



Ultrasonographic hemodynamics for prediction of poor liver regeneration induced by severe portal vein stenosis in rats

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Background: Insufficient portal vein blood flow, such as portal vein stenosis (PVS), plays a significant influence on liver regeneration. Early prediction of poor liver regeneration induced by severe PVS is critical. Ultrasound serves as a first-line imaging technique in diagnosing PVS based on the changes of portal vein hemodynamics. However, there is still no consensus on the criteria for evaluating the degree of PVS. Moreover, which degree of PVS can induce poor liver regeneration still is unclear. Therefore, it is essential to determine the stenosis degree that leads to significantly poor liver regeneration and to evaluate the value of ultrasonographic hemodynamics for predicting poor liver regeneration induced by severe PVS.

Methods: Rats were randomly subjected to sham operation rats group (SOR), PH group (group A), and PVS groups with mild, moderate, or severe stenosis flowing PH (groups B-D). PH group was set up a model of 70% hepatectomy, and PVS groups were produced by different degrees of partial portal vein ligation following PH. In the SOR group and PH group, the portal vein diameter (PVD) and portal vein velocity (PVV) were measured by Ultrasound at preoperative and postoperative 1, 3, 7, and 14 d. In PVS groups, PVD and PVV at the stenotic (PVD_s, PVV_s) and pre-stenotic (PVD_{pre}, PVV_{pre}) sites were also detected on 1, 3, 7, and 14 d after surgery, calculating the diameter stenosis ratio (DSR) and accelerating blood flow velocity ratio (AVR). Rats were sacrificed at 1, 3, 7, and 14 d post-surgery, and the expression of proliferating cell nuclear antigen (PCNA) and the liver regeneration rate (LRR) at 14 d were evaluated. The PVV_s, DSR, and AVR in the different groups were analyzed combined with the status of liver regeneration, and receiver operating characteristic (ROC) analysis was also applied to assess the value of PVV_s, DSR, and AVR in diagnosing severe PVS and the resulting poor liver regeneration.

Results: Seventy-two rat models of different degrees of PVS were successfully set up following 70% PH. The stenosis ratios (SRs) of each PVS group were 45.16%±3.44%, 59.21%±3.83%, and 69.56%±2.16%, respectively. Poor liver regeneration appeared to be significant when PVS was greater than 65% (group D), of which the LRR at 14 d was significantly lower compared to PH group (group A) and PVS groups with SR ≤50% (group B) and SR >50–65% (group C), respectively (all P<0.05). Meanwhile, PCNA expression of group D was significantly lower compared to group C at 1 d and groups A-C at 3 d (all P<0.05). Differences were also detected at 3 d between groups A and B and groups A and C (both P<0.05). Among PVS groups, PVV_s accelerated dramatically, with significant differences demonstrated between group D and groups B and C at 1 d, as well as group B and groups C and D at 3 d (all P<0.05). At 1, 3, and 7 d, DSR of groups C and D were significantly higher than that of group A (all P<0.05). At 1 and 3 d, AVR of group D was significantly higher than that of groups B and C (all P<0.05). ROC analysis showed the AUC of PVV_s at 1 d in diagnosing severe PVS was 0.84, while at 3 d, it was unable to differentiate from mild-moderate or severe PVS by PVV_s (P>0.05 vs. AUC =0.50). At 1 and 3 d, the AUC of DSR and AVR in diagnosing severe PVS were all greater than 0.80, comparatively much better in AVR (AUC >0.95). The best cut-off points of AVR at 1 and 3 d were 6.91 and 5.36, with the sensitivity and specificity respectively 100%, 91.67% at 1 d, and 100%, 83.33% at 3 d.

Conclusions: Poor liver regeneration could be significantly induced when PVS was greater than 65%. Ultrasound can well prove the changes of portal vein hemodynamics in different degrees of PVS in rats. The parameters PVV_s could be regarded as a valid index for diagnosing PVS but were not applicable for evaluating the stenosis degree. Comparatively, the parameters DSR and AVR, especially AVR, proved to be useful for differentiating severe PVS (>65%) in the early postoperative period, predicting the resulting poor liver regeneration.

Keywords: Liver regeneration; portal vein stenosis (PVS); ultrasound; hemodynamics; portal vein diameter (PVD); portal vein blood flow velocity (PVV)

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Introduction

Because of the severe shortage of donor's livers, living donor liver transplantation (LDLT) has undergone rapid development with the advancement in modern surgical techniques (1-3). Liver regeneration post-LDLT is a vital process of liver recovery, and the portal vein blood flow, accounting for 70–80% of the hepatic blood supply, provides a fundamental basis and a prerequisite for successful liver regeneration and survival. Insufficient portal vein blood flow, such as portal vein stenosis (PVS), is a significant complication after LDLT, especially in children. Mild PVS is conventional but does not affect liver function and regeneration. In patients with severe PVS, poor regeneration may occur, complicated with portal hypertension, small-for-size syndrome, or even liver failure. Early diagnosis and prompt treatment are critical (4-8).

As a non-invasive, cost-effective, and non-radioactive technique with bedside availability, ultrasound (US) serves as a first-line imaging modality to diagnose PVS in the early postoperative period and long-term follow-up. In general, PVS is suggested if the portal vein is regional stenosis, with blood flow aliasing and acceleration at the stenotic site. Most of the researchers believe that evident PVS can be diagnosed if the stenotic diameter is <2.5–3.5 mm or the diameter stenosis ratio (DSR) >50%, blood flow velocity (PVV) at the stenotic site (PVV_s) >150 cm/s or the accelerating velocity ratio (AVR) ≥ 3 (9-11). However, to date, unlike the criteria for grading carotid artery stenosis (12), there is still no consensus on the ultrasound diagnostic criteria for evaluating the degree of PVS. Moreover, which degree of PVS could induce poor liver regeneration still is unclear. In the clinic, the treatments are usually

provided when evident PVS was detected, accompanied by apparent symptoms such as abnormal transaminase level, blood coagulation disorders, portal hypertension, and so on. But in that case, irreversible damages of hepatocytes may already occur, and consequently, the grafts would be in the state of poor regeneration for a long-time even treatments are applied. Liver regeneration can be assessed by pathological and immunohistochemical tests such as the expression of proliferating cell nuclear antigen (PCNA), a kind of nucleoprotein only synthesized and expressed in proliferative cells, but these examinations are invasive and more costly (13,14). Therefore, there is a growing demand for the correct assessment of the degree of PVS, early predicting the possible poor liver regeneration induced by severe PVS noninvasively.

