

## Evaluation the value of markers for prediction of portal vein thrombosis after devascularization

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### ABSTRACT

**Aim.** To evaluate the value of D-dimer and P-selectin in cirrhotic portal hypertension (PHT) patients for prediction of portal vein thrombosis (PVT) after devascularization. **Material and methods.** 137 patients with cirrhotic PHT who undergone devascularization from January 2012 to April 2014 were retrospectively reviewed, all of them were divided into two groups (PVT group and non-PVT group) by Doppler ultrasonography (DU) examination. The level of D-dimer and P-selectin was tested during the peri-operative period. **Results.** 38 patients (27.7%) were found PVT by DU examination post-operatively. In contrast to the non-PVT group, the level of D-dimer and P-selectin in the PVT group was much higher significantly at 1, 3 and 7 days after devascularization ( $P < 0.05$ ). However, in the 15 days after surgery, the difference of P-selectin between the two groups was not significant ( $P = 0.260$ ). It was shown that the higher sensitivity of the two markers for PVT was D-dimer, the higher specificity belonged to P-selectin. The area under receiver operating characteristic (ROC) curve of P-selectin was the bigger of the two markers. When the two markers were combined to be used to diagnose PVT, the sensitivity was increased to 0.911, with a slight drop of specificity to 0.715, the area under ROC curve was 0.919. **Conclusion.** The level of D-dimer and P-selectin might be good candidate predictive markers for PVT in patients with cirrhotic PHT after devascularization. The combined test of the two markers can increase the value of prediction.

**Key words.** D-dimer. P-selectin. Combination.

### INTRODUCTION

Portal vein thrombosis (PVT) includes thrombosis in the portal vein, splenic vein and superior mesenteric vein or intrahepatic portal vein branches as they form an interactive vascular system without valves. PVT following devascularization occurs in 6.3 to 39.0% of patients and is mostly an unpredictable event.<sup>1,2</sup> Complete or partial occlusion of the portal venous system in cirrhotic portal hypertension (PHT) patients may determine liver ischemia, with consequent deterioration of liver function, and a sudden increase in portal pressure. Some complications of liver cirrhosis such as variceal hemorrhage may develop and death eventually may

occur.<sup>3-5</sup> It is therefore important to identify markers that can predict and prevent the occurrence of PVT.

D-dimer is a kind of fibrin degradation fragment, and is the molecular marker in the fibrinolysis cycle.<sup>7</sup> P-selectin is a cell adhesion molecule that is mainly produced by platelets and endothelial cells; it increases the activation of platelets and the adhesion of platelets to the endothelium.<sup>8</sup> Recently, D-dimer and P-selectin have been shown to play an important role in diagnostic indicator for thrombotic diseases.<sup>9,10</sup> In this study, we investigated whether D-dimer and P-selectin had a better predictive value to PVT after devascularization in patients with cirrhotic PHT.

### MATERIAL AND METHODS

#### Patients and study design

Two hundred sixty-seven consecutive patients who were diagnosed with hepatitis virus-related cirrhosis PHT between January 2012 to April 2014 in

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the 81st Hospital of PLA were included. The diagnoses of these patients were all confirmed by endoscopy and Doppler ultrasonography (DU). All of them underwent the surgery procedure. They accompanied with no serious cardiopulmonary diseases and concomitant chronic duodenal ulcers. Patients whose serum levels of D-dimer or P-selectin during the time of diagnosis and treatment were not available were excluded. Any patients who had been taking warfarin or Aspirin would have also been excluded. Accordingly, one hundred and thirty-seven patients were enrolled into this study, which was approved by our hospital. The characteristics of the patients including age, gender, Child-Pugh's score, preoperative biochemical tests were summarized in table 1. Biochemical tests were examined within 1-3 days preoperatively.

The study protocol conformed to the guidelines by the institutional Ethical Committee. All patients gave their informed consent to be included in the study. The research was carried out according to the principles of The Declaration of Helsinki.

