Fibrinolysis status in the Budd–Chiari syndrome in China

Zhang Ke*, Xu Hao, Wei Ning*, Zu Mao-heng and Fu Yu-fei

Pathogenesis and clinical characteristics of the Budd–Chiari syndrome (BCS) in Asia are somewhat different from the ones observed in Western countries. Obstruction of the inferior vena cava (IVC) or of the hepatic veins is caused to a greater extent by membranous webs than by thrombosis. Impaired fibrinolysis has been found in European patients with BCS, but its status in Chinese patients with this condition is still unknown. To explore the characteristics of fibrinolysis in BCS patients in this country, we measured the euglobulin lysis time (ELT) for overall fibrinolysis and the plasma levels of five fibrinolytic components in 65 Chinese patients with BCS and 43 healthy controls. In patients, ELTs were slightly shorter than in controls (mean, 293 vs. 357 min, $P<0.02$), tissue type plasminogen activator levels were higher than in controls (mean, 239 vs. 185 pg/ml, $P<0.01$), and plasminogen activator inhibitor 1 levels were lower than in controls (mean, 1.43 vs. 1.73 ng/ml, $P<0.001$). To explore BCS in more detail, we subgrouped the cases according to age, type of venous occlusion, Child-Pugh score, and thrombosis. As a result of this analysis, we found that young patients (age <30 years) had a longer ELT (mean, 440 min) than the older patient groups (30≤ age≤ 44, 45≤ age≤ 54, age>54 years; mean ELT = 242, 198, and 289 min, respectively, all $P<0.05$). The independent hepatic vein occlusion subgroup showed a longer ELT (mean, 367 min) than the combined hepatic vein and IVC or the independent IVC occlusion subgroup (mean ELT = 233 and 260 min, both $P<0.05$). ELT did not show significant differences between Child-Pugh class A and B subgroups (mean, 267 vs. 333 min, $P>0.05$). ELT in the subgroup without thrombosis was shorter than in controls (mean, 288 vs. 358 min, $P<0.05$), and in the subgroup with thrombosis, it was also slightly shorter than in controls, without reaching statistical significance (mean, 306 vs. 358 min, $P>0.05$). By and large, overall fibrinolytic potential was slightly increased in Chinese patients with BCS in this study, but fibrinolysis differed according to its baseline characteristics. Compared with the one seen in BCS patients from Western countries, BCS in China exhibits certain special changes in fibrinolysis and we were able to explain some of these changes. *Blood Coagul Fibrinolysis* 25:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

In Western countries, Budd–Chiari syndrome (BCS) is a life-threatening vascular disease, in which thrombosis develops in the hepatic veins, and its cause has been attributed to various thrombotic risk factors [1]. However, in Asian countries, such as China, obstruction of hepatic veins or inferior vena cava (IVC) is more frequently caused by membranous webs than by thrombosis [2]. Considering this special type of disease, the pathogenesis and the clinical characteristics of BCS may be different [3]. A study in Asia indicated that membranous webs may develop from an organized thrombus [4], but domestic scholars hypothesized some other possible origins [5,6]. Meanwhile, we also paid attention to thrombotic risk factors for BCS, such as factor V Leiden mutations, the prothrombin G20210A mutation, and other underlying diseases related to thrombosis, yet the majority of these gave negative results [7], and only resulted in spending considerable time and energy. However, following international research interests of BCS is now an advisable method. AQ1

To date, little attention has been paid in our country to the fibrinolytic system. The fibrinolysis pathway and its relation to thrombosis have been well studied [8]. As far as we know, scholars abroad set foot in this field to examine fibrinolysis in BCS patients from Western countries [9,10], and impaired fibrinolysis was found in patients with BCS [10]. Considering the geographical differences of BCS as we stated before, the fibrinolysis status in China may be different. To examine the fibrinolytic characteristics of BCS in this population, we conducted this study with the hope to unveil new information.

