DOI: 10.15825/1995-1191-2025-2-8-22

COMBINED SEQUENTIAL HYPOTHERMIC OXYGENATED AND NORMOTHERMIC MACHINE PERFUSION FOR LIVER TRANSPLANT FROM AN EXPANDED CRITERIA DONOR: FIRST CLINICAL APPLICATION IN RUSSIA

S.V. Gautier[1, 2], N.V. Grudinin[1], A.R. Monakhov[1, 2], M.A. Boldyrev[1], V.K. Bogdanov[1], D.M. Bondarenko[1], S.I. Zubenko[1], N.P. Mozheiko[1], M.G. Minina[1, 3], O.M. Tsirulnikova[1, 2]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Sechenov University, Moscow, Russian Federation

[3] Botkin Hospital, Moscow, Russian Federation

Objective: to analyze a clinical case series and evaluate the safety and efficacy of a sequential machine perfusion protocol combining dual hypothermic oxygenated perfusion (D-HOPE) and normothermic machine perfusion (NMP) for conditioning and viability assessment of liver grafts retrieved from expanded criteria donors (ECD) in routine clinical practice. **Materials and methods.** Between November and December 2024, two sessions of combined D-HOPE followed by NMP were conducted at Shumakov National Medical Research Center of Transplantology and Artificial Organs ("Shumakov Research Center") using liver allografts obtained from ECD after brain death. Following an initial period of static cold storage (SCS), machine perfusion was initiated using a circulatory assist device. A histidine-tryptophan-ketoglutarate (HTK)-based perfusate was used during the D-HOPE phase, while a red blood cell (RBC) suspension was used during the NMP stage. Throughout perfusion, temperature and hemodynamic parameters were continuously monitored and maintained. Laboratory parameters were assessed at designated intervals, in accordance with the institutional protocol developed at Shumakov Research Center. **Results.** Allograft #1 was deemed non-viable due to elevated lactate levels after 3 hours of perfusion and lack of glucose metabolism. The preservation times were as follows: SCS – 424 minutes, D-HOPE – 120 minutes, NMP – 300 minutes, totaling 844 minutes. Allograft #2 met the viability criteria and was successfully transplanted. Preservation times were: SCS – 260 minutes, D-HOPE – 124 minutes, NMP – 480 minutes, with a total preservation time of 884 minutes. Post-transplant peak levels of AST, ALT, and total bilirubin in the recipient were 922.5 U/L, 613 U/L, and 63.3 μmol/L, respectively. The only postoperative complication was acute kidney injury, managed with two sessions of hemodialysis. The patient was discharged after 14 days of hospitalization without need for readmission. At the time of writing, the patient is alive and complication-free, with a follow-up period of 3 months. **Conclusions.** Combined machine perfusion of liver grafts appears to be a safe and effective strategy to mitigate ischemia-reperfusion and preservation-related injury in liver transplantation. It also facilitates viability assessment of marginal liver grafts, reduces potential recipient complications, and expands the donor pool through the use of allografts from ECD.

Keywords: ex vivo perfusion, liver transplantation, machine perfusion.

INTRODUCTION

One of the most pressing and unresolved challenges in modern clinical transplantology is the significant mismatch between the number of patients on waiting lists and the availability of donor organs [1–3]. According to the Scientific Registry of Transplant Recipients (SRTR), at the end of 2022, there were 10,548 patients awaiting a liver transplant (LT) in the United States. During the same period, 12,862 patients were added to the waiting list, 13,638 were removed, and a total of 9,527 LTs were performed [3]. One of the primary strategies to expand the donor pool under these circumstances is the broader acceptance of organs from expanded criteria donors (ECDs), including those with significant steatosis, older donors, hemodynamically unstable donors, those in intensive care units for prolonged periods, and donors after circulatory death [4, 5]. However, such "marginal" organs have historically been associated with less favorable outcomes, including poorer recipient and graft survival rates [6–8, 26]. For example, severe macrovesicular steatosis of the allograft (>60%) has been shown to significantly increase the risk of primary nonfunction

Corresponding author: Mikhail Boldyrev. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (961) 974-59-55. E-mail: comex.ksb@gmail.com

(p = 0.002), acute kidney injury (p = 0.040), and the need for retransplantation (p = 0.012) [9]. The risk of graft loss (HR 2.3, 95% CI 1.7–3.0) and recipient death (HR 2.0, 95% CI 1.4–2.8) is approximately twice as high following transplantation of an organ from a donor after circulatory death (DCD), with biliary causes of graft loss occurring more frequently in the DCD group compared to the standard group (6% *vs* 1%, p = 0.04) [10]. It is increasingly clear that static cold storage (SCS), which has been the gold standard for donor organ preservation for over three decades, is no longer sufficient in the era of expanded donor eligibility. It does not provide an adequate level of protection against preservation-related injury for marginal allografts. For instance, one of the few randomized controlled trials on machine perfusion (MP) demonstrated that MP, compared to SCS, reduced the incidence of early allograft dysfunction by 14% (26% *vs* 40%; OR 0.61, 95% CI 0.39–0.96) and significantly decreased the occurrence of non-anastomotic biliary strictures by 12% (OR 0.36; 95% CI 0.14–0.94; p = 0.03) [14]. Dynamic or machine perfusion preservation not only protects marginal liver allografts from the deleterious effects of ischemia-reperfusion-preservation injury (IRPI) but also enables outcomes comparable to those achieved with standard donor organs, potentially increasing the number of usable organs for transplantation by 20% or more [11–14, 52].

Combined sequential machine perfusion is an actively developing method of perfusion conditioning for liver allografts [11, 23, 24]. This approach leverages hypothermic oxygenated machine perfusion (HOPE) to restore cellular ATP stores and minimize energy demands under hypothermic conditions, while clearing anaerobic metabolic byproducts such as succinate and NADH+. Following this, controlled oxygenated rewarming (COR) provides gradual, stepwise warming of the allograft under continuous oxygenated perfusion, further optimizing graft condition before proceeding to direct viability assessment during normothermic machine perfusion (NMP) [18, 11, 23]. Although the current evidence base remains limited, combined sequential perfusion is increasingly viewed as a highly promising development in machine perfusion and is steadily gaining a firm place in clinical transplantation practice.

The program for machine perfusion of liver transplants from ECDs has been actively developed at Shumakov National Medical Research Center of Transplantology and Artificial Organs since 2024. In this article, we present the first Russian clinical experience with the use of NMP as part of a combined HOPE-NMP protocol. In the first case, it was decided to abandon the use of the organ due to failure to meet viability criteria, while in the second case, the organ, having successfully met viability criteria, was transplanted into the recipient.

MATERIALS AND METHODS

Liver transplant

In all cases, liver allografts were used from ECDs as defined by Eurotransplant [25], with modifications by Shumakov National Medical Research Center of Transplantology and Artificial Organs (requiring the presence of one or more criteria):

- Donor age ≥65 years;
- Non-heart-beating donation (donation after circulatory death, DCD);
- Macrovesicular steatosis ≥40% (based on biopsy or visual assessment);
- Body mass index (BMI) ≥30;
- Donor blood sodium level ≥165 mmol/L;
- Intensive care unit (ICU) stay or mechanical ventilation (MV) duration >7 days;
- Predicted cold ischemia time ≥13 hours;
- AST >99 U/L;
- ALT >105 U/L;
- Total bilirubin >51 mmol/L;
- Need for adrenaline;
- Massive vasopressor support (norepinephrine >500 ng/kg/min);
- Periods of hypotension (mean arterial pressure <60 mmHg for 10 minutes or more);
- History of alcohol abuse or admission to ICU under the influence of alcohol;
- Donor risk index (DRI) >1.7 [26];
- Balance of Risk (BAR) score >18 [27].

Transplants from brain-dead donors were used in all cases. Multi-organ liver explantation from the donor was performed according to the standard technique in the Russian Federation [28]. No violations were identified during organ retrieval, transportation, or subsequent storage.

Pre-transplant preparation of the allograft was carried out according to a modified protocol. Initially, the portal vein was isolated, ligated, and cannulated using a 26 Fr cannula. Following portal vein cannulation, HOPE was initiated.

Given the requirement for subsequent NMP – which necessitates cannulation of both the portal vein and hepatic artery – HOPE was performed in dual mode (D-HOPE), perfusing through both vessels. It is important to note that while the immediate protective advantage of D-HOPE over standard HOPE at the hypothermic stage remains under investigation, we opted for dual cannulation to ensure readiness for NMP, which mandates both arterial and portal perfusion [29, 30].

