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BRIEF REPORT

# Surgical strategies to treat portal vein thrombosis during adult liver transplantation

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## Abstract

**Background** The incidence of portal vein thrombosis (PVT) at the time of liver transplantation (LT) may be variable and underestimated. Therefore, preoperative diagnosis and stratification of its extension is so relevant for adequate surgical planning. Revascularization of the portal vein graft becomes essential for graft and patient survival after LT. Early stages of PVT may be managed with eversion thrombectomy and end-to-end anastomoses. However, severe PVT (grades 3 and 4) poses significant challenges for patients requiring LT, resulting in more complex surgeries and higher complication rates. To address these complexities, various surgical techniques have been developed, including collateral alternative vessel utilization, renoportal anastomoses, mesoportal jump graft placement, cavoportal hemitranspositions, portal vein arterialization, or even multivisceral transplantation.

**Purpose** We herein describe the preoperative surgical planning as well as the different surgical strategies possible to treat portal vein thrombosis during LT.

**Conclusion** A comprehensive preoperative evaluation of PVT is crucial for accurately assessing its extent and severity. This information is vital for proper surgical planning, which ultimately prepares both the surgeon and the patient for potentially complex procedures during LT. The surgical alternatives presented in this technical report offer promising solutions for treating PVT during LT, making it a viable option for selected patients.

**Keywords** Liver transplantation · Portal vein thrombosis · Portal vein reconstruction · Liver hemodynamics · Postoperative complications

## Introduction

Portal vein thrombosis (PVT) is a common complication of end-stage liver disease and is triggered by decreased portal inflow from progressive liver cirrhosis and the increase of periportal lymphangitis and fibrosis [1]. The pathophysiology of PVT is complex and more frequent in patients with autoimmune, cryptogenic, and alcoholic cirrhosis, related to endothelial injury and thrombus formation [2]. PVT is commonly symptomless in patients with advanced cirrhosis due to splanchnic decompression through existing spontaneous portosystemic shunt. Revascularization of the portal vein graft is mandatory to ensure graft and patient survival after liver transplantation (LT). Since it is no longer an absolute contraindication for LT because of important technical innovations, surgical strategies for the revascularization of the portal vein graft depend on the extent of PVT. In 2000, Yerdel et al. [3] classified PVT into four grades according to its extent and the severity of the portal vein occlusion. Early PVT stages may resolve with eversion thrombectomy and end-to-end anastomoses [4]. However, severe PVT (grades 3 and 4) remains an intricate problem in patients requiring LT, mainly due to more complex surgeries and significantly higher complication rates [5]. Various techniques have been described to overcome these situations: collateral alternative vessel, renoportal anastomoses, mesoportal jump graft placement, cavoportal hemitranspositions, portal vein arterialization, or multivisceral transplantation (MVT). These

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strategies can be used as valuable alternatives for certain occasions during PVT.

We aim to describe the preoperative surgical planning as well as the different surgical strategies possible to treat portal vein thrombosis during LT.

## Preoperative surgical planning

The incidence of portal vein thrombosis (PVT) at the time of LT may be variable and underestimated. Therefore, preoperative diagnosis and stratification of its extension is so relevant for adequate surgical planning. Doppler ultrasonography (US) of the liver is a useful non-invasive and dynamic method modality for detecting PVT during cirrhotic patient work-up. This method can provide valuable information regarding the speed and direction of blood flow, diameter of the portal vein (PV), presence of thrombus, characterization of the vascular collateral system, and evaluation of vascular shunts. However, it is still an operator-dependent method, which can often underestimate the PVT and be difficult to categorize.

With the advent of multidetector computed tomography (MDCT), the entire vascular inflow and outflow hepatic system can be studied with high temporal and spatial resolution. The MDCT is an excellent non-invasive method for preoperative PVT staging and surgical planning and represents the most accurate conventional parameter in the characterization of PVT, with sensitivity and specificity respectively of 82% and 100% [6], as well as distinguish between bland and tumor thrombus, characterized by the enlargement of the vessel with endoluminal material which might have contrast enhancement on the arterial phase [7]. Through three-dimensional views and vascular reconstructions, PVT can be stratified, determining the extent of thrombosis in mesenteric veins (critical for surgical planning) and the identification of spontaneous portosystemic shunts (Fig. 1). Furthermore, it can show the presence collateral alternative vessels (potential inflow vascular sources) for revascularization of the portal vein graft, like the left gastric, middle colic, inferior mesenteric vein (IMV), or choledochal veins, or confirm the presence of renal-splenic shunts necessary for a succeeded renoportal anastomoses or anticipating the possibility for mesoportal jump graft placement by superior mesenteric vein (SMV) flow patency. Magnetic resonance imaging (MRI) with angiographic protocols can provide similar information to MDCT.

