

High plasma soluble thrombomodulin levels indicated poor prognosis of decompensated liver cirrhosis: a prospective cohort study

Xinhuan Wei^{a,*}, Xiaofei Du^{a,*}, Yali Liu^a, Jun Wu^b and Jing Zhang^a

Objective Hepatic sinusoidal endothelial injury is a prominent characteristic of liver cirrhosis. We determined plasma soluble thrombomodulin (sTM) levels in cirrhosis patients to evaluate the relationship between vascular injury and long-term prognosis.

Methods A prospective single-center study was performed. The participants were followed up for every 6 months or until death or transplantation. A chemiluminescent enzyme immunoassay was used to establish a baseline sTM.

Results Among the 219 patients with decompensated liver cirrhosis, 53.42% were caused by hepatitis B and hepatitis C. Plasma sTM levels were much higher in cirrhosis than in healthy controls and increased parallel with Child-Pugh classification ($P < 0.01$) and the amount of ascites ($P = 0.04$). After adjusting for sex, age, international normalized ratio, bilirubin, and other potential factors, multivariate Cox regression revealed that per TU/ml elevation of plasma sTM causes an increase of 8% in mortality, and per-SD elevation of thrombomodulin causes a 53% increase in mortality. As the mortality rates in low (5.90–12.60 TU/ml) and medium (12.70–18.00 TU/ml) sTM levels were similar, so we chose the cutoff of 18.00 TU/ml to divide into two groups, and K-M analysis indicated that patients with sTM >18.0 TU/ml demonstrated an additional 2.01 times death risk (95% CI, 1.13–7.93; $P = 0.01$) than those with sTM ≤ 18.0 TU/ml.

Conclusion Plasma sTM in cirrhosis was significantly increased in parallel with the severity of liver dysfunction. sTM elevation than 18 TU/ml indicated a poor prognosis of decompensated liver cirrhosis. *Eur J Gastroenterol Hepatol* 34: 1140–1146
Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.

Introduction

Liver sinusoidal endothelial cells (LSECs) form the wall of the hepatic sinusoids. In addition to acting as a barrier between hepatocytes and the bloodstream, LSECs have multiple essential roles, such as regulation of the vascular tone, inflammation, and thrombosis, autocrine and immune response, participation in liver fibrosis, liver generation [1,2], etc. In liver cirrhosis, extensive necrosis of hepatocytes, formation of regenerative nodules, structural remodeling of hepatic lobules and diffuse connective tissue hyperplasia result in the disorder of blood circulation in the liver, which is characterized by the shrinkage, occlusion, and distortion of the vascular bed, and

the compression of blood vessels by regenerative nodules and, thus, aggravating the damage of vascular endothelial cells. Because vascular endothelial injury is involved in the progression of liver cirrhosis and portal hypertension, there were several markers of endothelial injury, such as von Willebrand Factor (vWF), soluble P-selectin, soluble VE-cadherin, soluble thrombomodulin (sTM), etc. [3]. vWF was the most investigated factor and could predict the severity of cirrhosis and portal hypertension [4]. But vWF levels varied widely in healthy individuals due to genetic background. So extremely high values of vWF are more valuable in predicting the prognosis of cirrhosis than lower-level vWF. sTM is a well-known and reliable marker of endothelial injury in the early and advanced stages [5]. Thrombomodulin is an endothelial cell transmembranous glycoprotein acting as a receptor for thrombin and plays a key role in anticoagulation. TM was released into the bloodstream as sTM with four degraded forms and different molecular weights when the endothelial cell was stimulated by inflammation [6]. Previous studies showed that sTM was correlated with the incidence of coronary heart disease (CHD), renal failure, etc [6,7]. sTM levels were also found to be higher in acute and chronic liver failure, chronic hepatitis, and cirrhosis in several studies [8–10]. But these researches were limited by relatively small sample size (dozens of patients), and none of them was focused on the relationship of sTM with the outcome.

We conducted a prospective cirrhosis cohort and followed them for every 6 months to see if there was a link between baseline sTM and prognosis to find better cirrhosis progression markers.

European Journal of Gastroenterology & Hepatology 2022, 34:1140–1146

Keywords: decompensated liver cirrhosis, endothelial cells, mortality, thrombomodulin

^aThe Third Unit, Department of Hepatology, Beijing Youan Hospital, Capital Medical University and ^bDepartment of Clinical Laboratory, Beijing Jishuitan Hospital, Beijing, China

Correspondence to Jing Zhang, MD, The Third Unit, Department of Hepatology, Beijing Youan Hospital, Capital Medical University, No.8 Youanmenwai Street, Fengtai District, Beijing, 100069, China

Tel: +0086 13391859683; fax: +0086 10 63056962;

e-mail: zjyouan@ccmu.edu.cn

*Xinhuan Wei and Xiaofei Du contributed equally to the writing of this article.

Received 18 April 2022 **Accepted** 5 July 2022

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Patients and methods

Patient selection and enrollment criteria

This was a prospective observational study that was conducted in the future. From August 2016 to February 2021, hospitalized adult patients with decompensated liver cirrhosis were included in the study, with follow-up visits every 6 months or until death or liver transplantation. The study protocol was approved by Beijing Youan Hospital Ethics Committee [NO: 2016-13] in June 2016. All patients provided written informed consent. The research was performed according to the guidelines of the 1975 Declaration of Helsinki (6th revision, 2008). This study protocol was registered in Clinical Trials (ChiCTR1900025035).

Laboratory tests, ultrasound, or radiology imaging results were used to diagnose cirrhosis. Decompensated liver cirrhosis was marked by the development of overt clinical signs including ascites, bleeding, and hepatic encephalopathy. The exclusion criteria were as followed: hepatocellular carcinoma or any other malignancy, HIV-coinfections, with severe cardiac, respiratory, kidney diseases, or other severe diseases. Patients who did not have any follow-up information were also excluded. Age and sex-matched 50 healthy individuals were recruited from Beijing Jishuitan Hospital as the healthy control group.