The hepatic artery of the graft was isolated and cannulated with a 10 Fr cannula. Perfusion through the hepatic artery was initiated. Upon completion of the HOPE session, the graft was placed in a separate basin with ice chips and perfusion solution until it was ready for connection to the NMP. During this time, cannulation of

the biliary tract was performed using a 6 Fr probe, and the subhepatic section of the inferior vena cava (IVC) was cannulated with a 32 Fr cannula. The suprahepatic portion of the IVC was either clamped or tightly sutured.

All allografts exhibited standard vascular anatomy; however, in cases where an aberrant hepatic artery was present, a temporary or permanent anastomosis was created with the main artery to ensure adequate perfusion.

The grafts were weighed before perfusion, after completing the HOPE phase, and again after completing the NMP phase. Biopsies were taken before perfusion, after HOPE session, after the NMP session, and at the end of surgery.

Combined perfusion preservation

It should be noted that, at present, there is no universally accepted, validated algorithm for selecting the optimal method of perfusion preservation for liver allografts [31].

For perfusion, a standard set of consumables and equipment typically used for cardiopulmonary bypass during cardiac surgery was used. The material and technical support included the following: heart-lung machine Sorin Stockert S5 (LivaNova, UK), thermostatic regulating device Stockert 3T (LivaNova, UK), oxygenator Affinity NT (Medtronic, USA), thermoregulation and trunk line set.

The design of the perfusion circuit for "seamless" machine perfusion – meaning it does not require replacement of the tubing set when transitioning from D-HOPE to NMP – is an in-house development by Shumakov National Medical Research Center of Transplantology and Artificial Organs.

The circuit includes lines for perfusate supply to the portal vein and hepatic artery, a perfusate drainage line from the IVC of the graft, A drainage line from the organ container, and a cardiotomy reservoir.

During both stages, perfusion was conducted with flow adjustments to maintain appropriate perfusion pressures.

The first stage of combined machine perfusion consisted of a 2-hour D-HOPE session. The perfusate comprised 3 liters of HTK solution, supplemented with 150 mL of 25% human albumin to ensure adequate colloid osmotic pressure, and 2100 mg of the antioxidant acetylcysteine. Continuous recirculation of the perfusate was maintained at a flow rate of 1 liter per minute. Perfusate acid-base status (pH, pCO_2, pO_2, bicarbonate) was monitored every 30 minutes. Standard biochemical analysis of the perfusate was also performed at 30-minute intervals. A general view of the allograft during D-HOPE is presented in Fig. 1.

After the D-HOPE session, the allograft was disconnected from the perfusion circuit. The perfusion lines were then flushed with a 5% dextrose solution to remove residual perfusate. Following drainage of the dextrose solution, the circuit was refilled with the perfusate prepared for the NMP session. Recommended perfusion parameters are shown in Table.

After initiation of perfusate recirculation and achievement of the target temperature within the circuit, perfusate composition was adjusted based on the results of the initial acid-base analysis. Following this adjustment, the NMP session was commenced with a planned duration of at least 4 hours. Continuous infusion of heparin (1000 units/hour) and alprostadil (5–10 μg/hour) was maintained throughout the NMP session. The general appearance of the liver allograft during NMP is shown in Fig. 2, and bile secretion during NMP is illustrated in Fig. 3.

Thereafter, the acid-base sample was performed every 30 minutes throughout the perfusion. Standard biochemical analysis of the perfusate and acid-base analysis of bile were conducted 30 minutes after the start of perfusion

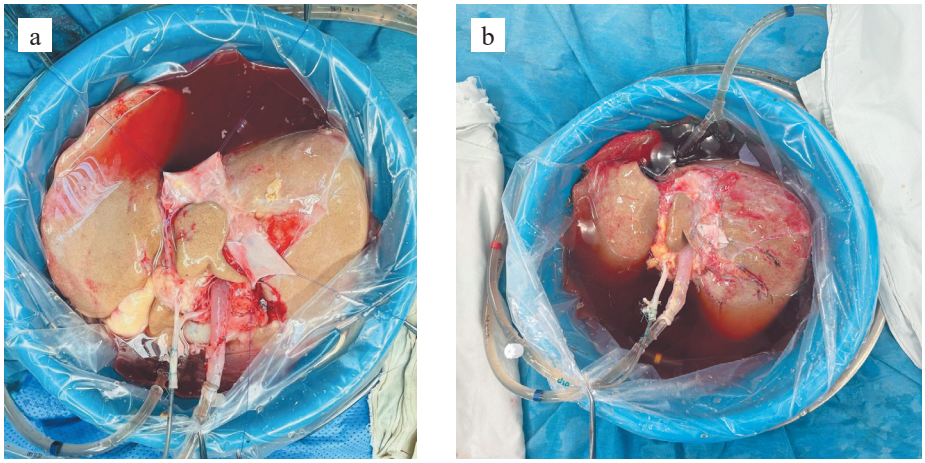

Fig. 1. Liver allografts during hypothermic oxygenated machine perfusion: (a) Case #1 – non-viable and subsequently rejected; (b) Case #2 – met viability criteria and was successfully transplanted

and subsequently every hour. Perfusion parameters – including flow rates, pressures, pump revolutions per minute, allograft temperature, graft consistency, perfusion homogeneity, oxygenation levels, and volume and characteristics of bile output – were continuously monitored, with data recorded every 30 minutes. All perfusion data were systematically entered into the perfusion protocol card developed at Shumakov National Medical Research Center of Transplantology and Artificial Organs.

Table

Recommended perfusion parameters for hypothermic oxygenated and normothermic machine perfusion of liver allografts

Parameter	HOPE [20, 63, 40]	NMP [23, 28, 40]
Perfusate temperature (°C)	8–10	36–38
Oxygenation level (pO_2, mmHg)	400–600	90–200
Flow, hepatic artery (mL/min)	40–70	>150–300
Flow, portal vein (mL/min)	300–400 (up to 500)	>500
Pressure, hepatic artery (mmHg)	20–25	60–70
Pressure, portal vein (mmHg)	3–5	10–13

Viability criteria

Assessment of liver allograft viability during the D-HOPE phase was not performed, as the determination of flavin mononucleotide (FMN) in the perfusate remains a promising but still investigational area requiring further precision studies [32, 33, 34]. The use of classical metabolic indicators (such as lactate, glucose, and pH) and markers of organ injury (LDH, AST, ALT) during hypothermic perfusion also remains under investigation and is not currently recognized as a validated method for viability assessment [34].

It should be emphasized that no universally accepted and validated criteria for viability assessment during NMP exist at present. Consequently, each group either develops their own criteria or relies on previously proposed standards [35, 36]. After a thorough review of the available literature, we adopted the VITTAL criteria developed and validated in the VITTAL study (Birmingham, UK) [20]. These criteria were modified to include mandatory qualitative bile analysis, based on the work of van Leeuwen and Matton [11, 37].

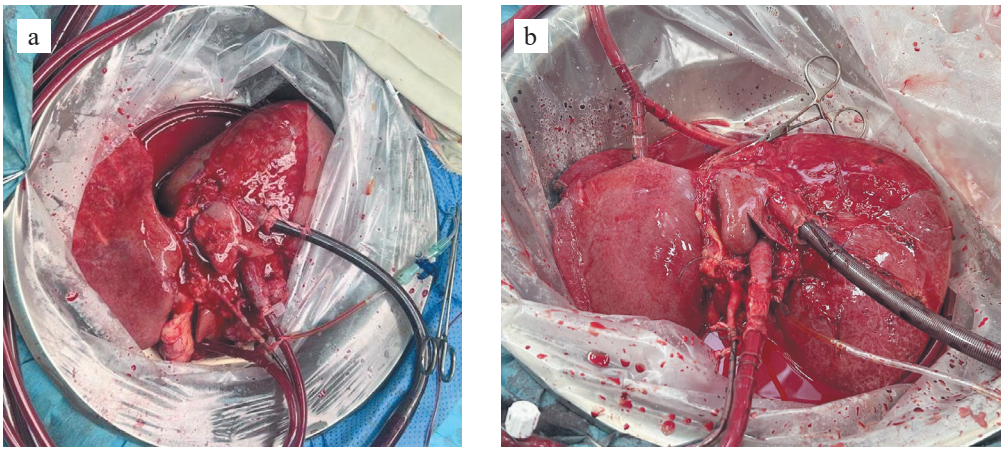

Fig. 2. Liver allografts during normothermic machine perfusion: (a) Case #1 – non-viable and subsequently rejected; (b) Case #2 – met viability criteria and was successfully transplanted

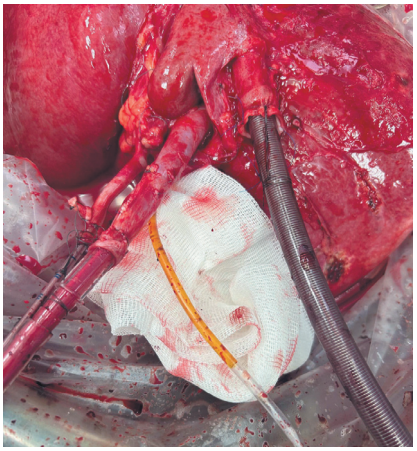

Fig. 3. Bile secretion by allograft in Case #1 during normothermic machine perfusion

Assessment of hepatocellular allograft link

Mandatory criterion: perfusate lactate level <2.5 mmol/L after 4 hours of perfusion; alternatively, a stable decrease in lactate was acceptable (lactate <2.5 mmol/L after 5 hours or <2.0 mmol/L after 6 hours).

