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Review Article

Assessing Donor Liver Quality and Restoring Graft Function in the Era of Extended Criteria Donors

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Abstract

Liver transplantation (LT) is the final treatment option for patients with end-stage liver disease. The increasing donor shortage results in the wide usage of grafts from extended criteria donors across the world. Using such grafts is associated with the elevated incidences of post-transplant complications including initial nonfunction and ischemic biliary tract diseases, which significantly reduce recipient survival. Although several clinical factors have been demonstrated to impact donor liver quality, accurate, comprehensive, and effective assessment systems to guide decision-making for organ usage, restoration or discard are lacking. In addition, the development of biochemical technologies and bioinformatic analysis in recent years helps us better understand graft injury during the perioperative period and find potential ways to restore graft function. Moreover, such advances reveal the molecular profiles of grafts or perfusate that are susceptible to poor graft function and provide insight into finding novel biomarkers for graft quality assessment. Focusing on donors and grafts, we updated potential biomarkers in donor blood, liver tissue, or perfusates that predict graft quality following LT, and summarized strategies for restoring graft function in the era of extended criteria donors. In this review, we also discuss the advantages and drawbacks of these potential biomarkers and offer suggestions for future research.

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Introduction

Liver transplantation (LT) is a life-saving treatment option for patients with end-stage liver disease. In recent decades, good short- and long-term outcomes after LT have been achieved because of improvements in surgical technologies and organ preservation.[1] Graft quality is believed to play a dominant role in early graft function and thereby dramatically influences graft survival and mortality after LT.[2–4] Over the last decade, the disparity between the need for LT and the organ shortage is widening, which leads to the expanded usage of grafts from the extended criteria donors (ECDs).[1] Traditionally, ECDs are donors with underlying medical diseases such as diabetes, or hypertension, advanced age, high-degree liver steatosis, prolonged ischemia time, pathogenic infection, prolonged intensive care unit stay, hypernatremia, and donation after circulatory death (DCD).[5–7] ECD graft quality is routinely considered inferior because of their increased rate of post-transplant complications, such as primary graft nonfunction (PNF),[2] early allograft dysfunction (EAD),[4] and ischemic-type biliary lesions (ITBLs)[8,9]

PNF is early graft loss after LT and requires emergency regrafting, which occurs following 2–10% of LTs.[10–12] ECDs include DCD donors[13] and those with severe steatosis,[14] prolonged ischemia time,[15–17] and high donor bilirubin level[18] sharply increase the risk of PNF, thereby reducing patient and graft survival. Unlike PNF, EAD represents marginal, usually reversible, graft function during the first postoperative week, and results in a higher morbidity and mortality.[4] Compared with 1–10% seen in donation after brain death (DBD) LT, the incidence of biliary complications after DCD LT is approximately 10–30%,[19–22] in which the time from asystole to cross-clamp is considered as a ma-

Keywords: Liver transplantation; Extended criteria donors; Graft quality; Assessment; Biomarkers; Restoration.

Abbreviations: ACR, acute cellular rejection; ALT, alanine transferase; AST, aspartate transferase; circRNAs, circular RNAs; DBD, donation after brain death; DCD, donation after circulatory death; DCs, dendritic cells; EAD, early allograft dysfunction; ECD, extended criteria donor; EV, extracellular vesicle; FMN, flavin mononucleotide; GcfDNA, graft-derived cell-free DNA; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; HMP, hypothermic machine perfusion; HO-1, Heme oxygenase-1; HOPE, hypothermic oxygenated perfusion; IRI, ischemia-reperfusion injury; ITBL, ischemic-type biliary lesion; KC, Kupffer cell; lncRNA, long noncoding RNA; LSEC, liver sinusoidal endothelial cell; LT, liver transplantation; MICA, major histocompatibility complex class 1 related chain A; miRNA, microRNA; MP, machine perfusion; mRNA, messenger RNA; MSC, mesenchymal stem cell; mtDAMP, mitochondria-derived damage-associated molecular pattern; NF-κB, nuclear factor-kappa B; NK, natural killer; NMP, normothermic machine perfusion; NODM, new-onset diabetes mellitus; PGE1, Prostaglandin E1; PNF, primary graft nonfunction; scRNA-Seq, single cell RNA sequencing; SCS, static cold storage; siRNA, small interfering RNA; Treg, regulatory T lymphocyte.

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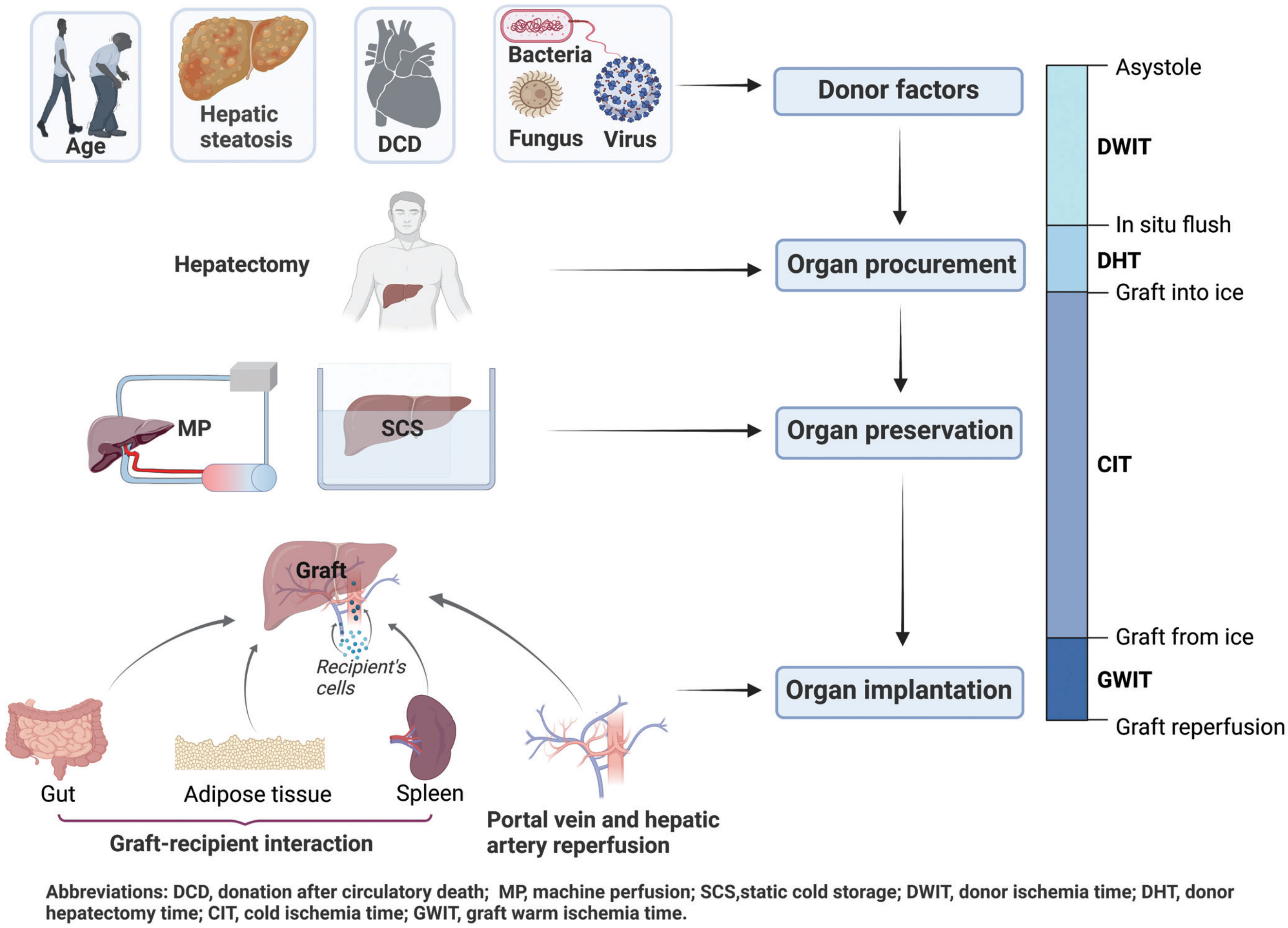

Fig. 1. Clinical factors that influence graft quality during the entire process of liver transplantation.

jor risk factor.[23] Moreover, advanced donor age, prolonged ischemia time, microvascular thrombosis, bile salt toxicity and immune injury may be the underlying mechanisms of the development of biliary complications.[24,25]

Therefore, ECDs should be well defined and precisely allocated to appropriate recipients. More importantly, in the era of ECD, effective systems need to be established to assess donor liver quality and guide the decision for organ usage or discard. Based on clinical risk parameters (Fig. 1), models like donor risk index,[2] Eurotransplant donor risk index,[26] and discard risk index[18] were constructed to evaluate the risk of graft failure or discard, serving as useful tools to make decisions for organ allocation.[2,26] However, those scoring models mainly focus on donor characteristics and cannot assess the degree of liver injury.[27] Furthermore, combining clinical parameters with advanced molecular profiles, imaging, or histopathology may contribute to the development of better systems. In recent years, with the rapid development of multi-omics, single cell technology, and bioinformatic analysis, significant achievements have been made in revealing the molecular profiles that are closely related to poor graft outcomes, and which can provide novel biomarkers for evaluation of graft viability.

Herein, we provide a review of potentially useful biomarkers in donor blood, liver tissue, and graft perfusate, which have been associated with impaired graft quality or predictive for the occurrence of EAD, PNF, and biliary complications after LT. In this review, we mainly focus on studies using human liver grafts. Given that the available biomarkers were insufficient in the field of LT, we also include experimental studies that have been performed in animal models. Furthermore, we summarize potential therapies for graft repairment during LT. Finally, we describe the pros and cons of the potential biomarkers, accompanied with suggestions for future graft assessment and restoration.

