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Liver graft preconditioning, preservation and reconditioning

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1609114 since 2016-11-10T23:24:53Z

Published version:

DOI:10.1016/j.dld.2016.06.031

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17 July 2024

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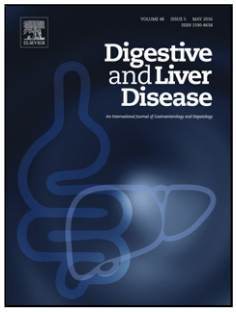

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PII: S1590-8658(16)30506-0
DOI: http://dx.doi.org/doi:10.1016/j.dld.2016.06.031
Reference: YDLD 3196

To appear in: *Digestive and Liver Disease*

Received date: 18-1-2016
Revised date: 2-5-2016
Accepted date: 27-6-2016

Please cite this article as: Gilbo N, Catalano G, Salizzoni M, Romagnoli R, Liver graft preconditioning, preservation and reconditioning, *Digestive and Liver Disease* (2016), http://dx.doi.org/10.1016/j.dld.2016.06.031

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Liver graft preconditioning, preservation and reconditioning

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Financial Disclosure: the authors of the manuscript have nothing to disclose

Word Count: 5,000 words

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Abstract

Liver transplantation is the successful treatment of end-stage liver disease; however, the ischemia-reperfusion injury still jeopardizes early and long-term post-transplant outcomes. In fact, ischemia-reperfusion is associated with increased morbidity and graft dysfunction, especially when suboptimal donors are utilized. Strategies to reduce the severity of ischemia-reperfusion can be applied at different steps of the transplantation process: organ procurement, preservation phase or before revascularization. During the donor procedure, preconditioning consists of pre-treating the graft prior to a sustained ischemia either by a transient period of ischemia-reperfusion or administration of anti-ischemic medication, although a multi-pharmacological approach seems more promising. Different preservation solutions were developed to maintain graft viability during static cold storage, achieving substantial results in terms of liver function and survival in good quality organs but not in suboptimal ones. Indeed, preservation solutions do not prevent dysfunction of poor quality organs and are burdened with inadequate preservation of the biliary epithelium. Advantages derived from either hypo- or normothermic machine perfusion are currently investigated in experimental and clinical settings, suggesting a reconditioning effect possibly improving hepatocyte and biliary preservation and resuscitating graft function prior to transplantation. In this review, we highlight acquired knowledge and recent advances in liver graft preconditioning, preservation and reconditioning.

Key words: liver transplantation, preservation, preconditioning, reconditioning

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Introduction

Liver transplantation (LT) is the ultimate treatment for end-stage liver disease, achieving substantial early and long-term results. However, the Ischemia-Reperfusion Injury (IRI) profoundly influences outcomes after LT, representing still a considerable challenge.

The success of LT has created a new dilemma: the organ supply does not satisfy a steadily increasing demand; to overcome this limit, the criteria for organ donation have become less restrictive by including grafts of suboptimal quality, such as those from Extended Criteria Donors (ECD) (i.e. mainly those with significant macrosteatosis, old donor age or prolonged hospitalization) or Donation after Circulatory Death (DCD)[1]. Unfortunately, suboptimal grafts are more susceptible to IRI and carry an increased risk of organ failure after transplantation[2]. It is now clear that IRI represents a major hurdle we must deal with to further improve LT outcomes, thus satisfying patients' needs.

Living donation, minimizing the duration of cold ischemia, may represent a solution to both IRI and shortage of donor grafts. The large experience accumulated by the Asian groups showed indeed that living donation can satisfactory replace a limited pool of deceased donors, achieving excellent survival[3,4]. However Western experience is still far from reaching comparable numbers in terms of both LT performed and patient survival, and considerable concerns regarding donor safety and post-transplant graft failure still exist. Hence, researchers have consistently focused on reducing the severity of IRI with interventions that can be applied at different steps of deceased donor LT (Figure 1).

The phase of procurement at the donor site offers the opportunity to treat the graft prior to the initiation of IRI, a concept known as organ *preconditioning*.

Maintaining the viability of the graft during the cold storage phase with dedicated solutions is probably the oldest attempt to reduce IRI, but the renewed interest in machine perfusion (MP) pushes towards new appealing scenarios in organ *preservation*.

Recent advances in experimental MP have prompted the possibility to assess graft viability and to resuscitate it immediately prior to LT, performing a real organ *reconditioning*.

Here, we review the acquired knowledge and highlight the emerging evidences on the effects of graft preconditioning, preservation and reconditioning in LT.

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1. Ischemia-Reperfusion Injury: pathogenesis and clinical implications

IRI inevitably occurs through the transplantation process from organ donation to graft revascularization. During the procurement, the graft is suddenly deprived of oxygen and cooled down with cold preservation solutions in order to slow metabolic processes. Yet, at 4°C metabolism is not fully stopped, ATP is progressively depleted and mitochondrial function dysregulated. After reperfusion, the production of reactive oxygen species, cytokines secretion, neutrophil infiltration and the impaired hepatic microcirculation provoke inflammation, cell death, loss of functioning parenchyma and ultimately organ failure[5,6]. Additionally, cholangiocytes are more susceptible to IRI and extended damage of the biliary epithelium is visible at the end of preservation of virtually all grafts[7,8]. Peribiliary vascular plexus and glands (containing the precursor's niche) are also damaged by microthrombi and necrosis, leading to impaired regeneration of the biliary epithelium. The physiological systems buffering the detergent effect of bile salts are dysregulated during the preservation phase, hence a toxic damage superimpose to the injuries of the biliary tree[9]. All these mechanisms are responsible of the development of Non Anastomotic biliary Strictures (NAS), a troublesome complication characterized by multiple stenosis of the bile ducts. Histological features of IRI include hepatocytes swelling, apoptosis, necrosis, sinusoidal endothelial cells detachment and polymorphonucleate infiltration[10].