### PVT diagnosis

DU (ACUSON Sequoia™ 512, Color Doppler equipment) examination of the portal vein was performed pre-/post-operationally,<sup>11</sup> and was performed in all cases by the same examiner to avoid interobserver variables. Spectral waveforms were obtained at measured angles of insonation of < 60°. The pre-operational examination was performed at three days before the surgery. The post-operational exami-

nation was performed at two, five, seven, fifteen, twenty-two and thirty days following the surgery. PVT was defined as the obstruction of more than 50% of the vascular lumen by a thrombus in the main portal vein, its large branches, or in the splenic vein. No patients had a diagnosed PVT prior to the operation. Then the samples were divided into two groups, including PVT group and non-PVT group.

### D-dimer and P-selectin determinations

Peripheral venous blood sample (15 mL) was collected from each patient who had fasted for at least 12 h and mixed with 3.8% sodium citrate (ratio 9:1). Sample was centrifuged for 10 min at 2,000 g, and the supernatant was stored at -80°C until use. D-dimer and P-selectin were measured by automated latex enhanced immunoassay in citrated plasma on instrumentation Laboratory (Coagulation Systems ACL 9000) and quantified using enzyme linked immunosorbent assay kits (ZhongShan Biotech, Beijing, China). Both of the two biomarkers were tested one day before surgery, the second test was performed in one day postoperatively. Thereafter, in three days, seven days and fifteen days after surgery, the test was performed again.

### Surgical technique

The surgical technique was applied as described by Zong, *et al.*<sup>12</sup> The same operative setup and standardized technique were applied for all patients.

**Table 1.** Preoperative clinical characteristics of cirrhotic patients.

	PVT (n = 38)	Non-PVT (n = 99)	t/ $\chi^2$ -value	P-value
Age, mean $\pm$ SD (years)	48.1 $\pm$ 7.1	45.2 $\pm$ 8.4	0.622	0.231
Gender				
Male	21	59	0.485	0.316
Female	17	40		
Child-Pugh's score	6.8 $\pm$ 1.3	7.0 $\pm$ 1.5	0.197	0.628
PT (sec)	14.7 $\pm$ 2.9	14.1 $\pm$ 2.3	0.206	0.595
TB ( $\mu$ mol/L)	26.3 $\pm$ 4.0	26.8 $\pm$ 4.7	0.146	0.742
ALT (IU/L)	29.1 $\pm$ 7.8	27.6 $\pm$ 6.3	0.323	0.382
AST (IU/L)	27.7 $\pm$ 7.1	28.5 $\pm$ 6.4	0.272	0.503
Operative time (min)	248.1 $\pm$ 71.2	231.0 $\pm$ 63.6	0.375	0.377
Intraoperative blood loss (mL)	634.6 $\pm$ 68.5	592.7 $\pm$ 89.3	0.571	0.259
FPP (cmH <sub>2</sub> O)	39.8 $\pm$ 7.6	36.3 $\pm$ 8.9	0.363	0.498

PVT: portal venous thrombosis. PT: prothrombin time. TB: total bilirubin. Alb: albumin. ALT: alanine aminotransferase. AST: aspartate aminotransferase. FPP: free portal pressure.

Procedures were performed by the same team of specialists in gastrointestinal and hepatobiliary surgery.

### Statistical analysis

All statistical analyses were performed using SPSS15.0 software (SPSS, Chicago, IL). Continuous data were expressed as mean values  $\pm$  standard deviation (SD). Significant differences between groups were determined by chi-squared analysis and unpaired Student's *t* test. Dichotomous variables were created out of continuous variables by using clinically important cutoff points. Receiver operating characteristic (ROC) curves, sensitivity (Sen), specificity (Spe), and positive and negative predictive values (PPV, NPV) were determined to assess the diagnostic value of D-dimer and P-selectin. *P*-values  $< 0.05$  were considered statistically significant.

