Received:
02 October 2018

Revised:
13 January 2019

Accepted:
04 February 2019

<https://doi.org/10.1259/bjr.20180847>

Cite this article as:

Xie P, Quan L, Xie S, Chen B, Wei K, Ren J, et al. Quantitative study of liver hemodynamic changes in rats with small-for-size syndrome by the 4D-CT perfusion technique. *Br J Radiol* 2019; **92**: 20180847.

FULL PAPER

Quantitative study of liver hemodynamic changes in rats with small-for-size syndrome by the 4D-CT perfusion technique

¹PEIYI XIE, ²LI QUAN, ²SIDONG XIE, ³BINGHUI CHEN, ¹KAIKAI WEI, ⁴JIE REN and ¹XIAOCHUN MENG

¹Department of Radiology, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

²Department of Radiology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

³Department of Radiology, The fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, China

⁴Department of Ultrasonics, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Address correspondence to:

Xiaochun Meng

E-mail: mengxch3@mail.sysu.edu.cn

Jie Ren

E-mail: renjieguangzhou@126.com

Peiyi Xie and Li Quan have contributed equally to this study and should be considered as co-first authors.

Objective: The microcirculatory hemodynamic changes of small-for-size syndrome (SFSS) are still unclear. In this study, they were investigated by four-dimensional CT perfusion (4D-CTP) technique.

Methods: The sham group, 50, 60, 70 and 80 % partial hepatectomy (PH) rat groups were established. At 1 hour (1h), 1 day (1 d), 3 days (3 d) and 7 days (7 d) post-operation, serological examination, 4D-CTP scan and histopathological examination were performed. One-way analysis of variance and the Kruskal-Wallis test were used for the comparison.

Results: Based on the diagnostic criteria of SFSS, the 80 % group was considered to be a successful model. In all the PH groups, portal vein perfusion and total liver perfusion peaked at 1h and declined at 1d and 3d. Both portal vein perfusion and total liver perfusion were significantly higher in the 80 % group than the sham group, 50 and 60% groups at 1h ($p < 0.05$), and 80 % group at 3d and 7d ($p < 0.05$). In the 50 and 60 % groups, hepatic artery perfusion decreased at 1h and maintained at a lower level until at 7 d;

whereas, in the 70 and 80% groups, it increased at 1h, then decreased and reached the lowest level at 7 d. No significant difference appeared in hepatic artery perfusion between any two groups at any time points. At all time points, hepatic perfusion index was lower in all the PH groups than the sham group. Significant differences in hepatic perfusion index appeared between the 80% group and the sham group at 1h and 1 d ($p < 0.05$).

Conclusions: The CTP parameters quantitatively revealed the microcirculatory hemodynamic changes in SFSS, which were further confirmed to be associated with histopathological injury. It is suggested that the hemodynamic changes in SFSS remnant liver can provide useful information for further revealing the mechanism of SFSS and may help for guiding the treatments.

Advances in knowledge: By using the 4D-CTP technique, the hepatic microcirculatory hemodynamic changes could be quantitatively measured *in vivo* for small animal research.

INTRODUCTION

Due to its poorer outcomes and higher morbidity, small-for-size syndrome (SFSS) after living-donor liver transplantation (LDLT) and extended partial hepatectomy (PH) has received increasing attention. Although the extensive portal hypertension and shear stress in the "small size" of the liver have been recognized as the primary initiating factors for SFSS,^{1,2} other factors associated with hepatic microcirculatory disarrangements, such as impaired hepatic venous

outflow, graft steatosis, recipient liver cirrhosis and pre-existing portal hypertension,^{3,4} also are known to contribute to the occurrence of SFSS. Under these conditions, SFSS even occurs in recipients with larger grafts (graft-to-recipient weight ratio: GW/RW > 0.8% or graft volume-to-spleen volume ratio: GV/SLV > 40%).⁵ An understanding of the actual microcirculatory hemodynamic changes of SFSS may aid its prevention and treatment.

Timely identification of SFSS is crucial for saving the graft after partial liver transplantation (PLT) or the remnant liver after extended PH. Up to date, the diagnosis of SFSS is mainly based on the acute clinical manifestations combined with small liver graft or remnant liver, with the exclusion of other causes. However, it is still a really challenging job, as there is no characteristic clinical manifestation in the early stage of SFSS. Elevated transaminase and jaundice present in various status with liver damage, such as rejection, recurrence of hepatitis, etc. Refractory ascites could indicate SFSS in the later stage, when irreversible liver damage has occurred in most cases. Besides these, the diagnosis of SFSS based on the small volume of the graft and remnant liver is not always reliable. Patients with severe fatty liver and advanced liver cirrhosis may suffer SFSS even with a large-volume graft or remnant liver.^{6,7} Needle biopsy definitely help for the diagnosis, but it is invasive and only represents the histopathology of the biopsy tissue. A series of studies^{8–10} have confirmed that the hemodynamic disorders of hepatic microcirculation play a key role in the SFSS onset and progression. At present, the most commonly used method for the evaluation of the hemodynamic status of hepatic microcirculation is indocyanine green removal tests, which only reflect the whole liver hemodynamic status and are unable to separate from arterial flow to portal blood flow.¹¹ Doppler ultrasonography is also a common used method for noninvasively revealing the elevated portal flow, portal velocity and the flow index of hepatic artery after PH and PLT, whereas all these index only reflect the hemodynamic in the large blood vessels rather than those in the liver tissue, and has not been considered as the reliably basis for the diagnostic of SFSS.¹²