Due to the diverse causes of hepatic diseases and the limitation in ethics, most research studies liver regeneration based on experimental animals. A rat model of 70% partial hepatectomy (PH) is a widely accepted liver regeneration research model, which is famous for its regeneration induction without causing fulminant hepatic failure (15). Partial ligation of the portal vein is commonly used for producing the PVS model (16). Therefore, this study used a combination of 70% PH and portal vein partial ligation to set up (PH + PVS) rat models. Based on the assessment of liver regeneration in the different degrees of PVS, the degree of PVS inducing poor liver regeneration was determined. Moreover, a comparative analysis was performed to evaluate the value of Ultrasound parameters PVV_s , DSR, and AVR for predicting poor liver regeneration induced by severe PVS. These findings will supply experimental evidence for further investigating PVS diagnosis and grading in the clinic.

Methods

Study subjects

One hundred and two healthy male Sprague-Dawley rats (200–400 g, 7–14 weeks of age, SPF grade) were purchased from Chengdu Dashuo Biotechnology Co., Ltd., and acclimatized for at least 7 days to laboratory conditions with a constant temperature and a 12 h light-dark cycle at the animal experiment center of West China Hospital.

The rats were randomly divided into five groups as follows: sham operation rats (SOR group, n=6), PH group (group A, n=24), and PVS groups with mild, moderate, and severe PVS after PH (group B, C, and D, n=24 respectively). Also, rats in A–D groups were further divided into four subgroups, based on the time after surgery (1, 3, 7, and 14 d), with 6 rats in each subgroup. The animal ethics committee approved all the rats and procedures of West China Hospital, Sichuan University. Rats were kept warm and had free access to food and water throughout the experiment.

Construction of rat models

Construction of 70% PH rat model

Rats were placed in the supine position after ether anesthesia, and then a 70% hepatectomy model was constructed based on methods described by Higgins *et al.* (15). Briefly, the middle lobe and left lateral lobe (about 68% of the total volume of the liver) were excised and weighed.

Construction of PVS model after PH

After PH, the portal vein was separated. A microvascular caliper was used to measure the portal vein diameter (PVD) at the site of pre-ligation, which was below the porta hepatis, and about 3 mm above the joint of the splenic vein and superior mesenteric vein. Based on pre-defined stenosis degrees, the diameter of the stenotic segment was calculated from the formula $SR = (1 - D_{stenosis}/PVD) \times 100\%$, and then the corresponding gauge of a medical needle was selected. The separated portal vein and the needle were ligated together by 3-0 silk suture, and afterward, the needle was removed slowly. The diameter of the stenotic segment of the portal vein was equaled to the outer diameter of the needle. Different degrees of PVS was induced using this method. In the end, 32,000 units penicillin and 5 mL NaCl (0.9%) was injected intraperitoneally in each rat before closing the abdominal cavity.

For the SOR group, neither hepatectomy nor portal vein

ligation was performed during surgery. For the PH group (group A), only 70% hepatectomy was conducted. For the PVS groups (groups B–D), different degrees of PVS were conducted following PH.

Ultrasound examination

Ultrasound was used to check the portal vein hemodynamic changes by a Philips iu22 ultrasound machine with a 5.0–12.0 MHz linear transducer. The rats were ether-anesthetized and placed in supine positions. The abdomen was shaved with an electric hair remover to minimize ultrasound attenuation. Ultrasound examination was performed by the same experienced physician, with rats supporting stable anesthesia.

In the SOR group and PH group (group A), PVD and PVV were measured by grayscale and Doppler ultrasound at preoperative and postoperative 1, 3, 7, and 14 d. In PVS groups (groups B–D), the PVD and PVV at the stenotic (PVD_s, PVV_s) and pre-stenotic (PVD_{pre}, PVV_{pre}) sites were measured, and then the DSR and AVR were subsequently calculated as follows: $DSR = (PVD_{pre} - PVD_s)/PVD_{pre} \times 100\%$, and $AVR = (PVV_s - PVV_{pre})/PVV_{pre}$. When measuring the velocities, based on the vessel diameter and direction, the Doppler sample volume should be adjusted to 0.5 mm, and the angle of insolation should remain constant at less than 60°. PVV_s should be detected at the stenotic site where the blood flow disturbance could be seen. All observation data were measured three times and averaged as the resultant values.