The standard care and data collection

The causes of cirrhosis include hepatitis B, hepatitis C, alcoholic liver diseases, and autoimmune hepatitis. The viral load was suppressed to below the lower detection limit in all hepatitis B patients who received antiviral therapy. All hepatitis C-associated cirrhosis achieved sustained viral response by direct-acting antiviral agents. That is, the viral load in all patients associated with hepatitis B virus (HBV)/hepatitis C virus (HCV) was undetected at the time of enrollment. All patients with alcoholic liver diseases were suggested to quit drinking and given nutritional support therapy. Immunosuppressants were used to treat autoimmune hepatitis unless there were contraindications. When necessary, drugs to lower portal vein pressure such as non-selective beta-blockers (NSBBs) were considered. Cirrhosis complications were treated according to guidelines.

Demography data, liver functions, and complications of cirrhosis were recorded from Electronic Medical Record System. The severity of cirrhosis was evaluated by Child-Pugh score, model of end-stage liver disease (MELD) score, and MELD-Na score. The MELD score was calculated as $9.57 \times \ln(\text{Serum creatinine}) + 3.78 \times \ln(\text{bilirubin}) + 11.2 \times \ln(\text{INR}) + 6.43 \times (0 \text{ for alcohol-related liver disease or for cholestatic liver disease; } 1 \text{ for all other causes})$ [11]. MELD-Na score was calculated using the following formula, MELD-Na score = MELD score + $1.59 \times (135 - \text{Na})$ with maximum and minimum Na values of 135 and 120 mmol/l [12]. According to West Haven criteria, hepatic encephalopathy was graded as minimal, grade I, grade II, grade III, and grade IV [13]. According to clinical practice guidelines, ascites were classified as mild, moderate, or large [14]. Patients were followed up regularly in the outpatient department or readmission to the hospital.

Plasma sTM determination

The blood samples were collected in vacuum tubes containing 3.2% trisodium citrate (Vacutainer; Becton–Dickinson, Meylan, France) and then centrifuged for 15 min at 1500 g. And the plasma was stored at -80°C within 2 h. sTM (TU/ml) was measured by chemiluminescent enzyme immunoassay using an automated immunoassay system (HISCL5000, Sysmex, Corollary reagent, Japan).

Statistical methods

Categorical data were summarized as numbers and percentages and were analyzed using the Chi-square test. Kolmogorov–Smirnov tests were performed for the normality test. Continuous variables were described as mean \pm SD or as medians (interquartile ranges) depending on whether they showed a Gaussian distribution, and intergroup comparisons were analyzed with Student's *t*-test or one-way analysis of variance, or Mann–Whitney U test. Spearman correlations were applied to analyze the relationship between sTM and clinical indexes. Smooth curve fitting was used to observe an association between sTM and living status. After accounting for significant confounders, we used multivariable Cox regression to investigate the independent relationship between sTM and cirrhosis patient mortality, and the hazard ratio (HR) and β were obtained. Three regression models were created to demonstrate the results' consistency. In the crude model, no cofounders were adjusted, and in model I and model II, cofounders that changed the estimates of sTM on mortality by more than 10% or were significantly associated with mortality were adjusted. Besides, to clearly show the influence, we calculated the per-SD increase. Given the differences in the baseline characteristics, propensity-score matching was used to identify a cohort of patients with similar baseline characteristics. A 1:5 matching protocol with a caliper width equal to 0.2 of the SD of the logit of the propensity score was used for matching. Moreover, for analysis of the mortality rates, Kaplan–Meier (K-M) survival curves and Cox regression were used. A sensitivity analysis was done to ensure our analysis' robustness. A stratification analysis was conducted to examine whether the association of sTM and overall mortality differed across various subgroups classified by sex, age, cause, Child-Pugh scores, ascites, hepatic encephalopathy, esophageal gastric variceal bleeding, white blood cell (WBC), bilirubin, and creatinine. Statistical analyses were performed using EmpowerStats software (<http://www.empowerstats.com>, X&Y solutions, Inc., Boston, Massachusetts, USA) and R (<http://www.R-project.org>) software. Graphs were performed with GraphPad Prism software version 5 (GraphPad Software, La Jolla, California, USA, 2012). $P < 0.05$ was considered statistically significant.

Results

Baseline clinical characteristics of the participants

A total of 377 cirrhosis patients were screened from August 2016 to February 2021. Because of their HIV coinfection, 23 patients were ruled out. In addition, 52 patients with other serious underlying diseases, such as

cardiopulmonary diseases, kidney diseases, etc., were excluded from the study. Sixty-four patients with compensated liver cirrhosis were also excluded. Finally, 238 cases were successfully enrolled and being followed up. Nineteen patients were lost to follow-up and excluded. Finally, after a median follow-up period of 9.57 (7.56–37.28) months, 219 cases were analyzed in the study (see Fig. 1 for the flowchart), 66.21% of whom were male. The causes of cirrhosis were mainly HBV and HCV infections (53.42%). Sixteen of them used NSBB to lower portal hypertension. Table 1 summarizes the clinical characteristics. We divided sTM tertile groups into low (5.90–12.60 TU/ml), medium (12.70–18.00 TU/ml), and high (18.10–46.20 TU/ml) groups according to their baseline plasma sTM. The mortality rates (including death and liver transplantation) in the tertile groups were 4.55%, 5.80%, and 15.48%, respectively, at the end of follow-up ($P = 0.04$). Patients in higher sTM groups had higher MELD scores, MELD-Na scores, Child-Pugh scores ($P < 0.01$), and a higher incident rate of moderate-large ascites ($P = 0.04$).

