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COMBINED MACHINE PERFUSION AND ISCHEMIA-FREE IMPLANTATION OF LIVERS FROM HIGH-RISK DONORS: FIRST EXPERIENCE IN RUSSIA

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Objective: to assess the efficacy and safety of combining sequential dual hypothermic oxygenated machine perfusion (DHOPE), controlled oxygenated rewarming (COR), and normothermic machine perfusion (NMP) with ischemia-free liver implantation (IFLI) for transplants obtained from high-risk expanded criteria donors (ECDs). **Materials and methods.** The study presents two cases of liver transplantation performed using the combined DHOPE-COR-NMP-IFLI protocol in May and June 2025 at the Shumakov National Medical Research Center of Transplantology and Artificial Organs. Liver allografts were procured from brain-dead ECDs. Perfusion was carried out using a cardiopulmonary bypass (CPB) machine following a period of static cold storage (SCS). The allografts were subsequently transplanted into recipients under continuous NMP after meeting viability criteria. The reproducibility and safety of the IFLI approach within the combined protocol were evaluated through descriptive analysis of donor characteristics, perfusion parameters, and intra- and postoperative outcomes in the recipients. **Results.** In both cases, the grafts met the established viability criteria despite pronounced macrovesicular steatosis (95% and 80%, respectively). In Case No. 1, all viability parameters were achieved after 4 hours of NMP. In Case No. 2, lactate clearance was suboptimal, reaching the acceptable threshold of 4.1 mmol/L only after 6 hours of perfusion. No post-perfusion syndrome or hemodynamic instability occurred in either recipient during graft reperfusion. Both recipients met the criteria for early allograft dysfunction, with cytolysis levels of 6562.9 and 1610.4 U/L, and 3822 and 2662 U/L, respectively. The recipients were discharged on postoperative days 17 and 34 without serious complications (Clavien–Dindo ≥IIIb). At 4- and 5-month follow-up, no transplant- or preservation-related complications were observed. **Conclusion.** The combined application of sequential machine perfusion (DHOPE-COR and NMP) with IFLI is a safe and effective dynamic preservation strategy. This approach enables the successful use of liver grafts from ECDs by minimizing ischemia–reperfusion injury.

Keywords: liver transplantation, machine perfusion, early graft dysfunction, expanded criteria donors.

INTRODUCTION

Expanding donor criteria is an effective strategy to address the growing gap between the number of organs available for transplantation and the number of patients in need [1, 2]. While broadening allograft eligibility helps reduce waiting-list mortality and partially mitigates organ shortages, it is also associated with less favorable transplant outcomes [3–5]. In this context, machine perfusion has emerged as a valuable tool, enabling the "resuscitation" of suboptimal organs affected by ischemia-reperfusion injury (IRI) and providing an opportunity to assess organ viability prior to transplantation [6–8].

Combined machine perfusion protocols, designed to compensate for the weaknesses of each individual technique, still leave one important challenge unresolved: the need for repeated static cold storage (SCS) after a normothermic machine perfusion (NMP) session, immediately before implantation [12]. The resulting "re-cooling" injury is becoming increasingly significant, especially as donor organs become more marginal. This sequence of repeated temperature fluctuations – re-cooling, brief cold ischemia, subsequent warm ischemia, and final reperfusion – creates additional stress on an already vulnerable graft [9–11].

Cirelli et al. describe two cases of liver transplantation from non-heart-beating donors with severe macrovesicular steatosis (>60% and >30%) after combined perfusion. Although both organs met the center's stringent viability criteria, one recipient required retransplantation

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on postoperative day 17, and the other developed acute kidney injury and acute respiratory failure. The authors emphasize that transplantation of severely steatotic livers remains a "risky endeavor", as their post-implantation behavior may be unpredictable even when NMP viability parameters appear acceptable [13].

Similarly, Patrono et al. report two cases of primary graft dysfunction (PGD) in allografts with 30% and 50% macrovesicular steatosis following NMP. Both grafts exhibited insufficient lactate clearance despite meeting other viability thresholds. The authors conclude that although transplantation of liver allografts with >30% steatosis may be feasible, viability assessment remains challenging, and liberalization of criteria can result in primary non-function [14, 15].

Based on studies on ischemia-free liver transplantation (IFLT), it can be assumed that complete elimination of ischemia enables the safe use of allografts with even 80–90% steatosis, with minimal injury to the graft. For centers where a full IFLT protocol is not technically feasible, reducing the procedure to ischemia-free liver implantation – i.e., omitting the donor-side perfusion stage – may represent a promising but insufficiently investigated compromise [16].

However, the studies published to date have employed isolated NMP after a preceding period of cold ischemia (an end-ischemic approach). This strategy inherently exposes the liver to IRI and does not provide adequate protection against the cumulative effects of SCS and subsequent reperfusion [17, 18].

In contrast, the use of a combined perfusion protocol incorporating dual hypothermic oxygenated perfusion (DHOPE) as a post–cold storage "resuscitation" stage, controlled oxygenated rewarming (COR) as a transitional phase between two temperature regimes, and NMP for comprehensive viability assessment, enables the relatively safe transplantation of high-risk allografts – including those from non-heart-beating donors – without compromising clinical outcomes [12].

Integrating the strengths of both approaches, namely DHOPE–COR–NMP sequence and the IFLI principles, would allow us to get the most out of each technique and ensure the safe transplantation of high-risk liver allografts. To date, however, we have not identified any studies investigating the combined application of these techniques.

DESCRIPTION OF CLINICAL OBSERVATIONS

In all reported cases, the liver allografts were referred to Shumakov National Medical Research Center of Transplantology and Artificial Organs (Shumakov Center) after being rejected by other transplant centers in accordance with the national allocation algorithm. The preservation protocol included the initial DHOPE, followed by COR, and concluded with NMP for viability assessment (the DHOPE–COR–NMP protocol). All grafts fulfilled the modified viability criteria established at Shumakov Center (Table 1), and were therefore deemed suitable for transplantation. Given the high-risk nature of these allografts and their individual characteristics, IFLI was selected as the optimal approach, instead of the conventional transition to SCS prior to implantation.

Combined sequential perfusion was carried out using the method we described earlier, which employs a cardiopulmonary bypass (CPB) machine and a custom perfusion circuit developed at Shumakov Center to ensure seamless perfusion, assembled from a standard CPB pump tubing set [19]. A modified histidine–tryptophan–ketoglutarate (HTK) solution served as the perfusate during the hypothermic phase (DHOPE + COR), while an erythrocyte-based perfusate was used during the NMP phase.

For perfusate drainage during IFLI, additional cannulation of the inferior vena cava (IVC) was performed via the subhepatic segment using 32–34 Fr cannulas.