Evaluation of liver regeneration

Assessment of the expression of PCNA

The rats in A–D groups were sacrificed at 1, 3, 7, and 14 d post-surgery, and the liver tissue specimens (2.0×1.0×1.0 cm³) were obtained. Then, the liver tissues were fixed in 10% neutral formalin, embedded in paraffin, made into slices at 5 μm. Immunohistochemical staining was performed to assess PCNA expression under high magnification based on the paraffin sections, and hepatocytes with a brown-yellow nucleus were considered positive for staining. Besides, about 100 mg of liver tissue was also harvested when rats were sacrificed. The adipose tissue and connective tissue on the surface were removed as much as possible, and then the liver tissue was placed in cryogenic vials and stored in liquid nitrogen. Western blotting was performed for semi-quantification of PCNA expression in liver tissues. For the

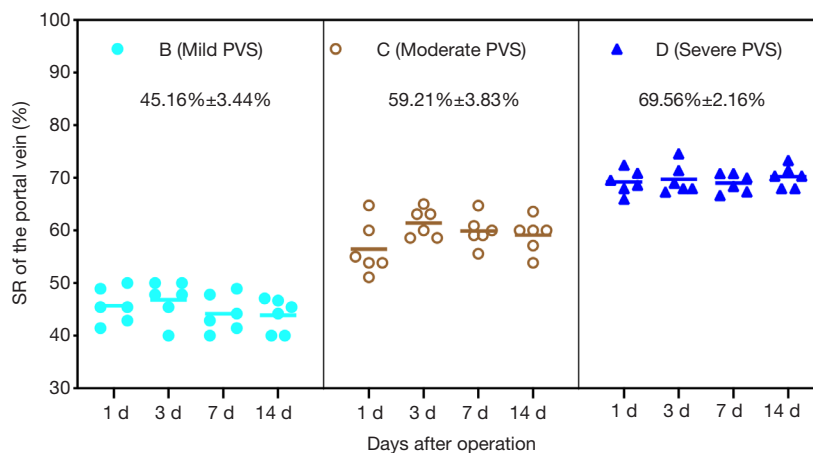


Figure 1 Box plot of the SR of the portal vein in the PVS groups. SR, stenosis ratio; PVS, portal vein stenosis.

SOR group, the rats were sacrificed at 14 d post-surgery, with its liver tissues obtained for PCNA examination.

Assessment of liver regeneration rate (LRR)

The residual liver was excised and weighed when rats sacrificed at 14 d post-surgery. LRR was calculated based on the equation: $LRR = D/E \times 100\%$, in which D represents the liver weight per 100 g of the body weight when sacrificed, and E represents the preoperative liver weight per 100 g of the body weight, which was calculated by (the excised liver weight/0.68) (17).

Statistical analysis

The analysis of variance (ANOVA) test was employed to study the differences in LRR, PCNA expression, and ultrasound parameters PVD, PVV, DSR, and AVR among groups. The least significant difference (LSD) test was used to compare the results pairs of groups with even variance, and the Dunnett-t3 test was used to test the data with uneven variance. Differences were considered significant if the P value <0.05. The receiver operating characteristic (ROC) curve was drawn, and the area under the curve (AUC), Std. Error, asymptotic Sig. (b), asymptotic 95% confidence interval, best cut-off, sensitivity, and specificity were calculated to evaluate the value of PVV, DSR, and AVR in diagnosing severe PVS, predicting the resulting poor liver regeneration. All statistical analyses were performed utilizing SPSS 25.0 and GraphPad Prism 8.

Results

Model construction

In this study, 72 rat models of different degrees of PVS were successfully set up following 70% PH. The SRs of each PVS group were $45.16\% \pm 3.44\%$, $59.21\% \pm 3.83\%$, and $69.56\% \pm 2.16\%$, respectively. In each PVS group, no statistical difference of SRs was observed at postoperative 1, 3, 7, 14 d (all $P > 0.05$, Figure 1).

LRR

The residual liver weight of the rats increased with time after PH. The LRR of 70% PH group and mild PVS group with $SR \leq 50\%$ on the 14th day both reached above 90%, which means the regenerated liver weight per 100 g bodyweight almost recovered to its original weight before surgery. Poor liver regeneration could be significantly induced when PVS was greater than 65% (group D), with the LRR of less than 80%. That was significantly lower compared to 70% PH group (group A) and PVS groups with $SR \leq 50\%$ (group B) and $SR > 50-65\%$ (group C), respectively (all $P < 0.05$, Figure 2).

The expression of PCNA

Immunohistochemical staining of PCNA demonstrated that only a few positive cells, with brown-stained nuclei, were seen in the SOR group. In the 70% PH group, the positive expression of cells increased at 1 d post-surgery, presenting

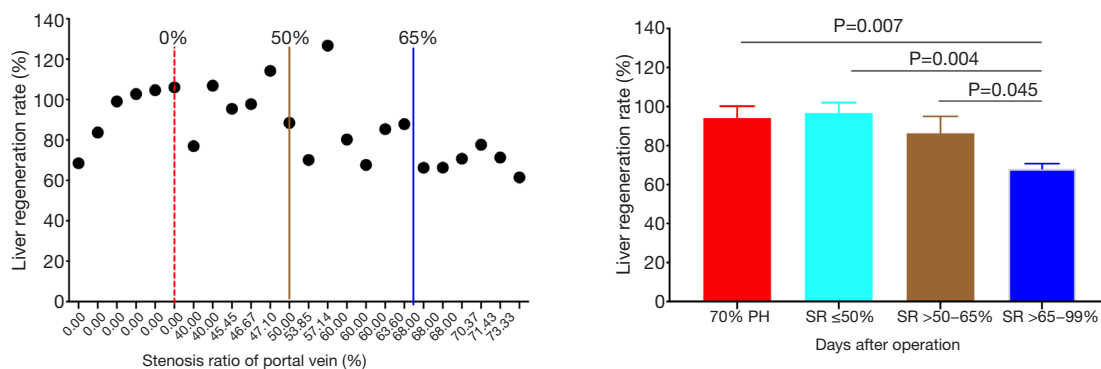


Figure 2 LRR in the distinct groups at 14 d post-surgery. LRR, liver regeneration rate.