## RESULTS

### Baseline characteristics

DU examination showed thirty-eight patients developed PVT post-operatively (38/137, 27.7%), the occurrence of PVT was in  $5.72 \pm 1.73$  days after devascularization, moreover, 86.8% PVT (33/38) occurred between day three and seven post-operationally.

There were no significant difference between the PVT group and the non-PVT group of the preoperative database including age, gender, Child-Pugh's score, blood routine biochemical tests, mean operative time, intra-operative blood loss and intra-operative freedom portal vein pressure. The database also including the level of D-dimer and P-selectin ( $P > 0.05$ ) (Table 2). The two groups were well balanced in the distribution of pre- and intra-operational characteristics.

### The level of D-dimer and P-selectin

As shown in table 2, in contrast to the non-PVT group, the level of D-dimer in the PVT group was much higher significantly in one day, three days, one week and fifteen days after devascularization ( $P < 0.05$ ). The similar situation could be found in the level of P-selectin. The significantly more altered was in the PVT group than in the non-PVT group postoperationally ( $P < 0.05$ ). However, in the fifteen days after surgery, the difference of P-selectin between the two groups was not significant ( $P = 0.260$ ).

Table 2. The level of D-dimer and P-selectin pre/post-operation.

	Pre-1		Post-1		Post-3		Post-7		Post-15	
	PVT	Non-PVT	PVT	Non-PVT	PVT	Non-PVT	PVT	Non-PVT	PVT	Non-PVT
D-dimer (ng/mL)	230.6 $\pm$ 58.4	218.2 $\pm$ 68.3	793.5 $\pm$ 65.8	362.1 $\pm$ 52.9	941.0 $\pm$ 67.2	391.8 $\pm$ 83.0	892.5 $\pm$ 82.5	398.5 $\pm$ 72.4	737.3 $\pm$ 59.5	351.5 $\pm$ 63.9
P-selectin (ng/mL)	29.1 $\pm$ 7.2	30.8 $\pm$ 6.1	77.2 $\pm$ 6.8	50.8 $\pm$ 5.3	106.7 $\pm$ 8.3	59.2 $\pm$ 6.7	97.3 $\pm$ 7.1	58.3 $\pm$ 7.0	47.9 $\pm$ 5.2	44.1 $\pm$ 6.2
P-value	0.206 <sup>a</sup>	0.383 <sup>b</sup>	0.041 <sup>a</sup>	0.048 <sup>b</sup>	0.018 <sup>a</sup>	0.022 <sup>b</sup>	0.039 <sup>a</sup>	0.036 <sup>b</sup>	0.048 <sup>a</sup>	0.260 <sup>b</sup>

PVT: portal venous thrombosis. Pre: preoperation. Post: postoperation. <sup>a</sup> Compare PVT with non-PVT group of D-dimer level. <sup>b</sup> Compare PVT with non-PVT group of P-selectin level.

### Value of D-dimer and P-selectin on PVT

By using the change in D-dimer level and P-selectin level as the diagnostic standard for PVT in cirrhosis patients after devascularization, we achieved the Sen, Spe, PPV and NPV in predicting incidence of PVT (Table 3). It showed that the higher Sen of the two markers for PVT was D-dimer, the higher Spe belonged to P-selectin. Based on the different changes in D-dimer, P-selectin levels and the false positive rates and Sen rates in different D-dimer, P-selectin levels, a ROC curve was plotted. Using the ROC analysis, the area under the ROC curve were 0.826 and 0.869, respectively. When the two markers were combined to diagnose PVT, the Sen was increased to 0.911, with a slight drop of Spe to 0.715, the area under the ROC curve was 0.919 (Figure 1).