Methods

Selection of cases and controls

Patients with BCS were recruited from the interventional radiology department of the affiliated hospital of Xuzhou Medical College. Xuzhou city is situated at the junction of the Jiangsu, Anhui, Henan, and Shandong provinces, and

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BCS has a relatively high incidence in this area [11]. Until now, more than 2000 patients with BCS were diagnosed and treated at this hospital, which in 2013 also became the BCS clinical center of the Jiangsu province. From April 2012 to September 2013, when patients with BCS were hospitalized, clinical data were collected and laboratory, radiology, MRI, and ultrasound assessments were performed. Baseline characteristics of BCS, such as age, Child-Pugh score, type of venous occlusion, and the presence of thrombosis, were examined. BCS was defined as a collection of portal hypertension symptoms caused by the obstruction of the hepatic vein and/or the IVC above the outflow opening of the hepatic vein, regardless of the cause of the obstruction [12]. However, this definition did not include obstruction caused by congestive heart failure or sinusoidal obstruction syndrome. All cases were selected at the first clinical visit and patients did not yet receive any anticoagulant therapy.

A total of 65 patients were enrolled in this study. After that, 43 healthy controls were selected in accordance with the ratio of the patients' sex and age. Healthy people, without a history of thrombosis or anticoagulant medication use, were included in the study. All BCS patients and controls are Asians.

Blood samples were collected from both patients and controls by venapuncture (the next morning, 8 a.m.) in tubes containing 0.109 mol of trisodium citrate. Plasma was prepared by centrifugation at 3000 rpm (revolutions per minute) for 10 min and then stored at −80°C until analysis. All subjects were told about this study and had signed an informed consent for voluntarily providing samples.

Measurement of fibrinolytic proteins in the plasma

We detected the levels of five different fibrinolytic proteins, including tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor 1 (PAI-1), plasminogen, α_2 antiplasmin (α_2-AP), and fibrinogen in both patients and controls. Plasma concentrations of t-PA antigen, PAI-1 antigen and activity, plasminogen antigen, and α_2-AP antigen were measured by an ELISA (t-PA Antigen & PAI-1 Antigen ELISA Kit, Shanghai Westang Biotech Co. Ltd., Shanghai, China; and PAI-1 activity, plasminogen Antigen & α_2-AP Antigen ELISA Kit, Shanghai Jianglai Biotech, Shanghai, China). All steps followed the manufacturer's instructions. The plasma fibrinogen level was measured by the clinical laboratory from this hospital using the blood coagulation analyser (SYSMEX-CA7000).

Measurement of overall fibrinolysis

The euglobulin contains important fibrinolytic factors, including t-PA, plasminogen, PAI-1, fibrinogen, and to a lesser extent α_2-AP. The test of euglobulin lysis time (ELT) used for the assessment of overall fibrinolysis has been described before [13]. As a reference, we mainly performed the following steps: 7.5 ml of distilled water and 120 μl of 1% acetic acid were added to a 15-ml conical centrifuge tube, then 0.5 ml of plasma was added; the tube was then moved to a 4°C refrigerator and on an ice bath for 10 min and then centrifuged for 5 min at 3000 rpm in order to precipitate the euglobulin; the supernatant was poured off and the tube was inverted on the filter paper to dry the euglobulin; we added 0.5 ml of borate buffer solution, pH = 9.0, and stirred the precipitate, and after it was dissolved, we placed the tube in a 37°C water bath for a few minutes and then added 0.5 ml of 0.025 mol/l $CaCl_2$ solution; we let the tube stand vertically for a few minutes until the clot formed, and then started the timer; finally, the clot was observed every 10 min, until it was completely lyzed to liquid, and at that point, we stopped the timer and recorded the ELT.

Compared with a modified method [14], this method was time-consuming and nonautomatized, but it was cost-effective and allowed us to easily observe the process dynamically. We determined ELTs successfully in all cases and controls using this method.