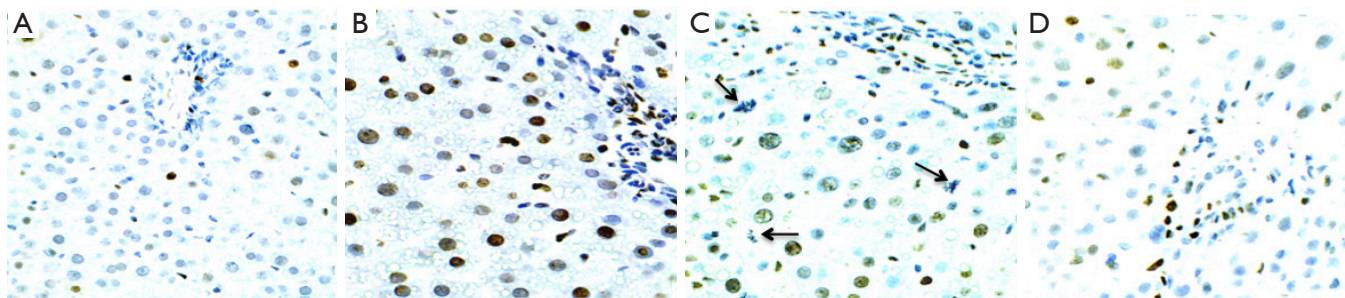


Figure 3 Immunohistochemical staining of PCNA in SOR group and PH group ($\times 400$). (A) SOR group; (B) PH group at 1 d; (C) PH group at 3 d; (D) PH group at 7 d, the negative reaction was observed in karyokinesis (arrow). PCNA, proliferating cell nuclear antigen; SOR, sham operation rats; PH, partial hepatectomy.

in the portal areas. At 3 d, the levels of PCNA-positive expression were still relatively high, with more expression in the central areas. At 7 d, the number of PCNA-positive cells decreased (*Figure 3*), while at 14 d, it was comparable to the levels in normal liver tissues. In PVS groups, the expression of PCNA showed similar trends with time. The negative reaction was observed in karyokinesis.

According to western blot analysis, PCNA expression appeared to be extremely low in the SOR group. In groups A-D, the expression had a similar trend with time. In brief, PCNA expression increased at 1 d after surgery, peaked at 3 d, and then gradually decreased at 7 and 14 d. At 3 d, the expression of PCNA in groups B and C were the highest, followed by group A, and then group D. More precisely, significant differences were detected between groups A and B and between groups A and C (both $P < 0.05$). The expression of group D was significantly lower compared to group C at 1 d and groups A, B, and C at 3 d (all $P < 0.05$). At 7 and 14 d, there were no statistical differences among groups (all $P > 0.05$, *Figures 4* and *5*).

Ultrasound examination

PVD and PVV in 70% PH group

In the 70% PH group, the residual liver and blood flow in the portal vein could be proved clearly by ultrasound. PVD started to increase at 1 d, peaked at 3 d, decreased gradually at 7 d, and recovered to the normal levels at 14 d. PVD at 3 d was significantly higher than 14 d PVD ($P < 0.05$). In contrast, PVV decreased to the lowest level at 3 d, increased gradually at 7 d, and then recovered similarly to normal levels at 14 d. PVV at 3 d was significantly lower than 1d and 14 d PVV (both $P < 0.05$). The results are shown in *Figure 6*.

Ultrasound PVV_s, DSR, and AVR in PVS groups

PVV_s: gray-scale ultrasound could prove the portal vein stenosis clearly, and Doppler ultrasound showed blood flow disturbance at the stenotic site with accelerated flow velocity (*Figure 7*). After surgery, PVV_s increased dramatically to the peak at 1 d, and then gradually decreased at 3 and 7 d. The

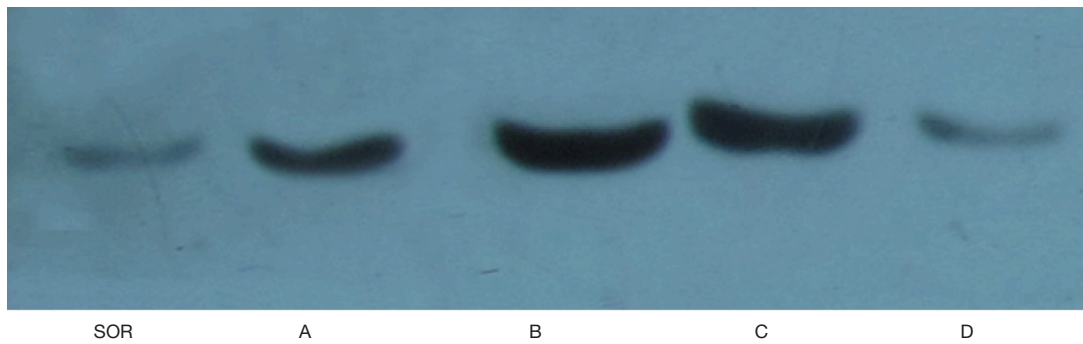


Figure 4 The expression of PCNA detected by western blot at 3 d post-PH in the distinct groups. PCNA expression in the SOR group was extremely low, and the expression of group D was significantly lower compared to groups A, B, and C. PCNA, proliferating cell nuclear antigen; PH, partial hepatectomy; SOR, sham operation rats.

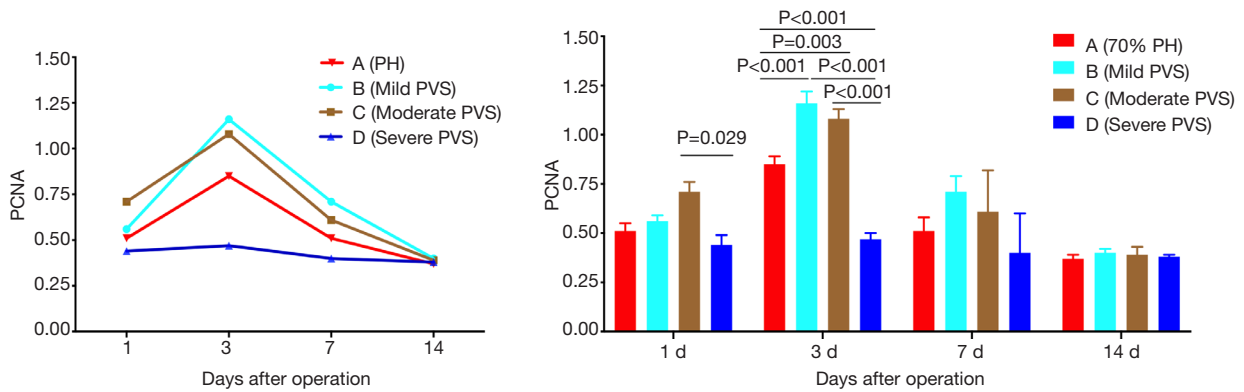


Figure 5 The expression of PCNA in the distinct groups. PVS, portal vein stenosis; PH, partial hepatectomy; PCNA, proliferating cell nuclear antigen.