The CT perfusion (CTP) technique has been used for measuring the hemodynamic changes of hepatic microcirculation in several liver conditions, including the cirrhotic liver disease,¹³ hepatic tumors,¹⁴ liver grafts after LT¹⁵ and treatment response following radiotherapy and chemotherapy,¹⁶ in which the hepatic arterial and portal perfusion in per unit volume of liver in unit time had been quantitatively measured respectively. And only few studies using CTP technique revealed the hepatic hemodynamic changes in SFSS. The roles of hepatic arterial and portal flow in the onset and progression of SFSS are still unknown. In recent years, a 320-row four dimensional CTP (4D-CTP) technique with a single scan coverage of 16 cm has become the latest technology to achieve 4D perfusion analysis of the whole organ. This technique not only overcomes the limitations of the previous CTP scan level but also enables coronal, sagittal and arbitrary plane cutting to truly achieve 4D perfusion analyses of the whole organ, which greatly improves the ability of hepatic perfusion studies.^{17,18}

In this study, the 4D-CTP technique was used to quantitatively examine the microcirculatory hemodynamic changes of SFSS. To avoid the influence of vascular complications, rejection and ischemia-reperfusion injuries after liver transplantation, 50, 60, 70 and 80% PH rat models were established in the present research. We analyzed the hemodynamic changes of whole liver microcirculation at different resection rates of remnant liver in combination with serological and histopathological results. This quantitative analysis of the impact of SFSS microcirculatory

hemodynamic disorder on liver injury will provide an objective basis for a non-invasive, early diagnosis of SFSS.

METHODS AND MATERIALS

Animals

All the experimental procedures involving animals were approved by the Animal Ethics Committee of Sun Yat-sen University.

A total of 144 Sprague–Dawley rats (age 7–8 weeks, weighing 200–250 g, with an equal number of males and females) were obtained from Guangdong Province Laboratory Animal Research Center (Guangdong, China) and randomly divided into the sham group, the 50, 60, 70, and 80% PH groups and the supplemental group ($n = 24/\text{group}$). At 1 hour (1 h), 1 day (1 d), 3 days (3 d) and 7 days (7 d) after operation, blood samples, 4D-CTP scans and histopathological examinations were performed on six rats from each group. Surgical operation, 4D-CTP scans and blood sampling were performed under 10% chloral hydrate anesthesia (intraperitoneal injection, 0.30–0.35 mg/100 g).

SURGERY

Based on the anatomic lobulation of rat liver, PHs with 50–80% resection were successfully carried out. The 50% PH group underwent resection of the middle lobe and the superior right lateral lobe. The 60% PH group received resection of the middle lobe, the superior right lateral lobe and the inferior right lateral lobe. The 70% PH group underwent resection of the left lateral lobe and the middle lobe, and the 80% PH group received resection of the left lateral lobe, the middle lobe and the inferior right lobe. These PHs were conducted on the four groups, resulting in groups of 50, 60, 70 and 80% PH rat models.¹⁹

An abdominal incision was conducted on rats in the sham group. Dissection was randomly conducted on the falciform ligament, coronary ligament, esophageal ligament, ligamentum hepatogastricum, ligamentum hepatoduodenale, and hepatorenal ligament. Engagement of the liver parenchyma was avoided. The abdomen was closed after 30 min.

Serological examination and tumor necrosis factor- α (TNF- α) immunoassay

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin (TB) concentrations were measured by an auto-analyser (Hitachi 7600, Japan).

TNF- α levels were tested by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech Life, 3607 Parkway Lane Suite, 200 Norcross, GA 30092, USA).

4D-CTP SCAN

In this study, all the 4D-CTP scans were performed on an Aquilion One 320-slice CT scanner (Toshiba, Japan). Dynamic volumetric scanning for 4D-CTP was performed with low-dose continuous collection mode. The scanning parameters were as follows: tube voltage 80 kV, tube current 100 mAs, 1.0 mm section thickness and 1.0 mm interval. The iodinated contrast agent (Iopamiron [300 mg I/mL], Shanghai) was injected manually into the tail vein of rats with 2 ml/kg body weight. CT scan was implemented

Figure 1. The 4D-CTP images of one rat in the 80% group at 1 h. Images A to D are axial HAP, PVP, HPI and CT plain images, respectively. Images E to H are coronal HAP, PVP, HPI and CT plain image, respectively. HAP, hepatic artery perfusion; HPI, hepatic perfusion index; PVP, portal vein perfusion.

while injection and the contrast agent is injected within 2 s. 41 series of volume data were collected within 60 s (0.5 s for each series and 1.0 s interval). The coverage range of the 4D-CTP scan was 99 mm for each rat.

