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*Original Article*

# Penicillar arterioles of red pulp in residual spleen after subtotal splenectomy due to splenomegaly in cirrhotic patients: a comparative study

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**Abstract:** Background: Following splenomegaly due to portal hypertension, pathologic characteristics include passive congestion and lymphoplasia. High venous pressure and hemodynamics can result in vascular proliferation and lymphoplasia, and promote splenic microcirculation and functional changes. The aim of this study was to determine the changes in penicillar arterioles (PAs) of red pulp in residual splenic tissue after subtotal splenectomy due to splenomegaly in cirrhotic patients to provide anatomic and physiologic evidence for reserved splenic surgery. Methods: Thirteen patients with splenomegaly due to portal hypertension, who were treated surgically, comprised the splenomegaly group. After 8 years, we obtained another specimen by puncture biopsy from the residual spleen group. We designated patients with splenic trauma as the control group. The morphology of PAs under light microscopy was facilitated by EVG staining and immunohistochemistry for CD34. Semi-thin sections were HE-stained. The ultrastructure of PA endothelial cells was observed under electron microscopy. Results: In the residual spleen group, diffuse distribution, tenuous elastic intima in the arterial wall, and continuity in PA of red pulp were seen under light microscopy. A significantly lower density and average cross-sectional area of PAs were observed in the residual spleen group compared with the splenomegaly and control groups ($P < 0.01$). A uniform mitochondrial matrix and a decreased number of ruptured cristae in PA endothelial cells were observed under electron microscopy. While there were some beneficial changes (splenic artery flow volume, portal venous diameter, and portal venous flow volume), the platelet and leucocyte counts were markedly increased in residual spleen. Conclusion: Subtotal splenectomy can eliminate the factors which precipitate splenomegaly (portal hypertension), improve the reconstruction of splenic capillaries, correct hypersplenism, and restore normal splenic function.

**Keywords:** Residual spleen, portal hypertension, splenomegaly, penicillar arteriole

## Introduction

The anatomy of the spleen is characterized by white and red pulp with its characteristic circulation, thereby determining the complexity of splenic hemodynamics [1]. The splenic arteries penetrate deep into the organ before dividing into trabecular arteries and dividing again into central arterioles. Each central arteriole gives rise to 15-30 radiating arterioles (penicillar arterioles [PAs]), and further dividing into smaller vessels entering the marginal zone and red pulp [2]. Each central artery is surrounded by a sheath of T-lymphocytes and B-lymphocyte follicles in the white pulp. PAs originate from the central arteries outside the white pulp. PAs have a diameter of 20-50 μm, and open into the venous sinuses and red pulp cords. The morphology is like penicillus, hence the term, PA [1]. Approximately 75% of the splenic volume is composed of red pulp. The splenic sinuses are the primary vascular structures of the spleen and account for approximately 30% of the red pulp. The splenic cord is meshwork that supports splenic sinuses and PAs can directly infuse into the cords [3]. The functions of the spleen (phagocytosis, immunity, hematopoiesis, and filtration) are dependent on arterioles and the venule filter bed [4]. Animal experiments have confirmed that open and closed circulations exist in the spleen [5]. Small portions of the blood in the red pulp splenic sinus

flow directly into the closed circulation. Most of the blood through the open circulation flows into the red pulp cords [6]. White pulp penetrates the arteries of the red pulp (pulp arterioles), and continues as arterial capillaries. Macrophages are packed around arterial capillaries to form a sheath (sheathed capillaries). Therefore, the arterial terminals of the red pulp are comprised of arterioles (= penicilli), arterial capillaries, and sheathed capillaries [7]. Most of the capillary terminals open into the splenic cords, and blood passes through the endothelial cell spaces on one side into the splenic sinusoids. Small portions of blood flow directly into the splenic sinuses via capillaries [8, 9]. Different birds have been found to have different distributions of PAs [10]. This showed that the spleen is a highly vascularized organ, and blood flow through the spleen is complex [11].