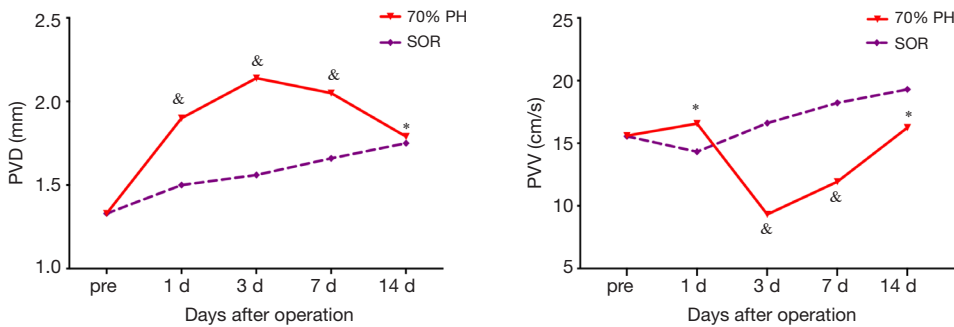


Figure 6 PVD and PVV in SOR group and PH group (&, $P < 0.05$ vs. SOR group; *, $P < 0.05$ vs. 3 d). PH, partial hepatectomy; PVD, portal vein diameter; SOR, sham operation rats; PVV, portal vein velocity.

PVV of 70% PH group was significantly lower compared to PVV_s of groups B–D at 1, 3, and 7 d and PVV_s of group D at 14 d (all $P < 0.05$). Among PVS groups, PVV_s of group D was significantly higher than that of groups B and C at 1 d,

and PVV_s of group C and D were significantly higher than that of group B at 3 d (all $P < 0.05$).

DSR: DSR in PVS groups were by the stenosis degree. Briefly, the DSR of group D was the highest, followed by

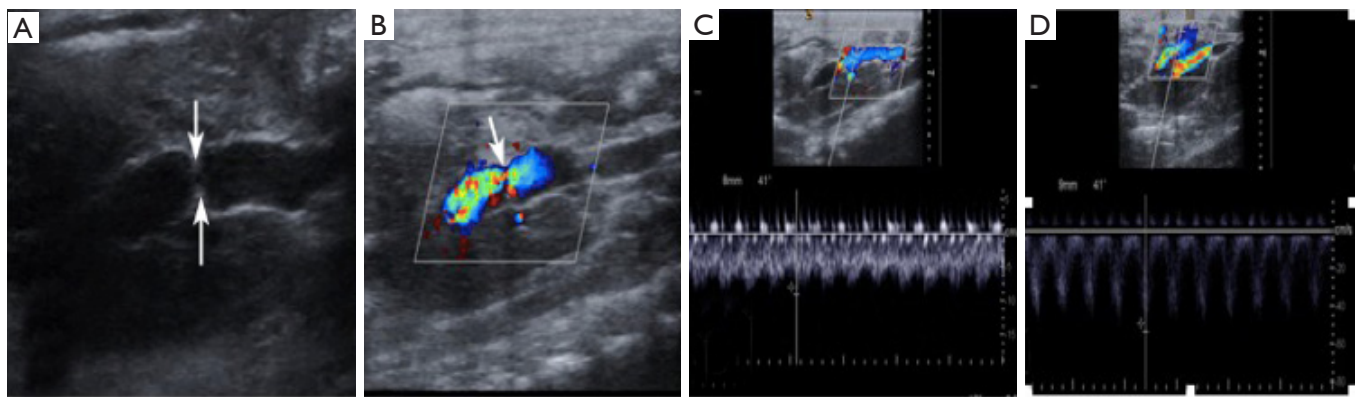


Figure 7 Ultrasound images in severe PVS rat. (A) Gray-scale ultrasound can clearly prove the stenotic site in the portal vein (arrow); (B) color Doppler ultrasound showed blood flow disturbance at the stenotic site (arrow); (C) spectral Doppler show decelerated flow velocity at the pre-stenotic site; (D) spectral Doppler showed accelerated flow velocity at the stenotic site. PVS, portal vein stenosis.

Table 1 Ultrasound parameters PVVs, DSR and AVR of the distinct groups at the same time point

US index	Group	1 d	3 d	7 d	14 d
PVV _s	A (70% PH)	16.57±2.36	9.95±1.52	11.60±1.65	14.85±1.62
	B (mild PVS)	53.92±9.44 ^a	37.17±6.31 ^a	26.12±1.36 ^a	33.93±5.65
	C (moderate PVS)	73.50±4.07 ^a	61.73±8.18 ^{a,b}	25.27±3.07 ^a	27.83±8.25
	D (severe PVS)	109.67±18.42 ^{a,b,c}	63.90±10.69 ^{a,b}	21.75±3.28 ^a	43.97±3.94 ^a
DSR (%)	B (mild PVS)	44.40±2.22	50.72±2.39	42.30±6.19	54.97±5.13
	C (moderate PVS)	61.16±4.73 ^b	64.39±3.03 ^b	61.57±4.84 ^b	62.07±5.34
	D (severe PVS)	67.54±1.75 ^b	69.16±1.84 ^b	65.74±3.73 ^b	66.50±1.43
AVR	B (mild PVS)	3.21±0.32	3.67±0.58	0.92±0.21	1.53±0.41
	C (moderate PVS)	5.54±1.00	4.32±0.77	2.10±0.73	2.24±1.07
	D (severe PVS)	16.58±3.23 ^{b,c}	8.65±1.62 ^{b,c}	1.31±0.45	3.81±0.74