All data were transferred to an Aquilion One Displaying Monitor (Toshiba, Japan) for post-processing. According to previous references,^{15,20,21} irregular regions of interest (ROIs) for the liver and spleen were manually drawn, which contained as much of the parenchyma as possible and avoided large blood vessels. Circular ROI were used for the aorta and portal vein, which were drawn as large as possible. All of the above ROIs avoided too close to the boundaries of the organs to avoid partial volume effects. And the border of the ROI was regulated manually on each slice. The time density curve (TDC) of each ROI was generated automatically. Based on the TDC of the aorta, portal vein, spleen and liver, the following perfusion parameters were calculated with the maximum gradient method: (1) hepatic artery perfusion (HAP) = $S_{L1}/\Delta A \times 60$ ^{15,22-24}; (2) portal vein perfusion (PVP) = $60 \times (S_{S1} \times S_{L2} + S_{S2} \times S_{L1}) / S_{S1} \times \Delta P$ ²²⁻²⁴; S_{L1} = the maximum slope of liver TDC prior to peak splenic enhancement; S_{L2} = the maximum slope of liver TDC after peak splenic enhancement; S_{S1} = the maximum slope of splenic TDC before peak splenic enhancement; S_{S2} = the maximum slope of splenic TDC after peak splenic enhancement; ΔA = peak aortic enhancement; ΔP = peak portal venous enhancement; (3) TLP = HAP + PVP; and (4) hepatic perfusion index (HPI) = $[HAP / (HAP + PVP)] \times 100\%$. Figure 1 shows the 4D-CTP images from one rat in the 80% group acquired at 1 h post-operation, which provides both axial and coronal perfusion images.

HAEMATOXYLIN AND EOSIN (H&E) STAINING

H & E staining was performed to evaluate liver injuries. Using a semi-quantitative histologic scoring system,²⁵ two investigators, blinded to the surgical data, recorded the sinusoidal damage score (including sinusoidal dilation and sinusoidal accumulation of necrotic cells), hepatocyte injury score (including single cell necrosis, confluent necrosis, small vacuolar transformation and eosinophilic globuli in the cytoplasm of hepatocytes, and hepatocyte edema) and liver damage score (the sum of the sinusoidal damage score and hepatocyte injury score) based on five random high-power fields of H&E slices.

KI-67 STAINING

A rabbit monoclonal anti human Ki-67 antigen (ZA0502, Beijing, China) was used to evaluate hepatocyte proliferation. 10 random fields in each slice (400 × magnification) were evaluated. Positive proliferating cells were counted and averaged by two researchers blinded to the surgery data. The proliferation index (PI) was calculated by dividing the number of positive proliferating cells by the sum of hepatocytes and expressed as percentage.

STATISTICAL ANALYSIS

All the CTP data were analyzed by two radiologists with over 5 years of experience in abdominal radiology. They were blinded to all surgery data and laboratory results. The intraclass correlation coefficient (ICC) judged the consistency between the two radiologists. The mean peak value and peak time of abdominal aortic enhancement of all rats were measured, and the 95% confidence intervals for the means were calculated. All rats beyond these ranges were excluded, and additional surgeries were performed in rats from the supplemental group. The HAP, PVP, total liver

perfusion (TLP) and HPI values of each group are expressed as the means $\pm$ standard deviation. Statistical analyses were performed with a statistical software package (SPSS, 13.0). CTP parameters, hepatic injury parameters and PI were compared by one-way analysis of variance and the Kruskal–Wallis test. Significance was established at $p < 0.05$ (2-tailed).

RESULTS

Clinical outcomes

All rats in the 50 and 60% groups survived after operation. In the 70% group, two rats died at 1 d and 2 d. In the 80% group, 11 rats died from 0 to 12 h ($n = 7$), 12 h to 1 d ($n = 3$) and 1 d to 3 d ($n = 1$). Additional PH surgeries were performed on rats from the supplemental group.

20 rats suffered massive ascites, including 3 in the 70% group (2 at 3 d and 1 at 7 d) and 17 in the 80% group (5 at 1 d, 6 at 3 d and 6 at 7 d). In the 80% group, five rats suffered massive hemorrhagic ascites, and 15 rats suffered weight loss of varying degrees, even up to 25% of body weight at 7 d in 1 rat. Two rats in the 80% group showed detectable jaundice at 3 d.

HEPATIC INJURY

Serological parameters

At 1 h, no significant differences in AST and ALT appeared between any two groups ($p > 0.05$). At 1 d, both AST and ALT reached peak levels in all of the PH groups, and these levels were significantly higher than those in the sham group ($p < 0.05$). Significant differences in AST and ALT also appeared between the 80 and 50% groups ($p < 0.05$). At 3 d, although AST and ALT levels declined slightly in all PH groups, they remained higher in the 80% group compared to the 50% group ($p < 0.05$) and higher in the 70 and 80% groups than the sham group ($p < 0.05$). At 7 d, AST and ALT levels further declined in all PH groups, but they still remained higher in the 80% group than the sham group and 50% group ($p < 0.05$).

Compared to the sham group, in the 50, 60 and 70% groups, TB increased slightly at 1 h, 1 d, 3 d and 7 d, but no significant differences appeared between any two groups ($p > 0.05$). In the 80% group, TB significantly increased at all the time points and peaked at 7 d. At 3 d and 7 d, TB was significantly higher in the 80% group than the sham group and the 50 and 60% groups ($p < 0.05$).

At 1 h, no significant differences appeared in TNF- α between any two groups ($p > 0.05$). In all the PH groups, TNF- α peaked at 1 d and declined at 3 d and 7 d. Both at 1 d and 3 d, TNF- α was significantly higher in the 80% group than the sham group and 50% group ($p < 0.05$). Except for these above mentioned differences, there was no significant differences in TNF- α between any other two groups ($p > 0.05$) (Supplementary Material 1).