Table 1

Criteria for liver graft viability during normothermic machine perfusion

Criteria for liver graft viability (assessment after 4–6 hours of normothermic perfusion)
Perfusate lactate <**4.5 mmol/L** after 4 hours of perfusion **OR**: **Stable decrease in lactate** with lactate **<4.5 mmol/L** after 6 hours
Presence of bile production AND: – pH difference (bile – perfusate) >**0.05, with bile pH ≥7.48**; – HCO_3^- difference (bile – perfusate) >**3.0 mmol/L, with bile HCO_3^- >18 mmol/L** – **(**Glucose difference (bile – perfusate) **<3.0 mmol/L** OR bile/perfusate glucose ratio **<0.67**) AND bile glucose **<16 mmol/L**
At least two of the following criteria:
Perfusate **pH >7.3** without continuous $NaHCO_3$ infusion;
Active **perfusate glucose metabolism** (reduction of high glucose levels and stabilization)
Stable hemodynamics: portal flow >**500 mL/min**, arterial flow >**150 mL/min**;
Homogeneous graft perfusion and soft parenchymal consistency

The suprahepatic segment of the vena cava was occluded with a Bulldog vascular clamp, leaving an adequate cuff to facilitate subsequent formation of caval anastomosis.

Ischemia-free liver implantation

Liver implantation during ongoing normothermic perfusion has been previously described by several authors and has remained largely unchanged since its introduction into clinical practice at Shumakov Center [10, 11]. At present, IFLI can be performed only with either the classical caval reconstruction involving replacement of the IVC or the standard technique with IVC preservation as described by A. Tzakis.

Before implantation, complete vascular integrity and hemostasis of the liver allograft parenchyma were confirmed. This step is essential, as any perfusate leakage could necessitate urgent volume replacement and potentially require temporary cessation of perfusion due to insufficient perfusate levels in the cardiotomy reservoir.

Following hepatectomy and preparation of the recipient's IVC cuff, the allograft was transferred into the abdominal cavity under continuous perfusion (Fig. 1).

Particular attention was given to the precise positioning of the Bulldog vascular clamp prior to initiating anastomosis formation, as improper placement could significantly complicate this stage of the procedure. The suprahepatic vena cava (or hepatico-caval) anastomosis was then constructed using the standard technique (Fig. 2).

Upon completion of the suprahepatic vena cava anastomosis, the Bulldog clamp was removed from the donor segment of the IVC to assess the integrity and hemostasis of the anastomosis.

Next, the portal vein was occluded with a vascular clamp, and portal perfusion was discontinued. From this point onward, perfusion of the allograft proceeded exclusively through the hepatic arterial system. A standard portal vein anastomosis was then performed (Fig. 3).

Next, following the same approach used for the caval anastomosis, the clamp was removed from the donor segment of the portal vein to verify the integrity and tightness of the anastomosis prior to reperfusion.

IVC decannulation was performed before initiating reperfusion, as this ensured a safe procedure without blood loss. To expose the retrohepatic portion of the IVC

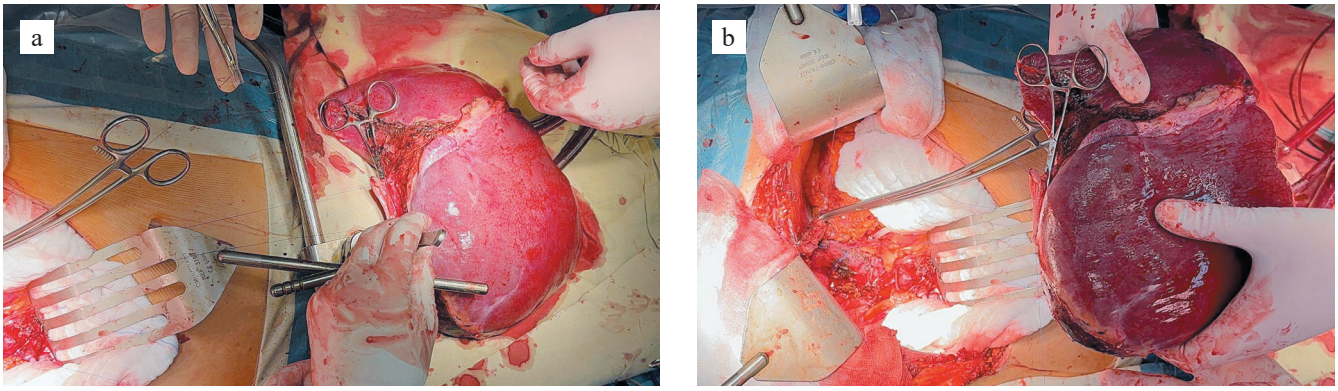

Fig. 1. (a) Liver allograft during ongoing normothermic machine perfusion; (b) preparation for ischemia-free liver implantation

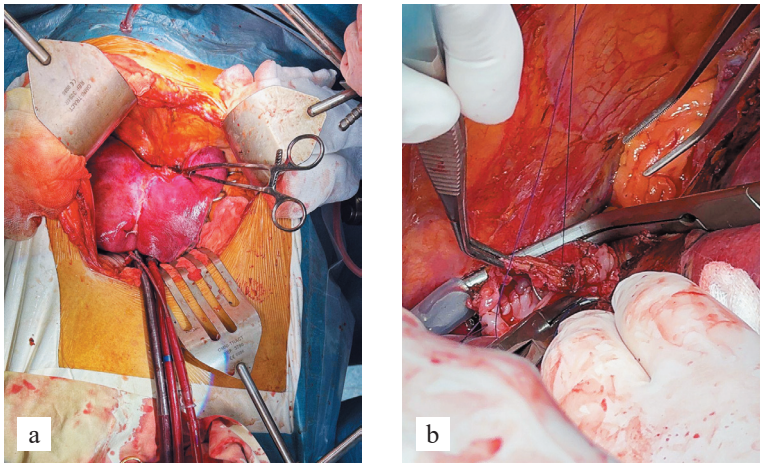

Fig. 2. (a) Beginning of the IFLI procedure; (b) caval anastomosis phase

and the cannula positioned in its subhepatic segment, the right lobe of the liver was gently rotated to the left. The opposing edges of the vein were then secured using mosquito clamps or other appropriate instruments; ligature holders could also be used. A stapling device or vascular clamp was pre-positioned on the subhepatic stump of the IVC, depending on the selected method for closing the stump or completing the caval reconstruction (Fig. 4).

In the observations described, a stapling device was used, as it minimized the duration of warm ischemia that would otherwise occur during manual suturing of the subhepatic portion of the IVC. Typically, due to tissue adhesion to the cannula, a small amount of traction was required to achieve IVC decannulation. Immediately after removal of the cannula, the IVC stump was either sutured or secured with a vascular clamp, and arterial perfusion was discontinued. The right lobe of the liver was then repositioned anatomically, followed by graft reperfusion and decannulation of the hepatic artery. From this point onward, liver transplantation proceeded according to the standard surgical technique.

