European Review for Medical and Pharmacological Sciences 2018; 22: 2742-2750

The relationship between the levels and function of endothelial progenitor cells and factor V Leiden and protein C deficiency in patients with primary Budd-Chiari syndrome

Z.-Y. GUAN, C.-W. YU, T. SONG, Y. GAO

Department of Vascular Surgery, First Affiliated Hospital of Bengbu Medical College, Bengbu, China

Abstract. – OBJECTIVE: Budd-Chiari syndrome (BCS) is a life-threatening hepatic disease characterized by hepatic venous obstruction at the level of hepatic vein, hepatic venules, or inferior vena cava. No evidence reported the relationship between the endothelial progenitor cells and the deficiency of factor V Leiden and protein C in patients with primary Budd-Chiari syndrome.

PATIENTS AND METHODS: We recruited participants between June 2014 and July 2015. For primary BCS group, 28 patients were collected. 20 patients were included in the NAFLD group. Another 73 healthy participants were recruited into the control group. None of the patients and participants had received interventional therapy or had undergone surgery prior to being recruited. Levels and functions of endothelial progenitor cells (EPCs) were examined. The factor V Leiden mutation, protein C deficiency and protein S deficiency were evaluated. Finally, the relationship between the levels and function of endothelial progenitor cells and factor V Leiden and protein C deficiency in patients with primary Budd-Chiari syndrome was analyzed.

RESULTS: The results showed that no significant differences were found between the BCS (and NAFLD) and control group considering age, sex, BMI, smoking ($p>0.05$ for variables). However, significant differences were observed in TG, TC, HDL-C, white blood cells, hemoglobin, ALT, AST, ALP, γ-GT, total bilirubin, and albumin ($p<0.05$ for variables). Compared with the healthy participants, significant downregulation was found in BCS and NAFLD patients regarding CD34+/CD45-, late outgrowth endothelial cells (OECs) colonies, OECs proliferation, and OECs tubulogenesis ($p<0.001$ for variables). Among the 28 BCS patients, factor V Leiden mutation (n=10, 35.71%, OR 12.67, 95% CI 5.24-27.93) and hereditary protein C deficiency (n=4, 14.29%, OR 7.48, 95% CI 2.02-21.43) were more prevalent than those in the control group. These results suggested that factor V Leiden mutation and protein C deficiency were major risk factors for BCS. Finally, we demonstrated that factor V Leiden and protein C deficiency may negatively regulate the OECs levels and functions in BCS patients.

CONCLUSIONS: It's important to improve the OECs levels and functions, and to prevent the deficiency of factor V Leiden and protein C in the treatment of BCS.

Key Words

Budd-Chiari syndrome (BCS), Endothelial progenitor cells (EPCs), Outgrowth endothelial cells (OECs), Factor V Leiden mutation, Protein C deficiency.

Introduction

Budd-Chiari syndrome (BCS) is a life-threatening hepatic disease characterized by hepatic venous obstruction at the level of hepatic vein, hepatic venules, or inferior vena cava[1,2]. BCS has been reported to occur in one out of a million in the United States, Europe, and ten out of a million in China[3]. Nevertheless, the pathogenesis remains unknown. In BCS patients, the vascular occlusion and membrane formation might be associated with the vascular endothelial injury[4-6]. The endothelial progenitor cells (EPCs) may play an important role in repairing the vascular endothelial injury[7-9]. According to the morphology, EPCs have been classified as the early EPCs (after 4 to 7 days) and the late outgrowth endothelial cells (OECs) (after 14 to 21 days of culture)[10]. The levels and functions of EPCs have been reduced and suppressed in primary BCS[11]. Factor V Leiden mutation and deficiencies of coagulation inhibitors have been found in BCS patients[12]. However, types of EPCs are abnormal in BCS patients. Moreover, the relationship between gene mutation and the functions of EPCs has not been explored. Given above, the purpose of this study was to investigate the number and function of early EPCs or OECs, and to analyze the relationship between gene mutation and EPCs levels and function.

Corresponding Author: Yong Gao, MD; e-mail: sgzynnprof@163.com

Patients and Methods

Patients

This study was approved by the Ethics Review Board of the First Affiliated Hospital of Bengbu Medical College. All patients signed the written informed consent. This research was carried out according to the principles of the Declaration of Helsinki. We recruited participants between June 2014 and July 2015. For primary BCS group, 28 patients were collected. For NAFLD group, data from 20 patients were recorded. Another 73 healthy participants were included into the control group. None of the patients and participants had received interventional therapy or had undergone surgery. The patients' baseline characteristics were shown in Table I.