The clinical manifestation of IRI can vary from immediate graft function with minimal damage to Early Allograft Dysfunction (EAD), Primary Non Function (PNF) and NAS. EAD is the result of the hepatocellular necrosis and it is biochemically evident as highly elevated transaminases and/or impaired synthetic function (elevated total bilirubin, prolonged coagulation) within the 1st week post-LT[11]. PNF is a life threatening condition in which the liver graft immediately fails on sustaining the physiological metabolic demands but a uniformly accepted definition is currently missing. Both graft and patient survival are severely reduced by the occurrence of EAD or PNF. NAS also represents a common cause of graft loss especially after transplantation of DCD grafts[12].

Strategies preventing IRI should reduce the incidence of its manifestations; clinical studies evaluating the efficacy of such strategies should therefore focus on transaminases peak post-LT, incidence of EAD/PNF, incidence of NAS and graft survival.

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2. Preconditioning (see Table 1)

Organ procurement offers a window for pre-treating the liver with several strategies. The most commonly attempted ones, are the Ischemic Preconditioning (IP) and the pharmacological preconditioning, i.e. administering medications effective against IRI.

2.1. *Ischemic preconditioning*

After the first report on the protective effect of transient run of IRI in the myocardium by Murray et *al.*[13], IP effect was evaluated and confirmed in different organs such as kidney and liver. The underlying protective mechanisms seem related to the increased production of protective mediators, antioxidants and inhibitor of apoptosis prior to the onset or IRI[14].
Different clinical trials have evaluated the effectiveness of IP applying different duration of ischemia and reperfusion. Koneru et *al*. first performed IP using a protocol consisting of five minutes of ischemia and five minutes of reperfusion before the beginning of cold ischemia. In a randomized trial involving 60 LTs no differences were found in surrogate or pathological markers of IRI[15]. Azoulay et *al*. used a protocol of ten minutes ischemia - ten minutes reperfusion and demonstrated a significant reduction of post-LT transaminase release. Despite this protective effect, IP significantly increased the incidence of EAD, although no impact on patient and graft survival was observed[16]. In our previous experience comparing the effect of IP (ten minutes ischemia – thirty minutes reperfusion) in both optimal and ECD grafts, post-transplant transaminases release was significantly reduced, especially in ECD grafts treated by IP, and the severity of histological IRI was improved. However, no difference was observed in functional recovery or incidence of EAD, although a trend towards better graft and patient survival was observed in a short-term follow-up (6 months). We concluded that, even if IP in suboptimal grafts showed a protective effect, it was not able to prevent the functional impairment often observed after LT with ECD grafts[17]. Degli Esposti et *al*. partly confirmed our findings analyzing steatotic and non-steatotic grafts undergoing IP. Steatotic grafts displayed reduction of necrosis and enhancement of autophagy after IP while no significant impact in non-steatotic grafts was observed[18]. Finally, a recent meta-analysis detected no improvement in post-transplant transaminases release, no differences in PNF, mortality, or retransplantation. Authors

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concluded that there are no evidences to support or to refute the use of IP during liver procurement[19].

The utility of IP in the setting of LT is far to be proven since clinical trials showed controversial results and data on the impact on long-term graft survival are still missing. Hence, IP should be used today only in the frame of experimental studies.

2.2. *Pharmacological preconditioning*

IRI has a complex pathogenesis where several harmful pathways are at play; inflammation is probably the most evident change and its severity is directly related to ischemia duration. Anti-inflammatory medication might be used to reduce IRI. Kotsch et *al*. tested the effectiveness of donor pulse infusion of methylprednisolone from the declaration of brain death (DBD) until organ procurement. In 100 DBD donors, the systemic concentration of pro-inflammatory cytokines (IL-6, IL-2, TNF-α) and the release of transaminases post-LT was reduced in donors randomly treated with steroids. Furthermore, mRNA quantification in biopsies taken at the beginning of the procurement showed a reduced expression of pro-inflammatory genes (TNF-α, IL-6, adhesion molecules and MHC class II). Untreated grafts more frequently experienced severe biopsy-proven acute rejection whilst no differences in incidence of PNF was observed[20]. Preconditioning with methylprednisolone in the donor seemed to counterbalance the pro-inflammatory effects of brain death and to effectively reduce the severity of inflammation after graft reperfusion.

Tacrolimus is an immunosuppressant that inhibits the activation of T lymphocytes and subsequently the release of chemokines; thus, tacrolimus exerts anti-inflammatory effects too. In a randomized trial involving 26 LTs, Kristo et *al.* evaluated the efficacy of tacrolimus infusion in the graft through the portal vein (1,500 ml of 5% albumin containing 20 ng/ml tacrolimus). Preconditioning with tacrolimus did not reduce transaminases release; markers of hepatic function (bilirubin, prothrombin time) did not differ between groups, thus tacrolimus preconditioning did not influence functional recovery. However, micro-array analysis showed significant reduction of genes related to inflammation in grafts pretreated with tacrolimus[21]. The effectiveness of tacrolimus preconditioning is therefore still unclear and should be investigated in further studies.

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Microcirculatory disturbances are related to direct damage of sinusoidal endothelial cells and platelet aggregation but also to decreased nitric oxide (NO), which is an endogenous vasodilator produced by nitric-oxide-synthase (NOS) whose function is impaired by IRI[22]. Restoration of NO content may be achieved with administration of inhaled-NO. In a randomized placebo-controlled trial, Lang et *al.* tested the efficacy of NO inhalation during the operative phase prior to graft reperfusion in 20 LTs. Reduction of transaminases release and functional recovery was significantly faster in recipients pretreated with NO, despite no differences in tissue markers of inflammation were observed[23]. Administration of NO during LT seemed therefore to improve functional recovery rather than directly reduce IRI, but survival analysis was not performed in the study.