Preoperative cine-portogram represents an invasive method to confirm or stratify the degree of PVT and achieve a dynamic study map of the collateral venous system and the presence of portosystemic pathways (Fig. 2). It is to

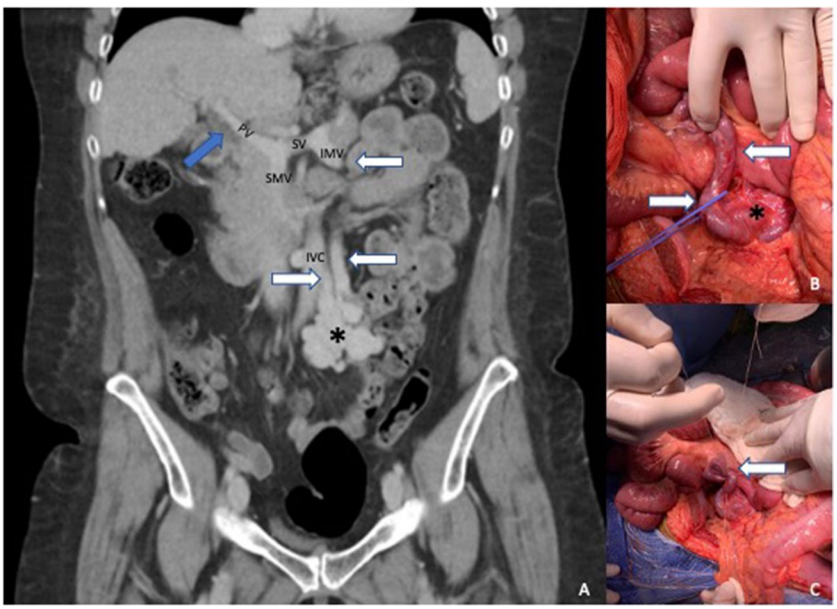


**Fig. 1** **A** MDCT coronal section in a patient with partial portal vein (PV) thrombosis (blue arrow). The white arrow marks the presence of a portosystemic shunt between the inferior mesenteric vein (IMV) and the inferior vena cava (IVC). The asterisk shows tortuous veins at the root of the mesentery in relation to the aortoiliac bifurcation with communication between the IMV and the IVC. SMV, superior mesenteric vein and SP, splenic vein. **B** Intraoperative identification of the spontaneous shunt between the inferior mesenteric vein and the inferior vena cava (white arrow). The asterisk shows tortuous veins at the root of the mesentery in relation to the aortoiliac bifurcation with communication between the IMV and the IVC. **C** Intraoperative ligation of the spontaneous mesocaval shunt, to avoid venous steal after hepatic reperfusion through the portal vein (white arrow)