EPC Isolation, Cultivation and Quantification

EPCs were detected according to cell culture methods as described previously[13]. After placement of an arterial sheath, 50 mL of whole blood were obtained for EPC isolation. Mononuclear cells (MNCs) were isolated by density gradient separation using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA). To obtain early EPCs, 3×10^7 isolated mononuclear cells per well were planted into culture plates and cultured in endothelial cell growth medium-2 (EGM-2MV, Sigma-Aldrich, St. Louis, MO, USA) containing hydrocortisone, hEGF, VEGF, hFGF-B, IGF-1, ascorbic acid, and 5% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA). Next, the non-attached cells were removed after 24 hours. The attached cells were determined for the capacity to ingest 1,1-dioctadecyl-3,3,39,39-tetramethylin-docarbocyanide-labeled acetylated low-density lipoprotein (acLDL) (Sigma-Aldrich, St. Louis, MO, USA) and to bind isothiocyanate Ulex Europaeus Agglutinin Lectin (UEA-1) (Sigma-Aldrich, St. Louis, MO, USA). After 7 days incubation, early EPC colonies were calculated. Four microscopic fields were assessed using a Nikon inverted microscope (200 × magnification). To obtain OECs, 2×10^7 mononuclear cells per well were seeded into the plates. Finally, the number of OECs colonies was quantified as above on the 21st day of *in vitro* culture. All the other materials were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Flow Cytometry

The EPCs were also quantified using flow cytometry as described previously[14]. The cells were allowed for assessment of $CD34^+/KDR^+$ population, early EPCs population ($CD34^+/CD45^+$), and OECs population ($CD34^+/CD45^-$)[15]. Briefly, the cells were isolated from whole blood using density gradient separation. These cells were gated by forward and side scatter to select mononuclear cells by removing erythrocytes, granulocytes, and cell debris. These obtained cells were incubated with the indicated antibodies against human antigens: phycoerythrin-Cy5-conjugated CD45 (BD Pharmingen, San Diego, CA, USA),

Table I. The clinical baseline characteristics of patients in this study.

	BCS (n = 28)	NAFLD (n = 20)	Control (n = 73)
Age (years)	41 ± 24	45 ± 27	46 ± 25
Gender (M/F)	15/13	9/11	36/37
Smoker (%)	10 (35.71%)	7 (35.00%)	26 (35.62%)
BMI (kg/m^2)	23.22 ± 2.19	22.45 ± 3.34	23.56 ± 2.61
TG (mg/L)	2.18 ± 0.63***	2.65 ± 0.56###	1.56 ± 0.68
TC (mg/L)	5.06 ± 0.81***	5.95 ± 0.83###	4.31 ± 0.66
LDL-C (mg/L)	1.11 ± 0.12	0.96 ± 0.15###	1.37 ± 0.13
HDL-C (mg/L)	3.25 ± 0.49***	3.76 ± 0.50###	2.17 ± 0.57
White blood cell ($\times10^9$/L)	4.92 ± 1.86***	4.67 ± 1.74###	3.38 ± 1.46
Hemoglobin (g/L)	127.00 ± 18.23***	114.12 ± 18.01###	84.02 ± 11.52
ALT(U/L)	25.63 ± 6.42***	23.23 ± 5.84###	14.70 ± 3.61
AST(U/L)	30.12 ± 8.17***	27.54 ± 9.42###	16.68 ± 7.71
ALP (U/L)	93.34 ± 20.79***	91.45 ± 18.63###	52.19 ± 11.14
γ-GT(U/L)	86.29 ± 21.06***	87.61 ± 17.94###	42.61 ± 11.85
Total bilirubin (μM)	25.45 ± 5.71***	23.97 ± 5.25###	13.46 ± 3.34
Albumin (g/L)	35.65 ± 4.22***	33.47 ± 5.18###	14.89 ± 3.27

BCS, Budd-Chiari Syndrome; NAFLD, nonalcoholic fatty liver disease; Control, the healthy participants; M/F, male or female; BMI: body mass index; TG: triglycerides; TC: total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; γ-GT, Gamma-glutamyltranspeptidase. The data were represented mean ± standard deviation (SD). ***$p<0.001$, BCS versus the control group; ###$p<0.001$, NAFLD versus the control group.

fluorescein isothiocyanate-conjugated CD133 (BD Pharmingen, San Diego, CA, USA), phycoerythrin-conjugated KDR (BD Pharmingen, San Diego, CA, USA), and fluorescein isothiocyanate-conjugated CD34 (BD Pharmingen, San Diego, CA, USA). Fluorescent isotype-matched IgG1 antibodies were used as the negative controls. Each analysis was conducted in duplicates and included 100,000 events.

EPC Proliferation Assay

EPC proliferation was determined by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich, St. Louis, MO, USA) as reported previously[16]. The EPCs were incubated with MTT (0.5 mg/mL, Sigma-Aldrich, St. Louis, MO, USA) for 4 h. The blue formazan was solubilized with dimethyl sulfoxide (DMSO). At last the absorbance was measured at 550/650 nm.