Potential biomarkers in donor blood

Donor serum alanine transferase (ALT), aspartate transferase (AST), total bilirubin, gamma glutamyl transpeptidase, and sodium concentration may reveal the underlying liver dysfunction and ischemic injury prior to graft procurement. Over the past decades, numerous studies have demonstrated that such laboratory disorders in donor blood are independent risk factors for early graft dysfunction following LT.[18,26,28] In recent years, novel biomarkers in donor blood have been found to useful for predicting graft outcomes. By analyzing data from over 10,000 nondiabetic donors, Ezekian *et al*.[29] showed that elevated donor serum hemoglobin A1c (HbA1c) >6.5% was associated with increased rate of PNF and decreased graft and patient survival. HbA1c is known to be a useful biomarker representing the average plasma glucose concentration within the last 3 months, serving as an early warning of diabetes. The liver undergoes glycogen deposition and hepatic steatosis resulting from diabetes.[30,31] Therefore, it is worth noting that HbA1c may be a valuable marker for further stratifying marginal graft quality. In a large prospective study of 815 participants, Piemonti *et al*.[32] identified increased serum donor interleukin 6 (IL6) and C-X-C motif chemokine ligand 10 (CXCL10) concentration as predictors of poor early graft function, graft failure and inferior graft survival after DBD LT. IL6 is responsible for transforming naïve B cells into mature plasma cells, as well as activating the production of IL17 to inhibit regulatory T lymphocyte (Treg) function.[32] Alternatively, CXCL10 is a useful chemoattractant for macrophages, natural killer (NK) cells and dendritic cells (DCs), thereby shaping initial immunity.[32] More interestingly, Pollara *et al*.[33] found that elevated circulating mitochondria-derived damage-associated molecular patterns (mtDAMPs) in donor plasma were associated with severe inflammation response and the development of EAD following DBD LT in a group of 55 recipients. The major source of mtDAMPs may be the mitochondria released from graft tissue or cell death during organ procurement, suggesting that mtDAMPs might quantitatively assess graft injury.

Potential biomarkers in donor grafts

The liver, a multifunctional organ in the body, is mainly engaged in metabolism, synthesis, storage, detoxification, and complex immune activities. After implantation, the donor graft becomes the new center of the recipient to perform those functions.[34] Therefore, the graft features could significantly regulate hepatic homeostasis and influence outcomes after LT (Table 1).[35–55] Donor grafts could be gained for histological assessment and quantification of liver injury during LT. Histopathology is the gold standard for the diagnosis of steatosis, fibrosis, necrosis, inflammation, and cellular infiltration in liver grafts. In our center, pretransplant, and post-reperfusion liver biopsies are routinely performed, offering valuable clues for graft quality assessment (Supplementary Table 1).[56] In addition, bile duct biopsies could provide valuable information to evaluate bile duct injury and predict graft outcomes. Dries *et al*.[57] proposed a scoring system (Supplementary Table 2), including biliary epithelium, mural stroma, peribiliary vascular plexus, thrombosis, intramural bleeding, peribiliary gland, and inflammation, to quantify bile duct injury.

Genetic variants

With the advent of genome-wide association studies and pretransplant genetic analysis, a series of genes and variants have been found to be susceptible to graft injury.[58] Heme oxygenase-1 (HO-1), a regulator of immune response, is considered to be cytoprotective gene of ischemia-reperfusion injury (IRI) during LT and is modulated by a single-nucleotide polymorphism A (-413) T.[35] Buis *et al*.[35] reported that, compared with recipients of a liver with an A-allele genotype (n=245), recipients of livers with an HO-1 TT-genotype (n=61) had dramatically elevated serum hepatic transaminases after LT and a higher incidence of PNF. HLA-C, which is the major inhibitory ligand for immunoglobulin-like receptors, inhibit the cytotoxic activity of NK cells, and therefore reduced liver inflammatory damage.[59] In a large LT cohort of 459 patients, Hanvesakul *et al*.[36] found that donor grafts with at least one HLA-C2 allele were associated with less incidence of graft dysfunction and rejection.

After LT, graft-derived cell-free DNA (GcfDNA), which is continuously released into recipient circulation because of cellular turnover, is a promising noninvasive biomarker to assess graft quality. Previous studies have showed that the elevated GcfDNA was a signal of early graft injury after LT, particularly acute cellular rejection.[37,60,61,38] For example, a prospective study conducted by Schutz *et al*.[38] demonstrated that GcfDNA increased by more than 50% 1 day following LT, probably because of the IRI. However, GcfDNA rapidly decreased to a median of <10% within 7–10 days without the recipient experiencing early graft injury over a 1 year observation period.[38] This suggested that GcfDNA may be a precise and superior biomarker to predict early graft dysfunction compared with conventional liver function tests.

RNAs

Protein-coding associated RNAs, for example messenger RNA (mRNA) and noncoding RNAs including microRNAs (miRNAs), circular RNAs (circRNAs) and long noncoding RNAs (lncRNAs) are believed to be reliable markers to evaluate graft injury because of their organ specificity. Nrf2 transcription factor, which is activated by reactive oxygen species, is known to protector against liver IRI via activating phase II antioxidants.[62] Zaman *et al*.[39] demonstrated that grafts (n=6) with increased Nrf2 mRNA expression before IRI were associated with lower liver injury. Interestingly, donors with low Nrf2 mRNA levels (n=8) were significantly older than those with high levels, suggesting that older grafts experienced severe IRI[39] and inferior graft quality. Additionally, Resch *et al*.[40] reported that high gene expression of the major histocompatibility complex class 1 related chain A (MICA) mRNA in zero hour biopsies (n=88) was associated with mild graft injury and prolonged graft survival. During LT, MICA had an important role in linking the innate and adaptive immune responses via interacting with NK cells, mucosal-associated invariant T, CD8+T cells, *et al*.[40] miR-22, a regulator of a series of pathways such as cell cycle, metabolism and kinase signaling, is relevant to cell survival, glucose metabolism, and protein translation.[41] Khorsandi *et al*.[41] rereported that low expression of graft miR-22 was associated with the incidence of PNF after DCD LT (n=21). Another study of 42 human LTs showed that high expression of donor graft miR-146b-5p was associated with the development of EAD.[42] Downregulation of miR-146b increased the production of tumor necrosis factor receptor-associated factor 6, which activated the nuclear factor-kappa B (NF-κB) pathway, and in turn enhanced Treg function.[42,63] In our previous study, we found that elevated donor graft miR-103 and miR-181 were significantly associated with the development of new-onset diabetes mellitus (NODM) in recipients following LT (n=30).[43] NODM not only increased the risk of biliary stricture and cholangitis but also resulted in poor graft survival, serving as an indicator of poor graft quality as well.[64] The two miRNAs targeted several genes related to glucose homeostasis and insulin signal transduction, which may have been the underlying mechanism.[43]

In a cohort of 115 human LTs, Wang *et al*.[44] reported that low levels of donor graft circFOXN2 and circNEXTIN3 that regulated miR-135b-5p and miR-149-5p and had roles in hepatic IRI were associated with the incidence of EAD. In a mice model of IRI (Qu *et al*.[65] identified 13 differentially expressed circRNAs (e.g., Chr3:83031528|83031748, Chr10:89473752|89483524) in postperfusion livers that were involved in more severe IRI in steatotic livers. In a rat LT model, Chen *et al*.[45] demonstrated that lncRNA LOC103692832 in rat grafts was related to early graft injury following LT that was mediated by the expression of apoptosis-related genes like *HMOX1* and *ATF3*. Nevertheless, the mechanisms of these potentially involved circRNAs and lncRNAs are still unclear, and further prospective or multi-

Table 1. Biomarkers from donor livers potentially useful for the prediction of graft outcomes following transplantation

Biomarker		Study	Sample	Model(s)	Group	Key point
Genetic variant	Heme oxygenase-1 A/T-allele genotype	Buis *et al*. (2008)[35]	Pretransplant biopsies	Human LT	A-allele genotype (n=245) vs. TT-genotype (n=61)	Graft with TT-genotype had elevated serum transaminases after LT and a higher incidence of PNF
	HLA-C2 allele	Hanvesakul *et al*. (2008)[36]	Pretransplant biopsies	Human LT	459 livers biopsies	Donor grafts with HLA-C2 allele were associated with less incidence of graft dysfunction
	GcfDNA	Levitsky *et al*. (2021)[37]	Recipient blood	Human LT	Normal function (n=94) vs. Acute dysfunction (n=68)	Elevated GcfDNA represented early graft injury after LT
	GcfDNA	Schutz *et al*. (2017)[38]	Recipient blood	Human LT	/	Elevated GcfDNA could predict early graft injury
RNA	Nrf2 mRNA	Zaman *et al*. (2007)[39]	Pretransplant biopsies	Human LT	14 donor liver biopsies	Higher Nrf2 mRNA expression before IRI were associated with lower liver injury
	MICA mRNA	Resch *et al*. (2021)[40]	Pretransplant biopsies	Human LT	88 liver biopsies	High expression of MICA mRNA could reduce graft injury
	MiR-22	Khorsandi *et al*. (2015)[41]	Post-reperfusion biopsies	Human DCD LT	PNF (*n*=7) vs. non-PNF (*n*=7)	Graft miR-22 was associated with PNF
	MiR-146b-5p	Li *et al*. (2017)[42]	1.5 hours after LT	Human LT	EAD (*n*=22) vs. non-EAD (*n*=20)	Graft miR-146b-5p was associated with EAD
	MiR-103 and miR-181	Ling *et al*. (2017)[43]	Pretransplant biopsies	Human LT	NODM (*n*=15) vs. non-NODM (*n*=15)	Graft miR-103 and miR-181 were significantly associated with the development of NODM
	CircFOXN2 and circNEXTIN3	Wang *et al*. (2021)[44]	Pretransplant biopsies	Human LT	EAD (*n*=29) vs. non-EAD (*n*=86)	Two circRNAs were associated with EAD
	LncRNA LOC103692832	Chen *et al*. (2019)[45]	12 hours after LT	Rat DBD LT model	/	Graft lncRNA LOC103692832 was related to early graft injury
Protein	Sirtuin 1	Nakamura *et al*. (2017)[46]	2 hours after LT	Human LT	51 liver biopsies	High graft Sirtuin 1 was associated with superior liver function
	Heme oxygenase-1	Nakamura *et al*. (2018)[47]	2 hours after LT	Human LT	51 liver biopsies	Enhanced Sirtuin 1 expression and protected against IRI
	YAP	Liu *et al*. (2019)[48]	3 hours after LT	Human LT	60 liver biopsies	Improved early liver function
	FGF15	Gulfo *et al*. (2020)[49]	Post-reperfusion biopsies	Rat DBD LT model	/	Low graft FGF15 was associated with more severe hepatic damage and inhibited regeneration
	CEACAM1	Nakamura *et al*. (2020)[50]	Pretransplant biopsies	Human LT	60 liver biopsies	Hepatic CEACAM1 could prevent early graft injury
	Hepatic occult collagen deposition	Hirao *et al*. (2021)[51]	Pretransplant biopsies	Human LT	Low level (*n*=140) vs. High level (*n*=54)	Increased risks of severe IRI and EAD

