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Original Article

Long-term outcomes of prophylactic splenocaval shunt plus devascularization in patients with high risk of bleeding from esophagogastric varices

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Abstract: Aim: To evaluate the effect, safety and long-term outcomes of prophylactic splenocaval shunt in patients with high risk of hemorrhage from esophagogastric varices. Methods: Clinical data of portal hypertension patients with high risk of variceal bleeding but no history of hemorrhage from January 2003 to December 2012 in Shaanxi Provincial People's Hospital were collected and analyzed. All patients underwent splenocaval shunt plus pericardial devascularization (Combined Group, n = 65), pericardial devascularization alone (PCDV Group, n = 50) or conservative therapy (Control Group, n = 30) respectively in order to prevent the first episodes of hemorrhage. Results: Operations were successfully performed in 115 patients, and no postoperative mortality occurred. Overall morbidity was 16.9% in combined group and 20.3% in PCDV group ($P > 0.05$). The bleeding rate was 6.0% in combined group, which were higher than those in PCDV group (20.5%, $P < 0.05$) and control group (53.3%, $P < 0.05$). However, the incidence of ascites (4.0%) in combined group was lower than that in PCDV group (25.6%, $P < 0.01$) and control group (28%, $P < 0.05$). Portal venous thrombosis in combined group was lower than that in PCDV group (10% *vs.* 33.3%, $P < 0.01$). And there was no significant difference in hepatic encephalopathy and survival among three groups in a follow-up of 30 to 120 months ($P > 0.05$). Conclusion: The prophylactic operation was recommended for patients with high risk of bleeding from esophagogastric varices, and splenocaval shunt plus pericardial devascularization is preferable.

Keywords: Portal hypertension, splenocaval shunt, devascularization

Introduction

Esophagogastric variceal bleeding is one of the most life-threatening complications in patients with portal hypertension. Up to 30% of initial bleeding are fatal, about 70% survivors still have high-risk rebleeding rate after the first variceal bleeding event and the mortality rate is approximately 20% within six weeks [1].

Endoscopic therapy is now the first-line intervention to control acute bleeding and prevent recurrent hemorrhage for patients with esophagogastric varices [2-4]. Though hemorrhagic hazards decreased, the disadvantages should not be ignored. Firstly, even though the therapy were performed every 2-3 weeks until variceal eradication, the rebleeding rate after endoscopic therapy was still as high as 23.1% to 50.0% [5-7]. Secondly, endoscopic therapy increased incidence and severity of portal hypertensive gastropathy, which was another important reason of gastrointestinal blood loss [8-10]. Finally, endoscopic therapy may lead to several complications, such as esophageal ulcers (16%-82%), esophageal fistula or perforation (2.9%-10.9%), dysphagia (20%-32%), annular esophageal stenosis (4%-20%), bacteremia, pleural effusion, and ascitic formation [4, 11-14].

Transjugular intrahepatic portosystemic shunt (TIPS) has been widely used since 1990s. The minimally invasive shunt has been shown superior to endoscopic therapy in reducing the variceal bleeding incidence in randomized con-

trolled trials [5, 6, 15-17]. However, the side effects are frequent and severe sometimes. Firstly, hepatic encephalopathy rate after TIPS may reach up to 9.1%-55.3%, thus it is not recommended to prevent the first episodes of variceal bleeding [18]. Secondly, the rebleeding rate after TIPS increase gradually and even up to 70% in 2 years due to the shunt dysfunction and stent occlusion [19]. Even though the membrane-covered stent, a better alterative to the bare stent, has shown better clinical outcome in fields of decreasing TIPS dysfunction and keeps higher patency rate, the hepatic encephalopathy and rebleeding rates following TIPS are not decreased [20]. Thirdly, hypersplenism-related thrombocytopenia or pancytopenia cannot be corrected by TIPS [21, 22]. The latter increases the risk of bleeding from varices and gastropathy, and prevents the patients with hepatitis B or C, the main reason of cirrhosis in China, from receiving further treatment due to the worsening white cell and platelet count.