EPCs Migration Assay

The migratory ability of early EPCs and OECs was measured in a modified Boyden chamber (8 μm pore size, Corning, Corning, NY, USA) by transwell assay[16]. Briefly, 4×10^4 EPCs were seeded in the upper chamber of 24-well transwell plates with a polycarbonate membrane (8 μm pores) covered by serum-free medium. Another special medium (50 ng/mL VEGF) was added to the lower chamber. After incubation for 24 h, the membrane was washed with phosphate-buffered saline (PBS) for three times and fixed with 4% paraformaldehyde. Additionally, the upper side of membrane was wiped gently. After that, the membrane was stained using hematoxylin for 10 min. Migration of OECs was assessed by measuring the area containing migrated cells as a percentage of the total area in five random fields (100 x). The other materials were obtained from Sigma-Aldrich (St. Louis, MO, USA). The data were expressed as mean ± SD.

OECs Tube Formation

OECs tube formation assay was conducted as described previously[17]. Extra cellular (EC) Matrix gel solution was thawed overnight at 4°C and then mixed with extra cellular Matrix diluent buffer (Sigma-Aldrich, St. Louis, MO, USA). The solution was added into a 96-well plate (Corning, Corning, NY, USA) at 37°C. Then, OECs (1.0×10^4) were placed on matrix solution with EGM-2 MV medium and incubated at 37°C for 20 h. Tube formation was observed under an inverted light microscope (100×). Four typical fields were taken. The results were expressed as mean ± SD.

Assessment of Factor V Leiden Mutation, Protein C Deficiency, and Protein S Deficiency

The factor V Leiden mutation, protein C deficiency, and protein S deficiency were evaluated according to the previous studies[12,18,19]. Briefly, the presence of mutations in the factor V gene (1691, G→A) was determined. In view of potential liver failure and anticoagulant treatment of patients with BCS, the presence of hereditary deficiencies of protein C, and protein S could only be estimated by modified criteria as compared with the controls. The relative risks of BCS compared with the healthy people were estimated as the odds ratio (OR) and the 95% confidence interval (CI)[20].

Statistical Analysis

All data were expressed as mean ± SD for numeric variables and expressed as number or percentage for categorical variables. $p<0.05$ was considered significant. Differences in baseline characteristics of underlying diseases, and categorical variables were compared using independent *t*-test and x^2-test. The comparison between groups was done using One-way ANOVA test followed by Least Significant Difference (LSD). The Statistical Product and Service Solutions (SPSS) 19 (IBM, Armonk, NY, USA) and GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA) software packages were used for statistical analysis.

Results

Patients' Baseline Characteristics

Information regarding age and gender of the three groups was shown in Table I. Their baseline characteristics were presented in Table I. No significant differences were found between the BCS (NAFLD) and control group regarding age, gender, BMI or smoking ($p>0.05$ for these variables, Table I). However, significant differences were observed in TG, TC, HDL-C, white blood cells, hemoglobin, ALT, AST, ALP, γ-GT, total bilirubin and albumin ($p<0.05$ for these variables, Table I).

Characterization of EPCs

The initially planted cells were round (Figure 1A). On the 7th day, the cells began to be clusters, namely early EPCs (Figure 1B). Then, OECs appeared as clusters on 21st day after plating and showed cobblestone appearance (Figure 1C). Both types of EPCs took up DiI-acLDL and showed lectin binding affinity (Figure 1D-E).