Presence of *two or more* of the following additional criteria:

– Bile production totaling at least 5 mL, with 4 mL or more produced during the final hour of perfusion, and ideally exceeding 10 mL per hour;

– Perfusate pH >7.3 without the need for continuous infusion or boluses of sodium bicarbonate;
– Evidence of glucose metabolism (progressive reduction in glucose concentration, response to glucose boluses, and insulin infusion);
– Stable portal and arterial flow rates (>500 mL/min and >150 mL/min, respectively);
– Uniform parenchymal perfusion with soft organ consistency.

Evaluation of the cholangiocellular link of the allograft

Viability assessment of the cholangiocellular link was based on the presence of two or more of the following criteria:

– Bile pH >7.48, with a pH difference between bile and perfusate >0.05;
– Bicarbonate (HCO_3^-) concentration in bile >18 mmol/L, with a HCO_3^- difference between bile and perfusate >3.0 mmol/L;
– Glucose concentration in bile <16 mmol/L, with a glucose difference between bile and perfusate <–3.0 mmol/L, or a bile-to-perfusate glucose ratio <0.67;

If the allograft failed to meet the viability criteria, perfusion was discontinued and the organ was used for research or discarded. If viability criteria were achieved, perfusion was continued with ongoing monitoring of all parameters. In parallel, the recipient was prepared and brought to the operating room, where standard anesthetic management was provided, followed by hepatectomy. Upon retrieval of the recipient's native liver, perfusion of the donor graft was stopped, the graft was cooled with ice chips, and flushed with Custodiol solution through the portal vein and hepatic artery (3 and 2 liters, respectively), after which it was transferred for implantation.

Liver transplantation and postoperative period

Liver transplantation was performed using the technique of hepatectomy with either preservation or replacement of the recipient's own IVC, depending on intraoperative and anatomical considerations. The postoperative period included a 1-day stay in the ICU, followed by transfer to a specialized transplant ward.

Induction immunosuppression was initiated with a pulse dose of methylprednisolone, followed by rapid tapering over the next 4 days. Tacrolimus, as the main component of maintenance immunosuppression, was started on postoperative days 2–3, with a target blood level of 7–8 ng/mL. Mycophenolic acid or mycophenolate mofetil was introduced after normalization of the complete blood count.

Comprehensive laboratory and instrumental monitoring was conducted daily during the first 7 postoperative days, and then every other day during the second week after transplantation.

Acute graft rejection was suspected based on laboratory abnormalities (elevations in total bilirubin, aminotransferases, and cholestasis markers) after excluding other causes (including vascular complications) and was confirmed by percutaneous liver biopsy.

Acute kidney injury (AKI) was diagnosed according to the following KDIGO criteria [39].

1. Rise in serum creatinine of ≥0.3 mg/dL within 48 hours;
2. Rise in serum creatinine to ≥1.5 times (≥50%) the baseline, which is known or presumed to have occurred within the prior 7 days;
3. Urine output <0.5 mL/kg/hour for 6 hours.

Early allograft dysfunction (EAD) was recorded according to the criteria proposed by K. Olthoff et al. [38]:

1. Total bilirubin >171 μmol/L on postoperative day 7;
2. International normalized ratio (INR) >1.6 on postoperative day 7;
3. AST or ALT >2000 U/L within the first 7 postoperative days.

Primary nonfunction (PNF) was diagnosed according to the UNOS criteria [40]:

Death or retransplantation within the first 7 postoperative days, associated with AST >3000 U/L and at least one of the following:

1. INR >2.5;
2. Acidosis (arterial pH <7.30, venous pH <7.25) or lactate >4 mmol/L.

The diagnosis of ischemic non-anastomotic cholangiopathy (NAC) of the liver graft was established using a combination of clinical, laboratory, and instrumental evaluation methods. Clinical signs included pruritus and jaundice, while laboratory indicators focused on elevated markers of cholestasis, specifically gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP).

In patients presenting with clinical or laboratory abnormalities suggestive of NAC, magnetic resonance cholangiopancreatography (MRCP) was performed to confirm or exclude the diagnosis.

In cases where MRCP findings indicated NAC without corresponding clinical or laboratory signs, the condition was classified as asymptomatic NAC.

Donors

Liver allografts from ECDs who were brain-dead were used. These organs had been previously declined by all transplant centers and were subsequently included in the machine perfusion preservation program. A brief description of the donors is provided below.

Case #1. Donor G. Male, 58 years old. BMI 34.3. Cause of death: hemorrhagic stroke (subarachnoid hemorrhage). Time in hospital and ICU: 1 day. Laboratory parameters: creatinine: 102 mmol/L, AST: 60 U/L, ALT:

45 U/L, total bilirubin: 20 μmol/L, plasma sodium level: 139 mEq/L. The liver allograft exhibited dense consistency and pronounced steatosis, with visual assessment indicating more than 60% involvement. Histological examination confirmed macrovesicular steatosis ranging between 65–70%. The weight of the liver allograft prior to perfusion was 3090 grams. Static cold preservation time upon arrival at Shumakov National Medical Research Center of Transplantology and Artificial Organs was 424 minutes.

Case #2. Donor N., female, 59 years old, BMI 45.8. Cause of death: hemorrhagic stroke (subarachnoid hemorrhage). Effective circulatory arrest lasted 15 minutes at the prehospital stage. Time in hospital and ICU: 3 days. Laboratory parameters: creatinine 90 μmol/L, AST 26 U/L, ALT 28 U/L, total bilirubin 9.9 μmol/L, plasma sodium level 136 mEq/L. The liver allograft demonstrated dense consistency and moderate steatosis upon visual inspection, with steatosis estimated at more than 30%. However, pathomorphological evaluation revealed a discrepancy: macrovesicular steatosis was 5–10%, while microvesicular steatosis was 55–60%. Weight of liver allograft prior to perfusion was 1910 grams. Static cold storage time at the moment of organ admission to the clinic was 260 minutes.

Recipient

In Case #2, the graft was transplanted to a recipient with an identical ABO blood group and compatible anthropometric indices. The recipient was a 53-year-old patient suffering from liver cirrhosis due to chronic HBV and HDV infection, with a MELD 3.0 score of 21 points. It is important to note that the patient experienced recurrent diuretic-resistant hydrothorax and ascites, requiring intensive diuretic therapy (spironolactone 300 mg/day, torasemide 40 mg/day) and frequent hospitalizations at Shumakov National Medical Research Center of Transplantology and Artificial Organs for laparocentesis and thoracocentesis procedures.

Perfusion parameters and viability assessment

Viability assessment of the liver allograft at the D-HOPE phase was not performed. NMP parameters are presented in Fig. 4.

Case #1. During the D-HOPE session, significant cytolysis was observed at 30 minutes into perfusion (ALT: 1500 U/L, AST: 1600 U/L) and further increased at 60 minutes (ALT: 3260 U/L, AST: 6440 U/L). Perfusate lactate at 60 minutes was 3.8 mmol/L, while perfusate glucose was 9.6 mmol/L. The pO_2 difference between inflow and outflow was 269 mmHg (inflow pO_2: 448 mmHg; outflow pO_2: 179 mmHg). It should be noted that these data were collected purely for subsequent retrospective analysis and did not influence real-time management decisions.

During the NMP phase, although a decrease in perfusate lactate was initially observed – reaching a minimum of 2.1 mmol/L at 180 minutes – a subsequent rise to 4.4 mmol/L occurred at 4 hours of perfusion. In addition, despite continuous infusion and periodic boluses of insulin, perfusate glucose level remained high, indicating impaired glucose metabolism and poor hormonal responsiveness in the allograft – a recognized indicator of non-viability [41, 42].

Bile production peaked early at 30 minutes of perfusion (4 mL) but subsequently declined and failed to meet the target values. Other hepatocellular viability parameters remained within normal limits. However, persistent high levels of cytolysis enzymes (max AST: 4650 U/L, ALT: 1950 U/L) – a factor considered in viability assessment by several groups [43] – raised additional concerns. Notably, the cholangiocellular viability parameters remained within normal limits.

The weight of the organ at the end of perfusion was practically unchanged, measuring 3067 g (compared to 3090 g pre-perfusion).