Post-transplant period

The postoperative management included an initial stay in the intensive care unit for up to 12 hours, after which patients were transferred to a specialized transplant unit for early rehabilitation in accordance with the local protocol. All recipients received standardized medical care based on the center's established guidelines, which included:

– Administration of a pulse dose of methylprednisolone with subsequent rapid withdrawal or transition to oral therapy in patients with high immunological risk. In such cases, therapy was supplemented with basiliximab or thymoglobulin depending on the patient's immunological risk profile.
– Early initiation of calcineurin inhibitors with targeted trough levels of 6–8 ng/mL, along with mycophenolic acid, provided that complete blood count parameters were within acceptable limits.
– Routine ultrasound examination of the liver graft with Doppler flowmetry, as well as biochemical tests, complete blood count, coagulation profile, and calcineurin

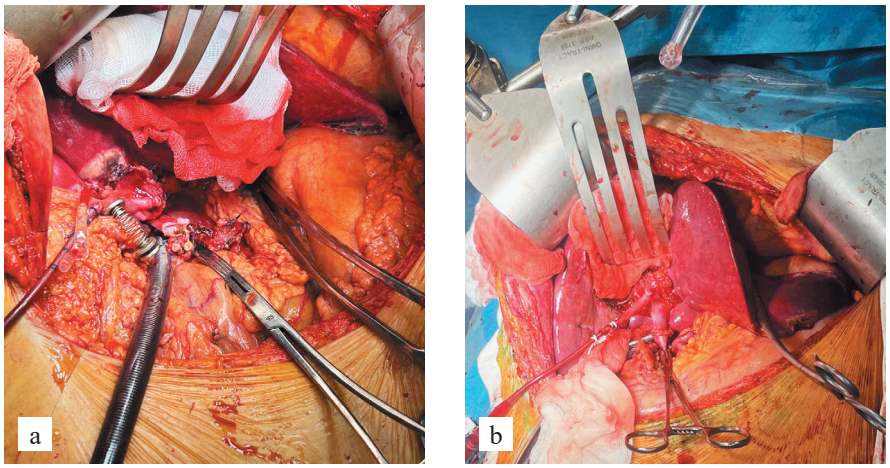

Fig. 3. IFLI procedure: (a) formation of (a) and formed (b) portal vein anastomosis

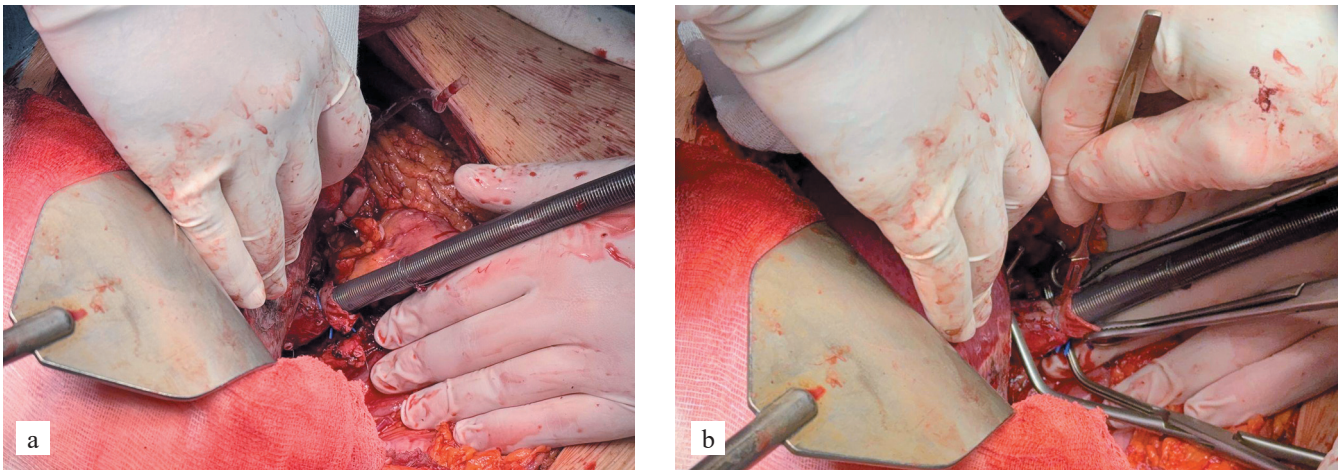

Fig. 4. (a) Visualization of the subhepatic section of the inferior vena cava with a return cannula and (b) its subsequent decannulation

inhibitor level measurement. These evaluations were performed daily during the first postoperative week, three times per week during the second week, and subsequently as clinically indicated.

An important component of postoperative management was intensive perioperative and postoperative monitoring. The following definitions were used as the primary criteria for identifying potential post-transplant complications:

– Acute graft rejection was suspected based on laboratory signs of increased cytolysis and cholestasis after excluding other potential problems, including vascular complications. The diagnosis was confirmed by percutaneous biopsy.
– Early liver graft dysfunction was determined according to the criteria proposed by K. Olthoff [20], defined as the presence of at least one of the following:
  - Serum bilirubin >171 μmol/L on postoperative day (POD) 7;
  - International normalized ratio (INR)> 1.6 on POD 7;
  - Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2000 IU/L within the first 7 postoperative days.
– Primary graft failure was diagnosed in accordance with UNOS criteria [22], acute kidney injury was defined using KDIGO criteria [21].
– Ischemic cholangiopathy (IC) was diagnosed based on a combination of clinical symptoms (pruritus, jaundice), laboratory indicators of cholestasis (elevated gamma-glutamyl transferase and alkaline phosphatase), and instrumental findings (magnetic resonance cholangiopancreatography, MRCP). If clinical or laboratory abnormalities were present, instrumental examination was performed to confirm or exclude IC. In cases where instrumental signs of IC were present without accompanying clinical or laboratory abnormalities, the condition was classified as asymptomatic IC.
– Post-reperfusion syndrome was defined according to Aggarwal et al. [32] and Hilmi et al. [33] as a ≥30% decrease in mean arterial pressure lasting more than 1 minute within the first 5 minutes after reperfusion, the occurrence of asystole or hemodynamically significant arrhythmias (such as ventricular fibrillation), or the need to initiate vasopressor therapy during or immediately after reperfusion.

Characteristics of donors and liver allografts

Characteristics of donors and liver allografts are summarized in Table 2.

Macroscopic appearance of the liver before perfusion is shown in Fig. 5 (a – case No. 1, b – case No. 2).