Surgical operations, including devascularization and shunt, are often the final choice to control variceal hemorrhage. Pericardial devascularization (PCDV), a simple but effective homeostatic method, is widely used in China. The procedure results in a high rebleeding rate, but encephalopathy rate is relative low [23-25]. In contrast to devascularization, portosystemic shunts, which have been favored in the Western and India, were found to have a higher encephalopathy rate and a lower rebleeding rate [26-28].

And now it still remains controversial whether the prophylactic surgery is necessary or not for the patient with high risk of hemorrhage but without a history of acute bleeding. Supporters think that the prophylactic intervention could decrease expected bleeding hazards and improve survival [29]. However, some scholars maintains that less than 50% patients with cirrhosis could suffer from hemorrhage, and thus it is unsuitable for each patient with esophagogastric varices to receive surgical operation [30]. We have previously reported a new portal blood flow-preserving procedure, i.e, small diameter splenocaval shunt plus devascularization, performed in our wards in recent years for the treatment of variceal hemorrhage from cirrhosis. The postoperative mortality, rebleeding and encephalopathy rate were 3.3%, 5.1% and 6.67%, respectively, indicating it is a good surgical choice for long-term control of variceal rebleeding [31].

In this study, we firstly evaluated the necessity of prophylactic splenocaval shunt in preventing the first episodes of hemorrhage in patients with high risk of hemorrhage by comparing bleeding rate between surgical and conservative patients. Secondly, we assessed the efficacy, safety, and outcome between different surgical approaches, i.e, splenocaval shunt plus devascularization and devascularization procedure alone.

Materials and methods

From January 2003 to December 2012, 145 patients with a high risk of esophagogastric variceal bleeding but without a history of hemorrhage were divided into three groups. 65 and 50 patients underwent small diameter proximal splenocaval shunt plus PCDV (Combined Group) and pericardial devascularization alone respectively (PCDV Group). The other 30 cases were not willing to receive surgical or endoscopic therapy, but received conservative therapy, such as propranolol, proton pump inhibitor, or mucosal protective agent (Control Group).

The diagnosis of portal hypertension and classification of liver function was based on clinical features, biochemical tests, endoscopic findings, ultrasonic findings, and radiologic findings, etc. Prior to the surgery, gastroscopy needed to be performed to confirm the presence or absence of esophageal or gastric varices and the congestive gastropathy. Red signs were defined as localized redish mucosal areas or spots on the mucosa above a varix. All patients were grouped according to Child-pugh classification and the varices were graded according to Conn's classification [32]. All operation is performed by a fixed surgical team. Because no endoscopic therapy was given in our wards, we had no endoscopic therapy patients as control. This study was approved by the ethics committee of Shaanxi provincial people's hospital and all patients gave their informed consent before their inclusion in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The characteristics of patients in three groups were given in **Table 1**.

Table 1. Patients' demographic, clinical, and preoperative investigative data

	Combined Group (*n* = 65)	PCDV Group (*n* = 50)	Control Group (*n* = 30)
Age (year)	32-60	31-58	28-54
Gender			
Male	38	31	18
Female	27	19	12
Etiology			
HBV-related	60	44	28
HCV-related	4	3	2
Alcohol related	1	3	-
Child pugh classification			
Grade A	28	20	13
Grade B	37	30	17
Grade of varices			
Grade II	3	2	4
Grade III-IV	62	48	26
Reasons for operation			
II varices + red signs	3	2	-
III-IV varices	62	48	-
PHG	25	19	-
Severe hypersplenism	60	48	-
Procedure information			-
Operating time (h)	4.0±1.5	3.1±1.2	-
Postoperative hospital stay (d)	12.8±1.9	13.5±2.0	-

HBV: viral hepatitis B; HCV: viral hepatitis C; PHG: portal hypertension gastropathy.