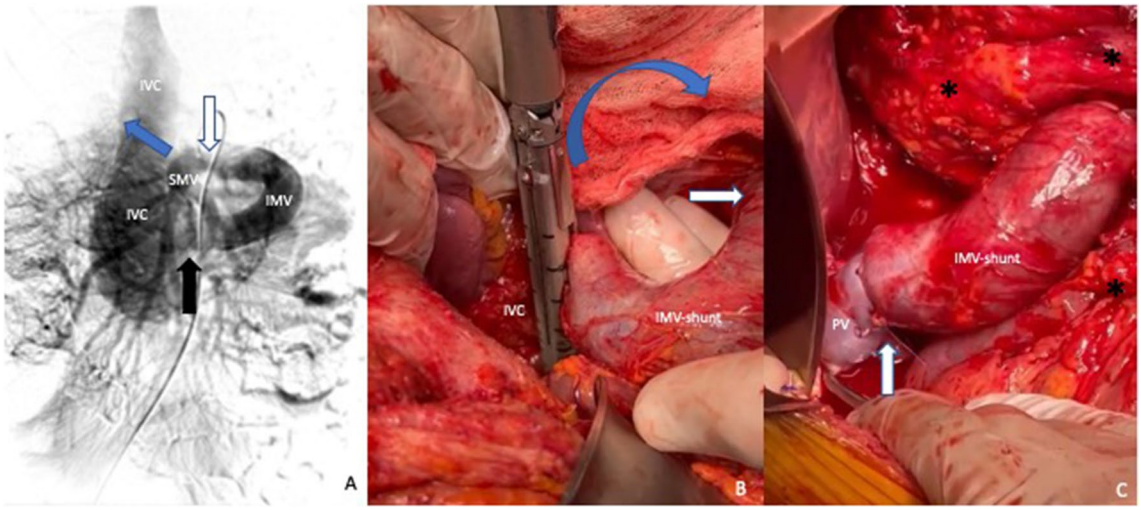


**Fig. 2** 36-year-old patient with a diagnosis of congenital liver cirrhosis, complete thrombosis of the portal vein and repeated variceal bleeding. At 15 years of age, a shunt was performed between the inferior mesenteric vein (IMV) and the inferior vena cava (IVC). **A** Selective angiographic examination of the superior mesenteric artery with portogram venous return. It shows total thrombosis of the portal vein (the blue arrow marks the place that the portal vein would occupy), patent superior mesenteric vein (SMV) with splanchnic flow derived through a large shunt between the IMV (white arrow marks the origin) and IVC (black arrow marks the outlet in the IVC). **B** Intraoperative dissection of the shunt (IMV-shunt), the white arrow marks the origin, and the use of a mechanical suture to dismantle the shunt in its distal portion. Then, liver reperfusion will be performed with the same IMV-shunt, rotating it 180 degrees (blue arrow) passing it through the transmesocolic route. **C** anastomosis (white arrow) between IMV-shunt (recipient) with portal vein (donor PV) to reperfuse the liver. IMV-shunt is noted through the transverse mesocolon (black asterisk)

highlight that intraoperative PV venography can be done to assess PV anatomy and obtain a dynamic characterization of the mesoportal and tributaries system, through the inferior mesenteric vein, the middle colic vein, or ileocolic vein. This method can demonstrate portal steal syndrome and help to take aggressive intraoperative actions, like ligation of large portal venous tributaries or dilated collateral vein (Fig. 1) or even PV stent placement [8].

## Surgical alternatives

Portal vein thrombosis is classified into 4 grades according to its extent and the severity of luminal occlusion by the thrombus [3]. Grades I and II represent < 50% and > 50% stenosis up to occlusion of the main PV trunk, correspondingly, with or without marginal extension to the SMV in both stages. Grades III and IV are characterized with more severe forms, with occlusion of the main PV and the proximal SMV for grade III and distal SMV occlusion, as well, for grade IV, without any collateral vessel. Hibi T. et al. have interestingly categorized their study based on the portal reperfusion strategy employed. They have identified two distinct variants: the "Physiological PVT Group" denotes instances where the original portomesenteric venous circulation was successfully reinstated to its natural state. Conversely, the "Non-physiological PVT Group" encompasses scenarios where the restoration of normal portal blood flow to the liver graft was not achievable. This subgroup includes procedures such as cavoportal hemitransposition, renoportal anastomosis, or portal vein arterialization.

### Surgical strategy for PVT grades 1 and 2

Eversion thrombectomy is the typical technique for removal of PV thrombus in this scenario [9, 10]. The PV should be extensively dissected to determine the extension of the PVT. The PV is clamped and transected high in the hepatic hilum and carefully retracted to permit a correct visualization and dissection of the thrombus in a plane between the thrombus and the endothelium. The PV should be flushed at end of the thrombectomy to measure optimal blood flow and remove residual clots (Fig. 3). Portal venous inflow can be improved with intraoperative ligation of large portal venous tributaries or dilated collateral veins (Fig. 1). In certain circumstances where the thrombus is well organized, the intima can be separated from the media vein wall or even may require a segmental resection of the stenotic vein tract. An end-to-end anastomosis may then be performed. Portal vein thrombosis can result in the shrinkage or hardening of the portal vein, which might require a patch to expand the vein. This procedure aims to decrease the likelihood of future thrombosis events [4]. Even more, the interposition of a cadaveric graft (iliac vein, internal jugular vein, ovarian vein) or

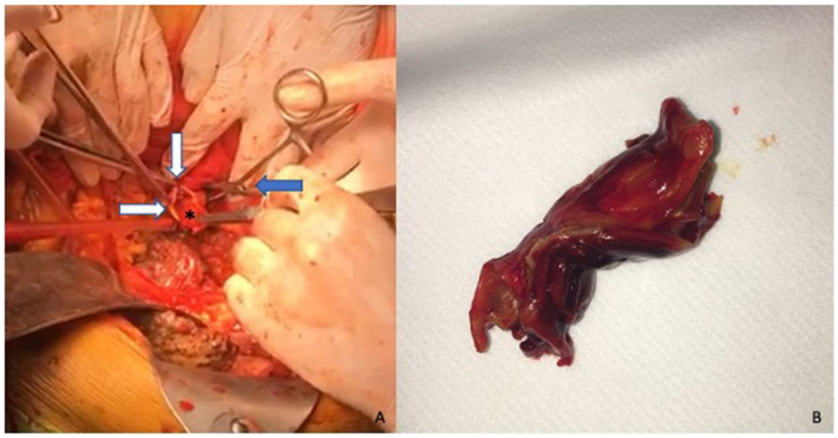


**Fig. 3** **A** Eversion thrombectomy of the portal vein. The PV is clamped (blue arrow) and transected high in the hepatic hilum and carefully retracted to allow a correct visualization and dissection of the thrombus in a plane between the thrombus (asterisk) and the PV endothelium (white arrows). The PV should be flushed at end of the thrombectomy to measure optimal blood flow and remove residual clots. **B** Organized thrombus that was removed from the portal vein