(continued)

Table 1. *(continued)*

Biomarker		Study	Sample	Model(s)	Group	Key point
Metabolite	Lysophospholipids, bile acids, phospholipids, sphingomyelins, and histidine metabolism products	Cortes *et al*. (2014)[52]	Pretransplant biopsies	Human LT	EAD (n=48) vs. non-EAD (n=48)	Predictors for EAD
	Lactate and phosphocholine	Faitot *et al*. (2018)[53]	Pretransplant biopsies	Human LT	EAD (n=7) vs. non-EAD (n=35)	Predictors for EAD
single cell RNA sequencing	A pro-inflammatory phenotype of KCs and a subset of DCs	Yang *et al*. (2021)[54]	24 hours after LT	Rat steatotic LT model	Fatty graft (n=3) vs. Control graft (n=3)	A pro-inflammatory phenotype of KCs that highly expressed colony-stimulating factor 3 and a subset of DCs with high expression of XCR1 were enriched in the steatotic grafts
	A dynamic transcription profile	Wang *et al*. (2021)[55]	Grafts gained from preprocurement, at the end of organ preservation and 2 h after reperfusion	Human DBD LT	n=1	Showed a dynamic transcription profile of intrahepatic cells during LT

DBD, donation after brain death; DCD, donation after circulatory death; DCs, dendritic cells; EAD, early allograft dysfunction; GcfDNA, graft-derived cell-free DNA; IRI, ischemia-reperfusion injury; KCs, Kupffer cells; LT, liver transplantation; MICA, major histocompatibility complex class 1 related chain A; NODM, new-onset diabetes mellitus; PNF, primary nonfunction.

center studies with larger samples are needed to verify the results.

Proteins

Sirtuin1, a histone/protein deacetylase that regulates inflammatory responses, cellular aging, and stress resistance, has an important role in autophagy induction involved in liver IRI.[66] A previous study showed that high Sirtuin1 expression in grafts post-reperfusion sharply inhibited proinflammatory cytokine levels accompanied by superior liver function and improved patient survival.[46] HO-1 is a rate-limiting enzyme that converts heme to biliverdin, free iron, carbon monoxide, and has anti-inflammatory and anti-oxidative activitiy.[47] In addition, Nakamura *et al*.[47] showed that high HO-1 levels in post-reperfusion liver biopsies (n=51) were associated with good liver function, dramatically enhanced Sirtuin1/LC3B expression, and protected against hepatic IRI by inducing autophagy. Notch1, a highly conserved transmembrane receptor, has been shown to reduce cellular apoptosis or necrosis and inflammatory response.[55] Kageyama *et al*.[44] demonstrated high Notch1 expression in grafts was correlated with low serum ALT levels, consistent with alleviated liver damage. In addition, Liu *et al*.[48] found that high graft YAP expression after LT was linked with well-preserved histopathology and improved liver function at 1–7 days following LT. YAP is an effector of Hippo pathway and regulates cell proliferation and apoptosis and maintains hepatic homeostasis. FGF15, which is secreted from the ileum following inflammatory stimulation, binds to Fgfr4/Klb, which is followed by downregulation of CYP7A1 expression and inhibition of bile acid synthesis and activation of the Hippo pathway to upregulate YAP levels.[49] In a rat DBD LT model, Gulfo *et al*.[49] reported ed that low FGF15 levels in grafts was associated with more severe hepatic damage and inhibited regeneration that was mediated by increased CYP7A1 and decreased YAP levels.

The use of ECD grafts has raised the incidence of graft dysfunction, which ranges from reversible dysfunction, known as EAD, to irreversible dysfunction or PNF. Therefore, biomarkers to predict EAD and PNF are necessary in the era of ECD. CEACAM1 is a glycoprotein involved in hepatocyte differentiation and regeneration and regulation of insulin clearance, serving as a bridge between hepatic injury and metabolic homeostasis.[50] Low CEACAM1 expression in human donor liver biopsies (n=60) was recognized as an independent predictor of EAD.[50] In a large study cohort (n=194), Hirao *et al*.[51] found that liver grafts with high occult collagen deposition were of increased risk of severe IRI and EAD, highlighting the effect of occult fibrosis on post-transplant outcome. In addition, Kurian *et al*.[68] investigated several upregulated signaling pathways including NF-κB and targets such as CXCL1, IL1, TRAF6, TIPARP, TNFRSF1B, as predictors of EAD. Kornasiewicz *et al*.[69] used graft proteomics to identify 21 significantly differentially expressed proteins in patients with (n=3) and without PNF (n=6). The proteins were mainly associated with mitochondrial oxidative phosphorylation or vital for the adenosine triphosphate-dependent turnover of proteins.

Metabolites

Cortes *et al*.[52] used metabolomic profiling of 124 graft biopsies to identify significantly increased lysophospholipids, bile acids, phospholipids, sphingomyelins, and histidine metabolism products that were predictors for EAD. Based on the metabolic features, an EAD predictive model was established and further determined in a validation set (n=24) to

Table 2. Potential biomarkers of graft function that are found in graft perfusates

Biomarker	Study	Model	Group	Key point
Bile production	Pavel *et al*. (2019)[77]	NMP	5 discarded human DCD livers	Earlier production of bile and higher bile flows during NMP were linked to better bile duct histology
Biliary bicarbonate, pH, and glucose	Matton *et al*. (2019)[78]	NMP	23 human donor livers	High biliary bicarbonate and pH and low glucose were associated with bile duct injury
Bile/perfusate glucose ratio and bile/ perfusate Na^+ ratio	Linares-Cervantes *et al*. (2019)[5]	A porcine DCD LT model; NMP	/	Bile/perfusate glucose ratio≤0.7 and bile/ perfusate Na^+ ratio ≥1.1 were correlated with successful LT
CDmiRs	Verhoeven *et al*. (2013)[79]	Human LT; SCS	Grafts developed ITBL (*n*=20) vs. Grafts without biliary strictures (*n*=37)	CDmiRs could be predictive of bile duct injury and ITBL
miR-122	Selten *et al*. (2017)[80]	Human DCD/ DBD LT; SCS	EAD (*n*=35) vs. non-EAD (*n*=48)	High miR-122 level could predict EAD
FMN	Muller *et al*. (2019)[81]	Human DCD/ DBD LT; HOPE	53 donor livers	High FMN level could predict severe graft dysfunction following LT
D-dimer	Karangwa *et al*. (2017)[82]	NMP	12 discarded human livers	High D-dimer level was associated with graft damage
NGA2F	Verhelst *et al*. (2018)[83]	Human DCD/ DBD LT; SCS	PNF (*n*=3) vs. non-PNF (*n*=63)	Increased NGA2F level could predict PNF

CDmiR, cholangiocyte-derived miRNA; DBD, donation after brain death; DCD, donation after circulatory death; EAD, early allograft dysfunction; FMN, flavin mononucleotide; HOPE, hypothermic oxygenated perfusion; LT, liver transplantation; NGA2F, agalacto core-alpha-1,6-fucosylated biantennary glycan; NMP, normothermic machine perfusion; PNF, primary graft nonfunction; SCS, static cold storage.

have 91% sensitivity and 82% specificity. Likewise, Faitot *et al*.[53] reported that lactate concentrations >8.3 mmol/g and phosphocholine concentrations >0.646 mmol/g were significantly associated with EAD. In our previous study, we identified metabolic profiles containing 57 dramatically differentially expressed metabolic features that were enriched in 24 common pathways including fatty acid, alanine, aspartate, thiamine, and riboflavin metabolism, the urea cycle, and ammonia recycling in PNF grafts.[28] Graft metabolites and clinical characteristics were combined to develop a PNF predictive model derived from eight selected metabolic variations including achillicin, 3-hydroxypropanal, 3-oxododecanoic acid glycerides, and dopexamine in combination with clinical parameters including donor total bilirubin >2 ng/mL, graft weight >1.5 kg, cold ischemia time >10 h, graft warm ischemia time >60 m. The model had an area under curve of 0.930 for predicting PNF.[28]

Single cell technology

Recent advances in single cell RNA sequencing (scRNA-Seq) allow investigation of the transcriptomic landscape of single cells in organisms and have increased our understanding of the heterogeneity and relevance between cells. In a rat LT model, Yang *et al*.[54] identified 11 kinds of cells in grafts and drew a single cell map of IRI after steatotic LT by scRNA-Seq. More importantly, they found a pro-inflammatory phenotype of Kupffer cells (KCs) that highly expressed colony-stimulating factor 3 and a subset of DCs with high expression of XCR1 that were enriched in steatotic grafts, suggesting their participation in fatty graft IRI.[54] In addition, Wang *et al*.[55] described a dynamic transcription profile of intrahepatic cells during LT by performing scRNA-Seq of grafts at preprocurement, at the end of organ preservation, and 2 h after reperfusion. They also found that a cluster of KCs that highly expressed TNFAIP3 interacting protein 3 after reperfusion, protected grafts against liver IRI.[55] We believe that as research on scRNA-Seq deepens, it may provide a deeper understanding of mechanisms related to liver IRI during LT, identify grafts at increased risk of IRI and develop strategies to protect organ against liver damage. In a study published on BioRxiv, we established a graft-tolerant mouse LT model and identified two stages of graft recovery, which included an acute and stable phases.[70] We also found that the interaction between $CD206^+MerTK^+$ macrophages and $CD49a^+CD49b^-$ NK cells regulated metabolic and immune remodeling of the graft.[70]