Statistical analysis

As the values of t-PA, PAI-1, plasminogen, α_2-AP, fibrinogen, and ELT we calculated displayed a normal distribution, the means of each parameter between cases and controls, and between case subgroups, were compared by the Student *t* test or *t*' test according to the results of Levene's test for equality of variances. The associations between fibrinolytic parameters in cases and liver function indices, and the association between ELT and the fibrinolytic components, were assessed using Spearman's rank correlation test. A *P* value less than 0.05 was considered to be statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, Version 18.0, SPSS Inc, Chicago, Illinois, USA) and scatter diagrams of the indices were drawn on the software GraphPad Prism, version 5.0.

Results

Baseline data

Baseline characteristics of the 65 patients with BCS are summarized and shown in Table 1. The characteristics of these 65 patients that were recruited into the study were

Table 1 Baseline data of BCS patients ($n = 65$)

Characteristics of BCS patients	Value
Median age, year (range)	44 (17–74)
Male sex	41
Type of venous occlusion	
Hepatic vein occlusion	25
IVC occlusion	19
Combined hepatic vein and IVC occlusion	21
Concurrent hepatic vein, IVC or portal vein thrombosis	17
Child-Pugh Score	
Class A	34
Class B	28
Class C	3

BCS, Budd–Chiari syndrome; IVC, inferior vena cava.

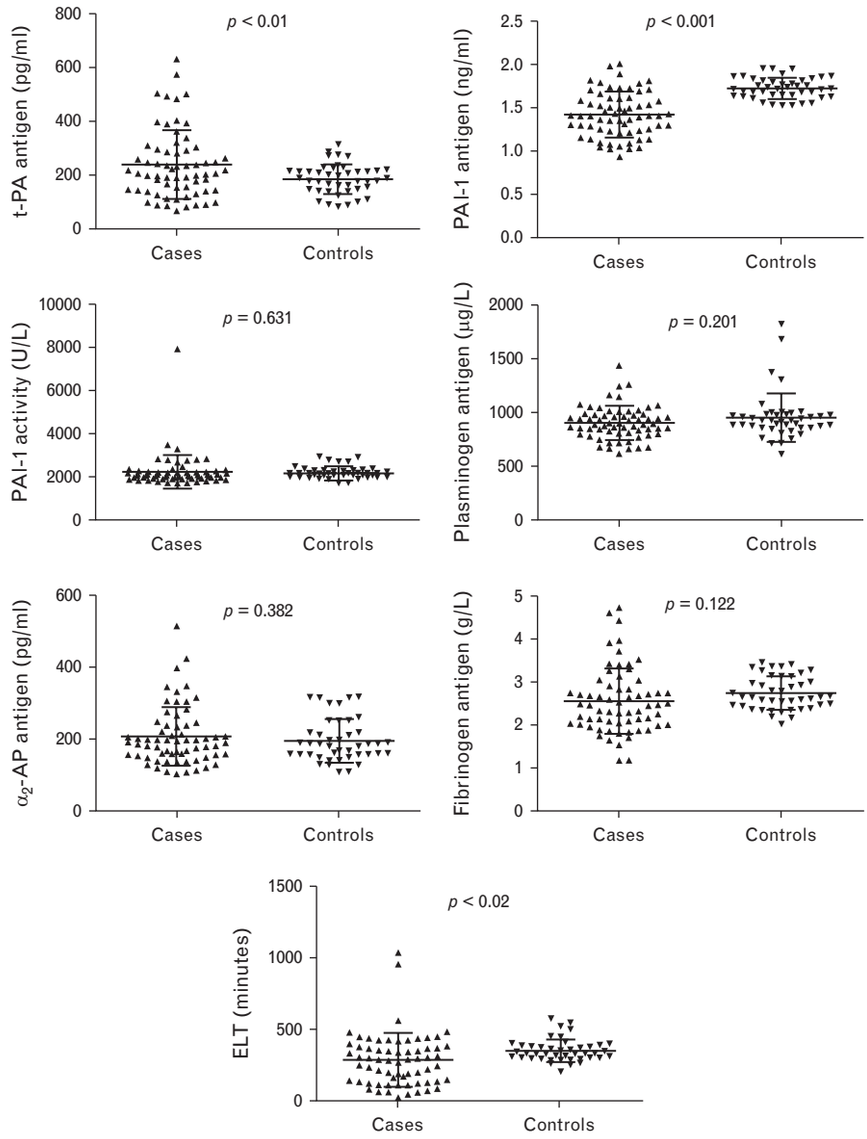
similar to those of the participants from the BCS group that we reviewed before [2], indicating that the current study group was representative. The age and sex of the patients and controls were comparable. The median age of the patients at diagnosis was 44 years (range, 17–74 years) and 41 of the patients were males. Concurrent hepatic vein, IVC, or portal thrombosis was present in 26% of the patients.