Table 2. Postoperative complications related to procedure

Complications	Combined Group (*n* = 65)	PCDV Group (*n* = 50)	Total
Pneumonia	3 (4.6%)	2 (4.0%)	5 (4.3%)
Abdominal abscess	1 (1.5%)	1 (2.0%)	2 (1.7%)
Stomach leakage	-	1 (2.0%)	1 (0.8%)
Pleural effusion	5 (7.7%)	6 (12.0%)	11 (9.6%)
Wound liquefaction	2 (3.0%)	-	2 (1.7%)
Total	11 (16.9%)	10 (20.0%)	21 (18.3%)

Indications for the combined surgery were as follows: (1) Severe varices (III-IV) of esophagus and/or gastric funds, including large tumor and rapidly enlarging varices. (2) Medium (II) varices with red signs [33]. (3) Severe portal hypertensive gastropathy (PHG), i.e. mosaic-like mucosal pattern with red, point lesions and/or cherry-red spot or black-brown signs. (4) Hypersplenism accompanied with the blood platelet count less than 30×10^9/L. (5) Patients with Child A or Child B classification. (6) Patients refused to receive other therapies. The exclusive criteria were as follows: (1) Age > 60 years. (2) Patients with Child C status. (3) General condition was poor to receive operation. (4) Patients complicated with primary liver cancer. (5) Portal hypertension secondary to malignant disease. (6) Thrombosis in portal system.

The criteria for PCDV were asfollows:(1)Nosuitablespleen vein for shunt, for example, the diameter of the spleen vein near splenic hilum was less than 6 mm; (2) Thrombosis in portal vein system; (3) Free portal pressure (FPP) less than 30 cmH_2O after splenectomy; (4) Regional portal hypertension. The surgical procedures were seen in our previous report [31].

After surgical operation, the platelet count was monitored and low molecule heparin or warfarin was routinely administrated to prevent thrombosis in splenocaval shunt stoma or in portal vein system. If the platelet was more than 300×10^9/L, or the thrombosis were seen in the portal vein or stoma, the anti-coagulation therapy was used continuously until the platelet count decreased less than 300×10^9/L or the thrombosis disappeared.

Statistical analysis

Statistical analysis was carried out with SPSS version 18.0. Analysis was done in aspect of the postoperative mortality (defined as death within 30 days after operation), the rate of complications, incidence of residual and recurrent varices, the bleeding (defined as hematemesis and/or melena with homodynamic instability or

Table 3. Changes of FPP in surgical groups (cmH_2O, $\bar{x}\pm s$)

Groups	Abdomen opening	Splenic artery ligation	Splenoectomy	PCDV	Shunt
Combined group (n = 28)	36.5±2.3	33.0±1.8	31.0±2.4	34.1±1.8	31.8±1.0[b,c]
PCDV group (n = 20)	37.0±2.5	32.5±2.0	29.8±1.9	33.1±1.9[a]	——
t	0.7162	0.9059	1.8570	1.7977	
P value	0.4775	0.3697	0.0697	0.0788	

Note: Compared preoperative: [a]($P < 0.05$), [b]($P < 0.01$); Compared to PCDV group: [c]($P < 0.05$).

Table 4. Long-term results of varices and bleeding

Results	Combined Group (n = 49)	PCDV Group (n = 39)	Control Group (n = 30)
Varices			
Residual	3 (6.1%)	6 (15.4%)	-
Recurrent	1 (2.0%)	4 (10.2%)	-
Aggrravated	-		21 (70.0%)
Gastropathy	3 (6.1%)	10 (25.6%)	-
Rebleeding reasons			
Varices	1 (2.0%)	6 (15.4%)	11 (36.7%)
Gastropathy	1 (2.0%)	2 (5.1%)	5 (16.7%)
Gastritis	1 (2.0%)	-	-

a drop in the hemoglobin level by > 20 g/L in 24 hours), the encephalopathy rate, late mortality, and survival. Survival was calculated using the Kaplan-Meier method. Differences between groups were assessed by Student's t test (ordinal variables) or the Chi-square (categorical variables) test. Risk factors in univariate analysis were entered into logistic regression analysis to identify its role in the onset of gastrointestinal bleeding. $P < 0.05$ was regarded as statistically significant.