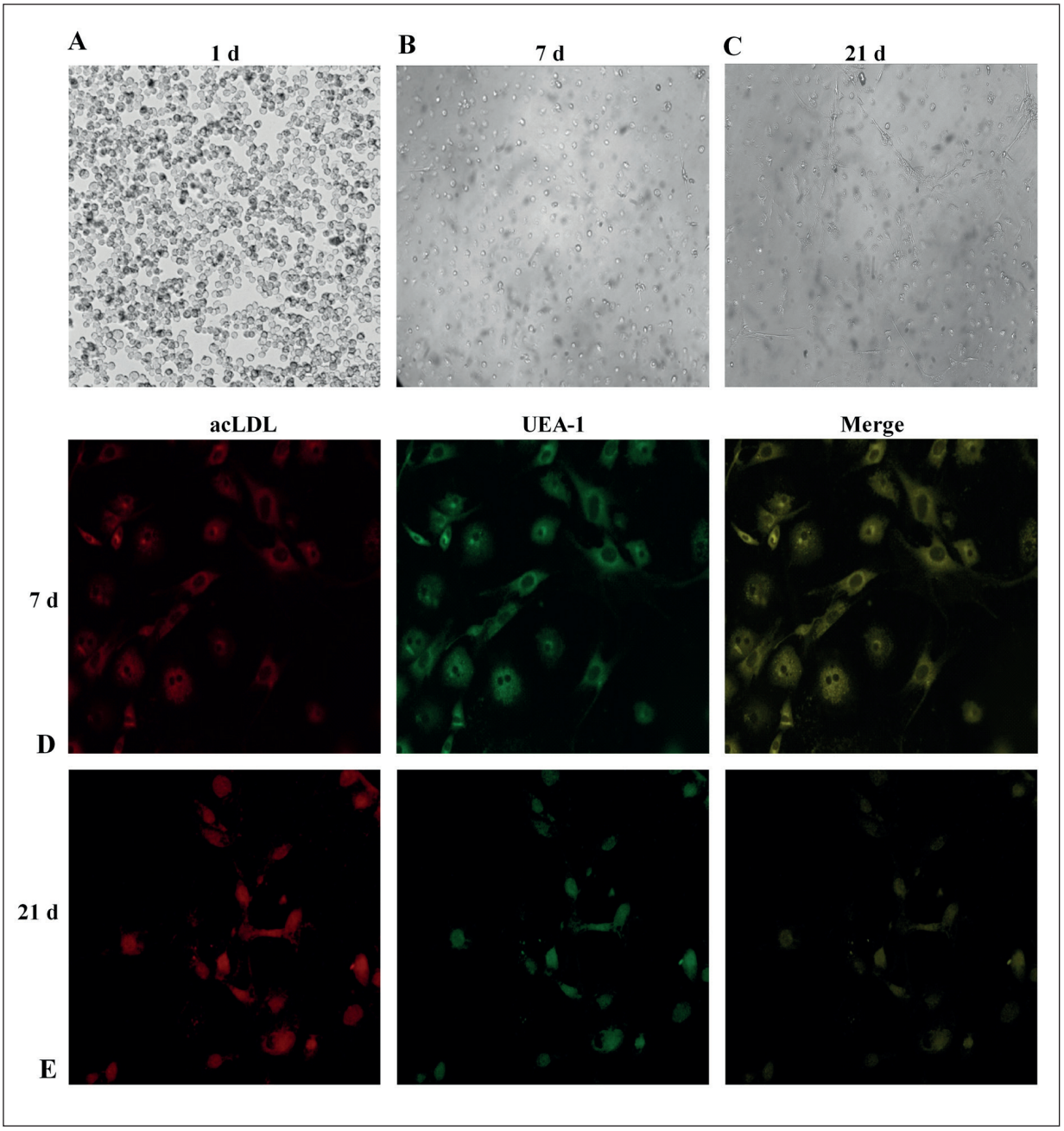

Figure 1. Characterization of endothelial progenitor cells. **A**-**C**, Representative fields of isolated cells on the initial, seventh day (early EPCs) and the twenty first day (OECs, outgrouth endothelial cells). **D**-**E**, The cells on the seventh and the twenty first day were cultured with UEA-1 and acLDL. All the photos were observed on a microscope (x100).

The Number and Function of EPCs in BCS, NAFLD and the Control Group

Compared with the healthy participants, significantly downregulation of $CD34^+/CD45^-$, OECs colonies, OECs proliferation, and OECs tubulogenesis were found in patients with BCS and NAFLD ($p<0.001$ for these variables, Table II). Nevertheless, no differences were found regarding early EPC colonies, $CD34^+/KDR^+$, $CD34^+/CD45^+$, early EPC migration, and OEC migration in the three groups ($p>0.05$ for these variables, Table II). Together, number of EPC was higher and function of EPC was better in BCS and NAFLD patients than in the control group.

Table II. The EPC levels and function in the three groups.

	BCS (n = 28)		NAFLD (n = 20)		Control (n = 73)	
EEPC levels						
CD34+/KDR+(%)	0.66±0.05		0.68±0.04		0.71±0.06	
CD34+/CD45+(%)	0.20±0.02		0.22±0.01		0.21±0.02	
CD34+/CD45-(%)	0.05±0.003***		0.07±0.002###		0.09±0.004	
Early EPCs colonies	5.04±1.15		5.10±1.21		5.08±1.36	
OECs colonies	2.29±0.64***		2.83±0.58###		4.37±0.87	
EPC function	**Early EPCs**	**OECs**	**Early EPCs**	**OECs**	**Early EPCs**	**OECs**
Proliferation	0.68±0.07	0.21±0.03***	0.71±0.04	0.23±0.05###	0.75±0.07	0.49 ± 0.06
Migration	16.48±5.12	7.56±2.13***	15.73±2.24	8.15±1.84###	17.73±1.65	13.76±2.01
Tubulogenesis		13.79±2.18***		13.41±1.95###		24.94±2.37

BCS, Budd-Chiari Syndrome; NAFLD, nonalcoholic fatty liver disease; Control, the healthy participants; EPC, endothelial progenitor cell; EPCs, endothelial progenitor cells; OECs, outgrouth endothelial cells. The data were expressed mean ± SD; ***p<0.001, BCS versus the control group; ###p<0.001, NAFLD versus the control group.

Factor V Leiden Mutation, Protein C Deficiency, and Protein S Deficiency in Patients with Budd-Chiari Syndrome

Among the 28 BCS patients, factor V Leiden mutation (n = 10, 35.71%, OR 12.67, 95% CI 5.24-27.93) and hereditary protein C deficiency (n = 4, 14.29%, OR 7.48, 95% CI 2.02-21.43) were more prevalent than those in the control group (Table III). However, protein S deficiency was not found in the BCS patients. Collectively, factor V Leiden mutation and protein C deficiency were major risk factors for BCS.