Portal hypertension is primarily caused by an increase in resistance to portal outflow, and secondly by an increase in splanchnic blood flow, which worsens and maintains the increased portal pressure. Increased portal inflow plays a role in the hyperdynamic circulatory syndrome, a characteristic feature of portal hypertensive patients [12-14]. In portal hypertension, splenomegaly not only involves spleen congestion, as traditionally thought, but also enlargement and hyperactivation of the splenic lymphoid tissue, as well as increased angiogenesis and fibrogenesis [1, 15]. Splenomegaly is accompanied by increased splenic blood flow volume and number of splenic blood vessels. The number of arterioles is greater than venules [16]; however, splenic hemodynamics associated with the splenic microcirculation and congestion have not been elucidated [17]. Some scholars have opined that splenomegaly can result in vascular structure changes in the red pulp and impact arterial-venous blood flow [18]. The aim of the current study was to conduct a morphologic comparative analysis of PAs in red pulp of residual splenic tissue after subtotal splenectomy due to splenomegaly in cirrhotic patients to provide anatomic and physiologic evidence in support of splenic preservation surgery.

## Material and methods

Ethical approval of the study protocol was obtained from the Human Research Ethics Committee of Weifang Medical University (Weifang, China). All individuals provided written informed consent before enrollment in the study.

*Study population*

Our studies relating to subtotal splenectomy began in 1984. As of 2012 we have conducted 752 subtotal splenectomies (preserving the lower pole, normal spleen size of splenic tissue, splenic omentum, and splenocolic vessels for the purpose of blood supply); there were 117 cases of splenic trauma and 635 cases of splenomegaly due to portal hypertension. Thirteen tissue samples were collected from patients in the 89th Hospital of the People's Liberation Army (Weifang, China). The 13 patients (7 males and 6 females; mean age, 31 years; age range, 26-36 years) had splenomegaly and cirrhosis who had undergone subtotal splenectomies plus fixation of the posterior sternal omentum majus. Patients were confirmed to have cirrhosis after hepatitis B infection; HBV-DNA was negative and patients were classified as class A or B according to the Child-Pugh classification. Cirrhosis was accompanied by hypersplenism with light or moderate varicose veins of the lower esophagus, and the fibrosis level in the spleen was III. Color Doppler ultrasound was used before surgery and 8 years after surgery to measure the size of the spleen. The preoperative long diameter was 48 ± 5 cm, the transverse diameter was 30 ± 4 cm, and the pachy-diameter was 10 ± 2 cm. The post-operative long diameter was 11 ± 1 cm, the transverse diameter was 7 ± 1 cm, and the pachy-diameter was 4 ± 1 cm. Spleen organization was obtained by operating on the splenomegaly group. Patients who underwent splenic puncture to check for spleen organization 8 years after surgery formed the residual spleen group. Thirteen patients (7 males and 6 females; mean age, 30 years; age range, 28-37 years) who underwent splenectomies, but who did not have splenomegaly formed the control group. Guided by color Doppler ultrasound, a hollow-needle biopsy was used to obtain samples of residual splenic tissue.

*Methods*

*Elastic-van Gieson staining, and immunohistochemistry:* Thirty-nine tissue samples were collected. Specimens were fixed in 10% formalin, dehydrated, embedded, and sliced. Each specimen was made into 15 slices (5 slices per