So, based on comprehensive assessment using the viability criteria of Shumakov National Medical Research Center of Transplantology and Artificial Organs, it was decided to abandon the use of the organ.

The D-HOPE and NMP time was 120 minutes and 300 minutes, respectively. Total machine perfusion time was 420 minutes, while total organ preservation time measured 844 minutes.

Case #2. The D-HOPE session demonstrated moderate cytolysis at 30 minutes (ALT: 645 U/L, AST: 890 U/L) and at 60 minutes (ALT: 799 U/L, AST: 1095 U/L). Perfusate lactate at 60 minutes measured 2.3 mmol/L, perfusate glucose was 8.2 mmol/L, and the pO_2 difference between inflow and outflow was 207 mmHg (inflow pO_2: 452 mmHg; outflow pO_2: 245 mmHg).

During the NMP phase, a slower pH normalization was noted compared to Case #1, although this did not require sodium bicarbonate boluses. Following an initial episode of hyperglycemia, glucose metabolism improved, with perfusate glucose levels stabilizing near physiologic values (8–12 mmol/L).

Lactate levels remained relatively elevated initially but showed a sharp decline at 4 hours of perfusion, reaching 2.6 mmol/L. Per protocol, due to the positive trend in lactate clearance, observation was continued. At 6 hours, lactate level further decreased to 2.3 mmol/L, and by 8 hours, it had reached 0.5 mmol/L, meeting viability criteria.

A consistently low level of cytolysis was also noted. Other hepatocellular and cholangiocellular viability parameters remained within normal limits throughout perfusion. Allograft weight after perfusion was 2000 g,

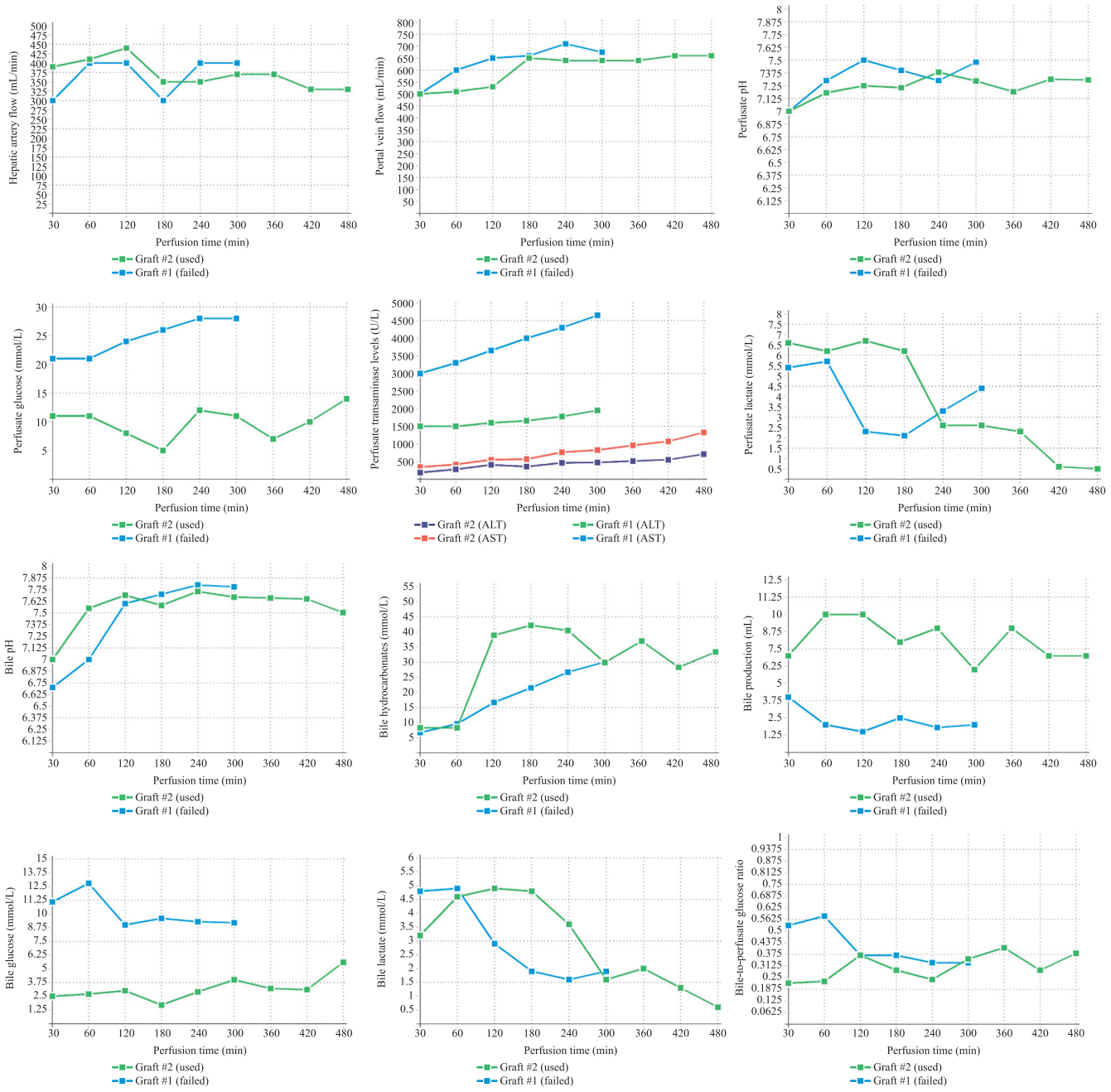

Fig. 4. Perfusion parameters of liver allografts in Case #1 (non-viable) and Case #2 (transplanted)

virtually unchanged from the pre-perfusion weight of 1910 g.

Based on these findings, the liver allograft was deemed viable. The D-HOPE time was 124 minutes, and the NMP time was 480 minutes. Total machine perfusion time was 604 minutes.

Liver transplantation

In Case #2, the LT operation lasted 290 minutes. Secondary warm ischemia time measured 20 minutes. Biliary ischemia time was 40 minutes, while total organ preservation time 884 minutes. After venous reperfusion of the graft, postreperfusion syndrome [44] did not develop. Moreover, there was no hemodynamic reaction to initiation of venous blood flow. Intraoperative blood loss was 200 mL, and transfusion included one unit of red blood cell mass.

Postoperative period

A schematic representation of the laboratory parameter dynamics during the postoperative period is shown in Fig. 5.

The peak AST level (922.5 U/mL) and ALT level (613 U/mL) were observed on postoperative day 1. The highest INR value (1.68) and total bilirubin level (63.3 μmol/L) were also recorded on postoperative day 1, followed by steady improvement. AKI developed on postoperative days 2–3, with peak creatinine of

400 μmol/L and urea of 29.2 μmol/L, requiring renal replacement therapy (RRT) via hemodialysis (two sessions). Following treatment, renal function fully recovered.

The total legnth of hospital stay was 14 days. During this period, due to the development of significant hydrothorax, pleural puncture and drainage were performed on the right side (postoperative day 1) and the left side (postoperative day 2). After adjustment of diuretic therapy, the hydrothorax regressed.

At the time of writing, the follow-up period was 3 months. The patient remains alive, has not been rehospitalized, and shows normal laboratory and instrumental parameters. It is noteworthy that IRPI was mild, and there were no signs of EAD, PNF, vascular or biliary complications.

Pathomorphologic study of liver allografts

In all cases, microscopic (using light microscopy) examination of allograft biopsies was mandatory at three stages: before perfusion, after completion of NMP, and before closure of the recipient's postoperative wound. Biopsies were obtained via an incisional method from the edge of both liver lobes, fixed in buffered 10% formalin, and submitted for pathomorphologic analysis.

– In *case #1*, the preperfusion (a) biopsy revealed, as previously noted, macrovesicular steatosis involving 65–70% of hepatocytes, hepatic fibrosis (stage F1 on the METAVIR scale), and diffuse, focal, moderate granular protein dystrophy of hepatocytes. The *postperfusion* (b) biopsy showed severe ischemia-reperfusion injury (IRI), characterized by diffuse focal hepatocyte necrosis within the parenchyma, predominantly in zones 1 and 3 of the hepatic acinus, accompanied by hemorrhages. Representative microphotographs are shown in Fig. 6.
– In *case #2*, the *preperfusion* (a) biopsy demonstrated diffuse focal large-droplet fatty degeneration of hepatocytes involving 5–10%, diffuse focal moderate granular protein dystrophy of hepatocytes, and hepatic fibrosis corresponding to stage F1–F2 on the METAVIR scale. The *postperfusion* (b) biopsy revealed moderate IRI of the liver. Similarly, the postreperfusion (c) biopsy pattern was consistent with moderate IRI, featuring subcapsular hepatocyte necrosis, most likely of compressive origin. Representative microphotographs are presented in Fig. 7.