cryopreserved vein grafts or a synthetic prothesis must be taken into to avoid a tension-free anastomosis. These situations may be more frequent in patients underwent living donor liver transplantation (LDLT).

## Surgical strategy for PVT grade 3 and 4

Complete PVT is generally associated with an increased risk of complications and a poor prognosis. Until recently, many transplant centers considered the presence of a PVT grade 3–4 to be a relative or absolute contraindication for LT.

During the surgical exploration, the initial option of eversion thrombectomy should not be ruled out since in many cases imaging studies can demonstrate extensive unorganized thrombi that can be removed by this surgical strategy. If adequate thrombectomy and optimal portal flow reconstitution cannot be achieved, creating a jump graft from SMV represents a very good resource for portal inflow reperfusion. In this scenario, preoperative surgical planning with adequate imaging can guide us to achieve this strategy. The SMV can be easily located at the root of the mesentery with the help of intraoperative ultrasound. An extensive dissection of the SMV facilitates the placement of clamps to later perform an end-to-side anastomosis, with the interposition of a cadaveric graft (iliac vein, internal jugular vein, ovarian vein) or cryopreserved vein grafts or a synthetic prothesis, which are usually carried transmesolic and remain positioned in an almost physiological situation (Fig. 4). We recommend using this surgical strategy before the an-hepatic phase to reduce ischemia times and corroborate the technical feasibility. Occasionally, a jump graft from SMV may not be feasible due to the presence of a complex collateral circulation around the vessel or flow insufficiency secondary to the presence of significant shunting systems and stealing flow syndrome. At this level, the left gastric vein, middle colic

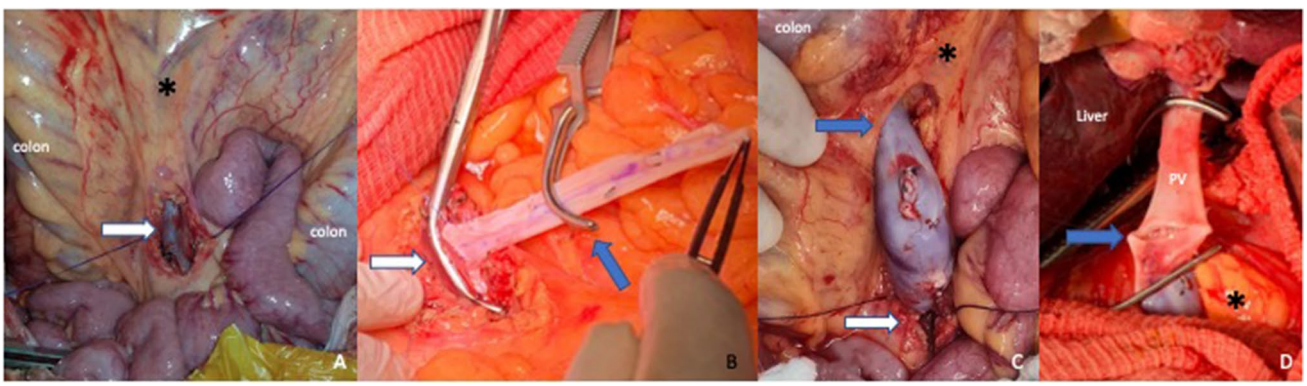


**Fig. 4** Cadaveric jump graft from superior mesenteric vein (SMV) to donor portal vein. **A** The SMV can be located at the root of the mesentery (white arrow); the asterisk marks the transverse mesocolon. **B** An extensive dissection of the VMS facilitates the placement of clamps to later perform an end-to-side anastomosis (white arrow), with the interposition of a cadaveric iliac vein graft (blue arrow), note that it is marked with blue fiber to guide the correct position of the graft. **C** The cadaveric iliac vein graft is brought into a cephalic position through a gap (blue arrow) over the transverse mesolon (asterisk) in an almost physiological situation. The white arrow shows the origin of the anastomosis between the SMV and the cadaveric graft. **D** End-to-end anastomosis between the cadaveric iliac vein graft (blue arrow) and the donor portal vein (PV) before liver reperfusion. The asterisk marks the transverse mesocolon