### DISCUSSION

The prevalence of PVT in cirrhotic PHT had been reported as values ranging from 0.6 to 2.1%.<sup>13</sup> However, PVT has been recognized as a potential complication following devascularization for PHT, some literatures showed a frequency from 6.3 to 39.0%.<sup>1,2</sup> In our study, the incidence of PVT after devascularization was 8.6%. PVT is often an occasional diagnosis in asymptomatic patients, but it may rapidly progress leading to massive thrombosis of intestinal veins, even fatal consequences.<sup>14</sup> Therefore, early diagnosis of PVT may be useful to prevent uneventful outcome. The early predictive indicator of postoperative PVT may be the most focus.

At present, some methods were performed to prevent the occurrence of PVT.

- Systemic or topical injection heparin for prophylaxis intraoperatively or immediate postoperatively.<sup>15</sup> But not all patients may occur PVT or accept the treatment of injection heparin.
- Anticoagulant therapy base on the testing platelet count, since this method reflects only the number of platelets, ignores the function of plate-

let count. Some reports showed that the level of platelet count was not more than the level of  $500 \times 10^9/L$  until portal vein thrombosis formation.<sup>16</sup>

- Imaging examination especially DU can provide information on the thromboses via echo analysis, detect the presence of post-stenotic dilatation, as well as flow defects and turbulence.

Although PVT can be accurately diagnosed by DU, the formation of thrombosis was a dynamic process, only when the appearance of PVT, the ultrasound can play a valuable role, so it can't predict thrombosis.

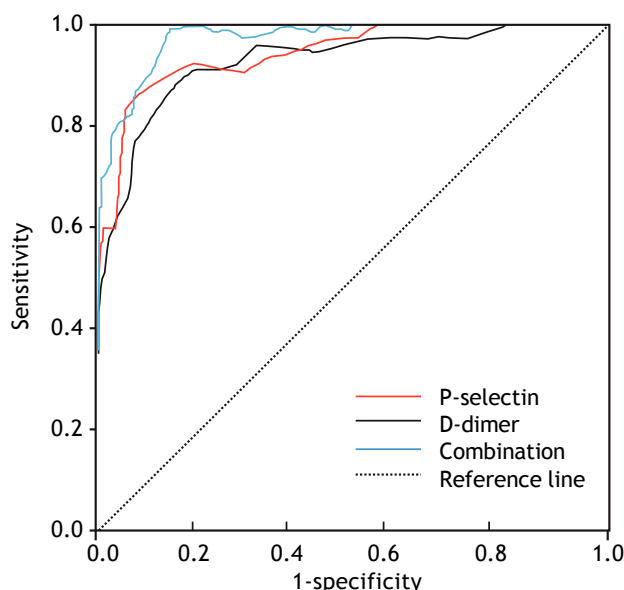
D-dimer is a small peptide fragments of early thrombosis in the role of fibrinogen degradation of plasmin formation, the presence of secondary fiber *in vivo* dissolved and specific marker of thrombosis.<sup>17</sup> Risch thought that D-dimer could be used as a diagnostic specificity of acute deep vein thrombosis indicators.<sup>18</sup> Palareti found the change of D-dimer level after anticoagulation therapy for patients may predict the risk of thrombosis recurrence.<sup>19</sup> In recent years, some studies have shown that D-dimer level as sensitive indicator for coagulation system and fibrinolytic system could predict the formation of PVT.<sup>20</sup> Our study showed that the specificity of D-dimer was 77.3% in predicting PVT incidence, the sensitivity was 84.6%, and it may be regarded as a stable and good indicator. Increased D-dimer after operation suggested that activation of coagulation *in vivo* and hepatic clearance of tissue plasminogen activator were related to the reduction of fibrinolysis inhibitor protein synthesis.

The study on deep venous thrombosis showed that P-selectin involved in platelet/endothelial cell adhesion and inflammation in vascular wall, which may lead to a new pathogenesis of venous thrombosis.<sup>21</sup> Blenn, *et al.* found that soluble P-selectin level in patients with deep vein thrombosis was significantly higher, which may be related to excessive platelet activation.<sup>22</sup> Myers's experiment showed that P-selectin could enlarge the effect of vein wall inflammation caused by thrombotic stimuli and promoting venous thrombosis. The higher level

Table 3. Evaluation the value for PVT of D-dimer and P-selectin.