Morphological scores of remnant livers

At 1 h, the sinusoidal damage score of all PH groups increased due to sinusoidal dilation, and significant differences between the sham group and each of the PH groups were observed ($p < 0.05$). This score was slightly higher in the 80% group than the other

PH groups ($p > 0.05$). Due to hepatocyte edema and reduced sinusoidal dilation, the sinusoidal damage scores decreased in all of the PH groups at 1 d, but the significant differences between the sham group and PH groups were lost ($p > 0.05$). At 3 d, scores slightly increased again in all PH groups due to necrotic cell accumulation in sinusoids. At 7 d, scores further increased and were significantly higher in the 80% group than the sham, 50 and 60% groups ($p < 0.05$) (Figures 2, 3A).

At 1 h, no significant differences in the hepatocyte injury score were observed between any two groups ($p > 0.05$). At 1 d, scores increased in all PH groups due to small vacuolar transformation of the cytoplasm, eosinophilic globuli in the cytoplasm, hepatocyte edema or necrosis. Significant differences in hepatocyte injury scores appeared between the sham group and each of the PH groups ($p < 0.05$), and, scores were higher in the 80% group than the other PH groups ($p > 0.05$). At 3 d, the hepatocyte injury score was significantly higher in the 80% group than the sham group and 50% group ($p < 0.05$), and scores were higher in the 60 and 70% groups than the sham group ($p < 0.05$). At 7 d, scores decreased in all of the PH groups due to hepatocyte regeneration. However, scores remained higher in the 80% group than the other PH groups ($p > 0.05$). (Figures 2, 3B)

At 1 h, the liver damage score increased in all of the PH groups and further increased at 1 d. Both at 1 h and 1 d, significant differences appeared between the sham group and each of the PH groups ($p < 0.05$) but not between the PH groups ($p > 0.05$). At 3 d, scores decreased in the 50% group and increased in other PH groups. Significant differences appeared between the 80% group and the sham, 50 and 60% groups ($p < 0.05$) and between the sham group and the 60 and 70% groups ($p < 0.05$). At 7 d, although the liver damage scores decreased in the 60, 70 and 80% groups, they were still significantly higher in the 80% group than the sham and 50% groups ($p < 0.05$). (Figure 3C)

Hepatocyte proliferation index

At 1 h, no significant differences in PI appeared between any two groups ($p > 0.05$). At 1 d, PI significantly increased in the 50%, 60 and 70% groups and was higher than that in the sham group ($p < 0.05$), whereas, in the 80% group, PI was slightly higher than that in the sham group ($p > 0.05$) and lower than those in other PH groups ($p > 0.05$). At 3 d, significant differences in PI existed between the sham group and each of the PH groups ($p < 0.05$) but not between any two of the PH groups ($p > 0.05$). However, all of the significant differences between two groups disappeared at 7 d ($p > 0.05$). Figure 4 shows the Ki-67 results of the sham group and 80% group at each time point.

Hepatic microcirculatory hemodynamic changes

According to criteria,²⁶ ICC indicated that the two radiologists strongly agreed on assessment (ICC = 0.688–0.830) in this study. For the PVP assessment (ICC = 0.830, $p < 0.001$), correlation was good. For the HAP assessment (ICC = 0.688, $p < 0.001$), correlation was moderate.

Based on the TDCs, the mean peak value and peak time of abdominal aortic enhancement were 167.62 ± 24.18 Hounsfield

Figure 2. HE staining of tissue samples from the sham group and 80% group. HE staining ($\times 200$ magnification). In the 80% group, obvious sinusoidal dilation appeared at 1 h post-operation; hepatocyte edema and vacuolar transformation appeared at 1 d and were more serious at 3 d; at 7 d, severe disruption of lobular architecture with extensive necrosis was detected. H&E, Haematoxylin and Eosin.

Unit and 20.22 ± 6.39 s, respectively, and the 95% confidence intervals were 163.27–171.97 Hounsfield Unit and 19.07–21.37 s, respectively. 11 rats exceeded these ranges and were excluded and replaced.

Changes in PVP and TLP in the PH groups

In all the PH groups, both PVP and TLP reached their peak levels at 1 h post-operation and then declined at 1 d. At 1 h, significant differences in PVP and TLP were observed between the 80% group and the sham, 50 and 60% groups ($p < 0.05$). At 1 d, significant differences in PVP and TLP remained between the 80% group and the sham group ($p < 0.05$), but disappeared at 3 and 7 d. At 3 d, PVP and TLP in the 50, 60 and 70% groups were close to those in the sham group. In the 80% group, these values decreased but remained slightly higher than those in the sham group ($p > 0.05$). At 7 d, PVP and TLP in all of the PH groups were close to those in the sham group. Additionally, in the 80% group, PVP and TLP at 1 h were significantly higher than those at 3 d and 7 d ($p < 0.05$), whereas, in the 50, 60 and 70% groups, no significant differences in PVP and TLP appeared between those at any two time points ($p > 0.05$). (Figure 5A and B)