It was postulated that sevoflurane, a volatile anesthetic that can activate NOS, might reduce IRI. Minou et *al*. randomly assigned 40 consecutive DBD donors to receive sevoflurane during liver procurement and observed a significant reduction of post-LT transaminases release. Furthermore, sevoflurane reduced the incidence of EAD selectively in steatotic grafts[24]. A recent multicenter randomized clinical trial, however, showed no advantage of sevoflurane anesthesia in LT. More than 100 recipients were assigned to receive inhaled sevoflurane during transplantation. The severity of hepatic injury (post-LT transaminase release) was similar in the study groups and no differences were observed in markers of hepatic function (bilirubin, prothrombin time). In contrast with the previous study, sevoflurane did not reduce the incidence of EAD, but the two protocols differed in timing of sevoflurane preconditioning (donor *vs*. recipient procedure)[25].

Hepatocyte injury after reperfusion is mediated by the release of reactive oxygen species with subsequent oxidative stress. Glutathione (GSH) is the major endogenous buffer involved in radical-free removal, whose synthesis is limited by the availability of precursors, especially cysteine. N-acetyl-cysteine (NAC) is a synthetic precursor that rapidly converts to cysteine into hepatocytes, therefore refurbishing GSH content. Several experimental and clinical studies evaluated the effectiveness of NAC administration prior to IRI, with contrasting results. In experimental settings, the vast majority of animal studies observed a significant reduction of transaminases release in animals pretreated with NAC[26]. However, different human trials reported either little protective or no effect of NAC preconditioning[27–30]. It must be observed that most of these trials considered small number of LTs and very few analyzed graft survival. Therefore, robust data on the effectiveness of NAC administration prior to LT are still missing.

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Hypoxia-inducible factor 1alpha (HIF-1alpha) is an oxygen sensible transcription factor that binds to its beta-heterodimer during acute hypoxia and promotes the expression of several genes related to metabolism, neo-angiogenesis and above all apoptosis/survival. The stabilization of the dimeric form of HIF-1 was shown to reduce transaminase release after IRI in animal experiments, via a mechanism probably involving iNOS pathway. However RNA transcription is impaired during ischemia and the restoration of blood flow during reperfusion coincides with the oxidation, degradation of the alpha subunit and ultimately destabilization of HIF heterodimers. Therefore the protection offered by HIF-1alpha signaling during IRI may be not good enough and therapeutic approaches have failed to reach the clinic[31].

IRI is the result of a complex cascade of inter-related events acting almost at the same time: tackling a single pathway administering a single medication might be not enough to effectively reduce IRI. Monbaliu et *al*. developed a multi-pharmacological IRI modulation, whose effectiveness was tested in a porcine DCD-LT model characterized by high PNF rate. Liver grafts were exposed to 45 minutes of warm ischemia (WI) before cold storage and were randomly treated or not with the pharmacological modulation. Modulated grafts were flushed with streptokinase (fibrinolytic agent) and epoprostenol (vasodilator) at the time of procurement; before, during and immediately after reperfusion of the liver, glycine (Kupffer's cell stabilizer), alpha1-acid-glycoprotein (anti-inflammatory), MAP-Kinase-inhibitor (pro-inflammatory cytokine generation inhibitor), alpha-tocopherol and glutathione (anti-oxidants), and apotransferrin (iron chelator) were administrated intravenously to the recipient animal. Transaminase release tended to be lower and oxidative stress, TNF-α concentration and bile salt-to-phospholipid ratio were significantly reduced in grafts treated with the modulation protocol. Notably, this multifactorial approach completely eliminated PNF, whose incidence was 55% in untreated animals. The multi-pharmacological modulation seems to be more effective compared to single-medication approaches, as it achieved the remarkable goal of preventing organ failure after transplantation[32]. If this effect will be confirmed in clinical settings, graft procured from high-risk donors could be transplanted in a safer manner.

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3. Preservation (see Table 2)

Static cold storage (SCS) is currently the standard for organ preservation but hypothermia alone does not ensure adequate graft viability. Preservation solutions are required to preserve homeostasis, supporting liver metabolism and delaying cell damage[33,34]. Several solutions are currently available: University of Wisconsin (UW), Histidine-Tryptophan-Ketoglutarate (HTK), Celsior (CS) and the most recent Institute Georges Lopez-1 (IGL-1). They all differ in terms of composition, ion balance, viscosity, osmolality and cost.

UW is a high viscosity colloid solution due to the presence of hydroxyethyl starch (HES), high potassium and low sodium concentration (125 mmol/L and 27 mmol/l, respectively)[35]. Despite its widespread use, UW carries some side effects: high viscosity might result in impaired flushing with microcirculatory disturbances; high potassium concentration might provoke cardiac arrest in the recipient upon liver reperfusion; finally, ischemic-type biliary lesions are not so rare[36].

HTK represents an alternative to UW whose advantages can be summarized in lower viscosity, lower potassium content (10 mmol/l, thus avoiding the risk of hyperkalemia) and better buffering properties[34]. CS has low viscosity, high sodium and low potassium concentration (100 mmol/l and 15 mmol/l, respectively) and combines the best aspects of both UW and HTK, retaining the buffering properties of HTK (histidine) and the presence of impermeants as in UW (lactobionic acid)[36,37]. Finally, in IGL-1 sodium and potassium concentrations are switched compared to UW ([Na+] = 120 mmol/l, [K+] = 25 mmol/l) and HES is replaced by polyethylene glycol resulting in lower viscosity[38]. These properties could improve liver wash-out during procurement and reduce IRI[39,40].

Identifying the best preservation solution is a difficult task, since randomized trials have shown conflicting results; however, some conclusions can be drawn from large series or clustering data from different trials. In a recent meta-analysis, Zuluaga et *al*. reported no differences in LT outcomes when UW was compared to CS or HTK. In particular, no differences were observed in terms of EAD, 1-year patient survival and occurrence of NAS[34]. Adam et *al.* partly confirmed this observation in their recent retrospective analysis of the European Liver Transplant Registry involving more than 48,000 LTs. Grafts preserved with UW, IGL-1 or CS showed similar 3-year graft survival; however, IGL-1 was associated with better preservation of partial liver grafts as

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shown by higher graft survival in this group. Notably, grafts preserved with HTK achieved significantly inferior survival three years post-LT when compared to grafts preserved with other preservation solutions and at multivariate analysis, HTK resulted an independent risk factor for graft loss[41].