Plasma fibrinolytic parameters

The level of t-PA antigen was increased in cases compared with controls (mean, 238.7 vs. 184.6 pg/ml, $P < 0.01$). In contrast, the PAI-1 antigen level was significantly decreased in patients compared with healthy controls (mean, 1.43 vs. 1.73 ng/ml, $P < 0.001$). Levels of PAI-1 activity, plasminogen antigen, α_2-AP antigen, and fibrinogen were slightly changed in cases compared with controls, yet this difference failed to reach statistical significance ($P = 0.631$, 0.201, 0.382, and 0.122, respectively). Scatters of each fibrinolytic parameter for all subjects are shown in Fig. 1.

As BCS can cause congestive damage of the liver, the synthesis of fibrinolytic proteins may be affected [1,15].

Fig. 1



Fibrinolytic parameters in cases and controls. Comparison of plasma levels of t-PA antigen (a), PAI-1 antigen (b), PAI-1 activity (c), plasminogen antigen (d), α_2-AP antigen (e), fibrinogen (f), and ELT (g) between patients with BCS ($n = 65$) and healthy controls ($n = 43$). Individual data points and their means and standard deviations are shown in the figure. α_2-AP, α_2 antiplasmin; BCS, Budd–Chiari syndrome; ELT, euglobulin lysis time; PAI-1, plasminogen activator inhibitor 1; t-PA, tissue type plasminogen activator.

Therefore, we evaluated the association between the fibrinolytic parameters and liver function parameters by using Spearman's correlation analysis. As a result, only aspartate transaminase and the prothrombin time exhibited a significant minor inverse correlation with plasminogen and fibrinogen, respectively. However, all correlation coefficients were lower than 0.3, indicating only a moderate relationship (Table 2).

Plasma fibrinolytic potential

As ELT was an important index representing overall fibrinolysis [13], we analyzed this parameter intensively. The mean of ELT in BCS patients was significantly lower than in controls (293 vs. 357 min, $P < 0.02$, Fig. 1g). To find out the factors associated with the ELT, we performed a correlation analysis between ELT and the fibrinolytic components in both cases and controls. The t-PA antigen appeared to be the most correlative to ELT, with a correlation coefficient of -0.686 in the control group and -0.387 in the case group (both $P < 0.01$). We also found a medium positive correlation between the PAI-1 antigen and ELT in the whole subject group ($r = 0.273$, $P < 0.01$).