Changes in HAP in the PH groups

In the 50 and 60% groups, HAP decreased at 1 h and was slightly lower than that in the sham group until the seventh day ($p > 0.05$). In the 70 and 80% groups, HAP increased and peaked at 1 h, then gradually decreased at 1, 3 and 7 d and reached its lowest level at 7 d. At 1 h and 1 d, HAP was higher in the 70 and 80% groups than the sham, 50 and 60% groups ($p > 0.05$), whereas, at 3 and 7 d, HAP was lower than that in the sham group ($p > 0.05$). However, no significant differences in HAP appeared between any two groups at any time point ($p > 0.05$). (Figure 5C)

Changes in HPI in the PH groups

In the 50 and 60% groups, the lowest HPI appeared at 1 h, which increased at 1 d, peaked at 3 d and decreased slightly at 7 d. In the 70% group, the lowest HPI also appeared at 1 h, which was close to that at 1 d; then, it increased and peaked at 3 d and decreased at 7 d. However, in the 80% group, the lowest HPI appeared at 1 d, which was slightly lower than the value at 1 h. HPI increased at 3 d and was maintained at 7 d. Although, at all time points, HPI was lower in all PH groups than the sham group, significant differences only appeared between the 80% group and the sham group at 1 h and 1 d ($p < 0.05$). (Figure 5D)

Correlation analyses of hemodynamic changes and liver damage score

At 1 h post-operation, there were positive correlations between sinusoidal damage score and PVP and TLP ($r = 0.766$, $p = 0.027$ and $r = 0.894$, $p = 0.007$, respectively). At 1 d post-operation, negative correlations were found between hepatocyte injury score, liver damage score and HPI ($r = -0.633$, $p = 0.049$ and $r = -0.608$, $p = 0.047$, respectively). At 3 d post-operation, there were negative correlations between hepatocyte injury score, liver damage score and HPI ($r = -0.663$, $p = 0.019$ and $r = -0.643$, $p = 0.033$, respectively).

DISCUSSION

Based on the combination of clinical outcomes, serological parameters and liver injury morphological scores,^{25,27,28} the 80% group can be considered a successful SFSS model in this study.

At 1 h post-operation, both PVP and TLP peaked in all PH groups, with significant differences between the 80% group and

Figure 3. Morphological scores of the remnant livers of all groups. (A) Sinusoidal damage score. * $p < 0.05$, at 1 h, all of the PH groups vs the sham group; at 7 d, the 70 and 80% group vs the sham group; + $p < 0.05$, the 80% group vs the 50 and 60% groups. (B) Hepatocyte injury score. * $p < 0.05$, at 1 d, all of the PH groups vs the sham group; at 3 d, the 60, 70 and 80% groups vs the sham group; + $p < 0.05$, the 80% group vs the 50% group. (C) Liver damage score. * $p < 0.05$, at 1 h and 1 d, all of the PH groups vs the sham group; at 3 d, the 60, 70 and 80% groups vs the sham group; at 7 d, the 80% group vs the sham group. + $p < 0.05$, at 3 d, the 80% group vs the 50 and 60% groups; at 7 d, the 80% group vs the 50% group. PH, partial hepatectomy.

A

B

C

the sham, 50 and 60% groups. The sinusoidal damage scores were also significantly higher in all PH groups than the sham group at this time point due to sinusoidal dilation, and it was higher in the 80% group than other PH groups but without significant difference. Moreover, there were positive correlations between sinusoidal damage score and PVP and TLP. However, none of the hepatocyte injury scores or serological parameters that reflected hepatocyte injuries were significantly changed in any of the PH groups. These results reconfirm that severe sinusoidal dilation caused by massive blood flow into the liver sinus may be the original reason and main cause of liver injuries in SFSS at a very early stage.

At 1 d, PVP, TLP and the sinusoidal damage score decreased rapidly. All of the significant differences in sinusoidal damage scores appeared at 1 h and disappeared at 1 d. AST, ALT and TNF- α increased significantly and peaked in all of the PH groups at 1 d and gradually decreased at 3 and 7 d. Significant differences between the 80% group and the sham and 50% groups existed in AST and ALT from 1 to 7 d and in TNF- α from 1 to 3 d. The hepatocyte injury scores also increased and peaked or almost peaked in all of the PH groups at 1 d and remained similar at 3 d but decreased at 7 d. Significant differences in hepatocyte injury scores only presented at 3 d between the 80% group and the sham and 50% groups. These results suggest that the significant increases in PVP and TLP and the liver injury caused by sinusoidal dilation lasted less than 1 d, and it will be better to perform portal inflow modulations before reperfusion to prevent excessive portal inflow and subsequent sinusoidal injuries instead of after SFSS occurs and to limit the application of these modulations to the short term to ensure adequate portal inflow to liver. From 1 d after surgery, hepatocyte injury contributed to the majority of the liver injury, and at 3 and 7 d, necrotic cell accumulation in sinusoids further aggravated the liver injuries, which were more obvious in the 80% group. Therefore, before this period, prophylactic treatments to protect hepatocytes and alleviate inflammation may contribute to recovery.

Compared to the rapid changes of AST, ALT and TNF- α , the change in TB was slow and mild post-operation. TB increased significantly only in the 80% group and peaked at 7 d, and it was significantly higher than in the sham, 50 and 60% groups at 3 and 7 d. Although TB was only measured for a period of 7 days, the increase in TB occurred obviously later than the changes in PVP and TLP and the sinusoidal and hepatocyte injuries. Therefore, we can reasonably conclude that the significant increase in TB is suggestive of severe liver injuries.