HTK was believed equivalent to UW for SCS but less expensive; nevertheless, recent findings highlighted HTK-associated disadvantages. In a large single-center series and a retrospective analysis of UNOS database, the survival of grafts from standard or ECD donors did not differ when grafts were preserved with HTK or UW; however, in subgroup analyses, HTK-preserved grafts achieved significant inferior survival after exposure to long-lasting cold ischemia or procurement from DCD donors[42,43]. Furthermore, two different clinical trials from Meine et *al*. and Gulsen et *al.* reported relevant increases in the incidence of NAS in patients receiving grafts from DCD donors preserved with HTK[44,45]. The reason why HTK is associated with worse outcomes after LT is not fully understood; the lack of antioxidants and oncotic agents might be an explanation. To summarize, the currently available evidence points towards the avoidance of HTK as a preservation solution for livers.

IGL-1 has been previously tested in rat model of isolated liver perfusion and in pig model of auto-transplantation, showing better preservation of cell integrity[39,40,46]. Dondero et *al*., in a recent randomized trial including fatty livers, failed to demonstrate IGL-1 superiority, proving that it is at least as effective as UW, with less expenses[38]. However, the outcome of steatotic grafts was not the primary endpoint of this study, which was probably underpowered to draw solid conclusions. Randomized clinical trials comparing IGL-1 to other preservation solutions are still missing, but the recent large-series analysis from Adam et *al*. confirmed good outcomes and postulated possible advantages for IGL-1[41]. Further investigation is therefore needed to confirm this hypothesis.

It is important to stress that all the currently available preservation solutions were developed with the purpose to prevent hepatocytes death over the ischemic storage phase. Other cell populations, including cholangiocytes, are probably not so well protected from IRI. Two recent studies focusing on the pathological aspects of the common bile duct, have indeed revealed that the bile duct epithelium is seriously injured at the end of SCS in up to 88% of cases[7,8]. These findings indicate that the bile duct of almost all grafts suffers relevant injury, which might be responsible

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for NAS development. These complications add relevant morbidity and significantly reduce long-term graft survival, representing a rising concern due to the increased transplantation of DCD grafts, burdened with markedly higher rates of biliary complications[47,48]. Improved bile duct preservation might be offered by MP, which has been associated with better preservation of the bile duct in experimental settings[49,50].

4. Reconditioning (see Table 3)

The concept of preserving organs by MP dates back to the 70's[51]; however, the simplicity and the increasing success of SCS limited its application until the last decade. These devices consist in closed circuits in which dedicated solutions are pumped into the liver through its vasculature (portal vein, inferior vena cava and hepatic artery), providing oxygen and nutrients. Heat-exchangers connected to the circuit refrigerate or rewarm the perfusate, and different types of perfusion can be performed: hypothermic, normothermic and sub-normothermic.

In order to improve early aerobic metabolism and function, addition of oxygen during SCS was attempted and proven effective in a pilot study in DCD-LT by Treckmann et *al.* in which humidified oxygen was persufflated in the graft via the hepatic veins[52]. This concept was subsequently confirmed and improved by both *oxygenated* hypothermic and normothermic machine preservation.

In principle, MP better supports graft metabolism by a continuous provision of nutrients and a wash-out effect of catabolites, thus avoiding ATP-depletion[53,54]. Biomarkers released in the perfusate might therefore reflect the functionality of the graft, the measurement of which might help clinicians to assess graft viability and decide whether transplant or discard the liver. Early preclinical studies have shown that the outcome of suboptimal grafts can be reconditioned to the better through a period of MP[55] thanks to the restoration of the ATP-balance, which would allow the energy-depleted cells to better cope with the metabolic stress upon reperfusion[56].

4.1. *Hypothermic Machine Perfusion*

Hypothermic Machine Perfusion (HMP) was the first reaching the clinic fostered by the great success with the kidney experience[57,58]. The group of Guarrera et *al*. was the first to successfully apply HMP throughout the preservation period in humans. In their matched-

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controlled series, they demonstrated the feasibility and some advantages of HMP over SCS: less severe IRI and reduction of the release of pro-inflammatory chemokines. However, they did not show improvements in major outcomes, such as EAD or survival[59,60]. In addition, this study presents some limitations, included the utilization of mostly good quality grafts which historically perform well after SCS. It is therefore not surprising that HMP-preserved good quality grafts displayed good function after LT. In a subsequent analysis, the same group preserved 31 ECD livers with HMP and no advantage was observed in this type of grafts: the rate of EAD was not reduced and 1-year patient survival was comparable, despite *post-hoc* analysis showed significant less NAS in HMP grafts[61]. Given these limited clinical applications, it is difficult to claim that HMP offers advantages over SCS, especially in suboptimal grafts. Large-size multicenter randomized clinical trials are therefore needed to acquire more evidences.

The research team from Zurich has been applying oxygenated hypothermic MP at the end of cold storage (so called HOPE) to recondition liver grafts after DCD donation[62,63]. In porcine DCD grafts, significant reduction of hepatocyte necrosis and transaminase release, recovery of ATP content and increased bile production was observed in recipients of HOPE-treated livers. With a survival analysis, authors showed that HOPE converted PNF into EAD, thus not fully recovering DCD grafts[62]. Nevertheless, in their clinical human experiences they observed a significant reduction of transaminase release, EAD and NAS in DCD grafts treated with end-ischemic HOPE compared to either DCD or DBD grafts conventionally preserved[63,64]. However, HOPE-treated grafts were exposed to significantly shorter cold ischemia (3 *vs*. 6.5 hours), which is a well-known risk factor for graft loss and biliary complications in DCD-LT[48,65]. Additionally, the non-randomized multi-center design of their studies is a limitation; further results from large randomized trials are therefore needed to clarify the effect of HOPE.