Potential biomarkers in perfusate

The donor graft and perfusate keep interplaying during preservation. Molecules including nucleic acids, proteins, and metabolites in perfusate may be associated with graft outcomes. In a review by Verhoeven *et al*.[71] in 2014, ALT, AST, lactate dehydrogenase, lactate, adenine nucleotide level, hyaluronic acid, thrombomodulin and inflammatory markers (e.g., hypoxia-inducible factor-1α, and tumor necrosis factor-α) in perfusate and perfusate pH were useful biomarkers to assess graft quality. Machine perfusion (MP) such as hypothermic machine perfusion (HMP), hypothermic oxygenated perfusion (HOPE), and normothermic machine perfusion (NMP) continuously inject the perfusion fluid into the graft blood vessels to form a circuit, mitigating IRI and maintaining cellular metabolism in graft.[72] So far, a series of current and ongoing clinical trials have shown that they were superior in reducing ischemic complications compared with static cold storage (SCS).[73–76] In addition, the development of detection technology and MP have facilitated the discovery of a series of novel perfusate biomarkers for graft viability evaluation and are summarized as below and in Table 2.[5,77–83]

Bile production and bile composition (e.g., bile glucose and Na^+) during NMP are useful biomarkers for graft synthesis function. Pavel *et al*.[77] restored five discarded DCD livers with NMP for 12 h and found that earlier production of bile and higher bile flows during NMP contributed to better bile duct histology. In addition, Matton *et al*.[78] showed that high biliary bicarbonate and pH, and low biliary glucose in human liver grafts (n=23) during NMP were significantly associated with high risk of bile duct injury. In a porcine LT model, Linares-Cervantes *et al*.[5] demonstrated that a bile/perfusate glucose ratio ≤0.7 and a bile/perfusate Na^+ ratio ≥1.1 within 4 h of NMP predicted graft survival after LT. Given that the role of donor graft miRNAs in predicting post-transplant outcomes, perfusate miRNAs may serve similarly. Furthermore, miRNAs have been shown to be stable in perfusate for at least 1 day.[79] Verhoeven *et al*.[79] showed that cholangiocyte-derived miRNAs (CDmiRs) in perfusate were predictive of bile duct injury and the development of ITBL. They also found that a significantly elevated hepatocyte-derived miRNA to CDmiRs ratio was associated with the incidence of ITBL. Moreover, Selten *et al*.[80] reported that both high miR-122 levels and a high miR-122/miR-222 ratio in SCS perfusate predicted the development of EAD and poor graft survival after LT in 83 recipients.

Flavin mononucleotide (FMN), a critical molecular of generating electrons for ubiquinone reduction in mitochondrial complex 1, was shown to be associated with mitochondrial injury.[81] Muller *et al*.[81] preserved 53 grafts with HOPE and demonstrated that a high perfusate FMN level after 30 m of HOPE was strongly linked to severe graft dysfunction. Wang *et al*.[84] infused 23 DCD livers with normothermic regional perfusion and found that the levels of perfusate FMN in transplantable grafts (n=15) were dramatically lower than those in nontransplantable grafts (n=8). D-dimer, a product of fibrin degradation, is a small protein fragment released during fibrinolysis. Karangwa *et al*.[82] preserved 12 discard donor livers with NMP and showed that D-dimer levels >3,500 ng/mL were significantly associated with graft liver injury, suggesting that it was predictive of poor graft function.

In a multicenter cohort study, Verhelst *et al*.[83] compared the glycome patterns in SCS perfusate in PNF (n=3) and non-PNF (n=63) groups and found that increased NGA2F, a single under galactosylated biantennary glycan, predicted the development of PNF with 100% accuracy. That highlighted the essential role of omics, especially the metabolomics, in discovering potential perfusate markers of poor graft function during LT.

Potential strategies for restoring graft function

In recent years, *in vivo* and *ex vivo* potential protective interventions that have been used to restore graft function are listed in Table 3.[85–102] During the process of *ex vivo* therapies, the role of MP is apparent because it provides a platform for graft preconditioning.

Gene therapy

Previous *in vivo* studies were performed to treat liver IRI by using small interfering RNA (siRNA). Jiang *et al*.[85] silenced toll-like receptor 4, a critical mediator of inflammation, in a hepatic IRI mouse model, resulting is significant reduction of serum transferases and histological injury. In another study, Zhao *et al*.[86] downregulated nuclear high-mobility group box 1 by transfecting mice with siRNA and found that it effectively inhibited the expression of serum inflammatory cytokines and protected the liver against IRI. Although the efficacy of hydrodynamic injection has been shown in these animal models, it is difficult to use in the clinic because of off-target effects. Recent studies of graft perfusates showed a potential to solve this problem. For example, Gillooly *et al*.[87] found that Fas siRNA directly added to the perfusate was successfully delivered to rat livers during HMP and NMP. This technology ensured that the siRNA only targeted the grafts, opening a new door for graft reconditioning. Antisense oligonucleotide, another gene modulation agent, was demonstrated to significantly reduce miR-122 expression and inhibit hepatitis C virus replication or reinfection after LT in a porcine LT model with NMP, further confirming the possibility of *ex vivo* gene therapy in grafts.[88]

Cell therapy

In vivo cell therapies such as tolerogenic DCs, Tregs, and mesenchymal stem cells (MSCs) have a role in immunomodulation. In a rat LT model, we innovatively treated acute rejection with a combination of galectin-1-induced tolerogenic DCs and apoptotic lymphocytes, which resulted in prolonged survival of the treated rats, with 37.5% surviving over 100 days, compared with untreated, all of which died within 14 days.[90] In a phase I clinical trial, Sanchez-Fueyo *et al*.[90] demonstrated that autologous Tregs transfer was safe and effective in reducing antidonor T cell responses after LT by intravenously administering autologous Tregs to the LT candidates. In addition, Shi *et al*.[91] found that human MSCs injection in LT recipients suppressed acute rejection and improved graft histology by upregulating the Treg/T help 17 cell ratio. Compared with *in vivo* treatment, *ex vivo* technology provides novel strategies for graft restoration. For instance, Verstegen *et al*.[92] showed in a porcine LT model that MSCs directly added to the perfusate during HOPE were effectively distributed to the porcine grafts, which continued to maintain their paracrine activity after distribution.

Extracellular vesicles

It has been reported that the above tolerogenic cells had the potential to undergo spontaneous malignant transformation.[103] Therefore, some investigators began to use MSC-, DC- and trig-derived extracellular vesicles (EVs) as alternatives to cell therapy. In *in vivo* mice and rat IRI models, MSC-derived EVs had a diverse set of functions including mitochondrial autophagy,[104,105] inhibition of immune response[106,107] and liver regeneration.[108,109] Zheng *et al*.[93] found in a rat IRI model that DC-derived EVs could protect liver against IRI through modulating differentiation of Tregs. In a rat LT model, Chen *et al*.[94] demonstrated that injection with Tregs-derived EVs after LT suppressed the proliferation of $CD8^+$ cytotoxic T cells and prolonged liver graft survival. Compared to the *in vivo* injection, the *ex vivo* technology has the potential to directly target donor grafts without concern for off-target effect. Rigo *et al*.[95] successfully delivered human liver stem cells-derived EVs into the rat livers during NMP, leading to less histological damage and lower levels of AST and lactate dehydrogenase in the treated group.

Anti-inflammatory agents

Liver IRI is characterized by the activation of pro-inflammatory responses. Therefore, adding anti-inflammatory agents to perfusate may regulate immune response and alleviate graft damage. In a porcine LT model, Goldaracena *et al*.[96] put alprostadil, n-acetylcysteine, carbon monoxide, and sevoflurane into the NMP perfusate, showing significantly decreased interleukin-6, tumor necrosis factor-α, and AST during NMP, and lower AST and bilirubin levels in serum after LT in the treated group.[96] In addition, Yu *et al*.[97] used Mcc950, which strongly inhibited the nucleotide-binding

Table 3. Potential therapies to restore donor liver function

Therapy	Study	Target	Model	Outcome
Gene therapy	Jiang *et al*. (2011)[85]	Toll-like receptor 4 siRNA	Mice-IRI *in vivo*	Reduce liver IRI
	Zhao *et al*. (2017)[86]	High-mobility group box 1 siRNA	Mice-IRI *in vivo*	Reduce liver IRI
	Gillooly *et al*. (2019)[87]	siRNA against the Fas receptor	Rats HMP and NMP	Absorbed by rat donor livers during HMP and NMP
	Goldaracena *et al*. (2017)[88]	Antisense oligonucleotide	Porcine LT NMP	Prevent HCV replication or reinfection after LT
Cell therapy	Peng *et al*. (2018)[89]	DC+ apoptotic lymphocytes	Rat LT *in vivo*	Prolong rat survival
	Sanchez-Fueyo A *et al*. (2020)[90]	Tregs	Human LT *in vivo*	Reduce antidonor T cell responses and play the potential role of graft rejection
	Shi *et al*. (2017)[91]	MSCs	Human LT *in vivo*	Suppress acute rejection and improve graft histology
	Verstegen *et al*. (2020)[92]	MSCs	Porcine LT HOPE	Absorbed by porcine grafts and continue to maintain paracrine activity after distribution
Extracellular vesicles	Zheng *et al*. (2018)[93]	EVs deprived from DCs	Rat IRI *in vivo*	Modulate differentiation of Tregs and protect liver against IRI
	Chen *et al*. (2019)[94]	EVs deprived from Tregs	Rat LT *in vivo*	Prolong liver graft survival
	Rigo *et al*. (2018)[95]	EVs deprived from human liver stem cells	Rats NMP	Absorbed by hepatocytes and reduce liver injury
Anti-inflammatory agents	Goldaracena *et al*. (2016)[96]	Alprostadil, n-acetylcysteine, carbon monoxide, and sevoflurane	Porcine LT NMP	Reduce liver injury
	Yu *et al*. (2019)[97]	mcc950	Porcine LT HMP	Reduce liver injury
Vasodilators	Hara *et al*. (2016)[98]	Prostaglandin E1	Rat LT NMP	Reduce liver injury and improve bile production, energy status, and rat survival
	Nassar *et al*. (2014)[99]	Prostacyclin analog (epoprostenol)	Porcine LT NMP	High bile production and good histopathology
	Echeverri *et al*. (2018)[100]	Endothelin1 antagonist (BQ123), epoprostenol, verapamil	Porcine LT NMP	High hepatic artery flow and reduce hepatocyte injury
Defatting	Nagrath *et al*. (2009)[101]	A cocktail*	Rat NMP	Decrease the intracellular lipid content of liver by 50% during 3 h perfusion
	Boteon *et al*. (2019)[102]	A cocktail* + L-carnitine	Human NMP	Decrease liver triglycerides by 38% and macrosteatosis by 40% over 6 h perfusion