To find something new about the fibrinolytic characteristics of BCS in China, we subgrouped the cases according to their baseline characteristics (age, Child-Pugh score, type of venous occlusion, and thrombosis) and made a comparison of ELTs among these subgroups. As a result, the young patients (age <30 years) had a longer ELT (mean, 440 min) than the older groups [mean, ELT = 242 ($30 \leq$ age ≤ 44), 198 ($45 \leq$ age ≤ 54), and 289 (age >54) min, respectively; all $P < 0.05$]. The independent hepatic vein occlusion subgroup showed a longer ELT (mean, 367 min) than the combined hepatic vein and IVC or independent IVC occlusion subgroup (mean, ELT = 233 and 260 min, both $P < 0.05$). ELT did not show significant differences between the Child-Pugh class A and B subgroups (mean, 267 vs. 333 min, $P > 0.05$). ELT in the subgroup without thrombosis was shorter than in controls (mean, 288 vs. 358 min, $P < 0.05$). ELT in the subgroup with thrombosis was also slightly shorter than in the controls, yet the difference did not reach a statistical significance (mean, 306 vs. 358 min, $P > 0.05$). All data are shown in Table 3.

Table 2 Associations of fibrinolytic parameters in BCS patients with parameters of liver function

	Spearman ρ correlation coefficient					
	t-PA	PAI-1 antigen	PAI-1 acitity	Plasminogen	α_2-AP	Fibrinogen
AST	0.202	0.043	−0.152	−0.273*	0.128	0.15
ALT	0.082	−0.117	−0.045	−0.227	0.083	0.06
Bilirubin	0.061	−0.089	−0.041	0.163	−0.105	−0.233
Albumin	−0.018	0.033	−0.144	−0.092	0.211	−0.106
PT	−0.136	0.064	0.076	−0.010	0.108	−0.279*

α_2-AP, α_2 antiplasmin; AST, aspartate transaminase; ALT, alanine aminotransferase; BCS, Budd–Chiari syndrome; PAI-1, plasminogen activator inhibitor 1; PT, prothrombin time; t-PA, tissue type plasminogen activator. *$P < 0.05$.

Table 3 Comparison of ELT in subgroups according to baseline characteristics of BCS

Baseline characteristics of patients	ELT (mean ± SD, minutes)
Age (years)	
<30	440 ± 253^{1}
31–44	242 ± 141^{2}
45–54	198 ± 118^{3}
>54	289 ± 104^{4}
Type of venous occlusion	
Hepatic vein occlusion	367 ± 239^{h}
IVC occlusion	260 ± 122^{i}
Combined hepatic vein and IVC occlusion	233 ± 124^{c}
Accompanied with thrombosis?	
Yes	306 ± 141
No	288 ± 199*
Child-Pugh Score	
Class A	267 ± 135
Class B	333 ± 232

BCS, Budd–Chiari syndrome; ELT, euglobulin lysis time; IVC, inferior vena cava; SD, standard deviation. P^{12}, $P^{13}$and $P^{14} < 0.01$; P^{hc} and $P^{hi} < 0.05$; $P^{*} < 0.05$ (compared with controls).

Discussion

This case–control study is the first study conducted in China to systematically explore the fibrinolysis status in patients with BCS. Even though a conclusion had been drawn that impaired fibrinolysis was linked to thrombotic tendencies in BCS patients from Europe [10], whether this is true in Chinese patients with BCS is still unclear. In the current study, we selectively and targetedly measured plasma concentrations of several fibrinolytic components (t-PA, PAI-1, plasminogen, α_2-AP, and fibrinogen). Meanwhile, as an overall index reflecting the whole plasma fibrinolytic potential, we performed the euglobulin lysis test *in vitro* to determine the ELT.

We have shown that the levels of individual components of the fibrinolytic pathway are somewhat changed in patients with BCS compared with healthy individuals. The concentration of the t-PA antigen was significantly higher in the BCS group, whereas that of the PAI-1 antigen was significantly lower (Fig. 1a and b). The activity of PAI-1 showed no statistical differences between the two groups. The plasminogen and α_2-AP antigen levels in BCS patients showed a small change, which failed to reach statistical significance.