Preliminary data suggested that HMP better preserves the biliary tree. In a rat model of LT with DCD grafts, Schlegel et *al*. showed that 1-hour HOPE was associated with significant reduction of intrahepatic fibrosis and cholangiocyte proliferation[66], although bile composition (reflecting cholangiocyte function) was not evaluated. Op den Dries et *al*. showed that HMP, applied as alternative to SCS, successfully prevents arteriolonecrosis of the peri-biliary plexus in pig DCD livers, thus ensuring better perfusion of the biliary tree. Yet, HMP failed to reduce injury to cholangiocyte function as showed by the analysis of the composition of bile produced during isolated reperfusion with autologous blood[49]. Authors claimed that the improvement of arterial

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perfusion would favor the recovery of post-ischemic bile ducts, and that this feature could not be observed during the short period of isolated reperfusion adopted in the study (2 hours). However, hypothermia reduces cell metabolism and pumping cold solution does not change this effect: HMP does not seem to couple better preservation with gain of function, despite the re-balanced energetic status. So, HMP applicability in LT remains unclear and large-size randomized clinical trials are required to further elucidate the potential of this new preservation technique.

4.2. *Normothermic Machine Perfusion*

With normothermic machine perfusion (NMP) the grafts is preserved in a functioning state, providing nutrients and adequate oxygen supply by means of blood-based preservation solutions. The concept of NMP is more appealing since it can completely avoid IRI if applied throughout the preservation phase. Furthermore, NMP allows extensive evaluation of the viability of grafts, opening the door to unique opportunities of intervention to be performed prior to LT.
Schön et *al*. were the first to experiment NMP in a pig model of transplantation[67], but the vast majority of the knowledge related to NMP comes from further studies of the Oxford group[68]. In a model of pig LT, Brockmann et *al*. compared the effectiveness of NMP and SCS on preserving both heart-beating and DCD grafts exposed to WI for 40 or 60 minutes. Post-LT transaminase release and severity of histological IRI were reduced in both types of grafts preserved with NMP, although the protective effect was less evident in DCD grafts exposed to 60 minutes WI, all of which experienced liver failure over time. Nevertheless, survival of DCD grafts exposed to 40 minutes WI and preserved with NMP was equal to that of heart-beating grafts preserved with the same technique. These results indicated the possibility to convert the outcome of high-risk livers (DCD) to that of 'standard' organs (heart-beating), although this result was observed with prolonged NMP[69]. Indeed, only 20 hours of preservation allowed a significant improvement of animal survival, showing that the protective effect of NMP might not be immediate but time-dependent. Interestingly, grafts developing functional failure after LT showed different behavior already during NMP preservation: some parameters such as transaminase release, bile production and vascular resistance could be used to predict post-transplant viability prior to LT.
Unfortunately, the addition of a short period of cold preservation in DCD grafts resulted detrimental in further experiments from the same group, since the subsequent application of

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NMP failed in protecting them from IRI. It must be noted, however, that in this setting all grafts were exposed to 60 minutes WI, in which NMP seems less effective[70]. A phase I human trial applying NMP during the preservation phase confirmed its safety and feasibility[71]; furthermore, the enrollment of a multi-center randomized clinical trial comparing NMP and SCS was completed and results are awaited.

Metabolic reconditioning reducing the fat content of steatotic grafts is another appealing characteristic of NMP. Jamieson et *al*. showed that fatty livers undergoing NMP progressively reduced steatosis over time and displayed nearly normal function[72]. The reduction of the percentage of steatosis in this model required a long run of MP, but this process can be accelerated by supplementing the perfusate with defatting medications. In fact, Nagrath et *al*. showed in obese rats that the addition of forskolin, hypericin, scoparone and visfatin could rapidly reduce the fat content by 50%[73]. If this effect would be confirmed in the clinical setting, NMP could increase the number of suboptimal steatotic grafts that could be considered for LT after reconditioning.

Minor et *al*. developed an intermediate approach of end-ischemic (sub)NMP in which the liver is slowly warmed up to 20°C. Controlled oxygenated rewarming (COR) has been tested in both animal and clinical studies and it was proved to better restore the energy balance, reduce the transaminase release and improve hepatic function when compared to SCS or HMP[74,75]. These preliminary studies suggested that smoothly increasing the temperature of the graft during MP may be more effective when compared to the abrupt rewarming observed during both NMP and graft reperfusion *in vivo*. This new concept deserves additional investigation,

Preliminary reports have suggested that NMP might better preserve the bile duct. Liu et *al*. using a porcine DCD model showed that 10 hours of NMP reduces injury to the biliary epithelium, as assessed by lower release of markers of cholangiocyte injury in bile (gamma-glutamyl-transferase and lactate dehydrogenase) and significantly milder lesions of the peri-biliary glands and vascular plexus at histology. Moreover, there were active proliferation of the biliary epithelium after 24 hours of isolated reperfusion and improved bile production compared to SCS preserved grafts[76]. This study suggested that NMP might promote bile duct regeneration after substantial injury, such as the one linked to DCD donation. Unfortunately, the model of isolated

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ex-vivo reperfusion adopted in the study could not monitor the development of NAS and these interesting findings still need confirmation in clinical settings.