or choledochal veins are common examples of potential shunting systems that can be used as vascular alternatives for liver inflow reperfusion (Fig. 5). Renoportal anastomosis (RPA) in patients with a patent surgical or spontaneous splenorenal shunt represents another surgical alternative [11, 12]. The left renal vein should be controlled before total hepatectomy but clamped and divided after caval reconstruction to prevent any splanchnic congestion and to preserve an optimal renal outflow. The RPA is performed either in an end-to-end (recipient left renal vein to donor PV) fashion or through the interposition of a venous/synthetic prothesis conduit between the left renal vein and the donor PV (Fig. 6). The RPA ensures an optimal congruence of the anastomosed vessels and almost a physiologic portal flow to the graft without the need to transect the inferior vena cava. However, it may affect renal function related to congestion of the left kidney.

Portocaval hemitransposition (PCHT) represents a surgical alternative in patients with diffuse PVT (grade 4) [13, 14] without any significant collateral vessel, in which the recipient IVC is used for portal inflow to the allograft, performing a direct end-to-end or end-to-side anastomosis with the PV of the donor (Fig. 7). A consequence of PCHT is complications resulting from increased systemic venous pressure (edema of the lower torso, renal failure, massive ascites, and thromboembolism). The calibration of the retrohepatic inferior vena cava may prevent this problem whenever a recipient has a patent splenorenal shunt (either spontaneous or surgical).

Portal vein arterialization (Fig. 8) is also a useful salvage method in extreme situations since complications

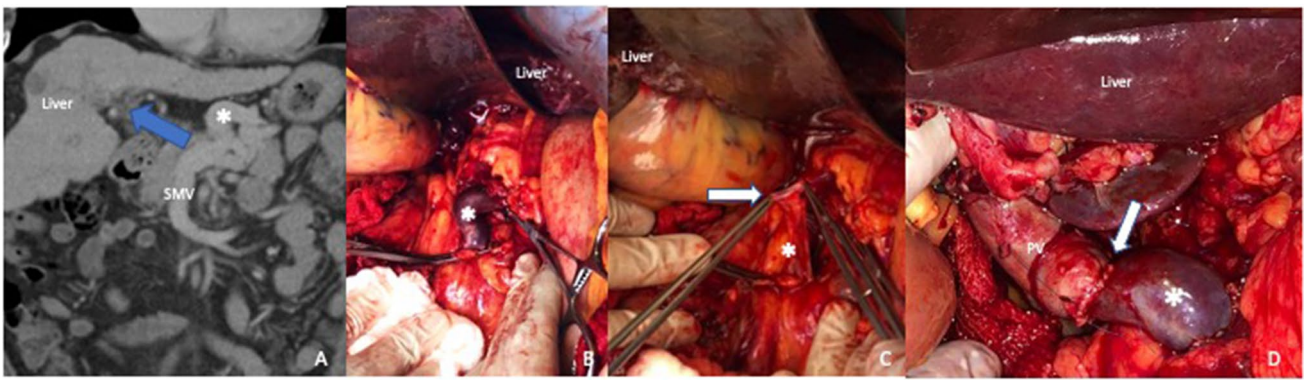


**Fig. 5** MDCT coronal section in a patient with complete portal vein thrombosis. **A** The blue arrow marks the place where the portal vein should be. SMV represents the superior mesenteric vein. The asterisk marks a dilated coronary vein that will be used to reperfuse the liver. **B** Dissection of the coronary vein (asterisk) throughout its course. **C** The coronary vein (asterisk) is sectioned in its distal portion (white arrow) and slightly rotated to be proximally anastomosed with the donor portal vein. **D** Hepatic reperfusion through the coronary vein. The white arrow shows end-to-end anastomosis between the coronary vein (asterisk) and the donor portal vein (PV)