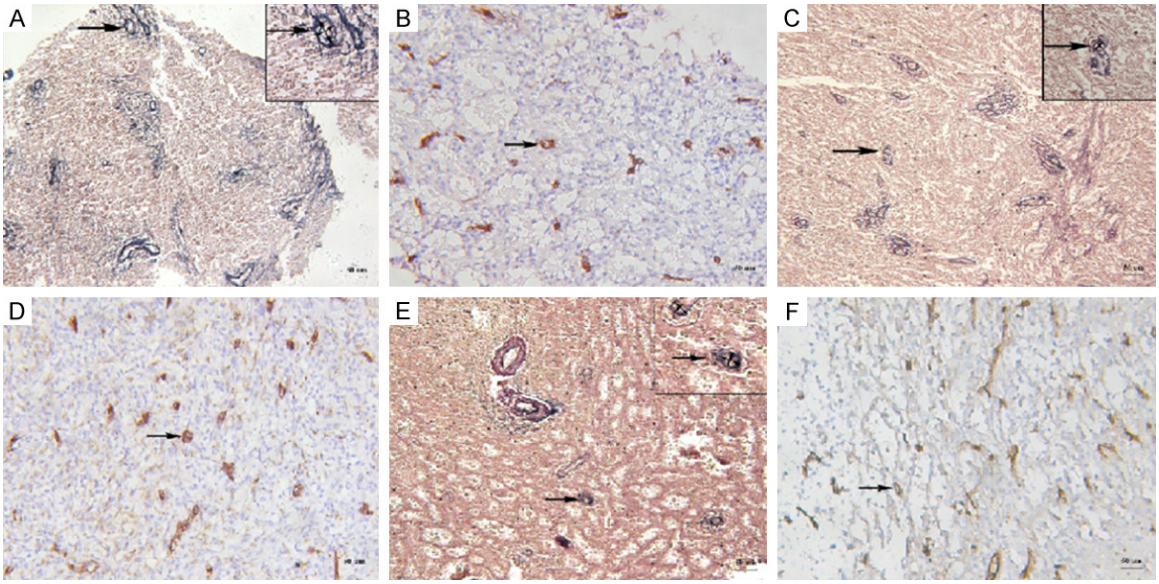


**Figure 1.** A: Penicillar arterioles (PAs) in residual spleen (arrow) (EVG staining, × 200 magnification, Insert, × 400 magnification). B: PAs in residual spleen (arrow) (immunohistochemistry for CD34, × 200 magnification). C: PAs in splenomegaly (arrow) (EVG staining, × 200 magnification, Insert, × 400 magnification). D: PAs in splenomegaly (arrow) (immunohistochemistry for CD34, × 200 magnification). E: PAs in normal spleen (arrow) (EVG staining, × 200 magnification, Insert, × 400 magnification). F: PAs in normal spleen (arrow) (immunohistochemistry for CD34, × 200 magnification).

group). Elastic-van Gieson (EVG) staining and immunohistochemistry for CD34 in the tissue sections were performed. Immunohistochemistry was performed as follows: antigen retrieval was performed using EDTA (pH = 8.5), 0.3% hydrogen peroxide, and goat serum to block the non-specific antigen. The Immunohistochemical SP method was performed; sections were then incubated overnight at 4°C with primary antibody (rat anti-human CD34 monoclonal antibody to label the endothelial cells), followed by incubation at room temperature for 30 min with biotinylated secondary antibody with DAB as the substrate, and stained with hematoxylin. The negative control group was studied using the same steps described above, but primary antibody was used instead of PBS.

*Quantitative assessment of PA:* After EVG staining, the immunohistochemical method (CD34) was used to identify PAs and slices were observed under light microscopy (BX51; Olympus, Tokyo, Japan) for histomorphologic analyses. Under light microscopy (× 400), Image-Pro Plus (Media Cybernetics, Silver Spring, MD) was used to calculate the PA counts. Five effective visual fields of each section in the red pulp were selected, the counts and section areas of PAs per pixel area in the aim area were calculated, and the mean was counted. Selection criteria for measuring the region were as follows: PA diameter, 6-50 μm; The internal elastic membrane and/or smooth muscle in vessel wall were observed; and venules were eliminated (irregular lumina, diameter of 50-200 μm). The immunohistochemistry kit was purchased from Zhongshan Corporation (Beijing, China) and the EVG kit was provided by the Beisuo Biotechnology Corporation (Shenzhen, China).

