
[9] M.T. Lau, W.K. So, P.C. Leung, et al., Fibroblast growth factor 2 induces E-cadherin down-regulation via PI3K/Akt/mTOR and MAPK/ERK signaling in ovarian cancer cells, PLoS One 8 (3) (2013) e59083.
[10] M.J. Wheelock, C.A. Buck, K.B. Bechtol, C.H. Damsky, Soluble 80-kd fragment of cell-CAM 120/80 disrupts cell-cell adhesion, J. Cell. Biochem. 34 (3) (1987) 187–202.
[11] M.M. Grabowska, B. Sandhu, M.L. Day, EGF promotes the shedding of soluble E-cadherin in an ADAM10-dependent manner in prostate epithelial cells, Cell. Signal. 24 (2) (2012) 532–538.
[12] S.B. Lee, A. Schramme, K. Doberstein, et al., ADAM10 is upregulated in melanoma metastasis compared with primary melanoma, J Invest Dermatol 130 (3) (2010) 763–773.
[13] A.J. Najy, K.C. Day, M.L. Day, The ectodomain shedding of E-cadherin by ADAM15 supports ErbB receptor activation, J. Biol. Chem. 283 (26) (2008) 18393–18401.
[14] M.M. Grabowska, M.L. Day, Soluble E-cadherin: more than a symptom of disease, Front. Biosci. 17 (5) (2012) 1948–1964.
[15] W. Schirrmeister, T. Gnad, T. Wex, et al., Ectodomain shedding of E-cadherin and c-Met is induced by Helicobacter pylori infection, Exp. Cell Res. 315 (20) (2009) 3500–3508.
[16] K.H. Lee, E.Y. Choi, M.S. Hyun, et al., Association of extracellular cleavage of E-cadherin mediated by MMP-7 with HGF-induced in vitro invasion in human stomach cancer cells, Eur. Surg. Res. 39 (4) (2007) 208–215.
[17] S.K. Johnson, V.C. Ramani, L. Hennings, R.S. Haun, Kallikrein 7 enhance pancreatic cancer cell invasion by shedding E-cadherin, Cancer 109 (9) (2007) 1811–1820.
[18] M.D. Covington, R.C. Burghardt, A.R. Parrish, Ischemia-induced cleavage of cadherins in NRK cells requires MT1-MMP (MMP-14), Am J Physiol Renal Physiol 290 (1) (2006) F43–F51.
[19] R. Kuefer, M.D. Hofer, C.S. Zorn, et al., Assessment of a fragment of ecadherin as a serum biomarker with predictive value for prostate cancer, Br. J. Cancer 92 (11) (2005) 2018–2023.
[20] I. Tsaur, K. Thurn, E. Juengel, et al., sE-cadherin serves as a diagnostic and predictive parameter in prostate cancer patients, J. Exp. Clin. Cancer Res. 14 (34) (2015) 43.
[21] Y. Okugawa, Y. Toiyama, Y. Inoue, et al., Clinical significance of serum soluble E-cadherin in colorectal carcinoma, J. Surg. Res. 172 (2) (2012) e67–e73.
[22] G. Hofmann, M. Balic, N. Danachi, et al., The predictive value of serum soluble E-cadherin levels in breast cancer patients undergoing preoperative systemic chemotherapy, Clin. Biochem. 46 (15) (2013) 1585–1589.
[23] J. Symowicz, B.P. Adley, K.J. Gleason, J.J. Johnson, Engagement of collagen-binding Integrins promotes matrix metalloproteinase-9-dependent E-cadherin ectodomain shedding in ovarian carcinoma cells, Cancer Res. 67 (5) (2007) 2030–2039.
[24] H. Streeck, D.S. Kwon, A. Pyo, et al., Epithelial adhesion molecules can inhibit HIV-1-specific $CD8^+$ T-cell functions, Blood 117 (19) (2011) 5112–5122.
[25] W. Schirrmeister, T. Gnad, T. Wex, et al., Ectodomain shedding of E-cadherin and c-Met is induced by Helicobacter pylori infection, Exp. Cell Res. 315 (20) (2009) 3500–3508.
[26] A. Sewpaul, J.J. French, T.K. Khoo, M. Kernohan, Soluble E-cadherin: an early marker of severity in acute pancreatitis, HPB Surg. 2009 (2009) 397375.
[27] B. Wang, J. Liu, Y. Xiong, et al., Soluble E-cadherin as a serum biomarker in patients with HBV-related liver disease, Clin. Biochem. 49 (16–17) (2016) 1232–1237.
[28] P.U. Patil, J. D'Ambrosio, L.J. Inge, et al., Carcinoma cells induce lumen filling and EMT in epithelial cells through soluble E-cadherin-mediated activation of EGFR, J. Cell Sci. 128 (23) (2015) 4366–4379.
[29] G. Fattovich, L. Covolv, S. Bibert, et al., IL28B polymorphisms, IP-10 and viral load predict virological response to therapy in chronic hepatitis C, Aliment. Pharmacol. Ther. 33 (10) (2011) 1162–1172.
[30] K. Neukam, A. Camacho, A. Caruz, et al., Prediction of response to pegylated interferon plus rivavirin in HIV/hepatitis C virus (HCV)-coinfected patients using HCV genotype, IL28B variations, and HCV-RNA load, J. Hepatol. 56 (4) (2012) 788–794.
[31] S.K. Tohra, S. Taneja, S. Ghosh, et al., Prediction of sustained virological response to combination therapy with pegylated interferon alfa and ribavirin in patients with genotype 3 chronic hepatitis C, Dig. Dis. Sci. 56 (8) (2011) 2449–2455.