Relationship Between Factor V Leiden Mutation, Protein C Deficiency and EPC Levels and Function in BCS Group

The data of BCS patients were classified into two groups: factor V Leiden mutation group and factor V Leiden WT group. The EPC levels and function, including $CD34^+/CD45^-$, OECs colonies, OECs proliferation, OECs migration, and OECs tubulogenesis, showed significant differences between factor V Leiden mutation patients and factor V Leiden WT patients (p<0.05 for these variables, Table IV). Similarly, the EPC levels and function, such as $CD34^+/CD45^-$, OECs colonies, OECs proliferation, OECs migration, and OECs tubulogenesis exhibited significant differences between protein C deficiency patients and protein C WT patients (p<0.05 for these variables, Table V). However, no significant differences were found between the mutation and WT patients regarding other variables of EPCs levels and function (p>0.05 for these variables, Table IV, Table V).

Discussion

Vascular disorders in BCS patients are associated with vascular endothelial injury and endothelial dysfunction[5,6,21,22]. Inflammation, repair reaction, and thrombus formation induced by continuous endothelial injury may promote the vascular occlusion and membrane formation[21,23,24]. It has been reported[25] that endothelial progenitor cells (EPCs) play an important role in re-endothelialization of the damaged endothelial layer. Downregulated levels and function of circulating EPCs significantly contribute to

Table III. Differences in prevalences of factor V Leiden mutation, protein C deficiency, and protein S deficiency in patients with Budd-Chiari syndrome (BCS) and control.

	BCS (%) (n = 28)	Control (%) (n = 73)	OR	95% CI
Factor V Leiden mutation	10 (35.71)	3 (4.11)	12.67	5.24-27.93
Protein C deficiency	4 (14.29)	1 (1.37)	7.48	2.02-21.43
Protein S deficiency	0 (0)	2(2.74)	–	–

BCS, Budd-Chiari Syndrome; Control, the healthy participants; OR, odds rate; CI, confidence interval.

Table IV. The EPC levels and function in the BCS patients with Factor V Leiden mutation.

	BCS (n = 10) Factor V Leiden mutation		BCS (n = 18) Factor V WT	
EPC levels				
CD34+/KDR+(%)	0.70 ± 0.05		0.68 ± 0.04	
CD34+/CD45+(%)	0.21 ± 0.04		0.19 ± 0.03	
CD34+/CD45-(%)	0.04 ± 0.003***		0.07 ± 0.004	
Early EPCs colonies	4.96± 1.15		5.11 ± 1.21	
OECs colonies	2.14 ± 0.47***		2.93 ± 0.58	
EPC function	**Early EPCs**	**OECs**	**Early EPCs**	**OECs**
Proliferation	0.65 ± 0.06	0.15 ± 0.02***	0.72 ± 0.04	0.31 ± 0.07
Migration	15.34 ± 3.97	5.83 ± 1.72***	16.55 ± 3.38	9.73 ± 1.98
Tubulogenesis		10.41 ± 2.06***		14.84 ± 2.16

BCS, Budd-Chiari Syndrome; WT, wide type; EPC, endothelial progenitor cell; EPCs, endothelial progenitor cells; OECs, outgrouth endothelial cells; ***p<0.001, BCS (factor V Leiden mutation) versus BCS (Factor V Leiden WT).

endothelial dysfunction[26]. Nowadays, it has been proved that EPCs express surface protein markers CD133$^+$, CD34$^+$, and KDR$^+$. Following the morphology and the cultured time, EPCs have been divided into the early EPCs and the late outgrowth endothelial cells (OECs)[10,15,27]. Early EPCs and OECs originate from different bone marrow born mononuclear cells. Briefly, early EPCs are from a heterogeneous population of CD45 positive (CD45$^+$) hematopoietic cells, including CD34$^+$/CD45$^+$ hematopoietic progenitors as well as CD45$^+$/CD14$^+$ monocytes[28]. However, OECs are derived from CD45 negative non-hematopoietic non-monocytes (CD45$^-$/CD14$^-$)[29,30]. The two kinds of cultured EPCs often exhibit different characteristics. OECs (not early EPCs) are able to directly form *in vitro* vascular networks[14] and construct *in vivo* perfused vessels[31]. Thus, these previous findings demonstrated that EPC levels and function play essential roles in vascular disorder of BCS.

It has been found that the number of EPCs is significantly higher in patients with liver cirrhosis compared with the healthy group[32,33]. In this study, the EPC levels were lower in BCS compared with NAFLD and the healthy participants. Meanwhile, we found that EPCs number was less in NAFLD than in the healthy people. Together, our results suggested that capacity of endogenous vascular repair was inhibited in primary BCS. Several mechanisms may contribute to reduction of EPCs in BCS patients and NAFLD participants. First,

Table V. The EPC levels and function in the BCS patients with protein C deficiency.