One cubic millimeter of fresh specimens from the splenomegaly and residual spleen groups were obtained. The specimens were immersed into glutaraldehyde within 1 min, then fixed with osmium tetroxide, washed with buffer solution, and dehydrated in a graded series of ethanol. The specimens were then immersed in propylene oxide solution for 30 min and propylene oxide: epoxy resin (Epon 812) for 2 h, embedded in epoxy resin, then placed into an oven at 45°C for 12 h, then 65°C for 48 h. Semi-thin sections (0.8 μm) were prepared for observation of PA location under light microscopy. Specimens were then made into ultrathin (70 nm) slices. The slices were immersed in a uranyl acetate saturated solution for 30

**Table 1.** Comparison of the density and average cross-sectional areas of PA among the three groups (n = 13, means ± SD)

| | Density (pieces/0.15 mm$^2$) | Average cross-sectional areas (pieces/μm$^2$) |
|---|---|---|
| Residual spleen | 7.63 ± 0.13[a] | 144.32 ± 16.53[c] |
| Splenomegaly | 9.53 ± 0.15[b] | 323.52 ± 99.31[d] |
| Control | 4.64 ± 0.15 | 185.01 ± 23.65 |

Compared with the splenomegaly and control group, [a]$P < 0.01$, [c]$P < 0.01$; compared with the control group, [b]$P < 0.01$, [d]$P < 0.01$.

min, and washed three times with double-distilled water for 15 min each wash. The slices were then immersed in a lead citrate solution for 15 min and washed three times with double-distilled water for 15 min each wash. The changes in PA endothelial cells (ECs) were observed using a Hitachi H-7500 transmission electron microscope.

Additionally, ultrasound and laboratory examinations were used to measure the splenic length, thickness, and average cross-sectional area, as well as hemodynamic and hematologic indices pre-operative and 8 years post-operative.

*Statistical analyses*

Data analyses were carried out using SPSS (v17.0; SPSS, Chicago, IL, USA). Values are the mean ± standard deviation (SD). Groups were compared by independent t-test and paired t-test. $P < 0.05$ was considered significant.

## Results

*Morphology and distribution of red pulp PAs*

In the residual spleen group, a diffuse distribution, tenuous elastic intima in the arterial wall, continuity in the PAs of the red pulp, surrounding elastic fibers with a lamellar or porous grid arrangement, and hyperplasia were noted under light microscopy (**Figure 1A**, **1B**). In the splenomegaly group, there was a diffuse distribution of the PAs in the red pulp, the number of PAs was increased, the elastic intima in the partial vessel wall was uneven, there was no continuity, elastic fibers had accumulated, the arrangement was disorganized, and radiating hyperplasia of surrounding elastic fibers were observed (**Figure 1C**, **1D**). In the control group, only a few PAs were distributed in the red pulp and splenic cord, and continuous elastic intima of the arterial wall was clearly observed. Hyperplastic elastic fiber was not found in the surrounding vessel wall (**Figure 1E**, **1F**).

*Quantitative analysis of red pulp PAs*

A significantly lower density of PAs was observed in the residual spleen group compared with the splenomegaly group, but a higher density of PAs compared with the control group ($P < 0.01$); the splenomegaly group also had a significant increase in density of PAs compared to the control group ($P < 0.01$). A significantly lower average cross-sectional area of PAs was noted in the residual spleen group compared with the splenomegaly and control groups ($P < 0.01$). Moreover, the splenomegaly group had a significantly greater average cross-sectional area of PAs than the control group ($P < 0.01$; **Table 1**).

*Ultrastructural changes of ECs in red pulp PAs*

An integrated nucleus and normal chromatin in ECs were observed in the splenomegaly group (**Figure 2A**). Partial swelling of the mitochondria, a light matrix, short and fewer mitochondrial cristae and even disappearance of mitochondria were observed. Partial flat mitochondrial cristae were flask-shaped and had vacuoles with a compact matrix; medullary degeneration was also noted (**Figure 2B**). In the residual spleen group, the mitochondrial matrix was uniform and decreased ruptured cristae in the PA ECs were observed under electron microscopy (**Figure 2C**, **2D**). In the control group, integrated nuclei and normal chromatin, mitochondrial cristae in a lamellar arrangement, continuous adventitia, a uniform matrix, and continuous intima were noted in ECs (**Figure 2E**, **2F**).