Plasma sTM level in liver cirrhosis

Plasma sTM level in liver cirrhosis patients was much higher than in healthy controls [16.20 (12.00–20.40) TU/ml vs. 8.3 (7.3–9.8) TU/ml; $P < 0.01$]. Plasma sTM level increased parallel with Child-Pugh grades and were 12.80 (10.60–17.60) TU/ml, 15.50 (12.20–19.90) TU/ml, and 18.50 (12.60–22.70) TU/ml in Child-Pugh A, B, and C, respectively ($P < 0.01$).

The relationship between sTM, laboratory indexes, and mortality

sTM was mild positive related to prothrombin time (PT) ($r = 0.15$; $P = 0.03$), INR ($r = 0.15$; $P = 0.03$), total bilirubin ($r = 0.19$; $P < 0.01$), MELD ($r = 0.22$; $P < 0.01$),

MELD-Na ($r = 0.21$; $P < 0.01$), creatinine ($r = 0.22$; $P < 0.01$), and WBC ($r = 0.23$; $P < 0.01$), while showing no significant relationship with neutrophils ($r = 0.08$; $P = 0.21$). The smooth curve demonstrated a positive relationship between sTM levels and mortality risk (Fig. 2).

The relationship of plasma sTM level with outcome

To investigate the prognostic significance of sTM, we used sTM as the independent variable and mortality as the dependent variable, whereas sex, age, INR, activated partial thromboplastin time (APTT), fibrinogen, WBC, bilirubin, albumin, and creatinine as covariates to adjust in multivariate regression analysis. To prove the stability of the results, sTM was described as the original values and per-SD values. In the crude model, none was adjusted, and in model I, sex, age, fibrinogen, and creatinine were adjusted, whereas in model II, sex, age, INR, APTT, fibrinogen, WBC, bilirubin, albumin, and creatinine were adjusted. Model II in the multivariate regression demonstrated that per TU/ml increment in sTM was associated with an increase of 8% in overall mortality, and an SD increase in sTM level was associated with a 53% higher risk of overall mortality (Table 2). After propensity-score matching, the effect of sTM remained HR = 1.07 [95% confidence interval (CI), 1.03–1.24; $P = 0.03$].

Subgroup analysis

Sex, age, etiology, Child-Pugh classification, ascites, hepatic encephalopathy, esophageal gastric variceal bleeding, WBC, bilirubin, and creatinine as stratification variables were used to observe the trend of effect sizes in these variables in subgroup analysis for mortality (Fig. 3). There was no significant difference in relationships between sTM and risks of mortality in the subgroup analyses (P -interaction > 0.05).

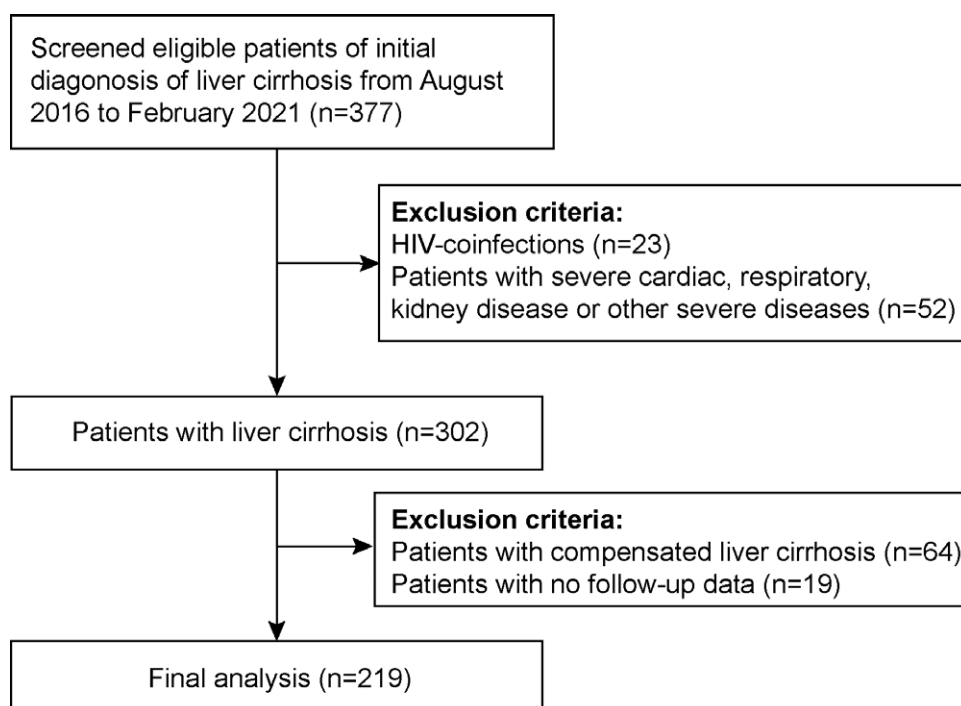


Fig. 1. Flowchart for patients' screening.

Survival analysis of plasma levels of sTM

In survival analysis, plasma sTM level in 199 survived patients was 15.60 (11.80–20.20) TU/ml, which was much lower than that in 20 patients of death or liver transplant [20.00 (14.00–24.60); $P < 0.01$]. As the mortality rates in low (5.90–12.60 TU/ml) and medium (12.70–18.00 TU/ml) tertile sTM levels were similar, so we chose the cutoff of 18.0 TU/ml to divide the patients into high sTM groups (18.10–46.2 TU/ml) and low sTM group (5.90–18.00 TU/ml). K-M survival curves showed that the high sTM group had significantly higher mortality (15.48%) than patients in the low sTM group (5.19%) (log-rank test: $P < 0.01$; Fig. 4). After adjustments for sex, age, INR, APTT, fibrinogen, WBC, total bilirubin, and albumin, patients in the high sTM group (>18 TU/ml) tend to demonstrate a significantly higher death risk (HR = 3.01; 95% CI, 1.13–7.93; $P = 0.01$).