Although hepatic artery vasospasm and necrosis of the bile duct has been revealed in allografts with SFSS by histopathology,²⁹ and restoration of hepatic artery flow has been proven to help improve the outcome,^{30,31} in this study, the absolute values of HAP in unit liver volume in the 70 and 80% groups did not decrease at 1 h and 1 d. Instead, HAP increased and peaked at 1 h, and it was higher than those in the sham, 50 and 60% groups at 1 h and 1 d. These results may be attributed to the significantly reduced liver volume and hepatic vascular bed. Afterward, although PVP and TLP significantly decreased at 3 and 7 d, in the 70 and 80% groups, HAP did

Figure 4. Ki-67 staining of tissue samples from the sham group and 80% group. Ki-67 detection ($\times 400$ magnification). Hepatic nuclei are stained brownish yellow (Arrow). Compromised hepatocyte proliferation appeared at 1 d in the 80% group, which was relieved at 3 d.

not increase as expected; it gradually decreased from 1 to 7 d and reached its lowest level at 7 d, which suggests that normal hepatic arterial buffer response was replaced by sustained arterial vasoconstriction. Therefore, in terms of employing adenosine to reverse the arterial vasospasm, a relatively long-term application may be more reasonable. However, the best time to apply adenosine and whether it would further aggravate sinusoidal damage with increased arterial inflow at the very early stage of SFSS require further verification. Furthermore, although no rats in the 50 and 60% groups exhibited SFSS or exhibited any signs of bile duct injuries, HAP in the 50 and 60% groups was decreased at all time points after surgery and was lower even than those in the 70 and 80% groups not only at 1 h and 1 d but also at 7 d. Therefore, whether arterial vasospasm always occurs after PH surgery with greater than 50% liver resection requires further investigation.

HPI reflects the proportion of arterial inflow to total blood inflow in the hepatic sinus, which is affected by both HAP and TLP. At 1 h post-operation, with the increase of liver resection ratio (50, 60, 70 and 80% group), the volume of remnant liver decreased, PVP and TLP gradually increased, which was significantly correlated with hepatic sinusoidal damage score. Also at this time point, the sinusoidal damage score was mainly caused by hepatic sinusoidal dilation, due to the impact of massive portal vein blood flow and shear damage. Based on the above conclusions, for extended PH and small-volume liver transplantation, we assume that prophylactic control of portal vein blood flow may be a crucial method for reducing SFSS damage or preventing SFSS. Unlike the previous study,²⁹ this study confirmed that the HAP in the 70, 80% group at 1 h post-operation did not decrease, but increased. The increase in TLP was further accelerated, and HAP was associated with hepatic sinus injury scores 1 h post-operation. Therefore, we assume that the treatment time for postoperative hepatic artery dilation

should be postponed appropriately. Many researchers believed that massive portal vein blood flow in SFSS patients can be regulated by hepatic artery cushioning mechanism, which leads to hepatic artery vasospasm, and even functional hepatic artery blood flow disappearance, further aggravates hepatocyte apoptosis and infarction. This finding has been confirmed through histopathological examination in patients with SFSS.^{29,30} However, in this study, the HAP did not decrease in all PH groups. The HAP in the 70, 80% group at 1 h post-operation did not decrease, but increased. Due to the increase in portal vein blood flow post-operation, the HPI of all PH groups was lower than that in the sham group. In the 80% group, that parameter was significantly lower, which suggests that HPI is a better reflection of the proportion of hepatic artery blood flow in microcirculatory hemodynamic changes compared to HAP. At 1 and 3 d post-operation, negative correlation was found between hepatocyte injury score, liver damage score and HPI. It is suggested that the treatment of hepatic artery blood flow should be improved at this stage.^{29,30}

In this study, compromised hepatocyte proliferation with lower PI was only detected in the 80% group at 1 d, and no significant differences in PI appeared between the 80% group and the other PH groups. At 3 d, PI was relieved with the decreases in PVP and TLP. At 7 d, in all the PH groups, hepatocyte proliferation returned to the level of the sham group, including the 80% group. Inhibited hepatocyte proliferation due to reduced synthetic function was detected in SFSS livers,³² but the results of the present study suggest that the reduced proliferation is a direct result of excessive portal inflow, rather than a reason for the progress of SFSS, which was quickly alleviated with the decline in portal inflow.

In this study, there are several limitations. First, CTP parameters were not obtained from the 13 dead rats that received 70 or 80%

Figure 5. The CTP parameters in all of the PH groups. (A) PVP. * $p < 0.05$, at 1 h and 1 d, the 80% group vs the sham group; + $p < 0.05$, at 1 h, the 80% group vs the 50 and 60% groups; (B) TLP. * $p < 0.05$, at 1 h and 1 d, the 80% group vs the sham group; + $p < 0.05$, at 1 h, the 80% group vs the 50 and 60% groups; (C) HAP; (D) HPI. * $p < 0.05$, at 1 h and 1 d, the 80% group vs the sham group. CTP, CT perfusion; HAP, hepatic artery perfusion; HPI, hepatic perfusion index; PH, partial hepatectomy; PVP, portal vein perfusion; TLP, total liver perfusion.