Results

Postoperative mortality and procedure-related complications

All patients had been operated electively and successfully. No postoperative mortality occurred and the procedure-related complications were seen in 21 (18.3%) cases. Pleural effusion, chest infection and abdominal abscess were the three highest complications and seen in 11 (9.9%), 5 (4.3%) and 2 (1.7%) respectively. Stomach leakage and wound liquefaction occurred in 1 patient of PCDV group and 2 patients of combined group respectively. All patients with complications recovered smoothly by the conservative managements. The complication rate was not significantly different between combined and PCDV groups (16.9% *vs*. 20.3%, $P = 0.70$) (**Table 2**).

FPP changes in surgical groups

FPP in combined and PCDV groups were (31.8±1.8) cmH_2O and (33.1±1.9) cmH_2O postoperatively, which were significantly lower than those before operation with (36.5±2.3) cmH_2O ($P < 0.05$) and (37.0±2.5) cmH_2O ($P < 0.05$). Compared to PCDV group, the FPP in combined group decreased more significantly ($P < 0.05$) (**Table 3**).

Long-term results of varices

Residual varices were defined as the decrease of varices less than 50% volume compared with the preoperative. Recurrence was defined as the reappearance of varices after the complete eradication of varices. Aggravation referred to the progress of varices, or new development of red signs. In 88 (76.5%) cases who underwent operation and followed-up, 3 (6.1%) had residual varices in combined group and 6 (15.4%) in PCDV group ($\chi^2 = 1.1457$, $P = 0.28$). At endoscopic follow-up, 5 cases (5.7%, 1 in combined group and 4 in PCDV group) of recurrent esophagus and gastric varices ($\chi^2 = 1.4169$, $P = 0.23$) were found. In combined group, 3 patients (6.1%) acquired congestive gastropathy, which was significantly lower than that in PCDV group (25.6%) ($\chi^2 = 6.5711$, $P = 0.01$). In control group, varices were found becoming larger gradually in 21 patients (70.0%), among which 3 patients had varices grade II at the first endoscopic examination, showed in **Table 4**.

Bleeding

In combined group, 3 patients (6.0%) happened postoperative hemorrhage, in which 1 was from gastric varices (2.0%), 1 from congestive gastropathy (2.0%) and 1 was drug-related. The

Table 5. Long-term results of liver disease-related complications in operative patients

Results	Combined Group (*n* = 49)	PCDV Group (*n* = 39)	Control Group (*n* = 14)*
Ascites	2 (4.0%))	10 (25.6%)	4 (28.0%)
Thromboses in portal vein	5 (10.2%)	13 (33.3%)	2 (14.1%)
Splenocaval stoma obstruction	1 (2.0%)	-	-
Encephalopathy	4 (7.8%)	3 (7.7%)	3 (21.4%)

Note: *Exclusive 16 patients who received endoscopic or surgical therapy.

Table 6. Reasons of deaths in follow-up

Reason	Combined Group (*n* = 49)	PCDV Group (*n* = 39)	Control Group (*n* = 14)
Rebleeding	1 (1.3%)	4 (5.0%)	1 (7.14%)
Hepatic failure	5 (6.3%)	3 (13.3%)	1 (7.14%)
Hepatic primary cancer	4 (6.3%)	3 (8.3%)	2 (14.2%)