	Cut-off value	Sen	Spe	PPV	NPV	AUC
D-dimer	500 ng/mL	0.838	0.760	0.813	0.807	0.826
P-selectin	80 ng/mL	0.793	0.906	0.919	0.763	0.869
Combination	—	0.911	0.715	0.747	0.886	0.919

Sen: sensitivity. Spe: specificity. PPV: positive predictive value. NPV: negative predictive value. AUC: area under the receiver operating characteristics curve. Combination, combined D-dimer, P-selection and platelet count.



**Figure 1.** The receiver operating characteristic (ROC) curve for diagnosis PVT by D-dimer and P-selectin.

P-selectin, the more inflammatory cells in vein wall, the bigger intravenous clot generated simultaneously in model mice.<sup>23</sup> Therefore, as an indicator of the degree or function of platelet activation, P-selectin can be regarded as a marker to evaluate the pro-thrombotic state, coagulation tendency and formation of thrombosis *in vivo*. Our study showed that compared with non-PVT group, the level of P-selectin was higher in PVT group post-operationally, especially in three days after surgery. When 80 ng/mL was set as a cut-off point, P-selectin had an 87% sensitivity and specificity for PVT.

As a dynamic process, the formation of thrombosis was monitored by our DU continuously. And the two indicators including D-dimer and P-selectin were tested continuously for assessing the predictive ability. DU examination showed the occurrence of PVT was  $5.72 \pm 1.73$  days (2-7 days) after devascularization. The two indicators showed significant difference between PVT group and non-PVT group in one, three and seven days after operation ( $P < 0.05$ ), however the differences could not be found in the fifteen days post-operatively. By this token, the best prediction PVT critical point should be set as early as possible, and should be lasted three days at least. From the ROC curve figure, the area under curve of P-selectin was the bigger. When the two indicators were combined together, the area may be up to 0.919, the value of early prediction can be improved. Then clinical interventions can be performed in time and safely.

## CONCLUSION

The level of D-dimer and P-selectin might be good candidate predictive markers for PVT in patients with cirrhotic PHT after devascularization. The combined test of the two markers can increase the value of prediction.

## ABBREVIATIONS

- **DU:** Doppler ultrasonography.
- **NPV:** negative predictive values.
- **PHT:** portal hypertension.
- **PPV:** positive predictive values.
- **PVT:** portal vein thrombosis.
- **ROC:** receiver operating characteristic.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

1. Winslow ER, Brunt LM, Drebin JA. Portal vein thrombosis after splenectomy. *Am J Surg* 2002; 184: 631-5.
2. Webster GJ, Burroughs AK, Riordan SM. Review article: portal vein thrombosis-new insights into aetiology and management. *Aliment Pharmacol Ther* 2005; 21: 1-9.
3. de Franchis R. Evolving consensus in portal hypertension. *J Hepatol* 2005; 43: 167-76.
4. Grace ND, Groszmann RJ, Garcia-Tsao G, Burroughs AK, Pagliaro L, Makuch RW. Portal hypertension and variceal bleeding: an AASLD single topic symposium. *Hepatology* 1998; 28: 868-80.
5. Garcia-Tsao G, Bosch J, Groszmann R. Portal hypertension and variceal bleeding, unresolved issues. Summary of an American Association for the study of liver disease and European Association for the study of the liver single-topic conference. *Hepatology* 2008; 47: 1764-72.
6. van der Bom JG, Heckbert SR, Lumley T, Holmes CE, Cushman M, Folsom AR, Rosendaal FR, et al. Platelet count and the risk for thrombosis and death in the elderly. *J Thromb Haemost* 2009; 7: 399-405.
7. Gosselin RC, Wu JR, Kottke-Marchant K, Peetz D, Christie DJ, Muth H, Panacek E. Evaluation of the Stratus CS Acute Care D-dimer assay (DDMR) using the Stratus CS STAT Fluorometric Analyzer: a prospective multisite study for exclusion of pulmonary embolism and deep vein thrombosis. *Thromb Res* 2012; 130: e274-e278.
8. Patel KN, Soubra SH, Bellera RV, Dong JF, McMullen CA, Burns AR, Rumbaut RE. Differential role of von Willebrand factor and P-selectin on microvascular thrombosis in endotoxemia. *Arterioscler Thromb Vasc Biol* 2008; 28: 2225-30.
9. Smith K. Endoscopy: ESD is associated with a moderate risk of deep vein thrombosis that may be determined by D-dimer levels. *Nat Rev Gastroenterol Hepatol* 2011; 8: 538.
10. Gremmel T, Ay C, Seidinger D, Pabinger I, Panzer S, Koppensteiner R. Soluble p-selectin, D-dimer, and high-sensitivity C-reactive protein after acute deep vein