Recipient characteristics

Transplants were performed in recipients who were compatible with donors according to the ABO blood group system and anthropometric parameters. Patients with high surgical or anesthesiological risk, those requiring emergency or urgent transplantation, and individuals with a high MELD score of 3.0 (>20 points) were excluded from consideration.

Case 1. Patient G., female, 34 years old, with liver cirrhosis secondary to autoimmune hepatitis and primary sclerosing cholangitis. BMI: 20.1; MELD 3.0: 16 points.

Case 2. Patient B., male, 51 years old, with liver cirrhosis due to HBV infection. BMI: 24.7; MELD 3.0: 13 points.

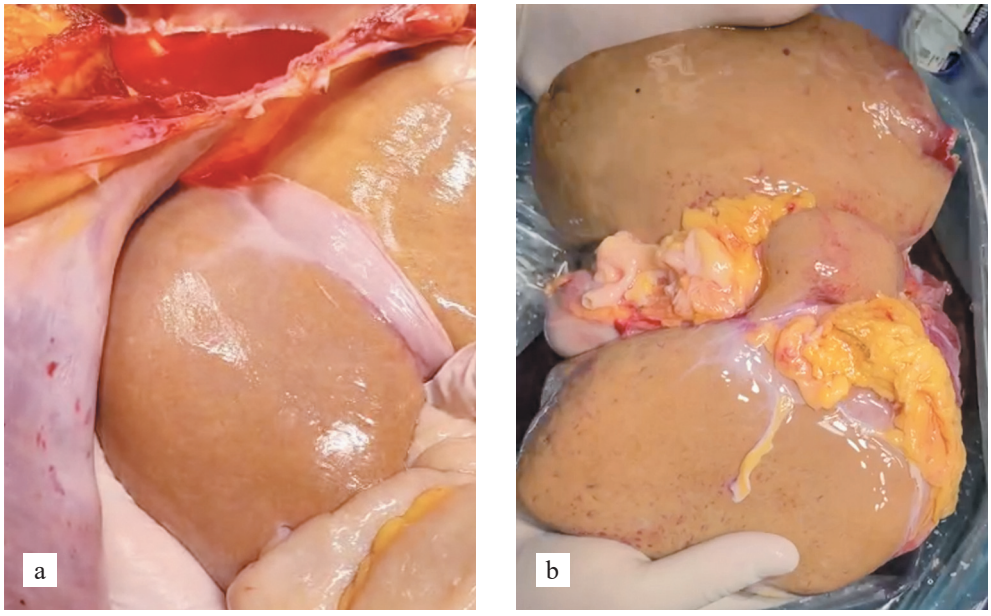

Fig. 5. Liver allografts before initiation of perfusion: (a) case No. 1 and (b) case No. 2

Perfusion parameters and viability assessment

The dynamics of the perfusate and bile parameters during NMP are presented in Fig. 6.

Combined perfusion was performed using a previously described technique [12], consisting of at least 90 minutes of DHOPE followed by a minimum of 60 minutes of COR, during which the liver allograft was gradually

Table 2

Main characteristics of recipients, donors, and perfusion

	Case 1	Case 2
	Donor	
Age	55	63
Gender	Male	Male
BMI	44.2	32.1
Donor type	DBD	DBD
Time in ICU	3 days	1 day
Donor Risk Index (DRI)	1.636	1.6
AST, U/L	143	38
ALT, U/L	86.2	31
Sodium, mmol/L	138	140
Total bilirubin, mmol/L	14.2	6.3
Creatinine, mmol/L	92.9	142
Macroscopic findings	Dense consistency, marked steatosis and fibrosis	Extremely soft consistency, marked steatosis
Microscopic findings	Macrovesicular steatosis 95%, liver fibrosis F-1 (METAVIR)	Macrovesicular steatosis 80%
	Transplant	
SCS, min	152	230
Weight before perfusion, g	1700	1930
Weight at the end of perfusion	1750	2300
	Perfusion	
DHOPE time, min	111	105
COR time, min	80	79
NMP time, min	707	595
IFLI time, min	70 *(as part of NMP)*	30 *(as part of NMP)*
Total perfusion time, min	898	779
Total storage time, min	1101	1009
Lactate at 4 hours, mmol/L	2.5	7.6
Lactate at 6 hours, mmol/L	3.6	4.1
	Indicators at 4 hours of perfusion and viability assessment	
ΔpH (bile–perfusate)	0.077	0.186
Bile pH	7.729	7.646
ΔHCO_3^- (bile–perfusate)	9.8	14.9
Bile HCO_3^-	43.5	36.5
Δ / ratio of glucose (bile–perfusate)	–2.5 / 0.42	–10.2 / 0.47
Bile glucose, mmol/L	1.8	9
Perfusate pH	7.652 *(without bicarbonate supplementation)*	7.46 *(without bicarbonate supplementation)*
Glucose metabolism	Yes (4.3 mmol/L)	No (19.2 mmol/L)
HA flow (mL/min)	610	420
PV flow (mL/min)	690	650
HA pressure (mmHg)	72	70
PV pressure (mmHg)	11	10
Transplanted	Yes	Yes

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; SCS, static cold storage; DHOPE, dual hypothermic oxygenated machine perfusion; COR, controlled oxygenated rewarming; NMP, normothermic machine perfusion; IFLI, ischemia-free liver implantation; HA, hepatic artery; PV, portal vein.

Fig. 6. Liver transplant perfusion parameters in case No. 1 and case No. 2

warmed to 16 °C. Viability and injury markers were not assessed during the DHOPE and COR phases.

After completing the hypothermic perfusion stages and replacing the perfusate with a red blood cell–based solution, NMP was initiated. During the first hour of NMP, the temperature was gradually increased from 20 °C to 34–36 °C, accompanied by a controlled rise in hepatic arterial and portal venous flow while maintaining target perfusion pressures [12].

Viability assessment was carried out using criteria developed and modified at the Shumakov National Medical Research Center of Transplantology and Artificial Organs, as summarized in Table 1.

Characteristics of the perfusate and key perfusion parameters are presented in Table 2.

Case 1 (Fig. 7). Despite the need for sodium bicarbonate infusion during the first hour of perfusion (a total of 250 ml) to stabilize pH, the pH showed a trend toward normalization and remained within the physiological range. The initial perfusate lactate level was 13 mmol/L, rising to a peak of 17 mmol/L at 90 minutes, followed by a gradual fall to 2.5 mmol/L by the 4-hour mark. A steady decrease in perfusate glucose levels was also observed, reaching a minimum of 4.3 mmol/L at 4 hours, which necessitated supplementation with 40% dextrose. Perfusion flows and pressures were maintained in accordance with protocol and fulfilled the center's established viability criteria.

During perfusion, bile production showed a steady increase: less than 2 ml during the first 2 hours, 4 ml in the third hour, and subsequently 6–9 ml per hour.