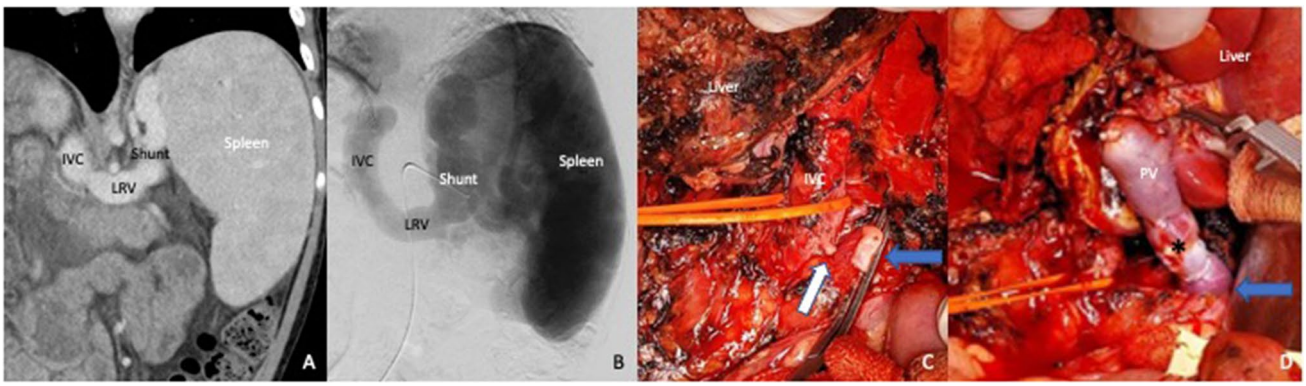


**Fig. 6** Renoportal anastomosis in a patient with complete thrombosis of the portal vein and superior mesenteric vein. **A** MDCT coronal slice shows splenorenal shunt (shunt) where all blood is drained to the inferior vena cava (IVC) via the left renal vein (LRV). **B** Dynamic angiographic study with venous return, shows splenorenal shunt (shunt) where all the blood is drained to the inferior vena cava (IVC) through the left renal vein (LRV). **C** Dissection and exposure of the infrahepatic IVC (IVC) that is repaired with an orange vessel loop. The white arrow marks the distal section of the left renal vein at its mouth in the IVC; the blue arrow shows the proximal portion of the left renal vein that will be used to reperfuse the liver. **D** Hepatic reperfusion through renoportal anastomosis. The blue arrow shows the left renal vein, anastomosed with the donor portal vein through the interposition of a cadaveric iliac vein graft (asterisk)

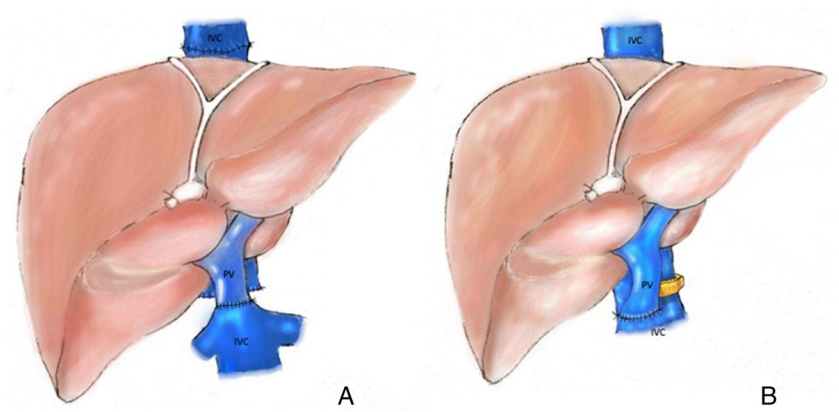


**Fig. 7** Schematics of portocaval hemitransposition. **A** The recipient inferior vena cava (IVC) is used for portal inflow to the allograft, performing a direct end-to-end side anastomosis with the donor portal vein (PV). **B** The recipient IVC is used for portal inflow to the allograft, end-to-side anastomosis with the PV of the donor. In certain cases, a partial clamping of the IVC is performed to modulate the flow (yellow mark)

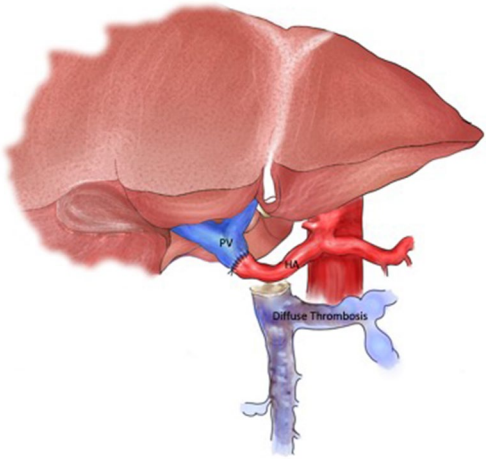


**Fig. 8** Portal vein arterialization scheme. PV indicates donor portal vein. HA, recipient hepatic artery. The schematic also shows a complete thrombosis (complete thrombosis) of the portal mesenteric axis

have been described, including right heart failure and liver allograft fibrosis [15]. Clinical data on long-term consequences are scarce and this method should be applied in particular situations.

Residual portal hypertension bleeding after these decongestive measures can be life-threatening during the postoperative period. Intraoperative splenectomy and gastric devascularization have been described as prophylaxis for postoperative bleeding with all the methods that we have described [16]. However, this conduct is disputed.