Compared to HMP, the protection induced by NMP seems more profound since significant reduction of PNF and improvement of post-transplant survival was achieved (at least in animal experiments). These two types of MP differ in a fundamental characteristic: the metabolic state of the liver. Granting full metabolism, NMP may allow to dispose of the injuries experienced during the ischemic phase with a first reperfusion round. Indeed, during NMP the liver is reperfused with a blood-based solution in the absence of alloimmunity interference; this might exhaust the burst of reperfusion-associated injuries and promote a less pro-inflammatory environment, improving function and outcome after transplantation. In fact, Izamis et *al*., in a metabolic fluxes analysis in rat livers, showed that in the first two hours of NMP, ischemic livers consume less oxygen compared to non-ischemic grafts, with enhanced glycogenolysis and anaerobic glycolysis. After two hours, the oxygen consumption equals and the metabolism aligns to that of fresh livers, remaining stable and self-adjustable thereafter[77]. So, ischemic livers show impaired function during the early phase of NMP but, at later stages, they seem to dispose of the previous damage and to change their metabolism improving their function. This hypothesis would cope with the observation that a long period of NMP is required to observe significant improvement in survival[69]. It might be questioned whether applying NMP for a time longer than 10 hours throughout the preservation phase would mean merely preserving or actually reconditioning the graft after ischemic injury. In this regard, NMP potential is even more enhanced by the possibility to treat grafts with medications, gene delivery or stem cell therapy, with the intent to completely recover damaged livers prior to transplantation[78,79]. It is clear that if this possibility would be confirmed, it will greatly help expanding the scarce resource of transplantable liver grafts.

Conclusions

Performing organ transplantation inevitably leads to IRI, which is a potential threat to the success of the procedure. The complex pathogenesis of IRI permits different approaches to prevent or reduce it. Attempts to precondition the graft before IRI onset can be performed either by induction of a transient period of ischemia-reperfusion or by administration of anti-ischemic

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medications. IP did not appear to influence short-term outcomes after transplantation, while the administration of some medications before LT showed promising results, although multi-pharmacological modulation of IRI was even more effective in early animal experiments. Preservation solutions are currently worldwide used for SCS with comparable results, except for HTK solution. In fact, large clinical series and meta-analyses suggest that this preservation solution should be avoided due to an increased risk of graft loss. MP not only represents a valid alternative to SCS, but also allows evaluation of graft viability before transplantation and might recover the injury induced by ischemia. This is a newly re-born area of interest, which needs further exploration before drawing ultimate conclusions. Currently available data seems to point toward a more efficient preservation of grafts with NMP. Furthermore, it offers the unique opportunity to treat the organ with novel therapies, converting high-risk suboptimal grafts into transplantable livers. If reconditioning of risky grafts would successfully translate into clinical practice, it will certainly represent a major progress in transplantation, offering a valuable tool to help rebalancing the impaired equilibrium between donor offer and transplant demand.

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FIGURE LEGEND

Figure 1. Possible strategies to tackle Ischemia-Reperfusion Injury throughout the liver transplantation process and proposed outcome measures in the recipient.

COR: Controlled Oxygenated Rewarming; EAD: Early Allograft Dysfunction; HOPE: Hypothermic Oxygenated Perfusion; HTK: Histidine-Tryptophan-Ketoglutarate; IGL-1: Institute George Lopez 1; NAS: Non-Anastomotic biliary Strictures; PNF: Primary Non Function; UW: University of Wisconsin solution

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Liver graft preconditioning, preservation and reconditioning

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Conflict of interest: the authors of the manuscript in line with the policy of the journal declare they do not have any conflict of interest to disclose.

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Table 1. **Summary of clinical and experimental studies considering preconditioning strategies.**

Study	Characteristics	Endpoints	Results
Ischemic Preconditioning			
Koneru et al. (2005) [7]	Randomized clinical trial IP 5 min ischemia 5 min reperfusion	62 LT IP vs. standard procurement	Safety	IP safe technique
			Liver function (post-LT AST, ALT, bilirubin, INR)	Comparable post-LT liver function
Azoulay et al. (2005) [8]	Prospective non-randomized clinical trial IP 10 min ischemia 10 min reperfusion	91 LT IP vs. standard procurement	Primary: incidence of IPF	Increased incidence of IPF
			Secondary: post-LT AST, ALT peak	Reduced post-LT AST, ALT peak
			Incidence of acute rejection	Reduced incidence of acute rejection
Franchello et al. (2009) [9]	Randomized clinical trial IP 10 min ischemia 30 min reperfusion	75 LT 64% ECD IP vs. standard procurement	Primary: post-LT liver injury (AST, ALT), function (INR, bilirubin, bile output)	Reduced liver injury in IRI in ECD Comparable liver function
			Histological severity of IRI	Reduced severity of IRI
			Secondary: incidence of infection complication	Reduced incidence of infection complications

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			6mo graft-survival subgroup analysis in ECD	Trend toward improved survival (confirmed in ECD)
Degli Esposti et al. (2011) [10]	Retrospective analysis of a prospective non-randomized trial	50 LT, 56% ECD (steatosis) IP vs. standard procurement	Primary: post-LT liver injury (AST, ALT) Secondary: incidence of necrosis (histology) acute rejection	Reduced injury in non-steatotic graft Reduced necrosis in steatotic graft Reduced incidence of acute rejection in steatotic graft IP induces autophagy in steatotic graft

Pharmacological Preconditioning

Kotsch, et al. (2008) [12]	Randomized clinical trial Methylprednisolone	100 LT Donor pulse infusion methylprednisolone	Post reperfusion release of pro-inflammatory interleukins Incidence of PNF Incidence of acute rejection	Reduced release of pro-inflammatory interleukins (IL2, IL6, TNF-alpha) Comparable incidence of PNF Reduced incidence and severity of acute rejection
Kristo et al. (2011) [13]	Randomized clinical trial Tacrolimus	26 LT Intraportal tacrolimus infusion (prior LT)	Primary: inflammatory response (genomic analysis) Secondary: post-LT liver injury (AST, ALT) and function (bilirubin,	Reduced expression of genes related to inflammation and immune response Comparable post-LT liver injury and function

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			PT)	
Lang et al. (2007) [15]	Randomized clinical trial Inhaled NO	20 LT Inhaled NO during LT	Primary: post-LT liver injury (ALT, AST), function (PTT, INR) Secondary: Tissue inflammation marker (histology)	Decreased post-LT liver injury, faster functional recovery No changes in inflammatory markers, reduced apoptosis
Minou et al. (2012) [16]	Randomized controlled trial Sevoflurane	60 LT Donor sevoflurane anaesthesia (DBD)	Primary: post-LT liver injury (AST, ALT) Secondary: incidence of EAD	Reduced post-LT liver injury Reduced incidence of EAD, especially in steatotic graft
Beck-Schimme et al. (2015) [17]	Multicentre randomized clinical trial Sevoflurane	98 LT Recipient sevoflurane anaesthesia	Primary: post-LT liver injury (AST) Secondary: incidence of EAD	Comparable post-LT liver injury Comparable incidence of EAD

DBD: donor after brain death; EAD: early allograft dysfunction; ECD: extended criteria donor; IP: ischemic preconditioning; IPF: initial poor function; IRI: ischemia-reperfusion injury; LT: liver transplantation; PNF: primary non-function.