Throughout perfusion, bile parameters remained within the thresholds indicative of cholangiocellular viability. Cytolysis markers during DHOPE were elevated but stable, with AST levels of 2008 and 2259 U/L and ALT levels of 2776.6 and 3016.4 U/L at 60 and 90 minutes, respectively. Overall, the liver allograft satisfied all established viability criteria and was considered suitable for transplantation.

Case 2 (Fig. 8).

Stabilization of pH was achieved only by the third hour of perfusion, with a total sodium bicarbonate infusion volume of 200 ml. Initial lactate level was 11.5 mmol/L, showing two peaks – 15 mmol/L at 30 minutes and 13.5 mmol/L at 2 hours – followed by a gradual decline to 7.6 mmol/L at 4 hours and 4.1 mmol/L at 6 hours. Despite continuous insulin infusion, glucose levels progressively increased and remained at 18–19 mmol/L until the end of perfusion. Bile secretion remained minimal throughout the procedure (2–2.5 ml/hour). Cytolysis markers at 60 and 90 minutes of DHOPE were 1829.7 and 2613.9 U/L (AST) and 504.5 and 824.8 U/L (ALT), respectively. The allograft fulfilled all established viability criteria except for glucose metabolism and was deemed suitable for transplantation.

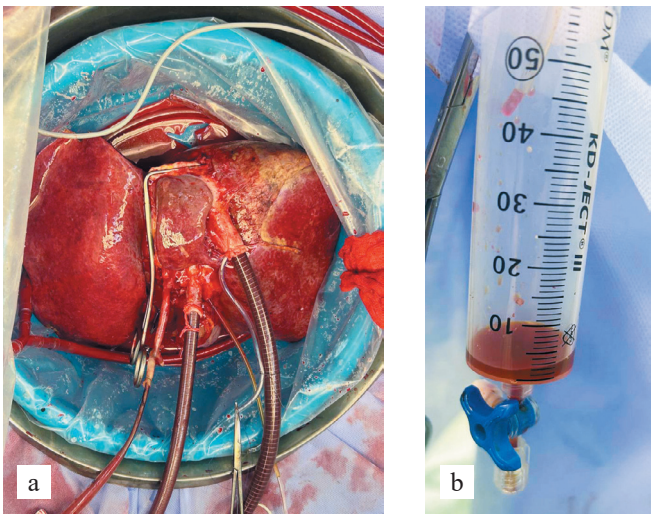

Fig. 7. (a) Liver allograft No. 1 and (b) bile secretion during NMP

Intraoperative liver transplantation characteristics

Case 1. Orthotopic liver transplantation was performed using the standard technique with IVC preservation according to A. Tzakis. Total operative time was 475 minutes, and biliary ischemia time was 70 minutes. Intraoperative blood loss was 700 ml, requiring transfusion of 2 units of red blood cells (RBCs) and 5 units of fresh frozen plasma (FFP). No postreperfusion syndrome or adverse hemodynamic response to reperfusion was observed. Arterial lactate level at the end of surgery was 2.4 mmol/L. Vasopressor therapy consisted of norepinephrine at 100 ng/kg/min.

Case 2. Orthotopic liver transplantation was also performed using the standard technique with IVC preservation according to A. Tzakis. Total operative time was 240 minutes, with a biliary ischemia time of 15 minutes. Blood loss was 1000 ml, and the patient received 2 units of RBCs and 2 units of FFP. No postreperfusion syndrome or hemodynamic instability during reperfusion was noted. Arterial lactate level at the conclusion of the procedure was 3.6 mmol/L, and vasopressor therapy consisted of norepinephrine at 90 ng/kg/min.

Postoperative period

The laboratory dynamics of the postoperative period in recipients are shown in Fig. 9.

Case 1. Peak AST and ALT levels reached 6562.9 U/L and 1610.4 U/L, respectively – meeting only one criterion for early allograft dysfunction. A rapid decline in

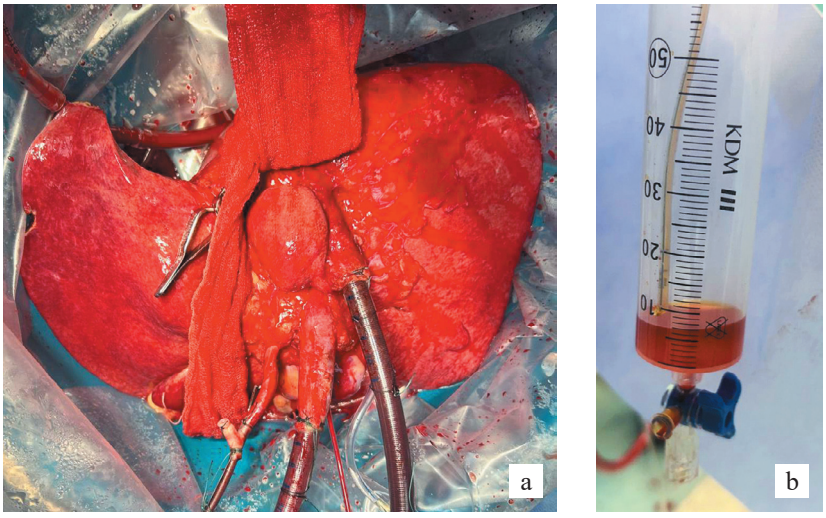

Fig. 8. (a) Liver allograft No. 2 and (b) bile secretion during NMP

transaminases was observed by postoperative day (POD) 2, with AST at 1267 U/L and ALT at 1212 U/L. The area under the curve (AUC) for AST was 5609.6 and for ALT 4353. Maximum total bilirubin level was 166 μmol/L on POD 6, followed by a steady decrease to normal values. Peak INR was 2.36 on POD 1. The postoperative course was uneventful, the patient was discharged on POD 17 without surgical or immunological complications. Two months after transplantation, the patient was readmitted due to Doppler-based findings suggestive of splenic artery steal syndrome. Splenic artery embolization was performed successfully. At the time of reporting, the follow-up period was four months.

Case 2. Peak AST and ALT levels were 3822 U/L and 2662 U/L, respectively, and constituted the only criterion fulfilled for early liver graft dysfunction. Similar to Case 1, AST and ALT values decreased rapidly by POD 2 (1429 and 2207 U/L, respectively), continuing to fall thereafter. The calculated AUC values were 4645 for AST and 8327 for ALT. The maximum total bilirubin level was 65.8 mmol/L on POD 1, followed by a steady decline to normal values. Peak INR was 3.11, also observed on POD 1. A postoperative hematoma in the subcutaneous tissue required evacuation, after which a vacuum-assisted wound system was applied on POD 3 and maintained for 7 days. The remaining postoperative course was uneventful. The patient was discharged on POD 34 without major complications. At the time of reporting, the follow-up period was 5 months.