	BCS (n = 4) Protein C deficiency		BCS (n = 24) Protein C WT	
EPC levels				
CD34+/KDR+ (%)	0.63 ± 0.04		0.69 ± 0.06	
CD34+/CD45+ (%)	0.22 ± 0.04		0.21 ± 0.05	
CD34+/CD45- (%)	0.05 ± 0.003***		0.08 ± 0.005	
Early EPCs colonies	5.12 ± 1.21		5.30 ± 1.33	
OECs colonies	2.05 ± 0.41***		2.87 ± 0.56	
EPC function	**Early EPCs**	**OECs**	**Early EPCs**	**OECs**
Proliferation	0.67 ± 0.07	0.17 ± 0.03***	0.69 ± 0.07	0.34 ± 0.05
Migration	16.72 ± 4.33	6.46 ± 1.80***	16.23 ± 4.79	9.86 ± 1.87
Tubulogenesis		11.25 ± 3.15***		15.72 ± 3.09

BCS, Budd-Chiari Syndrome; WT, wide type; EPC, endothelial progenitor cell; EPCs, endothelial progenitor cells; OECs, outgrowth endothelial cells; ***p < 0.001, BCS (protein C deficiency) versus BCS (protein C WT).

BCS and NAFLD related inflammation and oxidative stress are able to inhibit EPCs mobilization from bone marrow to peripheral blood[34-36]. Then, continuous vascular endothelial damage in BCS patients could destroy the supply of EPCs, thus resulting in reduction of EPCs number in BCS and NAFLD patients[37]. Finally, venous congestion often occurs in BCS, leading to portal hypertension, fibrosis, and cirrhosis[38]. Inflammatory factors, including TNF-α, NFκB, and HIF-1α, are able to decrease the survival time of EPCs[39].

To investigate the functions of EPCs in BCS patients and NAFLD, we analyzed the early EPCs and OECs proliferation, migration, and tubulogenesis. The data showed that the functions of OECs (not early EPCs) from the BCS and NAFLD group were significantly impaired compared with the healthy controls. However, no differences were found between the BCS and NAFLD group. This EPCs dysfunction in BCS and NAFLD may be triggered by the oxidative stress and inflammatory factors. These findings indicated that impaired EPCs functions would suppress their potential to efficiently repair vascular endothelial injury. Collectively, the reduced levels and function of EPCs, particularly the OECs, might be the leading cause of vascular endothelial damage in BCS and NAFLD.

According to the previous studies, BCS is closely associated with the deficiency of Factor V Leiden, protein C deficiency, and protein S. Human factor V is one of the several substances that helps blood clot. The anticoagulant protein could not bind normally to factor V mutation resulting in a hypercoagulable state, which increases abnormal and harmful blood clots[40]. The protein C is also one of the natural anti-coagulants of the hemostatic system, which is synthesized depending on vitamin K in liver[41]. During the formation of thrombin-thrombomodulin complex, protein C is converted into activated protein C, thus degrading and inactivating factor V by cooperating with cofactor protein S[42-44]. Consequently, protein C deficiency and protein S deficiency are considered as the risk factors of vein thrombosis in BCS[42-44]. In this investigation, these mutations were also demonstrated as important dangers in BCS patients. To further explore the role of EPCs in BCS, we determined the relationship between the EPCs functions and the above mutations. We divided the BCS patients into the following groups: factor V Leiden mutation and factor V WT, or protein C deficiency and protein C WT. Our data showed that patients with factor V Leiden mutation or protein C deficiency exhibited significant reduction of the EPC levels and function including $CD34^{+}/CD45^{-}$, OECs colonies, OECs proliferation, OECs migration, and OECs tubulogenesis compared with WT patients. However, no differences were found in early EPCs number and functions. Thus, factor V Leiden and protein C deficiency can downregulate the OEC levels and suppress the OEC function, thus leading to vascular endothelial injury and endothelial dysfunction in BCS patients.

Conclusions

The data in this study revealed that OECs levels are significantly decreased. OECs proliferation, migration, and tubulogenesis are inhibited in BCS and NAFLD. The OECs dysfunction may contribute to venous occlusion in patients with primary BCS. Impaired repair of endogenous vascular could enhance the progression and development of primary BCS and NAFLD. Meanwhile, we found that factor V Leiden and protein C deficiency may negatively modulate the OECs levels and functions in BCS patients. Together, improving the OECs levels and functions, and preventing factor V Leiden and protein C deficiency might ameliorate the patients with BCS.

Conflict of Interest

The authors have no conflict of interests to declare.