^a, P<0.05 vs. group A; ^b, P<0.05 vs. group B; ^c, P<0.05 vs. group C. PVV_s, portal vein velocity at the stenotic site; DSR, diameter stenosis ratio; AVR, accelerating velocity ratio; PH, partial hepatectomy; PVS, portal vein stenosis.

group C, and then group B. At 1, 3, 7 d post-surgery, DSR of group C and D were significantly higher than that of group A (all P<0.05), while at 14 d, no significant difference was observed among PVS groups (all P>0.05).

AVR: at 1 d post-surgery, AVR reached the peak, and then gradually decreased to the lowest levels until 7–14 d. AVR of group D was significantly higher than that of groups B and C at 1 and 3 d after surgery (all P<0.05), while there were no statistical differences among PVS groups at 7 and 14 d (all P>0.05).

All the results of PVV_s, DSR, and AVR in the distinct groups are showed in *Table 1* and *Figure 8*.

ROC analysis

In this study, poor liver regeneration could be significantly induced by severe PVS (SR >65–99%). According to the comparative analysis of portal vein hemodynamic changes in different degrees of PVS, ultrasound parameters PVV_s, DSR, and AVR of severe PVS group were significantly different from mild or moderate PVS groups, especially at 1 and 3 d post-surgery. Therefore, the ROC analysis was applied to assess the value of PVV_s, DSR, and AVR in differentiating severe PVS from mild-moderate PVS at 1 and 3 d, with mild-moderate PVS, defined as negative

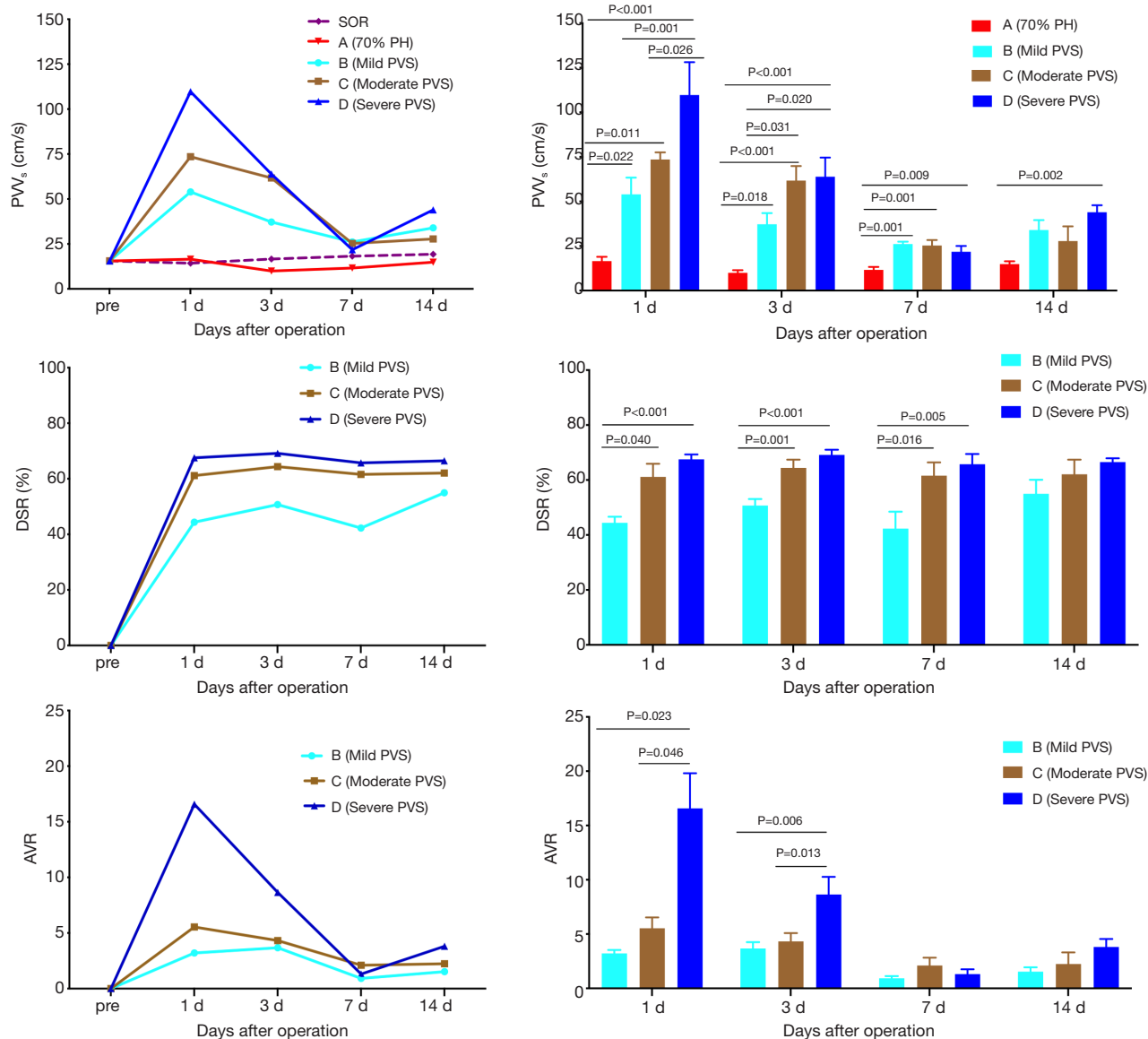


Figure 8 Ultrasound parameters PVV_s, DSR, and AVR in the distinct groups. SOR, sham operation rats; PH, partial hepatectomy; PVS, portal vein stenosis; PVV_s, portal vein velocity at the stenotic site; DSR, diameter stenosis ratio; AVR, accelerating velocity ratio.

results and severe PVS defined as a positive diagnosis.