Discussion

LSECs play a key role in the development and progression of liver cirrhosis. As a reliable endothelial cell injury marker, sTM was first discovered as a prognostic factor of liver cirrhosis in the prospective study. sTM levels increased significantly in liver cirrhosis patients compared with the healthy controls and paralleled with liver dysfunction. Patients with sTM higher than 18 TU/ml demonstrated a significantly higher death risk than those with lower sTM.

Endothelial cells produce the transmembranous glycoprotein TM. Except for the brain, TM is found in all human arteries, veins, capillaries, and lymphatics [15]. Acting as a scaffold for thrombin, TM accelerates the activation of protein C and accordingly accelerates degrading factor Va and factor VIIIa, thereby, restraining the coagulation reactions and restricting fibrin formation [16]. So, TM mediates the antifibrinolytic effect at a lower level and the profibrinolytic effect at a higher level. When endothelium cells were injured by inflammation, increased shear stress, or other factors, TM shed into plasm from the surface and formed sTM whose molecular weights were much lower than cellular TM [17]. There were 4–6 fragments of sTM [18] with different molecular weights but a similar anticoagulation function to TM [15,19]. The liver and kidney cleared sTM, and it was found in both plasma and urine [6].

Several studies verified that sTM was a sensitive marker of endothelial cell injury. Ishii *et al.* [18] found that when umbilical vein endothelial cells were treated with N-formyl-methionyl-leucyl-phenylalanine or lipopolysaccharide (LPS), sTM in the medium increased in a time-dependent manner and paralleled with the extent of cell damage. When culture mediums were supplemented with hydrogen peroxide, neutrophil proteases proteinase-3, elastase, or cathepsin G, Boehme *et al.* [5] discovered that sTM increased rapidly. The authors hypothesized that sTM was released rather than secreted by injured endothelial cells and that it could be used as both an early and advanced stage marker. Other studies observed similar results [20,21].

As a reliable marker of endothelial cell injury, sTM was found to be related to many vascular-associated diseases, such as CHD, renal failure, disseminated intravascular coagulation, vasculitis, etc. In a cohort of sepsis with and

without acute kidney injury, the plasma sTM was 23.6 U/ml vs. 15.6 U/ml, respectively, ($P < 0.001$) and served as an independent predictive factor rather than E-selectin, PAI-1, and protein C [22]. In a case-control study, sTM was found to have an inverse relationship with a CHD occurrence [7]. Aleksic *et al.* [17] found that lower sTM tertile amplified the CHD risk of higher fibrinogen. Ruff *et al.* [23] found that sTM level increased significantly paralleled from the control group, stable angina, and acute coronary syndromes group. A similar result was also reached by Mezaki T [24].

sTM is also a marker of LSECs injury. In a rat model, sTM and the expression of sinusoidal TM increased during acute liver injury induced by D-galactosamine, especially in the necrotic area and around the central vein, suggesting that sTM was related to endothelial injury and parenchymal necrosis [10]. Several studies had previously determined sTM in various liver diseases, but the results were inconsistent. In a case-control study, Biguzzi *et al.* found that sTM was elevated in hepatocellular carcinoma patients (42.1 ± 2.0 ng/ml) than in cirrhosis patients (28.3 ± 2.1 ng/ml; $P = 0.039$), and sTM level did not relate to the outcome of cirrhosis individuals [25]. Takano *et al.* [6] used two kinds of ELISA methods to detect plasma sTM. They found that sTM, including all fragments of sTM, increased in acute liver failure patients. sTM, on the other hand, remained stable in cirrhosis patients, whereas the ratio of smaller sTM to larger sTM decreased. In chronic hepatitis and early cirrhosis, the plasma sTM maintained similar to healthy controls [6].

All of the studies mentioned above failed to find a link between sTM and cirrhosis outcomes. The reason may be that the studies were cross-sectional studies or case-control studies with a relatively small number of patients. We enrolled 219 decompensated cirrhosis patients in our study and followed them up for every 6 months. All the results showed that sTM was related to liver dysfunction. First, in the low, medium, and high sTM tertile groups, the inflammation and liver function factors were all significantly increased, including bilirubin, albumin, and PT. In addition, the Child-Pugh score, MELD, and MELD-Na score also increased in the medium and high sTM tertile groups. Correlation study also found the positive relationships of sTM with PT, bilirubin, INR, MELD score, and MELD-Na score. These results all supported that sTM was correlated with liver dysfunction. As a marker of liver dysfunction, increasing sTM was found positively correlated with the death risk of cirrhosis. The correlation was very stable and not interfered with by other factors, such as sex, age, WBC count, etc. The cutoff value of 18.0 TU/ml could identify the patients with higher death risk. In this study, the sTM level in healthy controls was 8.3 (7.3–9.8) TU/ml, and according to the other studies, the normal range of sTM is 3.8–13.3 TU/ml [26]. When we chose the cutoff of 18.0 TU/ml to divide into two groups, the mortality rate was much higher in high sTM groups. This indicates the threshold effect of sTM relationship with mortality.