Changes in splenic length, thickness, and average cross-sectional areas, as well as hemodynamic and hematologic indices pre-operatively and 8 years post-operatively.

With respect to splenic length, splenic thickness, splenic average cross-sectional area, splenic artery flow volume, portal venous diameter, and portal venous flow volume, a significant difference was observed between the residual spleen and splenomegaly groups ($P < 0.001$). The platelet and leucocyte counts in

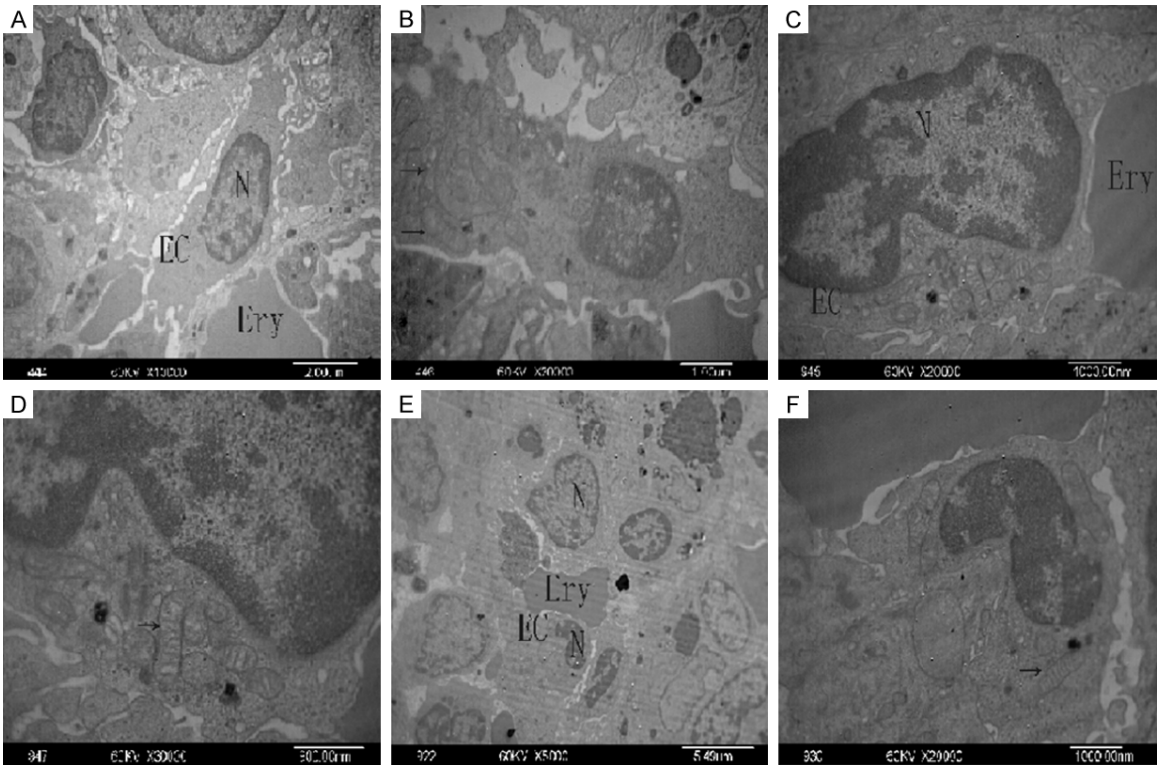


**Figure 2.** A: PA endothelial cells (ECs) in splenomegaly: an integrated cell nucleus (N), erythrocyte (Ery) (uranium-lead double staining, × 10000 magnification, Bars, 2.00 μm). B: PA ECs in splenomegaly: swelling mitochondria, shorter and fewer mitochondrial cristae (arrow) (uranium-lead double staining, × 20000 magnification, Bars, 1.00 μm). C: PA ECs in residual spleen: an integrated cell nucleus (N), erythrocyte (Ery) (uranium-lead double staining, × 20000 magnification, Bars, 1000.00 nm). D: PA ECs in residual spleen: Ruptured mitochondrial cristae, vacuolar and medullary degeneration (arrow) (uranium-lead double staining, × 30000 magnification, Bars, 900.00 nm). E: PA ECs in normal spleen: an integrated cell nucleus (N), erythrocyte (Ery) (uranium-lead double staining, × 5000 magnification, Bars, 5.49 μm). F: PA ECs in normal spleen: normal mitochondria (arrow) (uranium-lead double staining, × 20000 magnification, Bars, 1000.00 nm).

the residual spleen were increased markedly compared with the splenomegaly groups, and a significant difference was observed ($P < 0.001$; **Table 2**).

## Discussion

The mechanism underlying splenomegaly includes red pulp congestion, increased angiogenesis, fibrogenesis, hyperplasia, and hyperactivation of splenic lymphoid tissue [19]. Cavalli et al. [7] reported that enlargement in red pulp volume appears to be associated with a progressive lengthening of arterial terminals, which exhibit a length