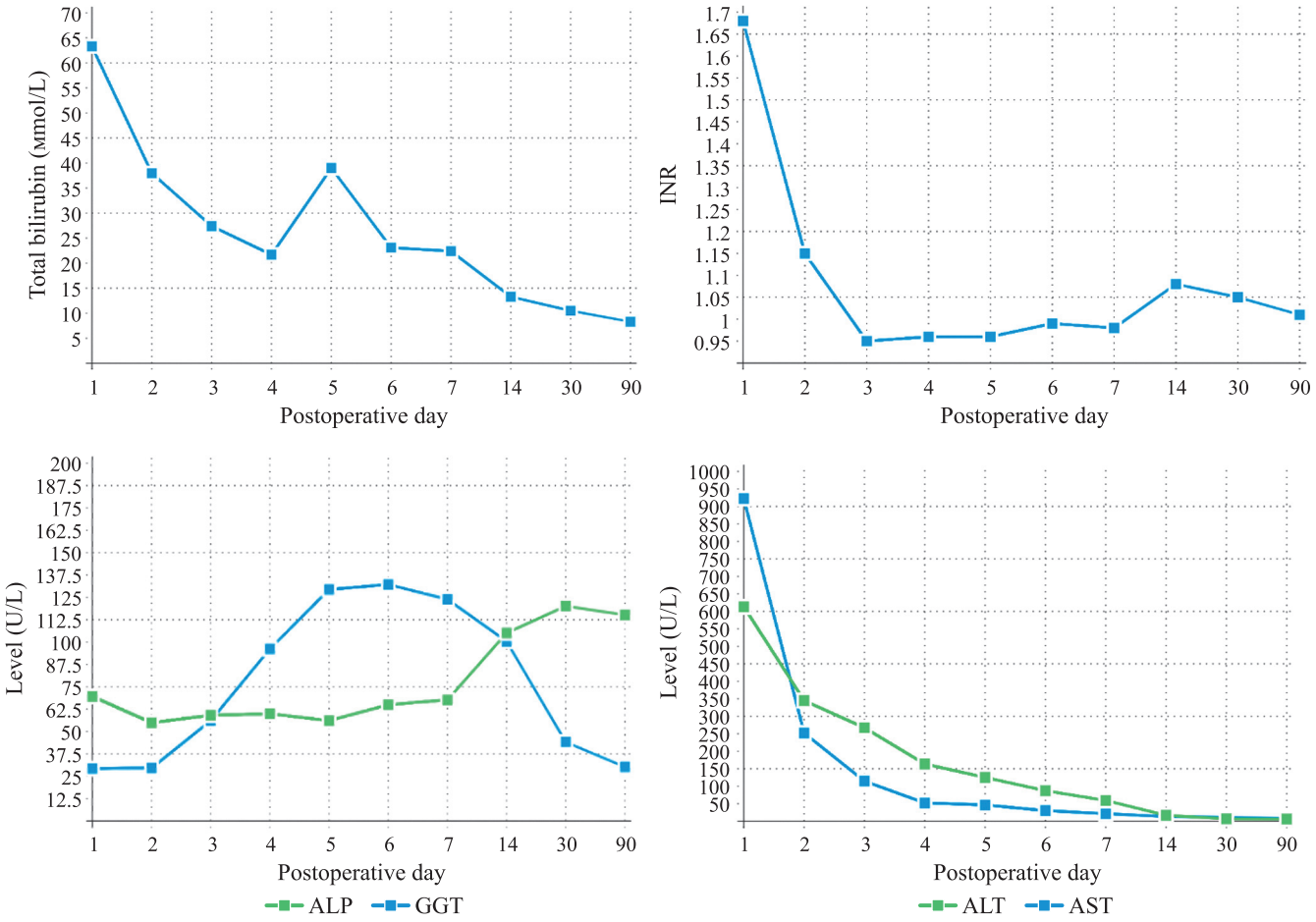

Fig. 5. Laboratory dynamics during the postoperative period in the liver recipient (Case #2)

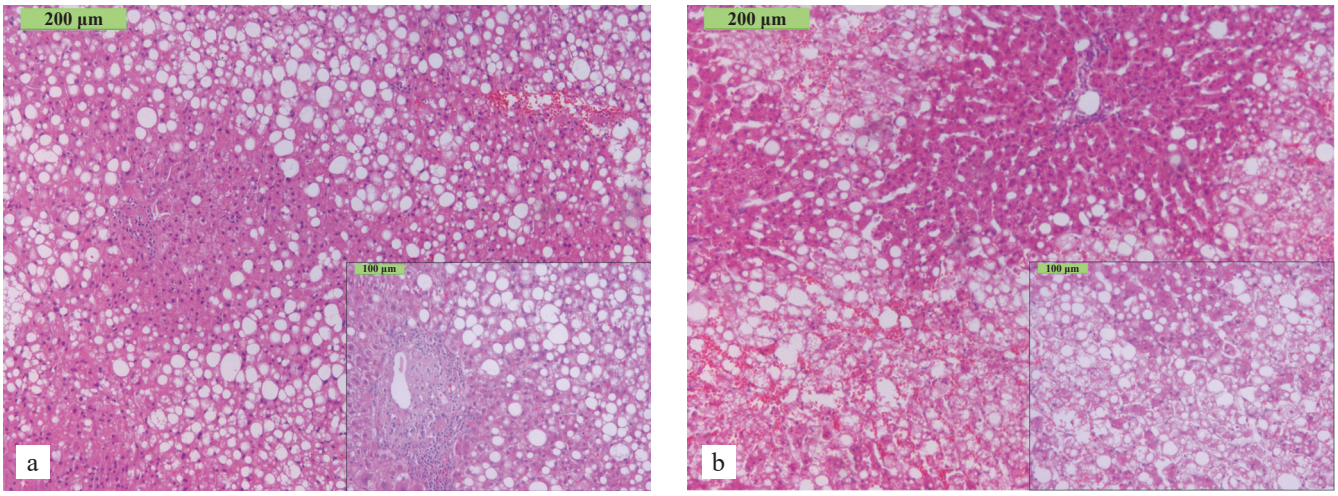

Fig. 6. Microphotographs of liver allograft biopsy in Case #1: (a) before machine perfusion, and (b) after perfusion. Histological description provided in the main text