Finally, MVT has also emerged as a proposal for diffuse PVT (grade 4), because liver substitution cures the underlying hepatopathy, while the healthy PV system provided by the small bowel graft cures the portal hypertension. However, in practice, MVT represents a highly complex procedure, associated with a high rejection rate, and in many countries, there are problems related to long waiting lists, restrictions of organ allocation policies, and the intrinsic risks of small bowel transplantation, make this surgical strategy difficult to implement. Despite this, Vianna et al. [17] reported patient and graft survival rates exceeding 70% at 5 years in 25 recipients with stage 4 PVT after MVT.

Table 1 resumes the advantages and disadvantages according to the different surgical strategies.

Regardless of the technique used to treat portal vein thrombosis, control of vascular flow is important during the intraoperative period. It can be carried out through non-invasive methods (US or flow meter) or invasive interventions (intraoperative portogram). In situations where there is a suspicion of vascular steal syndrome, utilizing an intraoperative portogram becomes particularly valuable as it enables accurate identification of the underlying cause, thereby facilitating targeted interventions as needed [8].

### Postoperative assessment

Doppler ultrasonography should be performed daily in the intensive care unit (ICU) and twice weekly once the patient was transferred to the ward. Anticoagulation can be started in ICU as soon as permitted by the clinical status (no evidence of postoperative bleeding), coagulation tests, and platelet counts. The rate of low molecular weight heparin can be adjusted according to the patient's weight and renal function. After discharge, transplants patients should receive long-term administration of aspirin (250 mg/d) as a prophylaxis against arterial thrombosis. The patency of anastomosis can be checked with US once a month, during the first 6 months, and if there is any diagnostic doubt, a MDCT is recommended.

## Results

From January 2012 to December 2021, 374 adult LT were performed at Hospital Italiano de Buenos Aires. During this period, 45 patients (12%) with grade 1 to 4 PVT received transplants; most PVT cases were managed via thrombectomy and end-to-end anastomosis. A dilated recipient left coronary vein was used in 6 patients. In one patient, hepatic

**Table 1** Advantages and disadvantages according to the different surgical strategies

| Surgical strategy | Advantages | Disadvantages |
|---|---|---|
| Eversion thrombectomy | - Typical technique for PVT grades 1 and 2<br>- Effective for non-organized thrombi<br>- Relatively simpler | - Limited applicability for PVT grades 3 and 4<br>- Ineffective for extensive or unorganized thrombi |
| Jump graft from SMV | - Maintains portal inflow using SMV as graft source<br>- Suitable for extensive organized thrombi | - Requires preoperative imaging and planning<br>- Possible challenges in identifying suitable SMV location |
| Alternate shunting systems | - Utilizes collateral vessels (e.g., left gastric, middle colic) for portal inflow<br>- Provides multiple technical options | - Presence of collateral circulation may vary<br>- May lead to altered vascular dynamics and congestion |
| Renoportal anastomosis | - Maintains almost physiological portal flow<br>- Avoids inferior vena cava transection<br>- Good vascular congruence | - Possible impact on renal function due to congestion<br>- Surgical complexity and potential for complications |
| Portocaval hemitransposition | - Uses recipient IVC for portal inflow<br>- Suitable for diffuse PVT without collateral vessels | - Increased systemic venous pressure-related complications<br>- Potential edema, renal failure, and thromboembolism |
| Portal vein arterialization | - Salvage method in extreme cases | - Complications like right heart failure and liver allograft fibrosis<br>- Limited long-term data on outcomes |
| Multivisceral transplantation | - Cures hepatopathy and massive portal hypertension<br>- Effective in some cases (complexity and availability vary) | - Highly complex procedure with a high rejection rate<br>- Limited implementation due to risks and organ allocation |

*PVT*, portal vein thrombosis; *SMV*, superior mesenteric vein; *IVC*, inferior vena cava

reperfusion was performed through the IMV. In addition, 14 patients required an extra-anatomical mesoportal jump graft and 4 patients received a non-physiological PV reconstruction, represented by RPA.

Thirty-two patients (72%) experienced postoperative complications. According to the Dindo–Clavien classification of surgical complications [18], severe morbidity (≥ IIIb) occurred in 16 patients (36%). Two patients died during the postoperative period. The first at 53 days after RPA related to septic complications and multiple organ failure and another patient whose liver had been reperfused via a coronary vein died 3 days after transplantation with primary graft failure.

## Discussion

The prevalence of PVT at the time of LT in patients with end-stage liver disease varies between 5 and 26% [7, 19]. The pathophysiology of pretransplant PVT is complex and the risk factors include male sex, race/ethnicity, a body mass index > 40 kg/m$^2$, diabetes mellitus, metabolic syndrome, nonalcoholic steatohepatitis, autoimmune hepatitis, hepatocellular carcinoma, and previously placed transjugular intrahepatic portosystemic shunt [20, 21]. PVT is commonly symptomless in patients with advanced cirrhosis due to splanchnic decompression through existing spontaneous portosystemic shunt [1].