density similar to normal spleens. Moreover, a different rearrangement of the various components of the arterial tree is present; specifically, the percentage and absolute volumes of arterioles and arterial capillaries show a higher increase than sheathed capillaries. Re et al. [20] reported that arteries, arterioles, and the periarterial lymphatic sheath (PALS) in patients with congestive splenomegaly and hypersplenism have an absolute volume and total length higher than controls. Moreover, patients with congestive splenomegaly and hypersplenism present with a luminal diameter significantly lower than normal. These findings demonstrate an increase in the white pulp arterial bed in congestive splenomegaly. Freeze-cracked surfaces and vascular casts of the spleen were observed using a scanning electron microscope (SEM) observation of the circulatory system of the human spleen. PAs were located in the red pulp, and some PAs returned to the marginal zone. The PAs usually followed a straight or gently curved course among the sinuses of the red pulp and opened into cordal spaces. Occasional arteries formed a labyrinthine structure of arterial channels, which directly connected with thin sinuses. The cur-

**Table 2.** Comparison of splenic size, heamodynamic and haematology indexes between the two groups (n = 13, means ± SD)

| | Splenomegaly | Residual spleen | *P* value |
|---|---|---|---|
| Splenic length (cm) | 47.777 ± 4.85 | 11.31 ± 1.11 | < 0.001 |
| Splenic thickness (cm) | 10.00 ± 1.68 | 3.62 ± 0.51 | < 0.001 |
| Splenic square area ($cm^2$) | 387.20 ± 101.09 | 33.05 ± 7.31 | < 0.001 |
| Splenic artery flow volume (ml/kg•min) | 7.51 ± 0.63 | 2.91 ± 0.03 | < 0.001 |
| Portal venous diameter (cm) | 1.42 ± 0.05 | 1.15 ± 0.04 | < 0.001 |
| Portal venous flow volume (ml/kg•min) | 19.71 ± 0.42 | 16.45 ± 0.43 | < 0.001 |
| Leucocytes (× $10^9$/L) | 2515.38 ± 494.72 | 4292.31 ± 377.41 | < 0.001 |
| Platelets (× $10^9$/L) | 50.90 ± 6.66 | 219.60 ± 33.01 | < 0.001 |