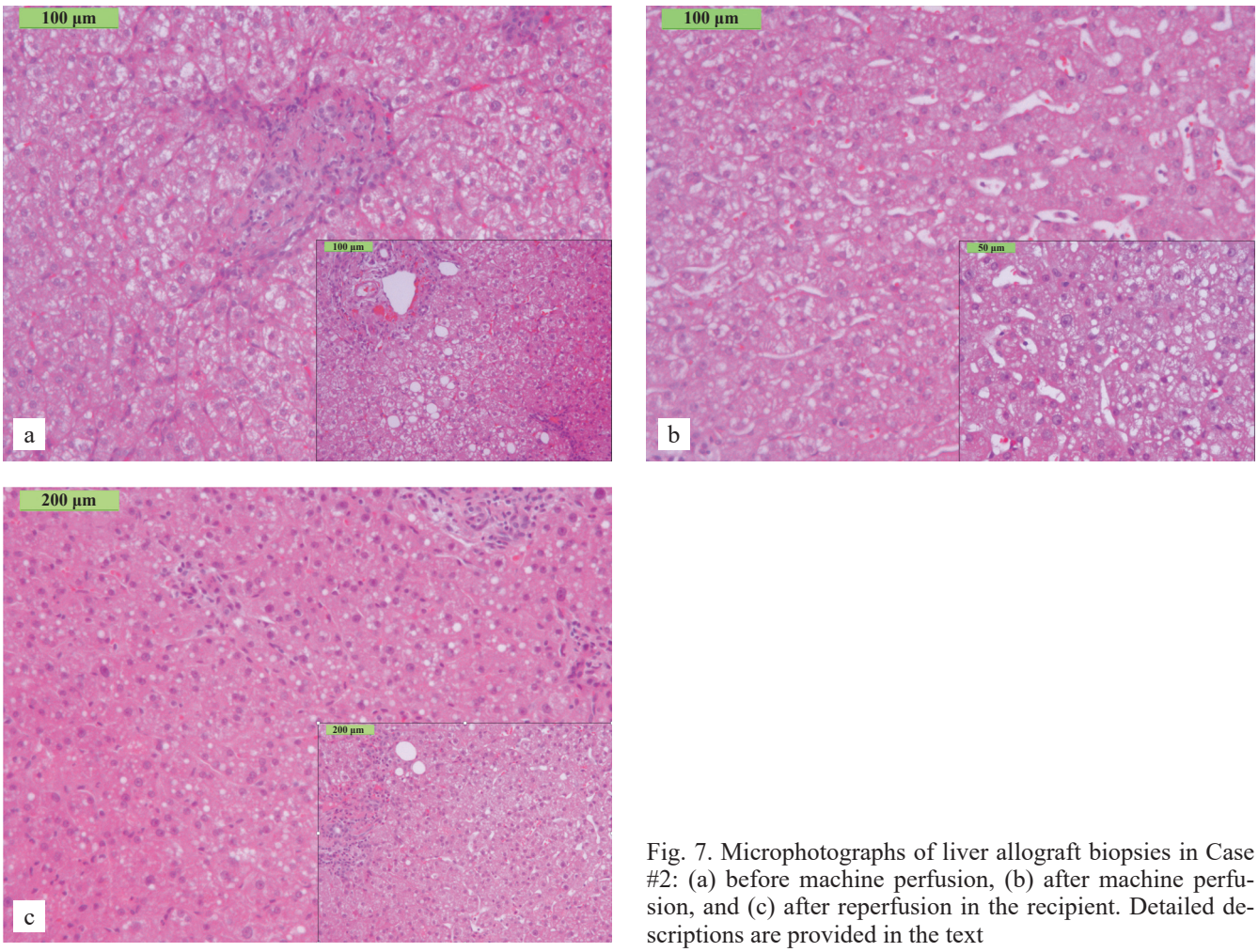

Fig. 7. Microphotographs of liver allograft biopsies in Case #2: (a) before machine perfusion, (b) after machine perfusion, and (c) after reperfusion in the recipient. Detailed descriptions are provided in the text

DISCUSSION

Machine perfusion is gaining prominence as a new gold standard for preserving liver allografts, especially those from ECDs. However, the isolated use of the main liver machine perfusion techniques – HOPE and NMP – despite their individual advantages, still presents a number of limitations [13–16]. For instance, assessment of liver graft viability during HOPE is restricted primarily to the measurement of a single validated marker, the concentration of FMN in the perfusate [17]. Moreover, the technical complexity of HOPE and the need for specialized equipment limit its availability to many centers,

thereby hindering broader adoption. While NMP offers extensive opportunities for comprehensive viability assessment, it does not fully eliminate IRPI due to normothermic reperfusion, although the injury is milder in an *ex vivo* setting [18–20].

Combined perfusion is a promising new direction in the development of liver allograft preservation, offering the opportunity to integrate the advantages of several machine perfusion techniques [11, 18, 23]. For instance, van Leeuwen et al., in a study involving combined perfusion of 54 allografts from high-risk donors (mean DRI 2.84, IQR 2.52–3.11), reported successful transplantation of 63% of organs, with 1-year graft and recipient survival rates of 94% and 100%, respectively. No cases of PNF were observed, and non-anastomotic biliary strictures developed in only one patient (3%). Two retransplantations were required due to chronic rejection (3%) and venous obstruction (3%) [11].

A sudden change in graft temperature from hypothermic (~4 °C) to normothermic (37 °C) conditions can induce "rewarming injury" or thermal injury, leading to further damage to the allograft [18, 21, 22]. To address this, the COR technique has been increasingly incorporated into combined perfusion protocols and has shown effectiveness when used as a standalone approach [21]. D. Hoyer, for example, reported a 50% reduction in peak AST levels (a surrogate marker for graft injury) in the COR group compared to the SCS group (AST 563.5 U/L *vs* 1204 U/L, P = 0.023) [22]. Nevertheless, there are no precise data on the efficacy of COR as part of combined protocols. Therefore, in the present cases, the combined perfusion protocol included only the "classical" HOPE and NMP stages.

At present, there is no universally accepted and validated algorithm for choosing the best perfusion strategy (isolated or combined, with various combinations) for each specific liver allograft [31]. This issue appears to be particularly pressing, as the routine application of "advanced" dynamic preservation techniques, including normothermic perfusion, to all organs meeting even a single expanded criteria donor (ECD) factor would be redundant and economically inefficient. On the other hand, the isolated use of hypothermic oxygenated perfusion for high-risk allografts (those presenting multiple risk factors) may be insufficient and could naturally lead to a higher incidence of complications. At Shumakov National Medical Research Center of Transplantology and Artificial Organs, the choice of perfusion method is made individually for each case, based on a comprehensive analysis of both donor and recipient factors. Continued experience accumulation and strengthened intercenter collaboration are expected to facilitate the development of more precise and standardized strategies for perfusion method selection in the future.

Assessment of liver graft viability remains a critical focus of contemporary research. In our work, we employed one of the most widely recognized viability assessment protocols, which demonstrated its effectiveness in the large VITTAL study [12, 20]. It is important to note that excessively stringent criteria may reduce the ability to identify potentially viable grafts, whereas overly liberal criteria increase the risk of post-transplant complications [35, 36]. For example, Panconesi et al. showed that using lactate clearance assessment at the sixth hour of NMP, only 13 (6.1%) of 213 allografts were classified as non-viable. In contrast, applying the Groningen or Brisbane criteria would have resulted in higher non-viability rates – 14.6% and 11.2%, respectively. The authors also highlighted that outcomes with so-called "lactate-high" allografts were comparable to those with lower lactate levels [51].

At the same time, as previously mentioned, studies by Mergental et al. revealed that the absence of mandatory qualitative bile assessment during viability evaluation led to ischemic cholangiopathy in four recipients [12, 20]. Retrospective analysis showed that, in three cases, non-anastomotic biliary strictures developed in recipients of DCD livers, where the bile produced during perfusion had low pH (<7.65) and low bicarbonate concentrations (<25 mmol/L) [12].

Based on these findings and a comprehensive review of the available literature, we modified the basic VITTAL protocol by incorporating mandatory assessment of biliary tree viability. It should be emphasized that, to date, there are no universally accepted criteria for liver graft viability assessment, and this remains an important area for further research and standardization.

We presented the machine perfusion of two liver grafts that had been rejected by all transplant centers due to their high degree of "marginality". Despite ex-vivo viability testing, the first allograft was ultimately deemed unsuitable for transplantation. The second graft met the viability criteria established by our center and was successfully transplanted into the recipient. This experience highlights the subjective nature of donor liver evaluation when based solely on initial clinical data.

The recipient's postoperative period was uneventful overall, despite the development of AKI, which may have been partially attributable to the patient's initially compromised condition and the use of high-dose diuretics prior to transplantation. Nevertheless, AKI resolved following two RRT sessions, and the total length of stay in the hospital was 14 days, which is consistent with the average in our center.

According to available data, the combined perfusion approach described here – particularly the application of the normothermic phase – is the first such experience in clinical practice in Russia. Thus, our observations demonstrate the high efficacy, safety, and reproducibili-

ty of the combined machine perfusion method for liver allografts obtained from ECDs.

The authors declare no conflict of interest.