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Table 2. **Summary of different clinical studies comparing preservation solutions.**

	Study	Characteristics	Endpoints	Results
Meine et al. (2006) [35]	Randomized clinical study	102 LT HTK vs. UW	Primary: incidence of PDF Secondary: graft survival, incidence of biliary complications	Comparable incidence of PDF Comparable graft survival Higher incidence of biliary complication in HTK HTK 25.8% vs. UW 8.6.% ($p<0.05$)
Ben Abdennebi et al. (2006) [31]	Animal experimental study (pig)	Porcine LT model IGL-1 vs. UW	Transaminase peak	Lower release of transaminase in IGL-1
Mangus et al. (2008) [33]	Retrospective clinical study	698 LT 62% ECD HTK vs. UW	primary: 3-mo mortality and graft loss 1-yr graft and patient survival. Secondary: incidence of biliary complications	Comparable 1-yr graft and patient survival, independently from graft type HTK may reduce incidence of biliary complication
Stewart et al. (2009) [34]	Retrospective clinical study	18 586 LT UNOS-database HTK vs. UW	Primary: graft survival Sub-analysis considering graft type (DCD vs. DBD) cold ischemia (short vs. long), donor age	HTK independent risk factor for reduced graft survival especially in DCD and prolonged cold ischemia.
Dondéro et al.	Randomized	140 LT	30-day allograft injury and	IGL-1 is as safe as UW

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(2010) [29]	clinical trial	IGL-1 vs. UW	function (AST, bilirubin, INR) 30-day incidence of HAT, PNF, NAS	
Garcia Gil et al. (2011) [24]	Randomized clinical trial	70 LT CS vs. UW	Primary: incidence of post-reperfusion syndrome Secondary: incidence of PNF, PDF	Lower incidence of post-reperfusion syndrome in CS CS 5.9% vs. UW 21.6 % ($p<0.05$) No PNF, no PDF
Gulsen et al. (2013) [36]	Retrospective clinical trial	35 LT from DCD grafts HTK vs. UW	incidence of biliary complication	Increased biliary complication with HTK HTK 76% vs UW 39% ($p<0.05$)
Lema Zuluaga et al. (2013) [25]	Meta-analysis	CS vs. UW HTK vs. UW CS vs. HTK	Primary: incidence of PNF, PDF Secondary: 4mo graft- and patient-survival incidence of ITBL	Comparable incidence of PNF, PDF Comparable graft and patient-survival Comparable incidence of ITBL
Adam et al. (2015) [32]	Retrospective clinical study	42,869 LT ELTR-registry HTK vs. UW CS vs. UW IGL-1 vs. UW	Graft survival	Comparable survival in UW, CS, IGL-1 Reduced survival in HTK HTK independent risk factor for graft loss

CS: Celsior solution; DBD: donation after brain death; DCD: donation after circulatory death; ELTR: European Liver Transplant Registry; HAT: hepatic artery thrombosis; HTK: Histidine-Tryptophan-Ketoglutarate; IGL-1: Institute George Lopez 1; ITBL: ischemia type biliary lesions; LT: liver transplantation; NAS: non-anastomotic biliary strictures; PDF: primary dysfunction; PNF: primary non-function; UNOS: United Network for Organ Sharing; UW: University of Wisconsin.

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Table 3. **Summary of clinical and experimental studies considering machine perfusion preservation and reconditioning.**

Study	Characteristics	Machine Perfusion	Endpoints	Results
Hypothermic				
Human Studies				
Guarrera et al. (2010) [51]	Non-randomized control-match clinical study	40 LT HMP vs. SCS	4-7 h HMP throughout preservation	Primary: incidence of PNF, EAD	No PNF in either groups, comparable incidence of EAD
				1-mo, 1-yr graft and patient survival	No differences in graft and patient survival
				Secondary: post-LT liver injury (AST, ALT), function (INR)	Reduced AST/ALT peak; comparable post-LT function
				Length of hospital stay	Reduced ICU and hospital stay
Henry et al. (2012) [52]	Non-randomized control-match clinical study	40 LT HMP vs. SCS	HMP throughout preservation (4-7h HMP)	Tissue expression of pro-inflammatory cytokines	Attenuated expression and release of pro-inflammatory cytokines
Dutkowski et al. (2014) [55]	Non-randomized	16 LT	2.4h SCS + 2h HOPE	Post-LT liver injury (AST,	Comparable release of liver enzymes after

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	control-match clinical study	HOPE vs. SCS DCD vs. DBD	vs. SCS	ALT), renal function, incidence of reperfusion syndrome 6-mo, 9-mo incidence of post-LT biliary complications	reperfusion, comparable incidence of kidney failure or reperfusion injury No incidence of biliary complications 6-mo or 9-mo post-LT in DCD treated with HOPE
Guarrera et al. (2015) [53]	Non-randomized control-match clinical study	61 LT ECD only HMP vs. SCS	HMP throughout preservation (4-7h HMP)	Primary: incidence of PNF, EAD, vascular complication. 1-yr graft and patient survival Secondary: incidence of biliary complication	Comparable incidence of PNF, EAD and vascular complication Comparable graft and patient survival Reduced incidence of biliary complications
Experimental Studies					
De Rougemont et al. (2009) [54]	Experimental animal study (pig)	Pig model of LT DCD 60min WI HOPE vs. SCS	HMP reconditioning 6h SCS + 1h HOPE vs. 7h SCS	Primary: serum (AST), tissue marker (ATP, glutathione) of liver injury, bile flow Secondary: animal	Reduced necrosis (AST), improved ATP and glutathione recovery, improved bile flow PNF not eliminated but converted to PDF