As is shown in *Figure 9* and *Table 2*, at postoperative 1 d, the AUC of PVV_s in diagnosing severe PVS was 0.84 ($P < 0.05$ vs. AUC = 0.50), and the best cut-off points were 81.20 cm/s, with a specificity up to 91.67% and a sensitivity of 66.67%. While at 3 d, it was unable to differentiate from severe or mild-moderate PVS by PVV_s ($P > 0.05$ vs. AUC = 0.50). Comparatively, the AUC of DSR and AVR at 1 and 3 d in differentiating severe PVS from mild-moderate PVS were all greater than 0.80 ($P < 0.05$ vs. AUC = 0.50), much

better in AVR (AUC > 0.95). The best cut-off points of DSR at 1 and 3 d were 57.43% and 66.06%, with the sensitivity and specificity respectively 100%, 75% at 1 d, and 83.33%, 83.33% at 3 d. About AVR, the best cut-off points at 1 and 3 d were 6.91 and 5.36, with the sensitivity and specificity respectively 100%, 91.67% at 1 d, and 100%, 83.33% at 3 d.

Discussion

The 70% partial hepatectomy (PH) model developed

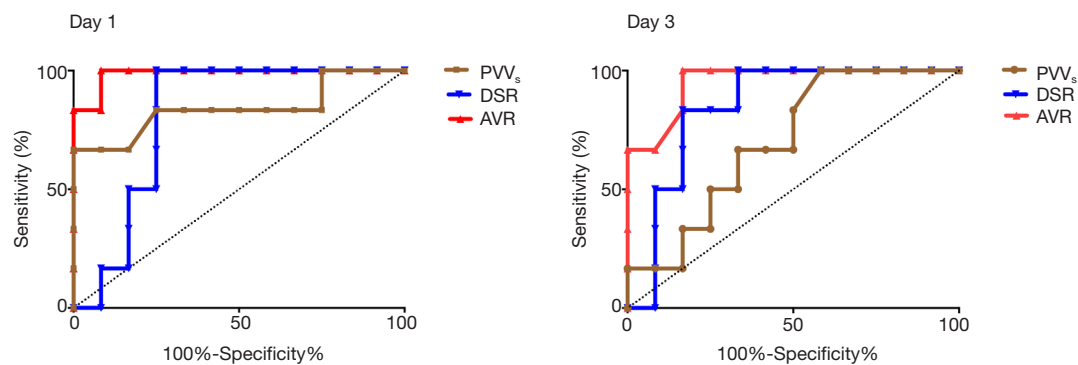


Figure 9 ROC curves of PVV_s, DSR, and AVR in differentiating severe PVS from mild-moderate PVS at 1 and 3 d post-surgery. ROC, receiver operating characteristic; PVV_s, portal vein velocity at the stenotic site; DSR, diameter stenosis ratio; AVR, accelerating velocity ratio; PVS, portal vein stenosis.

Table 2 Results of ROC analysis in differentiating mild-moderate and severe PVS

Day	US index	AUC	Std. error	Asymptotic Sig. (b)	95% confidence interval		Best cut-off	Sensitivity (%)	Specificity (%)
					Lower bound	Upper bound			
1 d	PVV _s	0.840	0.119	0.022	0.606	1.000	81.20 cm/s	66.67	91.67
	DSR	0.806	0.107	0.039	0.596	1.000	57.43%	100.00	75.00
	AVR	0.986	0.022	0.001	0.943	1.000	6.91	100.00	91.67
3 d	PVV _s	0.701	0.124	0.174	0.458	0.945	51.30 cm/s	66.67	66.67
	DSR	0.847	0.095	0.019	0.662	1.000	66.06%	83.33	83.33
	AVR	0.951	0.047	0.002	0.858	1.000	5.36	100.00	83.33

ROC, receiver operating characteristic; PVS, portal vein stenosis; PVV_s, portal vein velocity at the stenotic site; DSR, diameter stenosis ratio; AVR, accelerating velocity ratio.

by Higgins *et al.* is the classical model used for liver regeneration research (15). It is easy to construct and have high survival rates. Partial ligation of the portal vein is commonly used to induce portal vein stenosis (PVS) model (16). In this study, portal vein partial ligation was performed using different gauges of medical needles to induce rat models with different degrees of PVS following 70% PH. This (PH + PVS) model is easy to construct, stable, and ease of hemodynamic monitoring (18).

Liver regeneration occurs shortly after PH in rats, including regeneration of hepatocytes and reconstruction of hepatic tissue structures. Hepatocytes are in the G0 phase under normal physiological conditions. After PH, an orderly proliferation of residual liver cells can be triggered by injury-related factors. Hepatocytes start to enter the cell cycle firstly, with DNA synthesis peaking at 1 d, and afterward, cell division peaking at 3 d. After 1–2

cell cycles, the liver weight can return to a normal level at 7–14 d. The reconstruction of hepatic tissue structures depends on the proliferation of hepatic non-parenchymal cells, such as Kupffer cells and sinusoidal endothelial cells. In these cells, DNA synthesis generally peaks after 48 h post-PH. Proliferating cell nuclear antigen (PCNA) is a kind of nucleoprotein only synthesized and expressed in proliferative cells, and to aid in the assessment of the cell proliferation status in liver regeneration. In our study, the expression of PCNA after PH increased to the peak at 3 d, and the weight of regenerated liver recovered to near normal level at 14 d, following the regularity of liver regeneration (13,14,19–21).