Characteristics of the pathomorphological examination of the transplant

In all cases, microscopic (light microscopy) examination of the allograft was performed at three stages: before the start of perfusion, after completion of normothermic machine perfusion, and before suturing of the recipient's postoperative wound. A section of the bile duct was examined to assess conservation–ischemic injury both prior to perfusion and at the end of the surgery. Biopsies were obtained using an incisional technique from the edges of two liver lobes and from the distal portion of the common bile duct. Samples were fixed in 10% formalin and submitted for full pathomorphological examination.

An important observation is the *discordance* between the pathomorphological findings – including diffuse-focal and subtotal hepatocyte necrosis – and the clinically smooth postoperative course observed in both recipients. This discrepancy will be analyzed further in the *Discussion* section.

Case 1 (Fig. 10). *Preperfusion* biopsy showed moderate capsule sclerosis, diffuse-focal fatty degeneration (90–95%), involving medium and large-droplet degeneration of hepatocytes, fibrosis of the portal tracts and central vein walls, stage F1 fibrosis according to METAVIR.

Postperfusion biopsy, in addition to previously noted features, showed subcapsular and capsular hemorrhages, parenchymal edema, ruptured fat vacuoles in some areas, diffuse focal hepatocyte necrosis, and microhemorrhages within the parenchyma. The *postreperfusion* biopsy revealed subcapsular hepatocyte necrosis extending deep

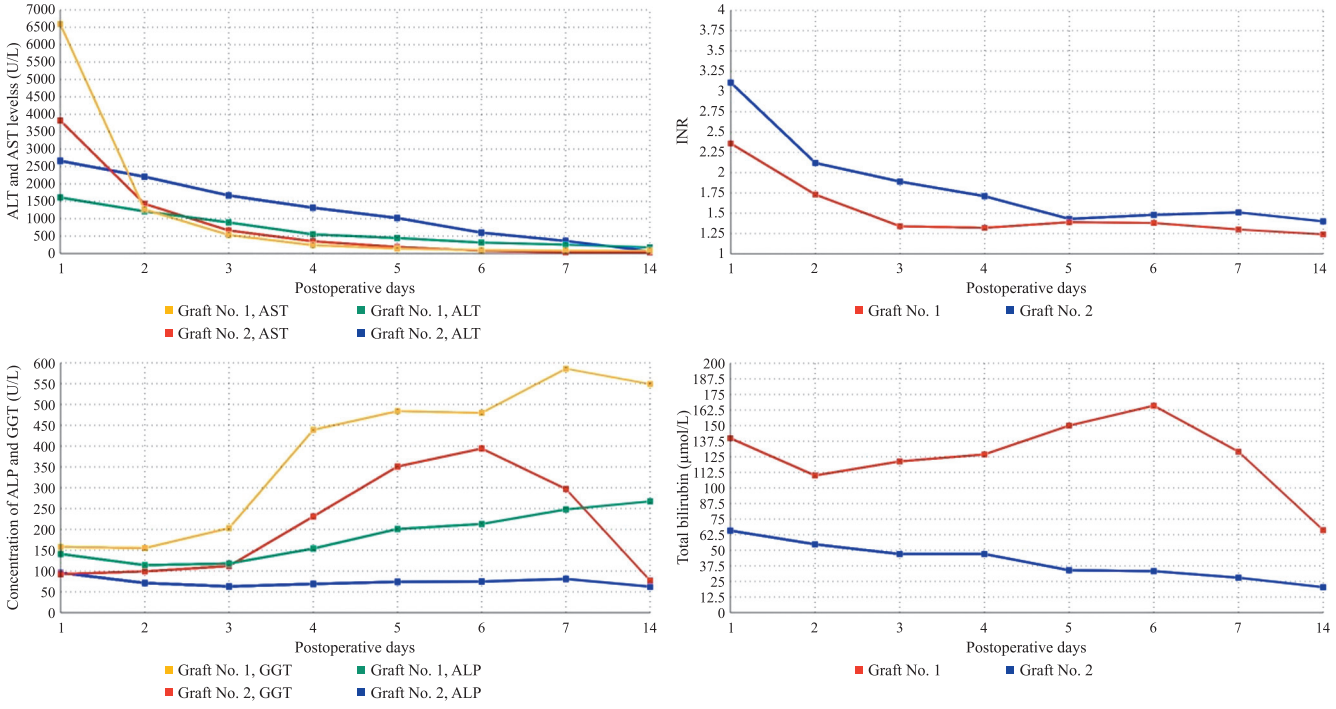

Fig. 9. Postoperative recipient parameters (case No. 1 and case No. 2.). Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase

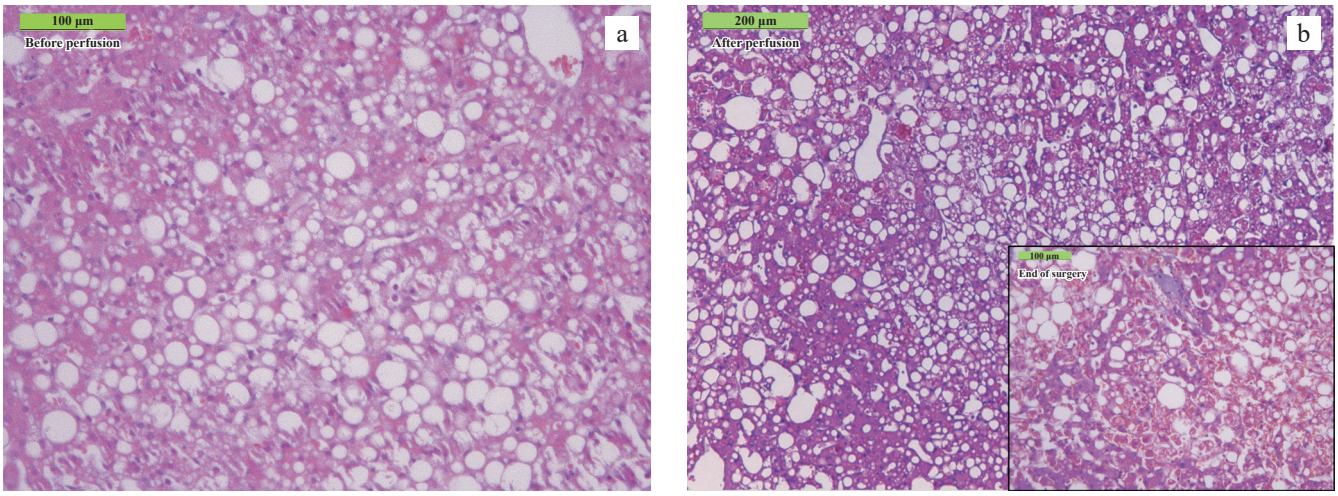

Fig. 10. Micrographs of a liver allograft biopsy in case No. 1 before and after machine perfusion. (a) Before perfusion (magnification 20×, H&E stain); (b) After perfusion (magnification 10×, H&E stain) and at the end of surgery (magnification 20×, Masson's trichrome stain). Detailed histological description is provided in the text