In theory, sTM should also act as a marker of portal hypertension. But sTM was only found correlated with the number of ascites and did not correlate with platelet count, esophageal gastric variceal bleeding, and hepatic

Table 1. Clinical characteristics of patients with liver cirrhosis

| sTM tertile (TU/ml) | All patients | Low sTM group (5.90–12.60) | Medium sTM group (12.70–18.00) | High sTM group (18.10–46.20) | P |
|-------------------------------------|---------------------|----------------------------|--------------------------------|------------------------------|-------|
| N | 219 | 66 | 69 | 84 | |
| Age (years) | 57.32 ± 10.96 | 55.00 ± 9.34 | 57.80 ± 10.39 | 58.76 ± 12.34 | 0.10 |
| Male (N/%) | 145(66.21%) | 45 (68.18%) | 42 (60.87%) | 58 (69.05%) | 0.52 |
| Causes (N/%) | | | | | |
| HBV | 70 (31.96%) | 23 (34.85%) | 24 (34.78%) | 23 (27.38%) | 0.26 |
| HCV | 47 (21.46%) | 18 (27.27%) | 17 (24.64%) | 12 (14.29%) | |
| Alcoholic | 63 (28.77%) | 18 (27.27%) | 17(24.64%) | 28 (33.33%) | |
| Autoimmune | 13 (5.94%) | 1 (1.52%) | 5 (7.25%) | 7 (8.33%) | |
| Others/unknown | 26 (11.87%) | 6 (9.09%) | 6 (8.70%) | 14 (16.67) | |
| WBC (×10 ⁹ /l) | 3.40 (2.29–4.96) | 2.73 (2.06–4.54) | 3.39 (2.20–5.00) | 3.75 (2.78–5.50) | 0.03 |
| Neutrophils (×10 ⁹ /l) | 2.09 (1.42–3.08) | 1.62 (1.07–2.73) | 1.84 (1.41–3.08) | 2.21 (1.63–3.32) | 0.01 |
| Hemoglobin (g/l) | 96.72 ± 26.81 | 97.54 ± 28.80 | 98.26 ± 26.15 | 94.82 ± 25.91 | 0.70 |
| Platelet (×10 ⁹ /l) | 65.00 (43.00–90.00) | 66.00 (42.25–84.25) | 66.00 (42.00–95.00) | 60.50 (45.75–91.25) | 0.47 |
| ALT (U/l) | 21.00 (12.00–29.00) | 17.00 (12.00–25.75) | 20.00 (13.00–29.00) | 22.50 (12.00–33.25) | 0.23 |
| AST (U/l) | 33.00 (24.00–50.00) | 28.50 (22.25–39.75) | 35.00 (23.00–52.00) | 35.00 (26.75–52.50) | 0.09 |
| Bilirubin (μmol/l) | 33.40 (22.05–62.75) | 29.45 (20.23–51.62) | 27.90 (22.10–45.50) | 42.05 (26.75–71.35) | <0.01 |
| Albumin (g/l) | 31.24 ± 5.00 | 32.24 ± 5.09 | 31.67 ± 4.77 | 30.12 ± 4.94 | 0.02 |
| Creatinine (μmol/l) | 64.00 (52.00–81.80) | 58.00 (51.00–74.97) | 60.00 (49.00–78.60) | 69.60 (57.50–86.50) | <0.01 |
| PT(s) | 15.60 (14.05–18.60) | 15.50 (14.35–17.40) | 15.50 (13.60–18.10) | 16.65 (14.28–21.10) | <0.01 |
| INR | 1.39 (1.25–1.65) | 1.38 (1.28–1.55) | 1.34 (1.21–1.62) | 1.48 (1.27–1.87) | <0.01 |
| Child-Pugh scores | 9.00 (7.00–11.00) | 8.00 (7.00–10.00) | 8.00 (7.00–9.00) | 10.00 (8.00–12.00) | <0.01 |
| Child-Pugh classification | | | | | |
| Child-Pugh A | 35 (15.98%) | 16(24.24%) | 13 (18.84%) | 6 (7.14%) | <0.01 |
| Child-Pugh B | 103 (47.03%) | 29 (43.94%) | 40 (57.97%) | 34 (40.48%) | |
| Child-Pugh C | 81 (36.99%) | 21 (31.82%) | 16 (23.19%) | 44 (52.38%) | |
| MELD scores | 8.41 (4.20–12.18) | 7.35 (3.92–11.11) | 6.68 (2.92–11.99) | 9.25 (6.93–16.17) | <0.01 |
| MELD-Na scores | 8.71 (4.57–15.24) | 8.56 (4.42–12.12) | 7.27 (3.03–11.99) | 9.81 (7.58–19.84) | <0.01 |
| Ascites | | | | | |
| None | 18 (8.22%) | 7 (10.61%) | 4 (5.80%) | 7 (8.33%) | 0.04 |
| Mild | 123 (56.16%) | 39 (59.09%) | 44 (63.77%) | 40 (47.62%) | |
| Moderate-large | 78 (35.62%) | 20 (30.30%) | 21 (30.43%) | 37 (44.05%) | |
| Hepatic encephalopathy | | | | | |
| No | 181 (82.65%) | 57 (86.36%) | 58 (84.06%) | 66 (78.57%) | 0.53 |
| Grade 1–2 | 34 (15.53%) | 9 (13.64%) | 9 (13.04%) | 16 (19.05%) | |
| Grade 3–4 | 4 (1.83%) | 0 (0.0%) | 2 (2.90%) | 2 (2.38%) | |
| Esophageal gastric varices bleeding | | | | | |
| No | 156 (71.23%) | 45 (68.18%) | 52 (75.36%) | 59 (70.24%) | 0.23 |
| Yes | 63 (28.77%) | 21 (31.82%) | 17 (24.64%) | 25 (29.76%) | |
| Clinical outcome | | | | | |
| Survive | 199 (90.87%) | 63 (95.45%) | 65 (94.20%) | 71 (84.52%) | 0.04 |
| Death/LT | 15(6.85%)/5(2.28%) | 2 (3.03%)/1(1.52%) | 2 (2.90%)/2(2.90%) | 11(13.10%)/2(2.38%) | |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, international normalized ratio; LT, liver transplantation; MELD, model of end-stage liver disease; PT, prothrombin time; TM, thrombomodulin; WBC, white blood cell.