In this study, PVD after PH started to increase at 1 d, peaked at 3 d, decreased gradually at 7–14 d, while PVV decelerated to the lowest at 3 d and recovered gradually at 7–14 d. The hemodynamic changes may be associated with

hepatocyte proliferation status and the changes of hepatic sinusoidal blood flow resistance in liver regeneration. After PH, the total blood volume to the whole liver was infused into the residual liver tissues. The reduction of sinusoidal capillary beds and the resulting blood flow resistance augmentation caused portal hypertension, with an increase in PVD and a decrease in PVV. At 3 d, blood flow resistance in the portal vein augmented further with peaks of hepatocytes mitosis and hepatic sinusoidal construction, resulting in PVD increasing to its highest and PVV decelerating to its lowest. At 7 and 14 d, with the completion of liver regeneration and restoration of liver volume, portal hypertension was alleviated, and so, the PVD and PVV gradually recovered to normal levels. Also, the changes in the blood flow volume of the portal vein during liver regeneration influenced PVD and PVV.

Enough blood flow in the portal vein is critical for liver regeneration. When severe PVS occurs, the blood supply to the liver can be reduced dramatically, leading to poor liver regeneration or even liver atrophy (4-8). However, which degree of PVS can induce poor liver regeneration still is unclear. In this study, poor liver regeneration was significantly induced when PVS was greater than 65%, with the LRR at postoperative 14 d respectively lower compared to 70% PH group and PVS groups with SR $\leq 50\%$ and SR $>50\text{--}65\%$, as well as the expression of PCNA at 3 d. The levels of liver regeneration were not significantly low when PVS $>50\text{--}65\%$, which may be because the reduction of portal vein blood flow had a negative correlation with liver regeneration in a particular range, with more active liver regeneration than non-PVS rats. Additionally, unlike the carotid artery, of which the mild, moderate and severe stenosis were respectively defined as $<50\%$ stenosis, $50\text{--}69\%$ stenosis, and $\geq 70\%$ stenosis to near occlusion (12), there is no explicit standard for grading PVS to date. In this study, it was found that the rats could hardly survive when PVS was greater than 75%. Based on the liver regeneration in different degrees of PVS, the severe PVS was defined as SR $>65\text{--}99\%$, which could induce significantly poor liver regeneration. Logically, the mild and moderate PVS were defined as SR $\leq 50\%$ and SR $>50\text{--}65\%$, respectively.

Imaging techniques play a vital role in the diagnosis of PVS. Angiography is the gold standard, but it is invasive and complex. Computed tomography (CT) causes radiation effects, and magnetic resonance imaging (MRI) is more costly (22,23). Ultrasound has been considered the primary noninvasive imaging modality to detect PVS, and the availability at the bedside and the absence of radiation

hazards make it an ideal first-line examination. PVS can be proved by Ultrasound, with regional stenosis of PVD and blood flow aliasing and acceleration at the stenotic site. The PVV at the stenotic site (PVV_s), the DSR, and the accelerating velocity ratio (AVR) are the most common measure to find PVS. Stenoses greater than 50% are considered hemodynamically significant. The more severe the stenosis is, the higher the PVV_s becomes (9-11). However, there is still no consensus on the ultrasound criteria for grading PVS, especially in diagnosing the severe PVS, which can induce significantly poor liver regeneration. In this study, the differences in PVV_s, DSR, and AVR of mild, moderate, and severe PVS varied by time. At 1 d post-surgery, PVV_s and AVR of severe PVS were significantly higher compared to mild and moderate PVS. At 3 d, PVV_s of moderate and severe PVS were both higher than that of mild PVS, and AVR of severe PVS still supported at a relatively higher level compared to mild and moderate PVS. At 7 and 14 d, there were no statistical differences among PVS groups. Similarly, DSR of moderate and severe PVS was higher than that of mild PVS at 1, 3, and 7 d, while at 14 d, no significant difference was observed among PVS groups. For these changes, besides the stenosis degree was a critical affected factor, the changes of blood flow resistance in different levels of liver regeneration may also influence the portal vein hemodynamics, as well as the blood flow volume. With the progress of liver regeneration, the differences between PVV_s, DSR, and AVR among PVS groups gradually diminished. Therefore, Ultrasound grading of PVS might better be performed in the early postoperative period, especially within three days, as showed in our experimental study. Also, PVV_s accelerated significantly higher than PVV of non-PVS rats, but the differences among PVS groups fluctuated dramatically with time.

Moreover, based on ROC analysis, it was unable to differentiate from mild-moderate or severe PVS by PVV_s at 3 d. Therefore, the PVV_s could be regarded as a useful index for diagnosing PVS but were not applicable for evaluating the stenosis degree. Comparatively, the DSR and AVR proved to be useful in diagnosing severe PVS (SR $>65\%$) and predicting the resulting poor liver regeneration, especially AVR (AUC >0.95). At 1 d, AVR >6.91 was proposed as the diagnostic criterion for severe PVS, the sensitivity and specificity were respectively up to 100% and 91.67%. Similarly, at 3 d, AVR >5.36 was regarded as the diagnostic criterion for severe PVS, the sensitivity and specificity were respectively up to 100% and 83.33%.

Conclusions

Poor liver regeneration could be significantly induced when PVS was greater than 65%. Ultrasound can well show the changes in portal vein hemodynamics in different degrees of PVS in rats. The parameters PVV_s could be regarded as a useful index for diagnosing PVS but were not applicable for evaluating the stenosis degree. Comparatively, the parameters DSR and AVR, especially AVR, proved to be useful for differentiating severe PVS (>65%) in the early postoperative period, predicting the resulting poor liver regeneration.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/atm.2020.04.21>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The animal ethics committee approved all the rats and procedures of West China Hospital, Sichuan University (No. 2019054A).

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