last one was a forty-year old female, lived in a remote area and she took excessive non-steroidal anti-inflammatory drugs for treatment of Influenza which led to uncontrolled hemorrhage and passed away three month postoperatively. We speculated that the reason for her death was the rupture of the esophagogastric varices because she had severe varices and portal hypertensive gastropathy before operation. In PCDV group, 8 patients (20.5%) had postoperative bleeding from varices in 6 (15.4%), congestive gastropathy in 2 (5.1%). The 7 patients with variceal bleeding (1 in combined group and 6 in PCDV group) were controlled successfully by endoscopic sclerotherapy. The other patients suffered from gastropathy (1 in combined group and 2 in PCDV group) were managed with propranolol and proton pump inhibitor. For the 30 cases in control group, hemorrhage occurred in 16 patients (11 from varices alone and 5 from varices plus PH, 53.3%) one to three times before they received therapeutic management. Among them, 6 received endoscopic sclerotherapy, 9 had surgery (6 for combined procedures and 3 for devascularization) for the first intervention in emergency or election. And one patient died of uncontrolled hemorrhage 2.5 years after he was diagnosed. The overall bleeding rate in control group was significantly higher than those received prophylactic surgery (53.3% vs. 12.5%, $P < 0.01$) and the rate in combined group was lower than that of PCDV group (6.0% *vs.* 20.5%, $\chi^2 = 2.9011$, $P = 0.042$), shown in **Table 5**.

Long-term results of liver disease-related complications

The incidence of hepatic encephalopathy were 7.8% (4/49) in combined group, 7.7% (3/39) in PCDV group, and 21.4% (3/14) in control group respectively ($\chi^2 = 0.0995$, $P = 0.75$). In patient undergone surgery, the symptoms were mild to moderate postoperatively and patients recovered with dietary and lactulose therapy and no case showed severe symptom or dropped into coma. However, symptoms in control group were often severe, repeated and needed to receive more effective therapy. There were 2 (4.0%) and 10 (25.6%) patients in combined and PCDV group respectively developed transient but aggravated amount of ascites postoperatively ($\chi^2 = 8.5708$, $P < 0.01$). One patient who had no ascites by ultrasound scan in combined group preoperatively developed uncontrolled ascites and hypoalbuminaemia after surgery. The symptoms lasted for 50 days before the disappearance of ascites. In control group, 4 patients (28.0%) with conservative treatment experienced gradually worsening ascites for the damaged liver function, which was higher than those in combined group ($P < 0.05$). Thromboses in portal vein were shown in 18 (20.5%) patients (5 in combined group and 13 in PCDV group, $\chi^2 = 7.1400$, $P = 0.01$), which was comparable to the control group with 14.2% (2/14) ($P > 0.05$). In combined group, 1 (2.0%) patients had splenocaval stoma thrombosis by Doppler ultrasound (**Table 5**).

Survival

Of the 49 patients in the combined group, 10 died during the follow-up, one of them died of bleeding, 5 of hepatic failure and 4 of hepatic primary cancer. In PCDV group, 10 patients died during the follow-up, among which 4 died of bleeding, 3 of hepatic failure and 3 of hepatic primary cancer. In control group, 4 died during the follow-up, among which 1 died of uncontrolled hemorrhage, 1 of hepatic failure and 2 of hepatic primary cancer (**Table 6**). The 1-, 3-,