Cocktail*, a combination of peroxisome proliferator activated receptor α ligand GW7647, peroxisome proliferator activated receptor δ ligand GW501516, pregnane X Receptor ligand hypericin, the constitutive androstane receptor ligand, the glucagon mimetic and cAMP activator forskolin, and the insulin-mimetic adipokine visfatin. DCs, dendritic cells; EVs, extracellular vesicles; HCV, hepatitis C virus; HMP, hypothermic machine perfusion; HOPE, hypothermic oxygenated perfusion; IRI, ischemia-reperfusion injury; LT, liver transplantation; MSCs, mesenchymal stem cells; NMP, normothermic machine perfusion; siRNA, small interfering RNA; Tregs, regulatory T lymphocytes.

domain leucine-rich repeat containing family pyrin domain containing 3 inflammasome, as an addition to the HMP perfusate in a porcine LT model. They found that Mcc950 significantly reduced inflammatory cytokines and histological injury, and prolonged long-term survival after LT.

Vasodilators

During the ischemic phase of LT, rapid adenosine triphosphate depletion and lack of blood flow result in mitochondrial dysfunction and liver sinusoidal endothelial cell (LSEC) injury.[110] After reperfusion, the injured LSECs not only produce insufficient vasodilators but also expressed P-selectin to accumulate platelets, which resulted in microcirculation disorder.[110] In a rat LT model, Hara *et al*.[98] inhibited the accumulation of platelets by adding prostaglandin E1 (PGE1) to the perfusate under normothermic conditions. PGE1 ameliorated serum liver enzymes and histologic necrosis, and significantly improved bile production and energy status. In addition, Nassar *et al*.[99] added a prostacyclin analog (epoprostenol) to NMP perfusate to preserve porcine livers and found that the use of prostacyclin analog led to high bile production and good histopathology. Furthermore, Echeverri *et al*.[100] compared the effects of endothelin1 antagonist (BQ123), prostacyclin analog (epoprostenol) and calcium channel antagonist (verapamil) to treat hepatic artery vasospasm induced by IRI in a porcine LT model. They demonstrated that grafts with BQ123 and verapamil treatment had higher hepatic artery flow and less hepatocyte injury compared with those treated with epoprostenol.

Defatting agents

Moderate to severe (>30%) macrosteatosis is a well-known risk factor for poor graft quality, making it necessary to defat prior to LT.[14] Nagrath *et al*.[101] treated rat fatty livers with a combination of six defatting agents normothermically and showed that the treatment could decrease the intracellular lipid content of rat liver by 50% after 3 h perfusion. Furthermore, Boteon *et al*.[102] assessed the efficacy of the above six agents combined with additional L-carnitine in defatting human livers with severe steatosis. They found that this method reduced liver triglycerides and macrosteatosis by 38% and 40% over 6 h NMP, enhanced metabolic parameters including increased urea and bile production, and downregulated biomarkers of liver injury (e.g., lower ALT and reduced inflammatory cytokines).

Other agents

In addition to the above agents, human atrial natriuretic peptide (hANP), heavy water, marine worm super hemoglobin (M101), glycine, relaxin, and polyethylene glycols have been found to alleviate liver injury.[111–116] Nigmet *et al*.[111] added hANP, a protective cardiovascular hormone for vascular endothelia, to SCS perfusate to preserve rat livers, showing that hANP supplementation decreased transaminase release, increased bile production, and protected sinusoidal architecture. In a porcine LT model, Alix *et al*.[113] added M101 to SCS perfusate and demonstrated that M101 significantly reduced blood levels of ALT, AST, and tumor necrosis factor α in recipients 3 days following LT. Moreover, Gassner *et al*.[114] used glycine, a simple amino acid that protected sinusoidal cells and hepatocytes, as an addition to NMP rat liver perfusate. They found less sinusoidal dilatation and tissue damage in the treated group.

Conclusions and perspectives

This review summarized and updated biomarkers in donor blood, liver tissue or graft perfusate to evaluate early graft injury (e.g., EAD, and PNF) and ITBL, and to identify potential therapies for graft repairment during the era of ECD. We focused on studies using human liver grafts and investigations of potential biomarkers involved in anti- or pro-inflammatory processes, which in turn shape immunity, regulate graft IRI, and further influence the development of EAD, PNF, or ITBL following LT. Given that relevant mechanisms of some molecules are lacking, further prospective studies and experiments are urgently needed to clearly understand their roles.

Although various biomarkers with available prognostic and diagnostic value in graft quality assessment have been widely explored, few are currently used in clinical practice. Current challenges associated with biomarker discovery research are as follows. Firstly, the sample sizes of these studies were small and mainly limited to single centers, suggesting that large multicenter cohorts or prospective randomized clinical trials are greatly necessary. Another problem is that the studies lack standardized endpoints and control groups.[117] Graft quality is commonly considered to be associated with early graft dysfunction or ITBL, yet other complications after LT (e.g., ACR, metabolic disorders, and graft steatosis or fibrosis) are still a matter of substantial debate. Therefore, we primarily summarized biomarkers predictive of EAD, PNF, and ITBL. Current studies mainly focus on finding biomarkers related to early graft injury, do not have prolonged follow-up and overlook long-term complications like ITBL. Importantly, the measurement of biomarkers should be rapid and easy and have high predictive specificity and sensitivity for graft quality. However, detection of potential biomarkers is costly and time consuming. Moreover, biomarkers need to be stable and measurable during graft procurement, preservation, and implantation.

Despite the availability of liver biopsies for histological assessment and quantification of liver injury during LT, they are invasive and only represent specific parts of the grafts. On the contrary, perfusates can be collected in large volumes and contain markers from the whole graft. In recent years, MP has constantly advanced, and it use in evaluation of graft viability has gradually increased. Nevertheless, different regions or centers have their own standards to determine graft quality.[78,118] More clear international guidelines that could guide the decision for organ usage, discard, or restoration prior to LT are recommended. In addition, we believe that MP could provide a platform for graft preconditioning, making it convenient to explore novel strategies for graft repair. Although high cost and the technical complexity limit wide usage of MP at its current stage, recently completed and ongoing clinical trials will make it an indispensable part of LT.[72,73]

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

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References

[1] Bodzin AS, Baker TB. Liver Transplantation Today: Where We Are Now and Where We Are Going. Liver Transpl 2018;24(10):1470–1475. doi:10.1002/lt.25320, PMID:30080954.
[2] 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;6(4):783–790. doi:10.1111/j.1600-6143.2006.01242.x, PMID:16539636.
[3] Agopian VG, Petrowsky H, Kaldas FM, Zarrinpar A, Farmer DG, Yersiz H, *et al*. The evolution of liver transplantation during 3 decades: analysis of 5347 consecutive liver transplants at a single center. Ann Surg 2013;258(3):409–421. doi:10.1097/SLA.0b013e3182a15db4, PMID:24022434.
[4] Lee DD, Singh A, Burns JM, Perry DK, Nguyen JH, Taner CB. Early allograft dysfunction in liver transplantation with donation after cardiac death donors results in inferior survival. Liver Transpl 2014;20(12):1447–1453. doi:10.1002/lt.23985, PMID:25179581.