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				survival	
Schlegel et al. (2013) [58]	Experimental animal study (rats)	Rodent model of LT DCD 30min WI HOPE vs. SCS	HMP reconditioning 4h SCS + 1h HOPE vs. 4h SCS	Primary: post-LT liver injury (AST, ALT), function (INR, factor V) Secondary: biliary injury and fibrosis	Reduced liver injury, improved function post-LT Reduced cholangiocytes proliferation, decreased biliary fibrosis
Op den Dries et al. (2014) [42]	Experimental animal study (pig)	Pig model of LT DCD 30min WI HMP vs. SCS	HMP throughout preservation (4h HMP)	Biliary epithelial injury (biliary LDH, γGT, histology) and function (biliary pH, bicarbonate)	Comparable biliary epithelial injury and function. Reduced necrosis of the peri-biliary vascular plexus
Normothermic					
Human Studies					
Ravikumar et al. (2016) [63]	Phase I non-randomized match-controlled clinical trial	60 LT NMP vs. SCS DBD and DCD (safety and feasibility)	NMP throughout preservation (3.5-18.5h)	Primary: 30d graft survival Secondary: peak AST/ALT, EAD, 6mo graft survival	Similar 30d graft survival Reduced peak AST, trend toward reduction of EAD incidence in NMP Similar 6mo graft survival
Experimental					

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Studies					
Schön et al. (2001) [59]	Experimental animal study (pig)	Pig model of LT DCD 60min WI NMP vs. SCS	NMP throughout preservation (4h NMP)	Post-LT liver injury (AST, ALT, histology), function (INR) Incidence of PNF, animal survival	Reduced liver injury, reduced necrosis, improved functional recovery Elimination of PNF, improved animal survival
Reddy et al. (2004) [62]	Experimental animal study (pig)	Pig isolated liver perfusion model DCD 60min WI	NMP reconditioning 24h NMP vs. 4h SCS + 20h NMP	Liver injury (AST, ALT, histology) and function (bile production, factor V, acid-base homeostasis, glucose metabolism)	Increased liver injury and necrosis after SCS Reduced bile production and impaired function after SCS NMP failed to resuscitate liver after SCS
Brockmann et al. (2009) [61]	Experimental animal study (pig)	Pig model of LT HBD DCD 40min WI DCD 60min WI NMP vs. SCS	NMP throughout preservation (5h NMP; 20h NMP)	Post- LT liver injury (AST, ALT, histology). Survival	Decreased liver injury and improved histology (40min WI – 20h NMP). No PNF, improved survival (40min WI – 20h NMP)
Nagrath et al. (2009) [65]	Experimental animal study (rat)	Rodent isolated liver reperfusion model Steatotic liver	NMP reconditioning	Perfusate fat content (triglycerides, VLDL), histology assessment of	Increased lipid peroxidation and export Reduced fat content by 50% (3h NMP)

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		Defatting cocktail		steatosis	
Jamieson et al. (2011) [64]	Experimental animal study (pig)	Pig isolated liver perfusion model Steatotic liver vs. Normal liver	NMP throughout preservation (48h NMP)	Liver function (bile production, factor V, acid-base homeostasis), fat content	Comparable function during isolated liver perfusion, Increased mobilization of triglycerides, reduction of fat vacuoles
Liu et al. (2014) [66]	Experimental animal study (pig)	Pig isolated liver perfusion model DCD 60min WI NMP vs. SCS	NMP throughout preservation (10h NMP)	Liver injury (AST, histology) Biliary epithelium preservation and regeneration (histology, Ki-67)	Reduced liver injury, improved histology Reduced injury of the peri-biliary gland and plexus, enhanced regeneration

DCD: donor after circulatory death; EAD: early allograft dysfunction; HBD: heart beating donor; HMP: hypothermic machine perfusion; HOPE: hypothermic oxygenated machine perfusion; LT: liver transplantation; NMP: normothermic machine perfusion; SCS: static cold storage; PNF: primary non-function; VLDL: very-low density lipoprotein; WI: warm ischemia

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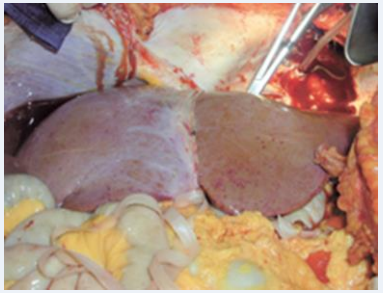
DONATION

PRECONDITIONING

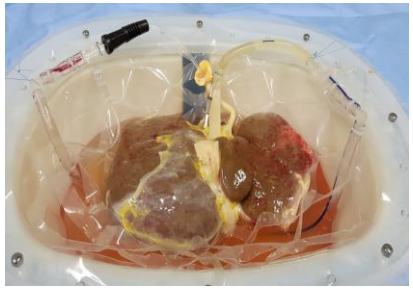
Ischemic
Pharmacological



STORAGE - TRANSPORTATION

PRESERVATION
UW/HTK/Celsior/IGL-1

RECONDITIONING
Hypothermic Machine Perfusion
Normothermic Machine Perfusion

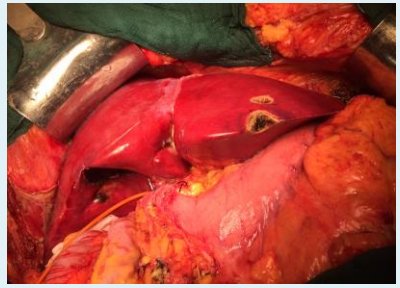


END-ISCHEMIC RECONDITIONING
HOPE
COR

TRANSPLANTATION

OUTCOME MEASURES

Transaminases peak
EAD/PNF
NAS
Graft survival



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