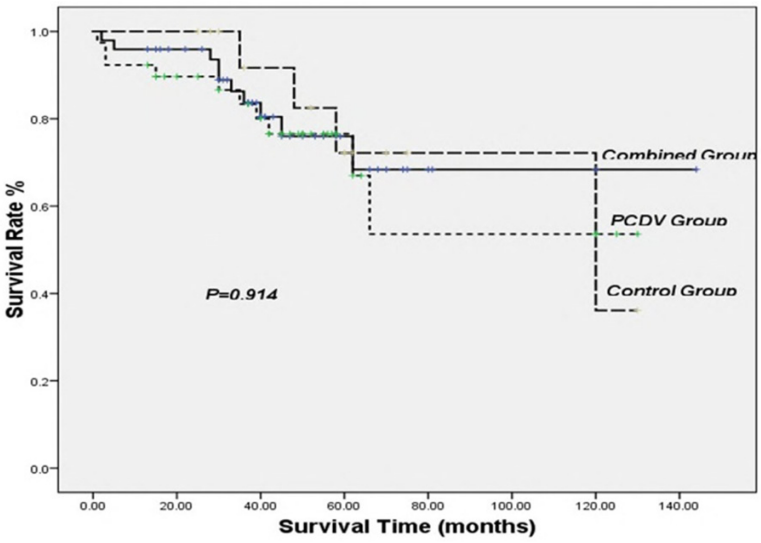

Figure 1. Survival curves, calculated by the Kaplan-Meier methods, for patients who received combined pericardial devascularization (PCDV) plus proximal splenocaval shunt, PCDV procedure alone or no treatment (numbers on curves represent surviving patient).

Table 7. Independent prognostic factors to the hemorrhage

	β	SE	*P*	Exp (B)	Odds ratio (95.0% CI)	
Surgical approach	-2.172	.896	.015	.114	.020	.661
Varices with or without red sign	2.031	.814	.013	7.618	1.546	37.529
Constant	-.617	.489	.207	.539		

5- and 10-year survival rate were 95.6% (47/49), 86.5% (32/37), 83.3% (10/12), and 66.7% (2/3) in combined group, 92.3% (36/39), 89.6% (26/29), 80.0% (8/10), and 66.1% (4/6) in PCDV group and 100.0% (14/4), 90.0% (9/10), 66.7% (4/6) and 50.0% (1/2) in control group, respectively ($P > 0.05$) (**Figure 1**).

Logistic regression analysis

In univariate analysis, etiology, Child-pugh classification, varices with or without red sign and surgical approach were prognostic factors to the onset of upper gastrointestinal bleeding. Meanwhile, varices with or without red sign (OR = 7.618; 95% CI: 1.546-37.529; $P < 0.005$) and surgical approach (OR = 0.114; 95% CI: 0.026-0.661, $P < 0.005$) were independent prognostic factors in logistic regression analysis (**Table 7**).

Discussion

By experiences in surgical treatment of portal hypertension, we thought that four principles must be addressed simultaneously for a successful surgical procedure, i.e, control or prevent variceal bleeding effectively, improve microcirculation and congestive status of the gastric mucosa, maintain hepatopetal perfusion, and reduce ascitic formation and volume. The small diameter splenocaval shunt plus devascularization was designed according to the principles mentioned above.

The main goal of the surgery for portal hypertension is to control and prevent bleeding from gastroesophageal varices. Our previous report showed that for patients with a history of variceal bleeding, the combined procedure was superior to devascularization alone in decreasing the postoperative rebleeding rate [31]. In present study, the procedure was used in the patients with high-risk but without a history of bleeding and there were no postoperatively mortality, indicating that the procedure was safe.

The controversial of whether prophylaxis is necessary for patient with varices has not been resolved. Primignani *et al.* reported that varices develops at a rate of 5% per year and 1/3 will be bleeding [8]. For patients with a hemorrhagic history but no treatment, the rebleeding rate was 60% and related death rate was about 35%-50% [18, 34]. However, for patients with high risk of bleeding, such as large varices, severe PHG, red sign, high spleen diameter/platelet count ratio, the bleeding rate may reach up to 45%-52% and even more [35, 36]. Inokuchi *et al.* reported in 1980s that prophylactic surgery decreased the expected bleeding rate about 80% and improve survival about 34% [29]. In present study, for such patients who received conservative therapy, the bleeding rate reached more than 50% and prophylactic surgery decreased the bleeding rate about 47% in combined group and 33% in devascularization group respectively. The above data dem-

onstrates that prophylactic intervention is necessary for patients with high risk of hemorrhage before the bleeding happened.