[5] Linares-Cervantes I, Echeverri J, Cleland S, Kaths JM, Rosales R, Goto T, *et al*. Predictor parameters of liver viability during porcine normothermic ex situ liver perfusion in a model of liver transplantation with marginal grafts. Am J Transplant 2019;19(11):2991–3005. doi:10.1111/ajt.15395, PMID:31012532.
[6] Nair A, Hashimoto K. Extended criteria donors in liver transplantation-from marginality to mainstream. Hepatobiliary Surg Nutr 2018;7(5):386–388. doi:10.21037/hbsn.2018.06.08, PMID:30498714.
[7] Zheng J, Xiang J, Zhou J, Li Z, Hu Z, Lo CM, *et al*. Liver grafts for transplantation from donors with diabetes: an analysis of the Scientific Registry of Transplant Recipients database. PLoS One 2014;9(5):e98104. doi:10.1371/journal.pone.0098104, PMID:24847864.
[8] Foley DP, Fernandez LA, Leverson G, Anderson M, Mezrich J, Sollinger HW, *et al*. Biliary complications after liver transplantation from donation after cardiac death donors: an analysis of risk factors and long-term outcomes from a single center. Ann Surg 2011;253(4):817–825. doi:10.1097/SLA.0b013e3182104784, PMID:21475025.
[9] Brunner SM, Junger H, Ruemmele P, Schnitzbauer AA, Doenecke A, Kirchner GI, *et al*. Bile duct damage after cold storage of deceased donor livers predicts biliary complications after liver transplantation. J Hepatol 2013;58(6):1133–1139. doi:10.1016/j.jhep.2012.12.022, PMID:23321317.
[10] D'Alessandro AM, Ploeg RJ, Knechtle SJ, Pirsch JD, Stegall MD, Hoffmann R, *et al*. Retransplantation of the liver—a seven-year experience. Transplantation 1993;55(5):1083–1087. doi:10.1097/00007890-199305000-00028, PMID:8497886.
[11] Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, *et al*. Risk factors for primary dysfunction after liver transplantation—a multivariate analysis. Transplantation 1993;55(4):807–813. doi:10.1097/00007890-199304000-00024, PMID:8475556.
[12] 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;17(5):1255–1266. doi:10.1111/ajt.14230, PMID:28199762.
[13] Coll E, Miñambres E, Sánchez-Fructuoso A, Fondevila C, Campo-Cañaveral de la Cruz JL, Domínguez-Gil B. Uncontrolled Donation After Circulatory Death: A Unique Opportunity. Transplantation 2020;104(8):1542–1552. doi:10.1097/TP.0000000000003139, PMID:32732830.
[14] Croome KP, Mathur AK, Mao S, Aqel B, Piatt J, Senada P, *et al*. Perioperative and long-term outcomes of utilizing donation after circulatory death liver grafts with macrosteatosis: A multicenter analysis. Am J Transplant 2020;20(9):2449–2456. doi:10.1111/ajt.15877, PMID:32216008.
[15] De Carlis R, Di Sandro S, Lauterio A, Botta F, Ferla F, Andorno E, *et al*. Liver Grafts From Donors After Circulatory Death on Regional Perfusion With Extended Warm Ischemia Compared With Donors After Brain Death. Liver Transpl 2018;24(11):1523–1535. doi:10.1002/lt.25312, PMID:30022597.
[16] Farid SG, Attia MS, Vijayanand D, Upasani V, Barlow AD, Willis S, *et al*. Impact of Donor Hepatectomy Time During Organ Procurement in Donation After Circulatory Death Liver Transplantation: The United Kingdom Experience. Transplantation 2019;103(4):e79–e88. doi:10.1097/TP.0000000000002518, PMID:30418426.
[17] Paterno F, Guarrera JV, Wima K, Diwan T, Cuffy MC, Anwar N, *et al*. Clinical Implications of Donor Warm and Cold Ischemia Time in Donor After Circulatory Death Liver Transplantation. Liver Transpl 2019;25(9):1342–1352. doi:10.1002/lt.25453, PMID:30912253.
[18] Rana A, Sigireddi RR, Halazun KJ, Kothare A, Wu MF, Liu H, *et al*. Predicting Liver Allograft Discard: The Discard Risk Index. Transplantation 2018;102(9):1520–1529. doi:10.1097/TP.0000000000002151, PMID:29485514.
[19] Dubbeld J, Hoekstra H, Farid W, Ringers J, Porte RJ, Metselaar HJ, *et al*. Similar liver transplantation survival with selected cardiac death donors and brain death donors. Br J Surg 2010;97(5):744–753. doi:10.1002/bjs.7043, PMID:20393979.
[20] Chan EY, Olson LC, Kisthard JA, Perkins JD, Bakthavatsalam R, Halldorson JB, *et al*. Ischemic cholangiopathy following liver transplantation from donation after cardiac death donors. Liver Transpl 2008;14(5):604–610. doi:10.1002/lt.21361, PMID:18433032.
[21] Abt P, Crawford M, Desai N, Markmann J, Olthoff K, Shaked A. Liver transplantation from controlled non-heart-beating donors: an increased incidence of biliary complications. Transplantation 2003;75(10):1659–1663. doi:10.1097/01.TP.0000062574.18648.7C, PMID:12777852.
[22] Pine JK, Aldouri A, Young AL, Davies MH, Attia M, Toogood GJ, *et al*. Liver transplantation following donation after cardiac death: an analysis using matched pairs. Liver Transpl 2009;15(9):1072–1082. doi:10.1002/lt.21853, PMID:19718634.
[23] Taner CB, Bulatao IG, Perry DK, Sibulesky L, Willingham DL, Kramer DJ, *et al*. Asystole to cross-clamp period predicts development of biliary complications in liver transplantation using donation after cardiac death donors. Transpl Int 2012;25(8):838–846. doi:10.1111/j.1432-2277.2012.01508.x, PMID:22703372.
[24] Hessheimer AJ, Cárdenas A, García-Valdecasas JC, Fondevila C. Can we prevent ischemic-type biliary lesions in donation after circulatory determination of death liver transplantation? Liver Transpl 2016;22(7):1025–1033. doi:10.1002/lt.24460, PMID:27082839.
[25] de Vries Y, von Meijenfeldt FA, Porte RJ. Post-transplant cholangiopathy: Classification, pathogenesis, and preventive strategies. Biochim Biophys Acta Mol Basis Dis 2018;1864(4 Pt B):1507–1515. doi:10.1016/j.bbadis.2017.06.013, PMID:28645651.
[26] Braat AE, Blok JJ, Putter H, Adam R, Burroughs AK, Rahmel AO, *et al*. The Eurotransplant donor risk index in liver transplantation: ET-DRI. Am J Transplant 2012;12(10):2789–2796. doi:10.1111/j.1600-6143.2012.04195.x, PMID:22823098.
[27] Flores A, Asrani SK. The donor risk index: A decade of experience. Liver Transpl 2017;23(9):1216–1225. doi:10.1002/lt.24799, PMID:28590542.
[28] Zhang X, Zhang C, Huang H, Chen R, Lin Y, Chen L, *et al*. Primary nonfunction following liver transplantation: Learning of graft metabolites and building a predictive model. Clin Transl Med 2021;11(7):e483. doi:10.1002/ctm2.483, PMID:34323420.
[29] Ezekian B, Mulvihill MS, Freischlag K, Yerokun BA, Davis RP, Hartwig MG, *et al*. Elevated HbA1c in donor organs from patients without a diagnosis of diabetes portends worse liver allograft survival. Clin Transplant 2017;31(9):e13047. doi:10.1111/ctr.13047, PMID:28667782.
[30] Stone BG, Van Thiel DH. Diabetes mellitus and the liver. Semin Liver Dis 1985;5(1):8–28. doi:10.1055/s-2008-1041754, PMID:3885402.
[31] Kotronen A, Juurinen L, Tiikkainen M, Vehkavaara S, Yki-Järvinen H. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. Gastroenterology 2008;135(1):122–130. doi:10.1053/j.gastro.2008.03.021, PMID:18474251.
[32] Piemonti L, Sordi V, Pellegrini S, Scotti GM, Scavini M, Sioli V, *et al*. Circulating CXCL10 and IL-6 in solid organ donors after brain death predict graft outcomes. Sci Rep 2021;11(1):6624. doi:10.1038/s41598-021-86085-6, PMID:33758270.
[33] Pollara J, Edwards RW, Lin L, Bendersky VA, Brennan TV. Circulating mitochondria in deceased organ donors are associated with immune activation and early allograft dysfunction. JCI Insight 2018;3(15):121622. doi:10.1172/jci.insight.121622, PMID:30089724.
[34] Ling Q, Xu X, Wang B, Li L, Zheng S. The Origin of New-Onset Diabetes After Liver Transplantation: Liver, Islets, or Gut? Transplantation 2016;100(4):808–813. doi:10.1097/TP.0000000000001111, PMID:26910326.
[35] Buis CI, van der Steege G, Visser DS, Nolte IM, Hepkema BG, Nijsten M, *et al*. Heme oxygenase-1 genotype of the donor is associated with graft survival after liver transplantation. Am J Transplant 2008;8(2):377–385. doi:10.1111/j.1600-6143.2007.02048.x, PMID:18093274.
[36] Hanvesakul R, Spencer N, Cook M, Gunson B, Hathaway M, Brown R, *et al*. Donor HLA-C genotype has a profound impact on the clinical outcome following liver transplantation. Am J Transplant 2008;8(9):1931–1941. doi:10.1111/j.1600-6143.2008.02341.x, PMID:18671674.
[37] Levitsky J, Kandpal M, Guo K, Kleiboeker S, Sinha R, Abecassis M. Donor-derived cell-free DNA levels predict graft injury in liver transplant recipients. Am J Transplant 2022;22(2):532–540. doi:10.1111/ajt.16835, PMID:34510731.
[38] Schütz E, Fischer A, Beck J, Harden M, Koch M, Wuensch T, *et al*. Graft-derived cell-free DNA, a noninvasive early rejection and graft damage marker in liver transplantation: A prospective, observational, multicenter cohort study. PLoS Med 2017;14(4):e1002286. doi:10.1371/journal.pmed.1002286, PMID:28441386.
[39] Zaman MB, Leonard MO, Ryan EJ, Nolan NP, Hoti E, Maguire D, *et al*. Lower expression of Nrf2 mRNA in older donor livers: a possible contributor to increased ischemia-reperfusion injury? Transplantation 2007;84(10):1272–1278. doi:10.1097/01.tp.0000288229.53064.e2, PMID:18049112.
[40] Resch T, Hackl H, Esser H, Günther J, Schwelberger H, Ritschl PV, *et al*. Expression of MICA in Zero Hour Biopsies Predicts Graft Survival After Liver Transplantation. Front Immunol 2021;12:606146. doi:10.3389/fimmu.2021.606146, PMID:34354697.
[41] Khorsandi SE, Quaglia A, Salehi S, Jassem W, Vilca-Melendez H, Prachalias A, *et al*. The microRNA Expression Profile in Donation after Cardiac Death (DCD) Livers and Its Ability to Identify Primary Non Function. PLoS One 2015;10(5):e0127073. doi:10.1371/journal.pone.0127073, PMID:25978529.
[42] Li C, Zhao Q, Zhang W, Chen M, Ju W, Wu L, *et al*. MicroRNA-146b-5p Identified in Porcine Liver Donation Model is Associated with Early Allograft Dysfunction in Human Liver Transplantation. Med Sci Monit 2017;23:5876–5884. doi:10.12659/msm.907542, PMID:29227984.
[43] Ling Q, Xie H, Li J, Liu J, Cao J, Yang F, *et al*. Donor Graft MicroRNAs: A Newly Identified Player in the Development of New-onset Diabetes After Liver Transplantation. Am J Transplant 2017;17(1):255–264. doi:10.1111/ajt.13984, PMID:27458792.
[44] Wang K, Wei X, Wei Q, Lu D, Li W, Pan B, *et al*. A two-circular RNA signature of donor circFOXN2 and circNECTIN3 predicts early allograft dysfunction after liver transplantation. Ann Transl Med 2020;8(4):94. doi:10.21037/atm.2019.12.132, PMID:32175387.
[45] Chen S, Fang H, Li J, Shi J, Zhang J, Wen P, *et al*. Microarray Analysis For Expression Profiles of lncRNAs and circRNAs in Rat Liver after Brain-Dead Donor Liver Transplantation. Biomed Res Int 2019;2019:5604843. doi:10.1155/2019/5604843, PMID:31828106.
[46] Nakamura K, Kageyama S, Ke B, Fujii T, Sosa RA, Reed EF, *et al*. Sirtuin 1 attenuates inflammation and hepatocellular damage in liver transplant ischemia/Reperfusion: From mouse to human. Liver Transpl 2017;23(10):1282–1293. doi:10.1002/lt.24821, PMID:28719070.
[47] Nakamura K, Kageyama S, Yue S, Huang J, Fujii T, Ke B, *et al*. Heme oxygenase-1 regulates sirtuin-1-autophagy pathway in liver transplantation: From mouse to human. Am J Transplant 2018;18(5):1110–1121. doi:10.1111/ajt.14586, PMID:29136322.
[48] Liu Y, Lu T, Zhang C, Xu J, Xue Z, Busuttil RW, *et al*. Activation of YAP attenuates hepatic damage and fibrosis in liver ischemia-reperfusion injury. J Hepatol 2019;71(4):719–730. doi:10.1016/j.jhep.2019.05.029, PMID:31201834.
[49] Gulfo J, Rotondo F, Ávalos de León CG, Cornide-Petronio ME, Fuster C, Gracia-Sancho J, *et al*. FGF15 improves outcomes after brain dead donor liver transplantation with steatotic and non-steatotic grafts in rats. J Hepatol 2020;73(5):1131–1143. doi:10.1016/j.jhep.2020.05.007, PMID:32422221.
[50] Nakamura K, Kageyama S, Kaldas FM, Hirao H, Ito T, Kadono K, *et al*. Hepatic CEACAM1 expression indicates donor liver quality and prevents early