Alhough t-PA is the activator of plasminogen, PAI-1 is an important inhibitor of the transformation of plasminogen into plasmin [16]. Research concluded that decreased t-PA and increased PAI-1 in the blood had a close relationship with venous thrombosis [17]. However, considering that only some of the BCS patients from China present with thrombosis as a complication, changes in t-P and PAI-1 may be different. The change in t-PA and PAI-1 in this study was explainable by the fact that in BCS patients, endothelial cells of the liver vasculature may be injured by the high pressure caused by venous obstruction and release t-PA and PAI-1 [18]. Excess t-PA can initiate fibrinolysis and protect against the formation of a fibrin clot [19]. However, the majority of the BCS patients in China always present a lengthy medical

history that can be causally linked to chronic liver disease, although t-PA has no obvious correlations with liver function parameters indicative of liver parenchyma injury (Table 2). However, as BCS is a liver vascular disease, t-PA clearance may be affected in patients with this condition [10,20]. As to the decreased PAI-1 antigen levels in BCS, there may be the result of elevated t-PA levels because PAI-1 can rapidly form a complex with t-PA and generate t-PA/PAI-1complexes [21]. This change was in line with a study showing that chronic liver disease always results in a high t-PA to PAI-1 ratio [22]. Levels of plasminogen, α_2-AP antigen, and fibrinogen were also measured, but the differences did not reach statistical significance between cases and controls, and therefore we did not pursue further analyses on the pathway of fibrinolysis.

The mean ELT was slightly lower in patients as compared with controls, and the difference reached statistical significance. We were able to draw the conclusion that a slightly increased overall fibrinolytic potential was present in the Chinese patients with BCS enrolled in this study. This slightly increased overall fibrinolysis can be partly explained by the low proportion of domestic BCS that is accompanied by thrombosis (about 9–12%) [23], but it also proved that this status cannot fully protect BCS patients from thrombosis. This is similar to a study that indicated that this status cannot fully protect liver disease patients from deep venous thrombosis [24]. In this study, a higher proportion of patients presented accompanying thrombosis (26%, Table 1), and this may be because of the small sample size.

We analyzed the relationship between the whole fibrinolytic potential and five fibrinolytic components, and found a medium inverse relationship between t-PA and ELT in both cases and controls ($r = -0.387$ and -0.686, respectively, both $P < 0.01$), and a medium positive correlation between PAI-1 antigen level and ELT in the entire subject group ($r = 0.273$, $P < 0.01$). The results were in line with a study about the relationships between ELT and the plasma levels of t-PA and PAI-1 [25].

More importantly, we found ELT in cases exhibiting a larger standard deviation than the controls (185 vs. 78 min, Fig. 1g). To accurately analyze and find more information about the characteristics of fibrinolysis in BCS patients from China, we compared ELTs among different subgroups according to the baseline characteristics (age, Child-Pugh score, type of venous occlusion, and thrombosis). The results revealed that ELT changed differently in these subgroups (Table 3) and we can, therefore, conclude that fibrinolysis in BCS patients differed according to these baseline characteristics. These results also indicated that overall fibrinolysis may be affected to a greater extent by the medical history and the extent of venous involvement in BCS patients from China, but it was not obviously affected by slightly abnormal liver function.

It is worth mentioning that ELT in the subgroup with thrombosis was slightly shorter than in controls, yet the difference did not reach statistical significance (mean, 306 vs. 358 min, $P > 0.05$). This indicated that thrombosis in BCS patients did not lead to overt secondary fibrinolysis. Instead, ELT in the subgroup without thrombosis was shorter than in controls (mean, 288 vs. 358 min, $P < 0.05$). Possibly an impaired feedback in triggering fibrinolysis resulted in thrombosis and maybe this part of patients had some similarities with the characteristics of fibrinolysis that are seen in patients from Western countries.

In conclusion, in this study, slightly increased fibrinolytic potential in Chinese patients with BCS was found by and large, but fibrinolysis showed differences, according to its clinical baseline characteristics. Although impaired fibrinolysis is considered to be a thrombotic risk factor in BCS patients from Western countries, changes in fibrinolysis in China had somewhat different characteristics and we were able to explain some of these changes.

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Conflicts of interest

None declared. AQ3

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