References

1) Potthoff A, Bahr MJ. Response to time to resize the role of liver transplant for Budd-Chiari syndrome. Liver Int 2015; 35: 2340-2340.
2) Crawford JM. Vascular disorders of the liver. Clin Liver Dis 2010; 14: 635-650.
3) Cheng DL, Xu H, Hua R, Xu K, Lv WF, Lu X, Du HT, Zhang QQ. Comparative study of MRI manifestations of acute and chronic Budd-Chiari syndrome. Abdom Imaging 2015; 40: 76-84.
4) Aydinli M, Bayraktar Y. Budd-Chiari syndrome: etiology, pathogenesis and diagnosis. World J Gastroenterol 2007; 13: 2693-2696.
5) MacNicholas R, Olliff S, Elias E, Tripathi D. An update on the diagnosis and management of Budd-Chiari syndrome. Expert Rev Gastroent 2012; 6: 731-744.
6) Murad SD, Plessier A, Hernandez-Guerra M, Fabris F, Eapen CE, Bahr MJ, Trebicka J, Morard I, Lasser L, Heller J, Hadengue A, Langlet P, Miranda H, Primignani M, Elias E, Leebeek FW, Rosendaal FR, Garcia-Pagan JC, Valla DC, Janssen HL; EN-Vie (European Network for Vascular Disorders of the Liver). Etiology, management, and outcome of the Budd-Chiari syndrome. Ann Intern Med 2009; 151: 167-175.

7) Wang ZC, Qi J, Liu LM, Li J, Xu HY, Liang B, Li B. Valsartan reduces AT1-AA-induced apoptosis through suppression oxidative stress mediated ER stress in endothelial progenitor cells. Eur Rev Med Pharmacol Sci 2017; 21: 1159-1168.
8) Kawasaki T, Nishiwaki T, Sekine A, Nishimura R, Suda R, Urushibara T, Suzuki T, Takayanagi S, Terada J, Sakao S, Tatsumi K. Vascular repair by tissue-resident endothelial progenitor cells in endotoxin-induced lung injury. Am J Respir Cell Mol Biol 2015; 53: 500-512.
9) Yang JX, Pan YY, Wang XX. Comments to the article "Diverse contribution of bone marrow-derived late-outgrowth endothelial progenitor cells to vascular repair under pulmonary arterial hypertension and arterial neointimal formation". J Mol Cell Cardiol 2017; 103: 139-140.
10) Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest 2000; 105: 71-77.
11) Huang R, Zhang QQ, Huang QX, Zu MH, Xu H, Zeng LY. Number and function of circulating endothelial progenitor cells in patients with primary Budd-Chiari syndrome. Clin Res Hepatol Gas 2017; 41: 139-146.
12) Janssen HL, Meinardi JR, Vleggaar FP, van Uum SH, Haagsma EB, van Der Meer FJ, van Hattum J, Chamuleau RA, Adang RP, Vandenbroucke JP, van Hoek B, Rosendaal FR. Factor V Leiden mutation, prothrombin gene mutation, and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case-control study. Blood 2000; 96: 2364-2368.
13) Wang JQ, Gu WP, Deng QQ, Huang Q, Wang AM, Liu SX, Tang HY, Liang Y, Yan JH, Ouyang S. Endothelial progenitor cell miR-126 promotes homing of endothelial progenitor cells within arterial thrombus in patients with cerebral infarction and its molecular mechanism. Eur Rev Med Pharmacol Sci 2018: 1078-1083.
14) Sieveking DP, Buckle A, Celermajer DS, Ng MK. Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. J Am Coll Cardiol 2008; 51: 660-668.
15) Timmermans F, Van Hauwermeiren F, De Smedt M, Raedt R, Plasschaert F, De Buyzere ML, Gillebert TC, Plum J, Vandekerckhove B. Endothelial outgrowth cells are not derived from CD133(+) cells or CD45(+) hematopoietic precursors. Arterioscler Thromb Vasc Biol 2007; 27: 1572-1579.
16) Li LF, Zhao XH. The effect of VEGF-induced connexin 43 on EPCs proliferation, migration and repair of injured vessels. J Am Coll Cardiol 2016; 68: C37.
17) Holnthoner W, Hohenegger K, Husa AM, Muehleder S, Meinl A, Peterbauer-Scherb A, Redl H. Adipose-derived stem cells induce vascular tube formation of outgrowth endothelial cells in a fibrin matrix. J Tissue Eng Regen Med 2015; 9: 127-136.
18) Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood-coagulation factor-v associated with resistance to activated protein-C. Nature 1994; 369: 64-67.
19) Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 88: 3698-3703.
20) Woolf B. On Estimating the relation between blood group and disease. Ann Hum Genet 1955; 19: 251-253.
21) Dang XW, Li LH, Xu PQ. Research status of Budd-Chiari syndrome in China. Int J Clin Exp Med 2014; 7: 4646-4652.
22) Sozer S, Fiel MI, Schiano T, Xu MJ, Mascarenhas J, Hoffman R. The presence of JAK2V617F mutation in the liver endothelial cells of patients with Budd-Chiari syndrome. Blood 2009; 113: 5246-5249.
23) Kage M, Arakawa M, Kojiro M, Okuda K. Histopathology of membranous obstruction of the inferior vena-cava in the Budd-Chiari syndrome. Gastroenterology 1992; 102: 2081-2090.
24) Afredj N, Metatla S, Faraoun SA, Nani A, Guessab N, Benhalima M, Bendib SE, Debzi N, Layaida K, Gamar L, Baiod N, Balamane M, Kaddache N, Bounab N, Kecili L, Boucekkine T. Association of Budd-Chiari syndrome and celiac disease. Gastroenterol Clin Biol 2010; 34: 621-624.
25) Werner N, Junk S, Laufs U, Link A, Walenta K, Bohm M, Nickenig G. Intravenous transfusion of endothelial progenitor cells reduces neointima formation after vascular injury. Circ Res 2003; 93: e17-24.
26) Huang PH, Chen YH, Chen YL, Wu TC, Chen JW, Lin SJ. Vascular endothelial function and circulating endothelial progenitor cells in patients with cardiac syndrome X. Heart 2007; 93: 1064-1070.
27) Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. Arterioscler Thromb Vasc Biol 2008; 28: 1584-1595.
28) Sharpe EE, Teleron AA, Li B, Price J, Sands MS, Alford K, Young PP. The origin and in vivo significance of murine and human culture-expanded endothelial progenitor cells. Am J Pathol 2006; 168: 1710-1721.
29) Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 2002; 109: 337-346.
30) Shantsila E, Watson T, Tse HF, Lip GY. New insights on endothelial progenitor cell subpopulations and their angiogenic properties. J Am Coll Cardiol 2008; 51: 669-671.
31) Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prchal JT, Ingram DA. Redefining endothelial progenitor cells via clonal analysis and hematopoieticstem/progenitor cell principals. Blood 2007; 109: 1801-1809.
32) Kaur S, Tripathi D, Dongre K, Garg V, Rooge S, Mukopadhyay A, Sakhuja P, Sarin SK. Increased number and function of endothelial progenitor cells stimulate angiogenesis by resident liver sinusoidal endothelial cells (SECs) in cirrhosis through paracrine factors. J Hepatol 2012; 57: 1193-1198.
33) Sieghart W, Fellner S, Reiberger T, Ulbrich G, Ferlitsch A, Wacheck V, Peck-Radosavljevic M. Differential role of circulating endothelial progenitor cells in cirrhotic patients with or without hepatocellular carcinoma. Dig Liver Dis 2009; 41: 902-906.