rent study revealed that three different modes of arterial terminals are available in the human spleen, as follows: arterial openings in the marginal zone which seem significant for presentation of antigens to the white pulp; openings into the cords of Billroth, which facilitate culling and pitting of blood cells; and direct connections with sinuses (closed circulation) which account for the quick blood flow through the spleen [21, 22]. Schmidt et al. [23] demonstrated the features of microcirculatory pathways in normal human spleen, as follows: 1) "arteriolar-capillary bundles" within lymphatic nodules and extensive branching of arterioles in the marginal zone (MZ); 2) the marginal sinus around lymphatic nodules; 3) the perimarginal cavernous sinus (PMCS) outside the MZ or immediately adjacent to the nodule itself; in which the PMCS receives flow via ellipsoid sheaths and MZ or directly from arterial capillaries, and drains into venous sinuses; 4) fast pathways for flow into venous sinuses via ellipsoid sheaths; 5) arterial capillary terminations in the reticular meshwork of the red pulp or MZ ("open" circulation) in which direct connections to venous sinuses also occur ("closed" circulation), although rarely; and 6) numerous open-ended venous sinuses in the MZ. The study revealed that arterial microvascular ends were traced in splenic cords [24, 25]. It is still controversial whether or not the human spleen harbors an open or closed microcirculation or both.

In this present study, the histological features of the PAs in the residual spleens which were preserved were discovered as follows. First, tenuous elastic intima in the arterial wall and continuity of red pulp cords, surrounding elastic fibers with lamellar or porous grid arrangement and hyperplasia were seen; however, destructive changes of uneven elastic intima in partial vessel walls in patients with splenomegaly were not observed. Second, quantitative analysis revealed that a significantly lower density of PAs was observed in the residual spleen group compared with the splenomegaly group, but higher compared with the normal spleen group. The splenomegaly and control groups were in agreement with that reported in the literature [7]. The results suggest changes in the quality and quantity of red pulp PAs due to splenomegaly, i.e., destruction of the elastic intima in the arterial wall and quantity were increased. Third, a uniform mitochondrial matrix and decreased ruptured cristae in PA ECs were observed under electron microscopy in residual spleen, and vesicular and medullary degeneration of mitochondria were not seen. These findings resemble our previous study (i.e., the mitochondrial structure of splenic sinusoid ECs of red pulp in residual spleen was normal) [26]. Our results indicate that the high-pressure conditions of the splenic pulp had returned, the vascular structure can appear as a remodeling phenomenon, and such changes may ameliorate splenic function. Through analysis of hemodynamic and hematologic clinical indices, we consider that morphologic changes in residual spleen PAs may relate to pressure degradation and a decrease in blood flow volume. In patients with splenomegaly, the splenic artery stem of blood supply is widened, and pressure and blood flow volume are increased; however, in patients with residual spleen, preserving the lower pole comes from the splenic omentum and splenocolic vessels for the purpose of blood supply, which presents a lower pressure and blood flow. There is an obvious difference in hemody-

namics between splenomegaly and residual spleen. We discovered that the average cross-sectional areas of PA in the residual spleen were significantly decreased compared with splenomegaly and normal spleen. Thus, PAs of residual spleen exhibit quantitative and qualitative differences; the differences reflect changes in splenic arterial perfusion. Megalosplenia due to portal hypertension belongs to an organ of hyperkinesis and hyporesistance. Unit volume enlargement of splenic red pulp can follow the increase in number of PAs to maintain splenic density and blood supply under a pathologic state. There was a contribution from the artery-capillary direct opening between the splenic sinusoids and surface area of the splenic cords. In addition, when an arteriovenous fistula was present, splenomegaly can give rise to hypersplenism. If resecting subtotal splenic tissues, like exsecting subtotal thyroid gland tissues in patients with hyperthyroidism, arterial perfusion decreased, which affected the PAs during splenic red pulp remodeling. Because our sample size was small, a multi-center large-sample study is needed. Nevertheless, this study provided interesting information; specifically, after subtotal splenectomy in cirrhotic patients with splenomegaly due to portal hypertension, residual spleen can result in microcirculatory and functional changes.

In conclusion, subtotal splenectomy can eliminate the initiation factors of splenomegaly due to portal hypertension, improve the reconstruction of splenic capillaries, correct hypersplenism, and reserve normal splenic function.

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## Disclosure of conflict of interest

The authors indicated no potential conflicts of interest.

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