Revascularization of the portal vein graft is mandatory to ensure graft and patient survival after LT. In the past, PVT used to be considered an absolute contraindication for transplantation due to its association with higher risk of complications and poor prognosis [5]. With the development of sophisticated surgical techniques and improved understanding of PVT management, the possibility of successful transplantation for patients with complete or diffuse PVT is becoming more realistic. This marks a significant advancement in the field, offering hope to those who were previously considered ineligible for transplantation due to this condition [5]. Accurate preoperative diagnosis and staging of PVT extension are crucial for determining the feasibility of transplantation and selecting the appropriate surgical approach [3]. Various non-invasive imaging modalities have been utilized to assess PVT, including US, MDCT, and MRI with angiographic protocols. Invasive methods like preoperative cine-portogram and intraoperative PV venography provide dynamic characterization and detailed mapping when needed [8, 22]. An integrated approach, combining the strengths of these imaging modalities, can significantly contribute to improved surgical planning and better outcomes in liver transplantation patients with PVT. In this manuscript, we present a comprehensive discussion on the surgical alternatives available for PVT grades 1 to 4, according to its extent and the severity of luminal occlusion by the thrombus [3]. We explore the current literature on each technique, highlighting their advantages, disadvantages, and potential complications. In grades 1 and 2, eversion thrombectomy is the standard technique for thrombus removal [9, 10]. We discuss the surgical steps involved, including portal venous

inflow improvement with ligation of large tributaries or collateral veins. Additionally, we explore the cases where segmental resection of the stenotic vein tract or the use of interposition grafts may be necessary, particularly in patients who underwent living donor liver transplantation. For grades 3 and 4, the management has evolved significantly. While eversion thrombectomy remains an option in selected cases, we delve into alternative strategies, such as creating a jump graft from the SMV. Furthermore, we discuss the use of complex collateral vessels like the left gastric vein, middle colic, IMV, or choledochal veins as vascular alternatives for portal inflow reperfusion. Renoportal anastomosis and PCHT are valuable surgical techniques for managing grade 3 and 4 PVT cases. While they can effectively restore portal blood flow and reduce portal venous pressure, they come with their specific benefits and potential complications [5, 11, 12]. We emphasize the importance of preoperative planning and imaging in guiding this approach [22]. It is crucial to address the complications associated with posterior PCHT that arise due to heightened systemic venous pressure. These complications can include edema of the lower torso, renal failure, massive ascites, and thromboembolism [5, 13, 14, 16]. To mitigate these issues, it is essential to focus on calibrating the retrohepatic IVC, particularly in recipients who possess a patent splenorenal shunt, whether it was created spontaneously or through a surgical procedure [5, 13, 14, 16]. In patients with an enlarged segment 1 of the liver, particularly significant in cases involving portal thrombosis, portal cavernoma, or pronounced portal hypertension coupled with peribiliary varices and a history of upper gastrointestinal surgery, the implementation of a temporary portocaval shunt could pose potential risks or, at the very least, might not serve its primary objective of reducing blood loss effectively. An alternative approach, the passive mesenterico-saphenous shunt technique, offers a means of achieving efficient portal decompression without requiring dissection of the portal pedicle [23].

Finally, portal vein arterialization and MVT offer potential solutions for certain medical conditions; their implementation requires careful consideration of organ allocation policies and the inherent risks associated with small bowel transplantation. Ongoing research and collaboration are necessary to ensure these approaches are used judiciously and ethically to benefit patients in need [15, 17].

## Conclusion

This comprehensive surgical manuscript highlights the evolving landscape of PVT management in adult LT patients. Previously considered a contraindication for transplantation, the advances in surgical techniques and improved understanding of PVT have opened new possibilities for successful transplantation in patients with diffuse PVT. Accurate preoperative diagnosis and staging of PVT extension are crucial for determining the feasibility of transplantation and selecting the appropriate surgical approach. The surgical alternatives presented in this technical report offer promising solutions for treating PVT during LT, making it a viable option for selected patients.

**Authors' contributions** The concept of the study was derived from M.D.S. This study was designed by M.D.S., V.A., J.M.U., J.M., E.D.S., J.P. The article was written by M.D.S. M.D.S. and J.W.U. prepared all figures. M.D.S., V.A., J.M.U., J.M., E.D.S., J.P. critically revised the manuscript. All authors have read and approved the manuscript.

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## Declarations

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Competing interests** The authors declare no competing interests.

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