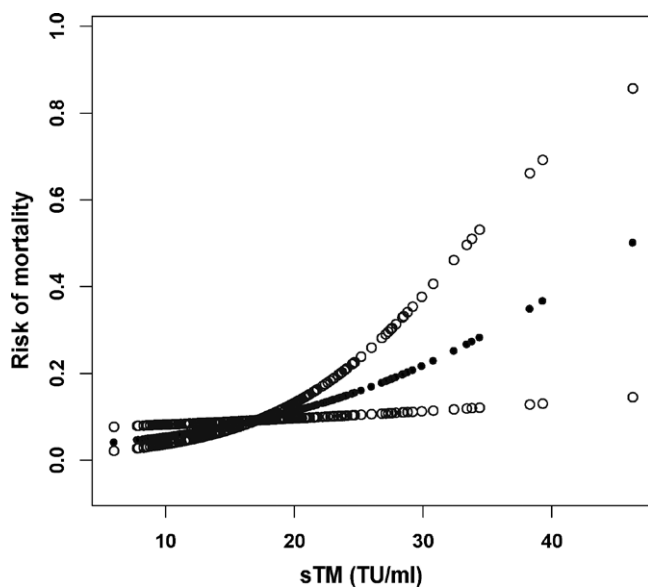


Fig. 2. Fitted curves of the soluble thrombomodulin and mortality risk relationship. The solid circle dots represent the smooth curve fit, and the hollow circle dots represent the 95% of confidence interval from the fit (logarithm-like hood ratio test, *P* < 0.01). sTM, soluble thrombomodulin.

Table 2. Multivariate Cox regression for soluble thrombomodulin and mortality

| Mortality | Nonadjusted | | Model I | | Model II | |
|-----------------|---------------------|------|---------------------|------|---------------------|------|
| | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P |
| sTM (TU/ml) | 1.08 (1.02–1.15) | 0.01 | 1.09 (1.02–1.17) | 0.01 | 1.08 (1.00–1.17) | 0.04 |
| Per-SD increase | 1.59 (1.17–2.15) | 0.01 | 1.53 (1.06–2.20) | 0.01 | 1.53 (1.00–2.32) | 0.03 |

In model I, sex, age, fibrinogen, and creatinine were adjusted, whereas, in model II, sex, age, INR, APTT, fibrinogen, WBC, bilirubin, albumin, and creatinine were adjusted.

CI, confidence interval; HR, hazard ratio; sTM, soluble thrombomodulin; WBC, white blood cell.

encephalopathy. Because sTM is cleared by hepatocytes, we hypothesized that the level of sTM was also affected by the liver function. Another finding in the study was the relationship between sTM and creatinine. The kidney also cleared sTM, indicating that the clearance rate of both the liver and the kidney had a significant impact on the sTM level. We deduced that sTM was a prognostic factor of survival mostly due to its relationship with liver function rather than portal hypertension.

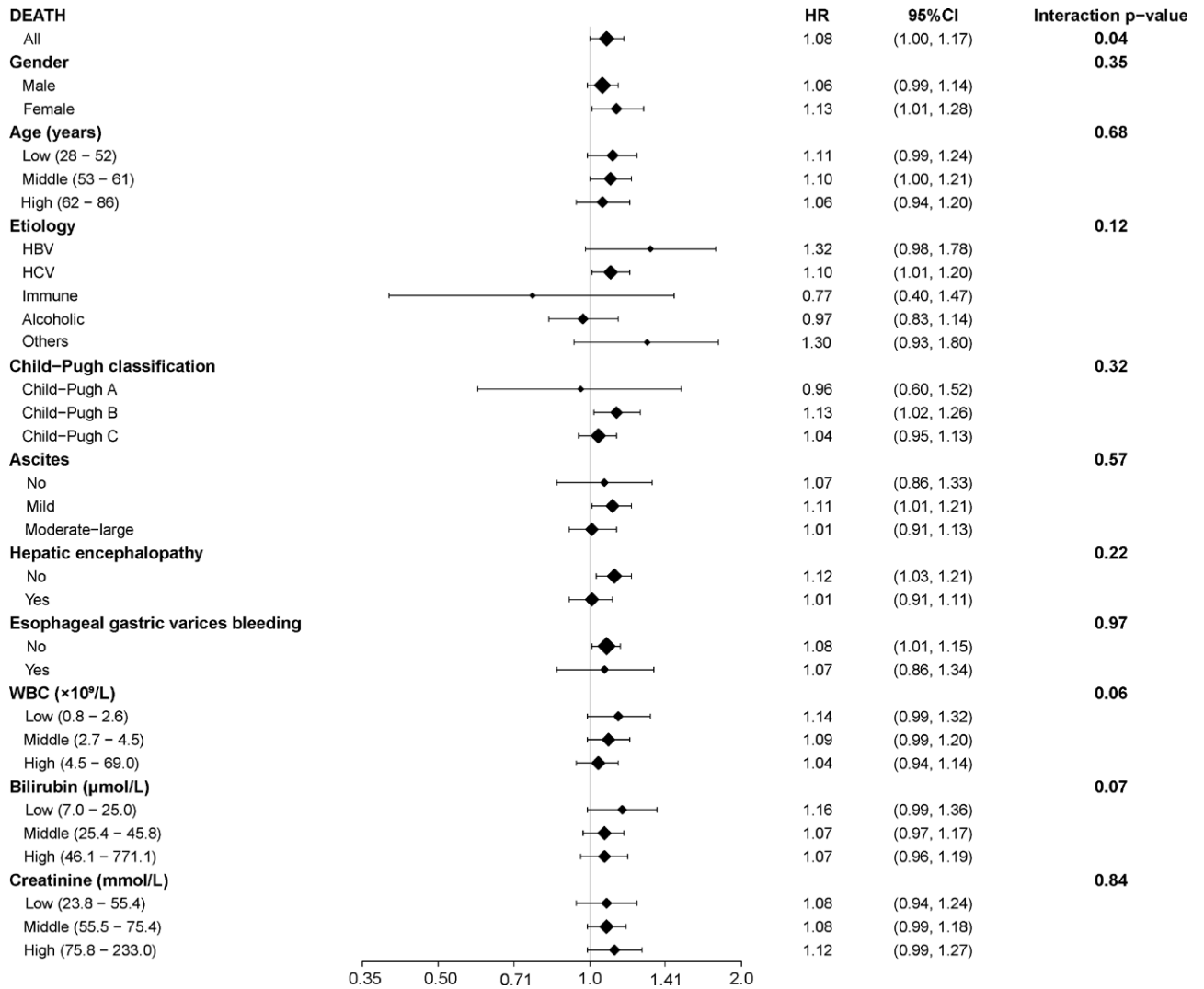


Fig. 3. Subgroup analysis of the association between soluble thrombomodulin and mortality. HBV, hepatitis B virus; HCV, hepatitis C virus; WBC, white blood cell.