- thrombosis of the lower limb. *J Vasc Surg* 2011; 54(6 Suppl.): 48S-55S.
11. Zong GQ, Fei Y, Chen J, Liu RM, Xu YF. Effects of selective double portazygous disconnection and devascularization on hemodynamics of the portal venous system. *Med Ultrason* 2014; 16: 291-7.
  12. Zong GQ, Fei Y, Chen J, Liu RM. Selective double disconnection for cirrhotic portal hypertension. *J Surg Res* 2014; 192: 383-9.
  13. Sobhonslidsuk A, Reddy KR. Portal vein thrombosis: a concise review. *Am J Gastroenterol* 2002; 97: 535-41.
  14. Reit MV, Burger JW, van Muiswinkel JM. Diagnosis and treatment of portal vein thrombosis following splenectomy. *Br J Surg* 2000; 87: 1229-33.
  15. Lisman T. Low molecular weight heparin in management and prevention of portal vein thrombosis. *Thromb Res* 2014; 134: 761-2.
  16. Jensen MK, de Nully Brown P, Lund BV, Nielsen OJ, Hasselbalch HC. Increased circulating platelet-leukocyte aggregates in myeloproliferative disorders is correlated to previous thrombosis, platelet activation and platelet count. *Eur J Haematol* 2001; 66: 143-51.
  17. Martin K, Kia L, Parikh-Neehar D, Kulik L, McMahon B. Heparin-induced thrombocytopenia testing is overutilized in cirrhosis & correlates with poor clinical outcomes. *Ann Hepatol* 2014; 13: 548-54.
  18. Risch L, Monn A, Lüthy R, Honegger H, Huber AR. The predictive characteristics of D-dimer testing in outpatients with suspected venous thromboembolism: a Bayesian approach. *Clin Chim Acta* 2004; 345: 79-87.
  19. Palareti G, Cosmi B, Legnani C, Tosetto A, Brusi C, Iorio A, Pengo V, et al. D-dimer testing to determine the duration of anticoagulation therapy. *N Engl J Med* 2006; 355: 1780-9.
  20. Cosmi B, Legnani C, Tosetto A, Pengo V, Ghirarduzzi A, Testa S, Prisco D, et al. Usefulness of repeated D-dimer testing after stopping anticoagulation for a first episode of unprovoked venous thromboembolism: the PROLONG II prospective study. *Blood* 2010; 115: 481-8.
  21. Ramacciotti E, Blackburn S, Hawley AE, Vandy F, Ballard-Lipka N, Stabler C, Baker N, et al. Evaluation of soluble P-selectin as a marker for the diagnosis of deep venous thrombosis. *Clin Appl Thromb Hemost* 2011; 17: 425-31.
  22. Blann AD, Noteboom WM, Rosendaal FR. Increased soluble P-selectin levels following deep venous thrombosis: cause or effect. *Br J Haematol* 2000; 108: 191-3.
  23. Myers DD, Hawley AE, Farris DM. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. *J Vase Surg* 2003; 38: 1075-89.