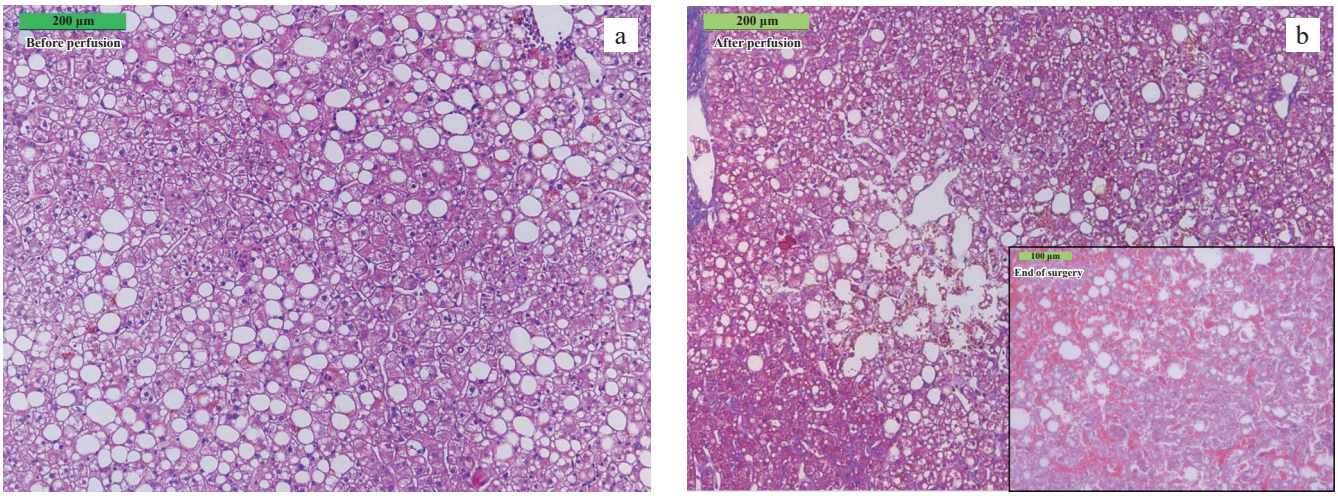

Fig. 11. Micrographs of a liver allograft biopsy in case No. 2 before and after machine perfusion. (a) Before perfusion (magnification 10×, H&E stain); (b) After perfusion (magnification 10×, Masson's trichrome stain) and at the end of surgery (magnification 20×, H&E stain). Detailed histological description is provided in the text

into the parenchyma with associated hemorrhages – most likely of compressive origin – as well as diffuse focal hepatocyte necrosis with hemorrhage. Bile duct biopsy revealed preserved peribiliary glands and biliary epithelium, with focal areas of epithelial desquamation.

Case 2 (Fig. 11). *Preperfusion* biopsy confirmed diffuse focal large-droplet fatty degeneration of hepatocytes involving up to 80%. Postperfusion biopsy showed subtotal hepatocyte necrosis and diffuse parenchymal hemorrhages.

Post-reperfusion biopsy also revealed diffuse focal hepatocyte necrosis accompanied by subcapsular and intraparenchymal hemorrhages, fibrosis of the central vein walls and portal tracts, and loose inflammatory infiltration within the portal areas. The bile duct biopsy showed no abnormalities and confirmed preservation of the peribiliary glands and biliary epithelium.

DISCUSSION

Minimizing or eliminating IRI is fundamental to expanding donor criteria, as IRI remains a major limitation to the maximal utilization of allografts from deceased donors [24, 25]. The concept of IFLT directly supports this principle. In a randomized controlled trial, Guo et al. compared outcomes in recipients of allografts preserved with IFLT (n = 32) versus SCS (n = 33). Early liver graft dysfunction, a key indicator of IRI, occurred in only 6% (n = 2) of patients in the IFLT group compared with 24% (n = 8) in the SCS group (p = 0.044). Post-reperfusion syndrome – another marker of preservation-related injury – developed in 9% (n = 3) of IFLT recipients versus 64% (n = 21) in the SCS group (p < 0.001) [16].

Further supporting these findings, He et al. reported the successful transplantation of a liver allograft with 85–95% macrovesicular steatosis using the IFLT technique,

reporting peak AST and ALT levels of only 375 and 123 U/L, and gamma-glutamyl transferase and alkaline phosphatase levels of 86 and 79 U/L, respectively [26].

Collectively, these results suggest that the complete avoidance of IRI enables the safe transplantation of virtually any organ – regardless of its suboptimal baseline condition – as long as it maintains adequate function in the donor prior to procurement.

However, despite its remarkable effectiveness in preventing IRI, IFLT has several technical drawbacks. Perfusion must begin at the donor hospital, and procurement procedure requires highly advanced surgical expertise, as it involves complete mobilization of the liver and inferior vena cava, along with meticulous dissection of the hepatoduodenal ligament. Furthermore, transporting the perfusion device with the organ continuously perfused to the recipient's hospital is logistically complex and labor-intensive [26]. To address these challenges, a simplified modification of IFLT – IFLI – which excludes the donor stage of machine perfusion has been proposed. IFLI prevents the recooling injury that occurs when an organ is re-cooled after completion of NMP but before implantation [10, 11, 27].

Chen et al. reported a significantly lower incidence of post-reperfusion syndrome in the IFLI group compared with the NMP group (8% vs. 58.8%, $p < 0.001$) and a higher frequency of primary graft dysfunction in recipients who did not undergo IFLI ($p = 0.041$). In a comparative study of IFLI ($n = 7$), NMP ($n = 7$), and SCS ($n = 14$), the same authors demonstrated reduced cytolysis in both perfusion groups relative to SCS ($p = 0.0015$ and $p = 0.016$ for AST and ALT, respectively), as well as a lower incidence of early dysfunction in the IFLI group compared with SCS ($p = 0.022$) and NMP ($p = 0.462$) [11]. Their use of fairly broad criteria for extended liver donation (Eurotransplant [29] and Vodkin et al. [28]) may partially explain the less pronounced results of IFLI in that cohort.

Notably, the avoidance of secondary warm ischemia may itself offer clinical benefits. In a study of 1,256 liver transplant recipients from brain-dead donors, Al-Kurd et al. reported a significantly lower risk of graft loss at both 1 and 5 years when secondary warm ischemia time was kept below 30 minutes [30]. Similarly, Sakamoto et al., analyzing outcomes in 67 living donor liver transplant recipients, found that secondary warm ischemia exceeding 48 minutes was a significant risk factor for the development of post-transplant biliary strictures ($p = 0.008$) [31].