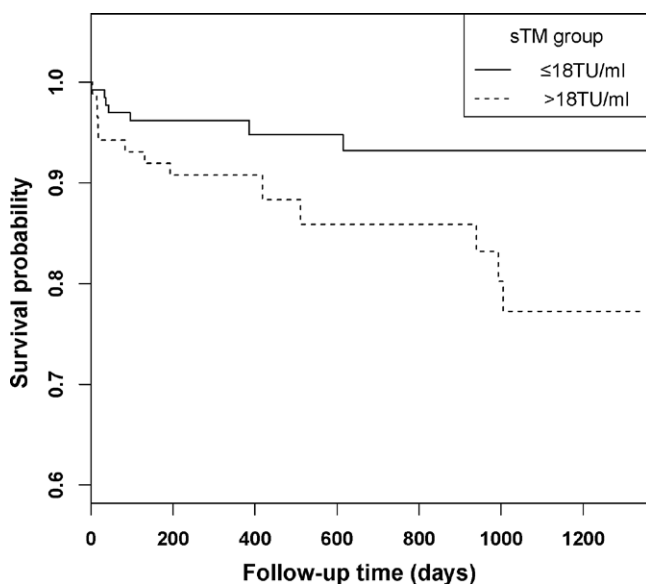


Fig. 4. Kaplan–Meier survival curves for the mortality. sTM, soluble thrombomodulin.

In the study, we did not aim to explore the reason for vascular endothelial injury. But the results showed that the amount of WBC and neutrophil ratio were significantly different in the three sTM groups. As Kume *et al.* [27] reported that TM antigen and activity levels were significantly decreased in sinusoidal cells isolated from LPS-treated rats and recombinant TM protects against LPS-induced liver dysfunction, which indicates that decreased sinusoidal TM levels may result in liver dysfunction. And La Mura *et al.* [28] also found that TM immunoreactivity was almost totally lost in endothelial cells 24 h after LPS injection, thus further confirming the involvement of the endothelium in the pathogenesis of liver damage induced by endotoxemia. Other researches also demonstrated that tumor necrosis factor [29] and other cytokines also aggravate vascular endothelial injury in liver cirrhosis, which can lead to the continuous increase of sTM level. Thus we speculated that inflammation may damage endothelial cells, thus resulting in an increase in sTM levels.

In cirrhosis, baseline plasma sTM was found to be significantly elevated and correlated with the severity of liver dysfunction and survival. But there was a paradox that higher

level sTM tends to promote anticoagulation which should alleviate disease progression. We guessed that elevated sTM could not totally reverse but may partly relieve the hypercoagulability tendency in the sinusoid. The balance still tends to coagulation rather than anticoagulation. However, in-vitro or in-vivo studies should be used to verify the deduction.

However, the study had some limitations. First, other vascular endothelial injury markers were not determined together, so there was no way to compare their clinical significance or see if combining them would be more effective. And patients with chronic hepatitis were not included in the study, even though vascular endothelial injury may occur before fibrosis. Besides, because this was a single-center study, our findings should be confirmed in other cohorts. Also, the samples were not tested in duplicates, which may result in inspection error, and were failed to monitor dynamically. And liver tissue pathology was not performed in this study, so the source of sTM is unclear and needs to be verified in further study. And the proportion of patients with NSBBs was small, so the effect of drug was not analyzed.

In conclusion, we clarified that baseline sTM was a useful marker to predict cirrhosis outcome through a prospective cohort with a relatively large sample size.

Acknowledgements

The authors would like to thank all patients involved in the study.

This study was supported by Capital's Funds for Health Improvement and Research (No.2020-1-3011) and Reform and development project of Beijing Institute of Hepatology (No. Y-2021KF-1).

All authors contributed to the study conception and design. J.Z. and Y.L. mainly contributed to the analysis and interpretation of data for the work. X.W. and X.D. mainly contributed to the data collection, analysis and first proof. J.W. was mainly responsible for the specimen detection. All authors read and approved the final manuscript.

Data sharing statement: the data are available from the corresponding author upon reasonable request.

Conflicts of interest

There are no conflicts of interest.

References

- Rafii S, Butler JM, Ding BS. Angiocrine functions of organ-specific endothelial cells. *Nature* 2016;529:316–325.
- Gracia-Sancho J, Caparrós E, Fernández-Iglesias A, Francés R. Role of liver sinusoidal endothelial cells in liver diseases. *Nat Rev Gastroenterol Hepatol* 2021;18:411–431.
- Budzyń M, Iskra M, Turkiewicz W, Krasiński Z, Gryszczyńska B, Kasprzak MP. Plasma concentration of selected biochemical markers of endothelial dysfunction in women with various severity of chronic venous insufficiency (CVI)-a pilot study. *PLoS One* 2018;13:e0191902.
- Ding XC, Ma WL, Li MK, Liu SW, Liu XY, Hai L, et al. A meta-analysis of the value of vWF in the diagnosis of liver cirrhosis with portal hypertension. *J Clin Transl Hepatol* 2019;7:3–8.
- Boehme MW, Galle P, Stremmel W. Kinetics of thrombomodulin release and endothelial cell injury by neutrophil-derived proteases and oxygen radicals. *Immunology* 2002;107:340–349.
- Takano S, Kimura S, Ohdama S, Aoki N. Plasma thrombomodulin in health and diseases. *Blood* 1990;76:2024–2029.
- Salomaa V, Matei C, Aleksic N, Sansores-Garcia L, Folsom AR, Juneja H, et al. Soluble thrombomodulin as a predictor of incident coronary heart disease and symptomless carotid artery atherosclerosis in the

Atherosclerosis Risk in Communities (ARIC) Study: a case-cohort study. *Lancet* 1999;353:1729–1734.