Without question, surgical procedure could decrease the rebleeding rate for patients who had history of hemorrhage. Whether prophylactic surgery could abate the expected bleeding rate or not? For patients who underwent prophylactic use of Surgira procedure, the postoperative bleeding rate was 1.5% in 10-year follow up in Japan [37]. In China, the bleeding rate after pericardial devascularization was 3.8%-21.09% [23-25]. In western countries, shunts are preferred for surgeons. The reported rebleeding rate was 10%-21% for Linton operation [38, 39], 5.5%-12% for Warren [40, 41], 5.0%-7.4% for Inokuchi shunt [42, 43]. In our study, the postoperative esophagogastric bleeding rate for patient undergoing combined procedures was 6.0%, which was lower than that in PCDV group. Furthermore, splenocaval shunt plus devascularization was a highly effective method to prevent the hemorrhagic risk from gastroesophageal variceal, which was comparable to the data mentioned above.

Though devascularization operation is widely accepted in China, the disadvantages are obvious. Firstly, the recurrence of varices increases gradually to adapt for the elevated free portal pressure in the prolonged follow-up. Secondly, devascularization might exacerbate congestive status and pathologic changes in the gastric mucosa [44, 45]. Incidence of gastropathy could go up to 90% in cirrhotic patients, which is correlated closely with the severity of varices [10]. In the present study, incidence of gastropathy was 38.5% preoperatively and the rate was still as high as 25.6% after devascularization. Both high incidence of residual or recurrent varices and exacerbated pathologic changes of gastric mucosa easily resulted to a high rebreeding rate [46, 47]. Finally, the development of the portal venous thrombosis would be inevitable after pericardial devascularization. Both past experiences and present studies demonstrated that a reasonable surgical operation can not only improve the microcirculation of the gastric wall, but also relieve the congestive status of the gastric mucosa, which is specially beneficial for patient with severe hypertensive gastropathy. This is why the combined procedure is the first surgical choice for patient with severe gastropathy in our department in recent years.

Postoperative encephalopathy is a severe complication in cirrhosis due to major loss of portal blood flow and damage directly to brain by endotoxin from bypassing of portal blood into systemic circulation. In this study, no patients had encephalopathy symptom preoperatively, the postoperative encephalopathy rate between two surgical groups had no significant differences. Hepatic encephalopathy rate did not increase in combined group maybe resulted from the small caliber splenocaval anastomosis which restricts the sharp loss of hepatopetal blood flow.

Ascites is a common complication in cirrhosis and should be controlled effectively. In present study, we have also observed a phenomenon that ascites disappeared more quickly and could be controlled more easily in the patients undergoing combined procedure than devascularization alone [31]. Ascites relieved more easily in combined group may because FPP is a little lower than that in devascularization group which reduce ascetic development postoperatively. Furthermore, the patency of splenocaval stoma may relieve the postoperative continuous increment of FPP. Hence we believed the combined procedure can not only keep the hydrostatical pressure of portal venous system no less than the upper limit of normal FPP, but also obtain the better outcome of controlling ascitic formation postoperatively.

In summary, we concluded that the prophylactic surgery can be recommended for the patients with high risk of esophagogastric bleeding to prevent the first episodes of hemorrhage in China. Splenocaval shunt plus devascularization is safe, highly effective and durable in preventing variceal bleeding, especially for the patients in rural areas where the endoscopy and TIPS facilities are not easily available. However, there were some limits in the study. Firstly, no endoscopic therapy patients were included as control group. Secondly, this was a retrospective study and the sample size was small. This needs further study.

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Disclosure of conflict of interest

None.

Authors' contribution

WWJ and LXD designed the research; WWJ, LXD, JQA, ZW and ZY performed the research; WWJ wrote the manuscript; ZL, JQA, ZW, CHL and DY collected data and contributed to analysis and interpretation of data; all authors revised the manuscript critically and approved its final version.

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