34) Gutierrez-Grobe Y, Gavilanes-Espinar JG, Masso-Rojas FA, Sanchez-Valle V, Paez-Arenas A, Ponciano-Rodriguez G, Chavez-Tapia NC, Uribe M, Mendez-Sanchez N. Metabolic syndrome and nonalcoholic fatty liver disease. The role of endothelial progenitor cells. Ann Hepatol 2013; 12: 908-914.
35) Sirker AA, Astroulakis ZM, Hill JM. Vascular progenitor cells and translational research: the role of endothelial and smooth muscle progenitor cells in endogenous arterial remodelling in the adult. Clin Sci 2009; 116: 283-299.
36) Tian ZL, Jia GL, Xi HL, Feng S, Wang XK, Li R. Investigation on etiology of hepatic venous obstruction Budd-Chiari Syndrome. Cell Biochem Biophys 2014; 70: 1803-1806.
37) Dimmeler S, Vasa-Nicotera M. Aging of progenitor cells: limitation for regenerative capacity? J Am Coll Cardiol 2003; 42: 2081-2082.
38) Hoekstra J, Janssen HLA. Vascular liver disorders (I): diagnosis, treatment and prognosis of Budd-Chiari syndrome. Neth J Med 2008; 66: 334-339.
39) Wilson GK, Tennant DA, McKeating JA. Hypoxia inducible factors in liver disease and hepatocellular carcinoma: current understanding and future directions. J Hepatol 2014; 61: 1397-1406.
40) De Stefano V, Leone G. Resistance to activated protein-C due to mutated factor-V as a novel cause of inherited thrombophilia. Haematologica 1995; 80: 344-356.
41) Chang JC. A thought on possible pathogenesis of ebola viral hemorrhagic disease and potential treatments: could it be thrombotic thrombocytopenic purpura-like syndrome? Ther Apher Dial 2016; 20: 93-98.
42) Tassin M, Llarena P, Laffaye F, Kaltenbach G. Protein C deficiency in patient with sepsis-associated disseminated intravascular coagulation and deep vein thrombosis. Case report. Arch Argent Pediatr 2013; 111: e28-e30.
43) Doganay S, Sonmezgoz F, Sen Y, Kocakoc E. Deep vein thrombosis associated with protein C and protein S deficiency: an unusual cause of acute abdomen. J Clin Ultrasound 2011; 39: 287-289.
44) Cho YP, Ahn JH, Choi SJ, Han MS, Jang HJ, Kim YH, Kim HJ, Kwon TW, Lee SG. Endovascular management of iliofemoral deep venous thrombosis due to iliac vein compression syndrome in patients with protein C and/or S deficiency. J Korean Med Sci 2004; 19: 729-734.