PH surgeries, and these rats may have experienced more serious injuries than the surviving rats. Therefore, these results may include some degree of bias. Second, we did not observe hemodynamic changes at different time points in the same animals, and we could not provide more accurate self-referential analyses. Finally, the PH rat models were employed to investigate the hemodynamic changes of small-for-size liver graft, and factors that affect liver hemodynamics after transplantation were not analyzed, including splanchnic hemodynamics, ischemia and reperfusion injuries, liver steatosis etc. which may further aggravate SFSS injuries. Further research in these fields will be performed in a step-by-step manner.

CONCLUSION

In this study, by using 4D-CTP technique, HAP, PVP and TLP in per unit of the remnant liver and the proportion of hepatic

arterial flow in the total inflow (the HPI) after PH were acquired, which not only confirmed the changes of PVP after the extended PH, but also reveal the increased HAP and decreased HPI within the short period after PH. It is suggested that the significant increased PVP may be the crucial factor for the onset of SFSS and the increased HAP with decreased HPI within the short period after PH may further aggravate liver injuries in SFSS.

ACKNOWLEDGMENT

The authors thank Xing Chen (TOSHIBA MEDICAL SYSTEMS (CHINA) CO., LTD.) for her technical support on CTP data post-processing. This study was supported by grants from the National Natural Science Foundations (81201090, 81371554, 81371655) and the Medical Scientific Research Foundation of Guangdong Province(A2018121).

REFERENCES

1. Yao S, Kaido T, Uozumi R, Yagi S, Miyachi Y, Fukumitsu K, et al. Is portal venous pressure modulation still indicated for all recipients in living donor liver transplantation? *Liver Transpl* 2018; **24**: 1578–88. doi: <https://doi.org/10.1002/lt.25180>
2. Badawy A, Hamaguchi Y, Satoru S, Kaido T, Okajima H, Uemoto S. Evaluation of safety of concomitant splenectomy in living donor liver transplantation: a retrospective study. *Transpl Int* 2017; **30**: 914–23. doi: <https://doi.org/10.1111/tri.12985>
3. Scatton O, Cauchy F, Conti F, Perdigao F, Massault PP, Goumard C, et al. Two-stage liver transplantation using auxiliary laparoscopically harvested grafts in adults: Emphasizing the concept of "hypersmall graft nursing". *Clin Res Hepatol Gastroenterol* 2016; **40**: 571–4. doi: <https://doi.org/10.1016/j.clinre.2016.03.002>
4. Raut V, Alikhanov R, Belghiti J, Uemoto S. Review of the surgical approach to prevent small-for-size syndrome in recipients after left lobe adult LDLT. *Surg Today* 2014; **44**: 1189–96. doi: <https://doi.org/10.1007/s00595-013-0658-6>
5. Gyoten K, Mizuno S, Kato H, Murata Y, Tanemura A, Azumi Y, et al. A novel predictor of posttransplant portal hypertension in Adult-To-Adult living donor liver transplantation: increased estimated Spleen/Graft volume ratio. *Transplantation* 2016; **100**: 2138–45. doi: <https://doi.org/10.1097/TP.0000000000001370>
6. Goldaracena N, Echeverri J, Selzner M. Small-for-size syndrome in live donor liver transplantation-Pathways of injury and therapeutic strategies. *Clin Transplant* 2017; **31**: e1288511 01 2017. doi: <https://doi.org/10.1111/ctr.12885>
7. Rajakumar A, Kaliamoorthy I, Rela M, Mandell MS. Small-for-Size syndrome: bridging the gap between liver transplantation and graft recovery. *Semin Cardiothorac Vasc Anesth* 2017; **21**: 252–61. doi: <https://doi.org/10.1177/1089253217699888>
8. Asakura T, Ohkohchi N, Orii T, Koyamada N, Tsukamoto S, Sato M, et al. Portal vein pressure is the key for successful liver transplantation of an extremely small graft in the pig model. *Transpl Int* 2003; **16**: 376–82. doi: <https://doi.org/10.1111/j.1432-2277.2003.tb00317.x>
9. Glanemann M, Eipel C, Nussler AK, Vollmar B, Neuhaus P. Hyperperfusion syndrome in small-for-size livers. *Eur Surg Res* 2005; **37**: 335–41. doi: <https://doi.org/10.1159/000090333>
10. Vollmar B, Menger MD. The hepatic microcirculation: mechanistic contributions and therapeutic targets in liver injury and repair. *Physiol Rev* 2009; **89**: 1269–339. doi: <https://doi.org/10.1152/physrev.00027.2008>
11. Hashimoto T, Miki K, Imamura H, Sano K, Satou S, Sugawara Y, et al. Sinusoidal perfusion in the veno-occlusive region of living liver donors evaluated by indocyanine green and near-infrared spectroscopy. *Liver Transpl* 2008; **14**: 872–80. doi: <https://doi.org/10.1002/lt.21460>
12. Vasavada B, Chen CL, Zakaria M. Using low graft/recipient's body weight ratio graft with portal flow modulation an effective way to prevent small-for-size syndrome in living-donor liver transplant: a retrospective analysis. *Exp Clin Transplant* 2014; **12**: 437–42.
13. Talakić E, Schaffellner S, Kniepeiss D, Mueller H, Stauber R, Quehenberger F, et al. CT perfusion imaging of the liver and the spleen in patients with cirrhosis: is there a correlation between perfusion and portal venous hypertension? *Eur Radiol* 2017; **27**: 4173–80. doi: <https://doi.org/10.1007/s00330-017-4788-x>
14. Wright KC, Ravoori MK, Dixon KA, Han L, Singh SP, Liu P, et al. Perfusion CT assessment of tissue hemodynamics following hepatic arterial infusion of increasing doses of angiotensin II in a rabbit liver tumor model. *Radiology* 2011; **260**: 718–26. doi: <https://doi.org/10.1148/radiol.11101868>
15. Bader TR, Herneth AM, Blaicher W, Steininger R, Mühlbacher F, Lechner G, et al. Hepatic perfusion after liver transplantation: noninvasive measurement with dynamic single-section CT. *Radiology* 1998; **209**: 129–34. doi: <https://doi.org/10.1148/radiology.209.1.9769823>
16. Kim SH, Kamaya A, Willmann JK. CT perfusion of the liver: principles and applications in oncology. *Radiology* 2014; **272**: 322–44. doi: <https://doi.org/10.1148/radiol.14130091>
17. Spira D, Schulze M, Sauter A, Brodoefel H, Brechtel K, Claussen C, et al. Volume perfusion-CT of the liver: insights and applications. *Eur J Radiol* 2012; **81**: 1471–8. doi: <https://doi.org/10.1016/j.ejrad.2011.04.010>
18. Laghi A, Multidetector CT. 64 slices) of the liver: examination techniques. *Eur Radiol* 2007; **17**: 675–83.
19. Martins PNA, Theruvath TP, Neuhaus P. Rodent models of partial hepatectomies. *Liver Int* 2008; **28**: 3–11. doi: <https://doi.org/10.1111/j.1478-3231.2007.01628.x>
20. Guan S, Zhao W-D, Zhou K-R, Peng W-J, Mao J, Tang F. CT perfusion at early stage of