- Alemán-Valls MR, González-Reimers E, Santolaria-Fernández F, Rodríguez-Martín JM, Díaz-Romero F, Raya-Sánchez JM. Lack of relationship between plasma thrombomodulin and portal hypertension in alcoholic liver disease. *Alcohol* 2000;20:205–206.
- Tacke F, Schöffski P, Trautwein C, Manns MP, Ganser A, von Depka M. Tissue factor and thrombomodulin levels are correlated with stage of cirrhosis in patients with liver disease. *Blood Coagul Fibrinolysis* 2001;12:539–545.
- Takatori M, Iwabuchi S, Ro S, Murayama M, Maeyama S, Uchikoshi T, et al. Increased serum levels and sinusoidal expression of thrombomodulin in acute liver damage. *Thromb Res* 1999;93:113–120.
- Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000;31:864–871.
- Biggins SW, Kim WR, Terrault NA, Saab S, Balan V, Schiano T, et al. Evidence-based incorporation of serum sodium concentration into MELD. *Gastroenterology* 2006;130:1652–1660.
- European association for the study of the liver. EASL clinical practice guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018;69:406–460.
- Biggins SW, Angeli P, Garcia-Tsao G, Ginès P, Ling SC, Nadim MK, et al. Diagnosis, evaluation, and management of ascites, spontaneous bacterial peritonitis and hepatorenal syndrome: 2021 practice guidance by the american association for the study of liver diseases. *Hepatology* 2021;74:1014–1048.
- Ishii H, Majerus PW. Thrombomodulin is present in human plasma and urine. *J Clin Invest* 1985;76:2178–2181.
- Maruyama I. Thrombomodulin, an endothelial anticoagulant; its structure, function and expression. *Jpn Circ J* 1992;56:187–191.
- Aleksic N, Wang YW, Ahn C, Juneja HS, Folsom AR, Wu KK. Assessment of coronary heart disease risk by combined analysis of coagulation factors. *Atherosclerosis* 2008;198:294–300.
- Ishii H, Uchiyama H, Kazama M. Soluble thrombomodulin antigen in conditioned medium is increased by damage of endothelial cells. *Thromb Haemost* 1991;65:618–623.
- Ohlin AK, Larsson K, Hansson M. Soluble thrombomodulin activity and soluble thrombomodulin antigen in plasma. *J Thromb Haemost* 2005;3:976–982.
- Boehme MW, Deng Y, Raeth U, Bierhaus A, Ziegler R, Stremmel W, Nawroth PP. Release of thrombomodulin from endothelial cells by concerted action of TNF-alpha and neutrophils: *in vivo* and *in vitro* studies. *Immunology* 1996;87:134–140.
- Boehme MW, Raeth U, Scherbaum WA, Galle PR, Stremmel W. Interaction of endothelial cells and neutrophils *in vitro*: kinetics of thrombomodulin, intercellular adhesion molecule-1 (ICAM-1), E-selectin, and vascular cell adhesion molecule-1 (VCAM-1): implications for the relevance as serological disease activity markers in vasculitides. *Clin Exp Immunol* 2000;119:250–254.
- Katayama S, Nunomiya S, Koyama K, Wada M, Koinuma T, Goto Y, et al. Markers of acute kidney injury in patients with sepsis: the role of soluble thrombomodulin. *Crit Care* 2017;21:229.
- Ruef J, März W, Winkelmann BR. Markers for endothelial dysfunction, but not markers for oxidative stress correlate with classical risk factors and the severity of coronary artery disease. (A subgroup analysis from the Ludwigshafen Risk and Cardiovascular Health Study). *Scand Cardiovasc J* 2006;40:274–279.
- Mezaki T, Matsubara T, Hori T, Higuchi K, Nakamura A, Nakagawa I, et al. Plasma levels of soluble thrombomodulin, C-reactive protein, and serum amyloid A protein in the atherosclerotic coronary circulation. *Jpn Heart J* 2003;44:601–612.
- Biguzzi E, Franchi F, Bucciarelli P, Colombo M, Romeo R. Endothelial protein C receptor plasma levels increase in chronic liver disease, while thrombomodulin plasma levels increase only in hepatocellular carcinoma. *Thromb Res* 2007;120:289–293.
- Ren W, Zhang J, Chen Y, Wen M, Su Y, Zhao Y, et al. Evaluation of coagulation, fibrinolysis and endothelial biomarkers in cirrhotic patients with or without portal venous thrombosis. *Clin Appl Thromb Hemost* 2020;26:1076029620982666.
- Kume M, Hayashi T, Yuasa H, Tanaka H, Nishioka J, Ido M, et al. Bacterial lipopolysaccharide decreases thrombomodulin expression in the sinusoidal endothelial cells of rats – a possible mechanism of intrasinusoidal microthrombus formation and liver dysfunction. *J Hepatol* 2003;38:9–17.
- La Mura V, Gagliano N, Arnaboldi F, Sartori P, Procacci P, Denti L, et al. Simvastatin prevents liver microthrombosis and sepsis induced coagulopathy in a rat model of endotoxemia. *Cells* 2022;11:1148.
- Coughlin SR. Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J Thromb Haemost* 2005;3:1800–1814.