REFERENCES

1. *Lewis A, Koukoura A, Tsianos GI, Gargavanis AA, Nielsen AA, Vassiliadis E.* Organ donation in the US and Europe: The supply *vs* demand imbalance. *Transplant Rev (Orlando)*. 2021 Apr; 35 (2): 100585. doi: 10.1016/j.trre.2020.100585. Epub 2020 Oct 11. PMID: 33071161.
2. *Langone AJ, Helderman JH.* Disparity between solid-organ supply and demand. *N Engl J Med*. 2003 Aug 14; 349 (7): 704–706. doi: 10.1056/NEJMe038117. PMID: 12917309.
3. *Kwong AJ, Kim WR, Lake JR, Schladt DP, Schnellinger EM, Gauntt K et al.* OPTN/SRTR 2022 Annual Data Report: Liver. *Am J Transplant*. 2024 Feb; 24 (2S1): S176–S265. doi: 10.1016/j.ajt.2024.01.014. PMID: 38431359.
4. *Goldaracena N, Cullen JM, Kim DS, Ekser B, Halazun KJ.* Expanding the donor pool for liver transplantation with marginal donors. *Int J Surg*. 2020 Oct; 82S: 30–35. doi: 10.1016/j.ijsu.2020.05.024. Epub 2020 May 15. PMID: 32422385.
5. *Moein M, Bahreini A, Razavi A, Badie S, Coyle S, Abedini M et al*. A Review of Long-Term Outcomes of Liver Transplantation Using Extended Criteria Donors in the United States. *J Surg Res*. 2025 Feb; 306: 561–569. doi: 10.1016/j.jss.2024.12.055. Epub 2025 Jan 31. PMID: 39892300.
6. *Zhang T, Dunson J, Kanwal F, Galvan NTN, Vierling JM, O'Mahony C et al*. Trends in Outcomes for Marginal Allografts in Liver Transplant. *JAMA Surg*. 2020 Aug 5; 155 (10): 926–32. doi: 10.1001/jamasurg.2020.2484. Epub ahead of print. Erratum in: *JAMA Surg*. 2020 Oct 1; 155 (10): 1002. doi: 10.1001/jamasurg.2020.4315. PMID: 32777009; PMCID: PMC7407315.
7. *Clavien PA, Harvey PR, Strasberg SM.* Preservation and reperfusion injuries in liver allografts. An overview and synthesis of current studies. *Transplantation*. 1992 May; 53 (5): 957–978. doi: 10.1097/00007890-199205000-00001. PMID: 1585489.
8. *Lozanovski VJ, Probst P, Arefidoust A, Ramouz A, Aminizadeh E, Nikdad M et al*. Prognostic role of the Donor Risk Index, the Eurotransplant Donor Risk Index, and the Balance of Risk score on graft loss after liver transplantation. *Transpl Int*. 2021 May; 34 (5): 778–800. doi: 10.1111/tri.13861. Epub 2021 May 1. PMID: 33728724.
9. *De Graaf EL, Kench J, Dilworth P, Shackel NA, Strasser SI, Joseph D et al*. Grade of deceased donor liver macrovesicular steatosis impacts graft and recipient outcomes more than the Donor Risk Index. *J Gastroenterol Hepatol*. 2012 Mar; 27 (3): 540–546. doi: 10.1111/j.1440-1746.2011.06844.x. PMID: 21777274.
10. *Callaghan CJ, Charman SC, Muiesan P, Powell JJ, Gimson AE, van der Meulen JH; UK Liver Transplant Audit.* Outcomes of transplantation of livers from donation after circulatory death donors in the UK: a cohort study. *BMJ Open*. 2013 Sep 3; 3 (9): e003287. doi: 10.1136/bmjopen-2013-003287. PMID: 24002984; PMCID: PMC3773642.
11. *Van Leeuwen OB, Bodewes SB, Lantinga VA, Haring MPD, Thorne AM, Brüggenwirth IMA et al*. Sequential hypothermic and normothermic machine perfusion enables safe transplantation of high-risk donor livers. *Am J Transplant*. 2022 Jun; 22 (6): 1658–1670. doi: 10.1111/ajt.17022. Epub 2022 Apr 18. PMID: 35286759; PMCID: PMC9325426.
12. *Mergental H, Laing RW, Kirkham AJ, Clarke G, Boteon YL, Barton D et al*. Discarded livers tested by normothermic machine perfusion in the VITTAL trial: Secondary end points and 5-year outcomes. *Liver Transpl*. 2024 Jan 1; 30 (1): 30–45. doi: 10.1097/LVT.0000000000000270. Epub 2023 Dec 15. PMID: 38109282.
13. *Dutkowski P, Polak WG, Muiesan P, Schlegel A, Verhoeven CJ, Scalera I et al*. First Comparison of Hypothermic Oxygenated PErfusion Versus Static Cold Storage of Human Donation After Cardiac Death Liver Transplants: An International-matched Case Analysis. *Ann Surg*. 2015 Nov; 262 (5): 764–770; discussion 770–771. doi: 10.1097/SLA.0000000000001473. PMID: 26583664.
14. *Van Rijn R, Schurink IJ, de Vries Y, van den Berg AP, Cortes Cerisuelo M, Darwish Murad S et al*. Hypothermic Machine Perfusion in Liver Transplantation – A Randomized Trial. *N Engl J Med*. 2021 Apr 15; 384 (15): 1391–1401. doi: 10.1056/NEJMoa2031532. Epub 2021 Feb 24. PMID: 33626248.
15. *Nasralla D, Coussios CC, Mergental H, Akhtar MZ, Butler AJ, Ceresa CDL et al*. A randomized trial of normothermic preservation in liver transplantation. *Nature*. 2018 May; 557 (7703): 50–56. doi: 10.1038/s41586-018-0047-9. Epub 2018 Apr 18. PMID: 29670285.
16. *Markmann JF, Vagefi PA, MacConmara MP*. Normothermic Machine Perfusion Increases Donor Liver Use. *JAMA Surg*. 2022 Aug 1; 157 (8): 742–743. doi: 10.1001/jamasurg.2022.1424. PMID: 35507357.
17. *Eden J, Thorne AM, Bodewes SB, Patrono D, Roggio D, Breuer E et al*. Assessment of liver graft quality during hypothermic oxygenated perfusion: The first international validation study. *J Hepatol*. 2025 Mar; 82 (3): 523–534. doi: 10.1016/j.jhep.2024.08.030. Epub 2024 Sep 7. PMID: 39251091; PMCID: PMC11830552.
18. *Van Leeuwen OB, Lantinga VA, Lascaris B, Thorne AM, Bodewes SB, Nijsten MW et al.* 'Back-to-base' combined hypothermic and normothermic machine perfusion of human donor livers. *Nat Protoc.* 2025. https://doi.org/10.1038/s41596-024-01130-8.
19. *Watson CJE, Kosmoliaptsis V, Randle LV, Gimson AE, Brais R, Klinck JR et al*. Normothermic Perfusion in the Assessment and Preservation of Declined Livers Before Transplantation: Hyperoxia and Vasoplegia-Important Lessons From the First 12 Cases. *Transplantation*. 2017 May; 101 (5): 1084–1098. doi: 10.1097/TP.0000000000001661. PMID: 28437389; PMCID: PMC5642347.