- hepatic diffuse disease. *World J Gastroenterol* 2005; **11**: 3465–7. doi: <https://doi.org/10.3748/wjg.v11.i22.3465>
21. Kanda T, Yoshikawa T, Ohno Y, Kanata N, Koyama H, Takenaka D, et al. CT hepatic perfusion measurement: comparison of three analytic methods. *Eur J Radiol* 2012; **81**: 2075–9. doi: <https://doi.org/10.1016/j.ejrad.2011.07.003>
 22. Miles KA, Hayball M, Dixon AK. Colour perfusion imaging: a new application of computed tomography. *Lancet* 1991; **337**: 643–5(91)92455-B [pii](London, England). doi: [https://doi.org/10.1016/0140-6736\(91\)92455-B](https://doi.org/10.1016/0140-6736(91)92455-B)
 23. Miles KA, Hayball MP, Dixon AK. Functional images of hepatic perfusion obtained with dynamic CT. *Radiology* 1993; **188**: 405–11. doi: <https://doi.org/10.1148/radiology.188.2.8327686>
 24. Blomley MJ, Coulden R, Dawson P, Korman M, Donlan P, Bufkin C, et al. Liver perfusion studied with ultrafast CT. *J Comput Assist Tomogr* 1995; **19**: 424–33. doi: <https://doi.org/10.1097/00004728-199505000-00016>
 25. Alexakis N, Gakiopoulou H, Dimitriou C, Albanopoulos K, Fingerhut A, Skalistira M, et al. Liver histology alterations during carbon dioxide pneumoperitoneum in a porcine model. *Surg Endosc* 2008; **22**: 415–20. doi: <https://doi.org/10.1007/s00464-007-9440-4>
 26. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med* 2016; **15**: 155–63. doi: <https://doi.org/10.1016/j.jcm.2016.02.012>
 27. Dahm F, Georgiev P, Clavien P-A. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. *Am J Transplant* 2005; **5**: 2605–10. doi: <https://doi.org/10.1111/j.1600-6143.2005.01081.x>
 28. Ikegami T, Masuda Y, Ohno Y, Mita A, Kobayashi A, Urata K, et al. Prognosis of adult patients transplanted with liver grafts. *Liver Transpl* 2009; **15**: 1622–30.
 29. Demetris AJ, Kelly DM, Eghtesad B, Fontes P, Wallis Marsh J, Tom K, et al. Pathophysiologic observations and histopathologic recognition of the portal hyperperfusion or small-for-size syndrome. *Am J Surg Pathol* 2006; **30**: 986–93. doi: <https://doi.org/10.1097/0000478-200608000-00009>
 30. Kelly DM, Zhu X, Shiba H, Irefin S, Trenti L, Cocieru A, et al. Adenosine restores the hepatic artery buffer response and improves survival in a porcine model of small-for-size syndrome. *Liver Transpl* 2009; **15**: 1448–57. doi: <https://doi.org/10.1002/lt.21863>
 31. Golriz M, Majlesara A, El Sakka S, Ashrafi M, Arwin J, Fard N, et al. Small for size and flow (SFSF) syndrome: an alternative description for posthepatectomy liver failure. *Clin Res Hepatol Gastroenterol* 2016; **40**: 267–75. doi: <https://doi.org/10.1016/j.clinre.2015.06.024>
 32. Troisi R, Praet M, de Hemptinne B. Small-for-size syndrome: what is the problem? *Liver Transpl* 2003; **9**: S1S1.. doi: <https://doi.org/10.1053/jlts.2003.50193>