Minimization of IRI and mitigation of its consequences can, as previously noted, be achieved through isolated or combined machine perfusion protocols. In our view, the DHOPE-COR-NMP protocol developed by the team at the University Medical Center Groningen represents the most effective strategy currently available [12]. Despite its strong potential – particularly for the utilization of high-risk organs from non-heart-beating (NHB) donors – its effectiveness remains limited when applied to allografts with significant macrovesicular steatosis (>30%).

Cirelli et al. reported two cases of liver transplantation using allografts from NHB donors with severe macrovesicular steatosis (>60% and >30%) following combined DHOPE-COR-NMP perfusion [13]. Although both grafts satisfied the center's stringent viability criteria (Groningen criteria), the clinical outcomes were suboptimal. In the first case, retransplantation was required on POD 17 due to persistent high-volume ascites (>10 L/day) and sustained vasopressor dependence. The authors attributed this to a 30% reduction in graft volume caused by rapid resolution of steatosis, which led to excessive length and subsequent kinking of the suprahepatic vena cava. The patient also experienced pronounced post-perfusion syndrome requiring norepinephrine therapy, acute kidney injury necessitating dialysis, and later kidney transplantation.

In the second case, the presence of lipopeliosis in the graft biopsy raised suspicion of "fat embolism" syndrome, which may have contributed to acute hypoxic respiratory failure and acute kidney injury. Based on these observations, the authors concluded that transplantation of organs with severe steatosis remains a risky endeavor, as graft behavior can remain unpredictable even when viability criteria are met during NMP.

In our study, we presented the outcomes of applying the combined DHOPE–COR–NMP–IFLI protocol, which by the time of reporting had become a routine component of our practice for high-risk donors. The cases described demonstrate the high efficacy and safety of machine perfusion in the transplantation of allografts with macrovesicular steatosis exceeding 80%, with no serious postoperative complications observed.

In our view, the markedly elevated peak AST and ALT levels likely reflect not the extent of IRI but rather the systemic entry of a substantial volume of transaminase-rich perfusate (more than 500 ml). This interpretation is supported by the relatively low area under the curve (AUC) for transaminase levels and their rapid decline as early as POD 2. Notably, the only instance of early liver graft dysfunction was identified solely on the basis of conventional biochemical criteria (AST/ALT peaks), without any clinical correlate or adverse effect on the postoperative course. This observation aligns with a broader shift in the field toward revising early dysfunction criteria in the era of dynamic perfusion preservation.

Graft function has remained stable for more than three months following transplantation, underscoring the satisfactory short-term outcomes achievable in highly steatotic allografts using this combined perfusion protocol.

In our practice, we apply viability criteria that are considerably broader than the classical, generally accepted standards. We believe that the sustained downward trend in lactate levels – rather than the absolute value

at any given time – is a far more meaningful indicator of graft viability. Moreover, we consistently observe that livers with a high degree of steatosis demonstrate a delayed lactate peak.

The IFLI stage is particularly critical, as any disruption in allograft perfusion can immediately convert the preservation strategy into one involving warm ischemia. Nevertheless, when the standard protocol for implantation under continuous normothermic perfusion is strictly followed, IFLI becomes a routine and reproducible method. The prolonged implantation time in Case 1 (70 minutes) was attributable to the recipient's anatomical features and technical difficulties associated with forming the caval anastomosis.

In all cases, we used the caval reconstruction with preservation of the IVC, creating a hepato-caval anastomosis between the suprahepatic IVC of the graft and a common cuff of the recipient's hepatic veins, following the technique of A. Tzakis. The classical approach, which includes complete replacement of the recipient's IVC, necessitates formation of the lower caval anastomosis only after graft reperfusion, because a cannula remains positioned in the subhepatic IVC to drain perfusate into the cardiotomy reservoir. Similarly, a modified implantation technique using IVC preservation as proposed by J. Belghitti appears impractical in the context of continuous perfusion, as complete isolation of the cavotomy field would disrupt outflow of perfusate through the IVC of the graft into the cardiotomy reservoir.

When preparing the graft for implantation, it is essential to retain an adequate length of the suprahepatic segment of the IVC above the vascular clamp to ensure proper anastomotic construction. Insufficient tissue may result in suture breakage and inadequate alignment of the vascular edges, whereas excessive wall length may create an overly long venous segment, increasing the risk of kinking or bending.

An important observation in our series is the discrepancy between the pathomorphological findings from post-perfusion and post-reperfusion biopsies and the favorable postoperative clinical course in both recipients. Despite diffuse–focal and subtotal hepatocyte necrosis, intraparenchymal and subcapsular hemorrhages, and inflammatory infiltration, the only criterion for early allograft dysfunction that was met was the elevation of cytolytic enzymes (AST and ALT) on POD 1. Given the rapid decline in their levels on subsequent days – and therefore the relatively low AUC – we propose, as previously noted, that these early enzyme surges are more likely attributable to the rapid entry of a large volume (>500 mL) of aminotransferase-rich perfusate into the systemic circulation during reperfusion ("bolus effect") rather than to extensive IRI of the graft itself.

Notably, neither recipient developed graft-specific complications typically expected with such a pathomorphological profile – such as primary graft non-function or severe early allograft dysfunction [40, 41].

In all cases, we performed an incisional (marginal) biopsy of the liver allograft. Several studies have shown that this technique provides a larger volume of tissue and can better detect – or even overestimate – the extent of pathomorphological processes compared with puncture (fine-needle) biopsy [34, 35, 37]. The main limitation of incisional biopsy is that the obtained fragment is taken from a marginal subcapsular area, where fibrosis and other alterations may be more pronounced than in deeper regions of the graft. This may lead to overdiagnosis and inaccurate assessment of the true prevalence of pathological processes [34–37]. For example, histologic examination of zones where the graft comes into contact with the perfusion container may reveal changes consistent with diffuse compression necrosis of hepatocytes rather than representing a widespread process throughout the parenchyma. Importantly, compression necrosis itself does not appear to impair allograft function or influence immediate postoperative outcomes [38, 39].

The relationship between pathomorphological findings and clinical outcomes under conditions of dynamic perfusion preservation requires further research to determine the optimal biopsy technique and to clarify its role in predicting postoperative outcomes.

Overall, IFLI combined with the DHOPE-COR-NMP protocol appears to be a promising strategy for dynamic preservation and implantation of liver allografts from extended-criteria, high-risk donors. Larger studies are needed to better define the potential limitations of this technique and its place within the current landscape of perfusion technologies.

The authors declare no conflict of interest.

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