transplantation injury. J Clin Invest 2020;130(5):2689–2704. doi:10.1172/JCI133142, PMID:32027621.

[51] Hirao H, Ito T, Kadono K, Kojima H, Naini BV, Nakamura K, *et al*. Donor Hepatic Occult Collagen Deposition Predisposes to Peritransplant Stress and Impacts Human Liver Transplantation. Hepatology 2021;74(5):2759–2773. doi:10.1002/hep.32030, PMID:34170562.

[52] Cortes M, Pareja E, García-Cañaveras JC, Donato MT, Montero S, Mir J, *et al*. Metabolomics discloses donor liver biomarkers associated with early allograft dysfunction. J Hepatol 2014;61(3):564–574. doi:10.1016/j.jhep.2014.04.023, PMID:24798621.

[53] Faitot F, Besch C, Battini S, Ruhland E, Onea M, Addeo P, *et al*. Impact of real-time metabolomics in liver transplantation: Graft evaluation and donor-recipient matching. J Hepatol 2018;68(4):699–706. doi:10.1016/j.jhep.2017.11.022, PMID:29191459.

[54] Yang X, Lu D, Wang R, Lian Z, Lin Z, Zhuo J, *et al*. Single-cell profiling reveals distinct immune phenotypes that contribute to ischaemia-reperfusion injury after steatotic liver transplantation. Cell Prolif 2021;54(10):e13116. doi:10.1111/cpr.13116, PMID:34469018.

[55] Wang L, Li J, He S, Liu Y, Chen H, He S, *et al*. Resolving the graft ischemia-reperfusion injury during liver transplantation at the single cell resolution. Cell Death Dis 2021;12(6):589. doi:10.1038/s41419-021-03878-3, PMID:34103479.

[56] Xia W, Ke Q, Wang Y, Feng X, Guo H, Wang W, *et al*. Donation after cardiac death liver transplantation: Graft quality evaluation based on pretransplant liver biopsy. Liver Transpl 2015;21(6):838–846. doi:10.1002/lt.24123, PMID:25824672.

[57] op den Dries S, Westerkamp AC, Karimian N, Gouw AS, Bruinsma BG, Markmann JF, *et al*. Injury to peribiliary glands and vascular plexus before liver transplantation predicts formation of non-anastomotic biliary strictures. J Hepatol 2014;60(6):1172–1179. doi:10.1016/j.jhep.2014.02.010, PMID:24560661.

[58] Yang JY, Sarwal MM. Transplant genetics and genomics. Nat Rev Genet 2017;18(5):309–326. doi:10.1038/nrg.2017.12, PMID:28286337.

[59] Parham P. MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol 2005;5(3):201–214. doi:10.1038/nri1570, PMID:15719024.

[60] Zhao D, Zhou T, Luo Y, Wu C, Xu D, Zhong C, *et al*. Preliminary clinical experience applying donor-derived cell-free DNA to discern rejection in pediatric liver transplant recipients. Sci Rep 2021;11(1):1138. doi:10.1038/s41598-020-80845-6, PMID:33441886.

[61] Kataria A, Kumar D, Gupta G. Donor-derived Cell-free DNA in Solid-organ Transplant Diagnostics: Indications, Limitations, and Future Directions. Transplantation 2021;105(6):1203–1211. doi:10.1097/TP.0000000000003651, PMID:33534526.

[62] Leonard MO, Kieran NE, Howell K, Burne MJ, Varadarajan R, Dhakshinamoorthy S, *et al*. Reoxygenation-specific activation of the antioxidant transcription factor Nrf2 mediates cytoprotective gene expression in ischemia-reperfusion injury. FASEB J 2006;20(14):2624–2626. doi:10.1096/fj.06-5097fje, PMID:17142801.

[63] Lu Y, Hippen KL, Lemire AL, Gu J, Wang W, Ni X, *et al*. miR-146b antagomir-treated human Tregs acquire increased GVHD inhibitory potency. Blood 2016;128(10):1424–1435. doi:10.1182/blood-2016-05-714535, PMID:27485827.

[64] Ling Q, Xie H, Lu D, Wei X, Gao F, Zhou L, *et al*. Association between donor and recipient TCF7L2 gene polymorphisms and the risk of new-onset diabetes mellitus after liver transplantation in a Han Chinese population. J Hepatol 2013;58(2):271–277. doi:10.1016/j.jhep.2012.09.025, PMID:23041303.

[65] Qu X, Zheng C, Wang B, Wang F, Sun X, Gao Y, *et al*. Comprehensive analysis of circular RNAs from steatotic livers after ischemia and reperfusion injury by next-generation RNA sequencing. FEBS Lett 2021;595(1):99–109. doi:10.1002/1873-3468.13960, PMID:33070312.

[66] Finkel T, Deng CX, Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. Nature 2009;460(7255):587–591. doi:10.1038/nature08197, PMID:19641587.

[67] Kageyama S, Nakamura K, Ke B, Busuttil RW, Kupiec-Weglinski JW. Serelaxin induces Notch1 signaling and alleviates hepatocellular damage in orthotopic liver transplantation. Am J Transplant 2018;18(7):1755–1763. doi:10.1111/ajt.14706, PMID:29464890.

[68] Kurian SM, Fouraschen SM, Langfelder P, Horvath S, Shaked A, Salomon DR, *et al*. Genomic profiles and predictors of early allograft dysfunction after human liver transplantation. Am J Transplant 2015;15(6):1605–1614. doi:10.1111/ajt.13145, PMID:25828101.

[69] Kornasiewicz O, Bojarczuk K, Bugajski M, Golab J, Krawczyk M. Application of a proteomic approach to identify proteins associated with primary graft non-function after liver transplantation. Int J Mol Med 2012;30(4):755–764. doi:10.3892/ijmm.2012.1062, PMID:22825711.

[70] Huang H, Zhang X, Chen H, Feng S, Zhang C, Chen R, *et al*. Decoding the single-cell landscape and intercellular crosstalk in the transplanted liver: a 4-dimension mouse model. bioRxiv 2021. doi:10.1101/2021.01.06.425562.

[71] Verhoeven CJ, Farid WR, de Jonge J, Metselaar HJ, Kazemier G, van der Laan LJ. Biomarkers to assess graft quality during conventional and machine preservation in liver transplantation. J Hepatol 2014;61(3):672–684. doi:10.1016/j.jhep.2014.04.031, PMID:24798616.

[72] MacConmara M, Vagefi PA. Machine Perfusion in Liver Transplantation. Adv Surg 2021;55:175–195. doi:10.1016/j.yasu.2021.05.013, PMID:34389091.

[73] 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;557(7703):50–56. doi:10.1038/s41586-018-0047-9, PMID:29670285.

[74] de Meijer VE, Fujiyoshi M, Porte RJ. Ex situ machine perfusion strategies in liver transplantation. J Hepatol 2019;70(1):203–205. doi:10.1016/j.jhep.2018.09.019, PMID:30409464.

[75] Marecki H, Bozorgzadeh A, Porte RJ, Leuvenink HG, Uygun K, Martins PN. Liver ex situ machine perfusion preservation: A review of the methodology and results of large animal studies and clinical trials. Liver Transpl 2017;23(5):679–695. doi:10.1002/lt.24751, PMID:28240817.

[76] Nickkholgh A, Nikdad M, Shafie S, Abbasi Dezfouli S, Mehrabi A, Eason JD, *et al*. Ex Situ Liver Machine Perfusion as an Emerging Graft Protective Strategy in Clinical Liver Transplantation: the Dawn of a New Era. Transplantation 2019;103(10):2003–2011. doi:10.1097/TP.0000000000002772, PMID:31022148.

[77] Pavel MC, Reyner E, Molina V, Garcia R, Ruiz A, Roque R, *et al*. Evolution Under Normothermic Machine Perfusion of Type 2 Donation After Cardiac Death Livers Discarded as Nontransplantable. J Surg Res 2019;235:383–394. doi:10.1016/j.jss.2018.09.066, PMID:30691820.

[78] 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;103(7):1405–1413. doi:10.1097/TP.0000000000002500, PMID:30395120.

[79] Verhoeven CJ, Farid WR, de Ruiter PE, Hansen BE, Roest HP, de Jonge J, *et al*. MicroRNA profiles in graft preservation solution are predictive of ischemic-type biliary lesions after liver transplantation. J Hepatol 2013;59(6):1231–1238. doi:10.1016/j.jhep.2013.07.034, PMID:23928409.