20. *Mergental H, Laing RW, Kirkham AJ, Perera MTPR, Boteon YL, Attard J et al*. Transplantation of discarded livers following viability testing with normothermic machine perfusion. *Nat Commun*. 2020 Jun 16; 11 (1): 2939. doi: 10.1038/s41467-020-16251-3. PMID: 32546694; PMCID: PMC7298000.
21. *Minor T, von Horn C, Zlatev H, Saner F, Grawe M, Lüer B et al*. Controlled oxygenated rewarming as novel end-ischemic therapy for cold stored liver grafts. A randomized controlled trial. *Clin Transl Sci*. 2022 Dec; 15 (12): 2918–2927. doi: 10.1111/cts.13409. Epub 2022 Oct 17. PMID: 36251938; PMCID: PMC9747115.
22. *Hoyer DP, Mathé Z, Gallinat A, Canbay AC, Treckmann JW, Rauen U et al*. Controlled Oxygenated Rewarming of Cold Stored Livers Prior to Transplantation: First Clinical Application of a New Concept. *Transplantation*. 2016 Jan; 100 (1): 147–152. doi: 10.1097/TP.0000000000000915. PMID: 26479280.
23. *Liu Q, Del Prete L, Ali K, Grady P, Bilancini M, Etterling J et al*. Sequential hypothermic and normothermic perfusion preservation and transplantation of expanded criteria donor livers. *Surgery*. 2023 Mar; 173 (3): 846–854. doi: 10.1016/j.surg.2022.07.035. Epub 2022 Oct 24. PMID: 36302699.
24. *De Vries Y, Matton APM, Nijsten MWN, Werner MJM, van den Berg AP, de Boer MT et al*. Pretransplant sequential hypo- and normothermic machine perfusion of suboptimal livers donated after circulatory death using a hemoglobin-based oxygen carrier perfusion solution. *Am J Transplant*. 2019 Apr; 19 (4): 1202–1211. doi: 10.1111/ajt.15228. Epub 2019 Jan 23. PMID: 30588774; PMCID: PMC6590255.
25. *Oosterlee A, Rahmel A.* Eurotransplant International Foundation Annual Report 2008. 2011.
26. *Feng S, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA et al*. Characteristics associated with liver graft failure: the concept of a donor risk index. *Am J Transplant*. 2006 Apr; 6 (4): 783–790. doi: 10.1111/j.1600-6143.2006.01242.x. Erratum in: *Am J Transplant*. 2018 Dec; 18 (12): 3085. doi: 10.1111/ajt.15155. PMID: 16539636.
27. *Martínez JA, Pacheco S, Bachler JP, Jarufe N, Briceño E, Guerra JF et al*. Accuracy of the BAR score in the prediction of survival after liver transplantation. *Ann Hepatol*. 2019 Mar-Apr; 18 (2): 386–392. doi: 10.1016/j.aohep.2019.01.002. Epub 2019 Apr 17. PMID: 31036493.
28. *Pogrebnichenko IV.* Effektivnoe ispol'zovanie pecheni mul'tiorgannogo donora dlya transplantatsii: dis. … kand. med. nauk. M., 2014; 143.
29. *Koch DT, Tamai M, Schirren M, Drefs M, Jacobi S, Lange CM et al*. Mono-HOPE Versus Dual-HOPE in Liver Transplantation: A Propensity Score-Matched Evaluation of Early Graft Outcome. *Transpl Int*. 2025 Feb 5; 38: 13891. doi: 10.3389/ti.2025.13891. PMID: 39974599; PMCID: PMC11835512.
30. *De Vries Y, Brüggenwirth IMA, Karangwa SA, von Meijenfeldt FA, van Leeuwen OB, Burlage LC et al*. Dual Versus Single Oxygenated Hypothermic Machine Perfusion of Porcine Livers: Impact on Hepatobiliary and Endothelial Cell Injury. *Transplant Direct*. 2021 Aug 5; 7 (9): e741. doi: 10.1097/TXD.0000000000001184. PMID: 34386578; PMCID: PMC8354629.
31. *Sousa Da Silva RX, Weber A, Dutkowski P, Clavien PA*. Machine perfusion in liver transplantation. *Hepatology*. 2022 Nov; 76 (5): 1531–1549. doi: 10.1002/hep.32546. Epub 2022 May 17. PMID: 35488496.
32. *Schlegel A, Muller X, Mueller M, Stepanova A, Kron P, de Rougemont O et al*. Hypothermic oxygenated perfusion protects from mitochondrial injury before liver transplantation. *EBioMedicine*. 2020 Oct; 60: 103014. doi: 10.1016/j.ebiom.2020.103014. Epub 2020 Sep 24. PMID: 32979838; PMCID: PMC7519249.
33. *Eden J, Breuer E, Birrer D, Müller M, Pfister M, Mayr H et al*. Screening for mitochondrial function before use-routine liver assessment during hypothermic oxygenated perfusion impacts liver utilization. *EBioMedicine*. 2023 Dec; 98: 104857. doi: 10.1016/j.ebiom.2023.104857. Epub 2023 Oct 31. PMID: 37918219; PMCID: PMC10641151.
34. *Patrono D, Catalano G, Rizza G, Lavorato N, Berchialla P, Gambella A et al*. Perfusate Analysis During Dual Hypothermic Oxygenated Machine Perfusion of Liver Grafts: Correlations With Donor Factors and Early Outcomes. *Transplantation*. 2020 Sep; 104 (9): 1929–1942. doi: 10.1097/TP.0000000000003398. PMID: 32769628.
35. *Groen PC, van Leeuwen OB, de Jonge J, Porte RJ.* Viability assessment of the liver during *ex situ* machine perfusion prior to transplantation. *Curr Opin Organ Transplant*. 2024 Aug 1; 29 (4): 239–247. doi: 10.1097/MOT.0000000000001152. Epub 2024 May 17. PMID: 38764406; PMCID: PMC11224566.
36. *Jeddou H, Tzedakis S, Chaouch MA, Sulpice L, Samson M, Boudjema K*. Viability Assessment During Normothermic Machine Liver Perfusion: A Literature Review. *Liver Int*. 2025 Feb; 45 (2): e16244. doi: 10.1111/liv.16244. PMID: 39821671; PMCID: PMC11740183.
37. *Matton APM, de Vries Y, Burlage LC, van Rijn R, Fujiyoshi M, de Meijer VE et al*. Biliary Bicarbonate, pH, and Glucose Are Suitable Biomarkers of Biliary Viability During *Ex Situ* Normothermic Machine Perfusion of Human Donor Livers. *Transplantation*. 2019 Jul; 103 (7): 1405–1413. doi: 10.1097/TP.0000000000002500. PMID: 30395120; PMCID: PMC6613725.
38. *Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J et al*. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl*. 2010 Aug; 16 (8): 943–949. doi: 10.1002/lt.22091. PMID: 20677285.
39. *Khwaja A.* KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract*. 2012; 120 (4): c179–c184. doi: 10.1159/000339789. Epub 2012 Aug 7. PMID: 22890468.
40. *Al-Freah MAB, McPhail MJW, Dionigi E, Foxton MR, Auzinger G, Rela M et al*. Improving the Diagnostic Criteria for Primary Liver Graft Nonfunction in Adults Utilizing Standard and Transportable Laboratory Parameters: An Outcome-Based Analysis. *Am J Transplant*.

2017 May; 17 (5): 1255–1266. doi: 10.1111/ajt.14230. Epub 2017 Apr 5. PMID: 28199762.

41. *Eshmuminov D, Becker D, Bautista Borrego L, Hefti M, Schuler MJ, Hagedorn C et al*. An integrated perfusion machine preserves injured human livers for 1 week. *Nat Biotechnol*. 2020 Feb; 38 (2): 189–198. doi: 10.1038/s41587-019-0374-x. Epub 2020 Jan 13. PMID: 31932726; PMCID: PMC7008032.
42. *Schurink IJ, Willemse J, Verstegen MMA, van der Laan LJW, de Jonge J*. Long-Term Perfusion of the Liver Outside the Body: Warming Up for *Ex vivo* Therapies? *Hepatology*. 2020 Oct; 72 (4): 1485–1487. doi: 10.1002/hep.31474. PMID: 33464565; PMCID: PMC7702161.
43. *Olumba FC, Zhou F, Park Y, Chapman WC; RESTORE Investigators Group.* Normothermic Machine Perfusion for Declined Livers: A Strategy to Rescue Marginal Livers for Transplantation. *J Am Coll Surg*. 2023 Apr 1; 236 (4): 614–625. doi: 10.1097/XCS.0000000000000555. Epub 2023 Jan 11. PMID: 36728302.
44. *Siniscalchi A, Gamberini L, Laici C, Bardi T, Ercolani G, Lorenzini L, Faenza S*. Post reperfusion syndrome during liver transplantation: From pathophysiology to therapy and preventive strategies. *World J Gastroenterol*. 2016 Jan 28; 22 (4): 1551–1569. doi: 10.3748/wjg.v22.i4.1551. PMID: 26819522; PMCID: PMC4721988.
45. *Parente A, Tirotta F, Pini A, Eden J, Dondossola D, Manzia TM Dutkowski P, Schlegel A*. Machine perfusion techniques for liver transplantation – A meta-analysis of the first seven randomized-controlled trials. *J Hepatol*. 2023 Nov; 79 (5): 1201–1213. doi: 10.1016/j.jhep.2023.05.027. Epub 2023 Jun 9. PMID: 37302578.
46. *Tingle SJ, Dobbins JJ, Thompson ER, Figueiredo RS, Mahendran B, Pandanaboyana S, Wilson C*. Machine perfusion in liver transplantation. *Cochrane Database Syst Rev*. 2023 Sep 12; 9 (9): CD014685. doi: 10.1002/14651858.CD014685.pub2. PMID: 37698189; PMCID: PMC10496129.
47. *Banker A, Bhatt N, Rao PS, Agrawal P, Shah M, Nayak M, Mohanka R.* A Review of Machine Perfusion Strategies in Liver Transplantation. *J Clin Exp Hepatol*. 2023 Mar-Apr; 13 (2): 335–349. doi: 10.1016/j.jceh.2022.08.001. Epub 2022 Aug 10. PMID: 36950485; PMCID: PMC10025749.
48. *Boteon YL, Laing RW, Schlegel A, Wallace L, Smith A, Attard J et al*. Combined Hypothermic and Normothermic Machine Perfusion Improves Functional Recovery of Extended Criteria Donor Livers. *Liver Transpl*. 2018 Dec; 24 (12): 1699–1715. doi: 10.1002/lt.25315. PMID: 30058119; PMCID: PMC6588092.
49. *Chen Z, Hong X, Huang S, Wang T, Ma Y, Guo Y et al*. Continuous Normothermic Machine Perfusion for Renovation of Extended Criteria Donor Livers Without Recooling in Liver Transplantation: A Pilot Experience. *Front Surg*. 2021 May 24; 8: 638090. doi: 10.3389/fsurg.2021.638090. PMID: 34109206; PMCID: PMC8180843.
50. *Chen Z, Wang T, Chen C, Zhao Q, Ma Y, Guo Y et al*. Transplantation of Extended Criteria Donor Livers Following Continuous Normothermic Machine Perfusion Without Recooling. *Transplantation*. 2022 Jun 1; 106 (6): 1193–1200. doi: 10.1097/TP.0000000000003945. Epub 2022 Sep 7. PMID: 34495016; PMCID: PMC9128617.
51. *Panconesi R, Shanmugarajah K, Satish S, Zhang M, Wehrle C, Jiao C et al*. Rethinking Lactate's Role as a Post-Transplant Outcome Predictor During Normothermic Machine Perfusion: Science or Speculation? *Am J Transplant*. 2025 Jan; 25 (1 Suppl 1): S126.
52. *Van Leeuwen OB, de Vries Y, Fujiyoshi M, Nijsten MWN, Ubbink R, Pelgrim GJ et al*. Transplantation of High-risk Donor Livers After *Ex Situ* Resuscitation and Assessment Using Combined Hypo- and Normothermic Machine Perfusion: A Prospective Clinical Trial. *Ann Surg*. 2019 Nov; 270 (5): 906–914. doi: 10.1097/SLA.0000000000003540. PMID: 31633615.

The article was submitted to the journal on 9.04.2025