[80] Selten JW, Verhoeven CJ, Heedfeld V, Roest HP, de Jonge J, Pirenne J, *et al*. The release of microRNA-122 during liver preservation is associated with early allograft dysfunction and graft survival after transplantation. Liver Transpl 2017;23(7):946–956. doi:10.1002/lt.24766, PMID:28388830.

[81] Muller X, Schlegel A, Kron P, Eshmuminov D, Würdinger M, Meierhofer D, *et al*. Novel Real-time Prediction of Liver Graft Function During Hypothermic Oxygenated Machine Perfusion Before Liver Transplantation. Ann Surg 2019;270(5):783–790. doi:10.1097/SLA.0000000000003513, PMID:31592808.

[82] Karangwa SA, Burlage LC, Adelmeijer J, Karimian N, Westerkamp AC, Matton AP, *et al*. Activation of Fibrinolysis, But Not Coagulation, During End-Ischemic Ex Situ Normothermic Machine Perfusion of Human Donor Livers. Transplantation 2017;101(2):e42–e48. doi:10.1097/TP.0000000000001562, PMID:27941437.

[83] Verhelst X, Geerts A, Jochmans I, Vanderschaeghe D, Paradissis A, Vanlander A, *et al*. Glycome Patterns of Perfusate in Livers Before Transplantation Associate With Primary Nonfunction. Gastroenterology 2018;154(5):1361–1368. doi:10.1053/j.gastro.2017.12.027, PMID:29309776.

[84] Wang L, Thompson E, Bates L, Pither TL, Hosgood SA, Nicholson ML, *et al*. Flavin Mononucleotide as a Biomarker of Organ Quality-A Pilot Study. Transplant Direct 2020;6(9):e600. doi:10.1097/TXD.0000000000001046, PMID:32904032.

[85] Jiang N, Zhang X, Zheng X, Chen D, Zhang Y, Siu LK, *et al*. Targeted gene silencing of TLR4 using liposomal nanoparticles for preventing liver ischemia reperfusion injury. Am J Transplant 2011;11(9):1835–1844. doi:10.1111/j.1600-6143.2011.03660.x, PMID:21794086.

[86] Zhao G, Fu C, Wang L, Zhu L, Yan Y, Xiang Y, *et al*. Down-regulation of nuclear HMGB1 reduces ischemia-induced HMGB1 translocation and release and protects against liver ischemia-reperfusion injury. Sci Rep 2017;7:46272. doi:10.1038/srep46272, PMID:28382970.

[87] Gillooly AR, Perry J, Martins PN. First Report of siRNA Uptake (for RNA Interference) During Ex Vivo Hypothermic and Normothermic Liver Machine Perfusion. Transplantation 2019;103(3):e56–e57. doi:10.1097/TP.0000000000002515, PMID:30418428.

[88] Goldaracena N, Spetzler VN, Echeverri J, Kaths JM, Cherepanov V, Persson R, *et al*. Inducing Hepatitis C Virus Resistance After Pig Liver Transplantation-A Proof of Concept of Liver Graft Modification Using Warm Ex Vivo Perfusion. Am J Transplant 2017;17(4):970–978. doi:10.1111/ajt.14100, PMID:27805315.

[89] Peng Y, Ye Y, Jia J, He Y, Yang Z, Zhu X, *et al*. Galectin-1-induced tolerogenic dendritic cells combined with apoptotic lymphocytes prolong liver allograft survival. Int Immunopharmacol 2018;65:470–482. doi:10.1016/j.intimp.2018.10.019, PMID:30390594.

[90] Sánchez-Fueyo A, Whitehouse G, Grageda N, Cramp ME, Lim TY, Romano M, *et al*. Applicability, safety, and biological activity of regulatory T cell therapy in liver transplantation. Am J Transplant 2020;20(4):1125–1136. doi:10.1111/ajt.15700, PMID:31715056.

[91] Shi M, Liu Z, Wang Y, Xu R, Sun Y, Zhang M, *et al*. A Pilot Study of Mesenchymal Stem Cell Therapy for Acute Liver Allograft Rejection. Stem Cells Transl Med 2017;6(12):2053–2061. doi:10.1002/sctm.17-0134, PMID:29178564.

[92] Verstegen MMA, Mezzanotte L, Ridwan RY, Wang K, de Haan J, Schurink IJ, *et al*. First Report on Ex Vivo Delivery of Paracrine Active Human Mesenchymal Stromal Cells to Liver Grafts During Machine Perfusion. Transplantation 2020;104(1):e5–e7. doi:10.1097/TP.0000000000002986, PMID:31609902.

[93] Zheng L, Li Z, Ling W, Zhu D, Feng Z, Kong L. Exosomes Derived from Dendritic Cells Attenuate Liver Injury by Modulating the Balance of Treg and Th17 Cells After Ischemia Reperfusion. Cell Physiol Biochem 2018;46(2):740–756. doi:10.1159/000488733, PMID:29621784.

[94] Chen L, Huang H, Zhang W, Ding F, Fan Z, Zeng Z. Exosomes Derived From T Regulatory Cells Suppress CD8+ Cytotoxic T Lymphocyte Proliferation and Prolong Liver Allograft Survival. Med Sci Monit 2019;25:4877–4884. doi:10.12659/MSM.917058, PMID:31258152.

[95] Rigo F, De Stefano N, Navarro-Tableros V, David E, Rizza G, Catalano G, *et al*. Extracellular Vesicles from Human Liver Stem Cells Reduce Injury in an Ex Vivo Normothermic Hypoxic Rat Liver Perfusion Model. Transplantation 2018;102(5):e205–e210. doi:10.1097/TP.0000000000002123, PMID:29424767.

[96] Goldaracena N, Echeverri J, Spetzler VN, Kaths JM, Barbas AS, Louis KS, *et al*. Anti-inflammatory signaling during ex vivo liver perfusion improves

the preservation of pig liver grafts before transplantation. Liver Transpl 2016;22(11):1573–1583. doi:10.1002/lt.24603, PMID:27556578.
[97] Yu Y, Cheng Y, Pan Q, Zhang YJ, Jia DG, Liu YF. Effect of the Selective NLRP3 Inflammasome Inhibitor mcc950 on Transplantation Outcome in a Pig Liver Transplantation Model With Organs From Donors After Circulatory Death Preserved by Hypothermic Machine Perfusion. Transplantation 2019; 103(2):353–362. doi:10.1097/TP.0000000000002461, PMID:30247318.
[98] Maida K, Akamatsu Y, Hara Y, Tokodai K, Miyagi S, Kashiwadate T, *et al*. Short Oxygenated Warm Perfusion With Prostaglandin E1 Administration Before Cold Preservation as a Novel Resuscitation Method for Liver Grafts From Donors After Cardiac Death in a Rat In Vivo Model. Transplantation 2016;100(5):1052–1058. doi:10.1097/TP.0000000000001127, PMID:26950723.
[99] Nassar A, Liu Q, Farias K, D'Amico G, Buccini L, Urcuyo D, *et al*. Role of vasodilation during normothermic machine perfusion of DCD porcine livers. Int J Artif Organs 2014;37(2):165–172. doi:10.5301/ijao.5000297, PMID:24619899.
[100] Echeverri J, Goldaracena N, Kaths JM, Linares I, Roizales R, Kollmann D, *et al*. Comparison of BQ123, Epoprostenol, and Verapamil as Vasodilators During Normothermic Ex Vivo Liver Machine Perfusion. Transplantation 2018;102(4):601–608. doi:10.1097/TP.0000000000002021, PMID: 29189484.
[101] Nagrath D, Xu H, Tanimura Y, Zuo R, Berthiaume F, Avila M, *et al*. Metabolic preconditioning of donor organs: defatting fatty livers by normothermic perfusion ex vivo. Metab Eng 2009;11(4-5):274–283. doi:10.1016/j.ymben.2009.05.005, PMID:19508897.
[102] Boteon YL, Attard J, Boteon APCS, Wallace L, Reynolds G, Hubscher S, *et al*. Manipulation of Lipid Metabolism During Normothermic Machine Perfusion: Effect of Defatting Therapies on Donor Liver Functional Recovery. Liver Transpl 2019;25(7):1007–1022. doi:10.1002/lt.25439, PMID:30821045.
[103] Carlson K, Kink J, Hematti P, Al-Adra DP. Extracellular Vesicles as a Novel Therapeutic Option in Liver Transplantation. Liver Transpl 2020;26(11):1522–1531. doi:10.1002/lt.25874, PMID:32844568.
[104] Yang B, Duan W, Wei L, Zhao Y, Han Z, Wang J, *et al*. Bone Marrow Mesenchymal Stem Cell-Derived Hepatocyte-Like Cell Exosomes Reduce Hepatic Ischemia/Reperfusion Injury by Enhancing Autophagy. Stem Cells Dev 2020;29(6):372–379. doi:10.1089/scd.2019.0194, PMID:31969065.
[105] Zhang L, Song Y, Chen L, Li D, Feng H, Lu Z, *et al*. MiR-20a-containing exosomes from umbilical cord mesenchymal stem cells alleviates liver ischemia/reperfusion injury. J Cell Physiol 2020;235(4):3698–3710. doi:10.1002/jcp.29264, PMID:31566731.
[106] Haga H, Yan IK, Borrelli DA, Matsuda A, Parasramka M, Shukla N, *et al*. Extracellular vesicles from bone marrow-derived mesenchymal stem cells protect against murine hepatic ischemia/reperfusion injury. Liver Transpl 2017;23(6):791–803. doi:10.1002/lt.24770, PMID:28407355.
[107] Yao J, Zheng J, Cai J, Zeng K, Zhou C, Zhang J, *et al*. Extracellular vesicles derived from human umbilical cord mesenchymal stem cells alleviate rat hepatic ischemia-reperfusion injury by suppressing oxidative stress and neutrophil inflammatory response. FASEB J 2019;33(2):1695–1710. doi:10.1096/fj.201800131RR, PMID:30226809.
[108] Nong K, Wang W, Niu X, Hu B, Ma C, Bai Y, *et al*. Hepatoprotective effect of exosomes from human-induced pluripotent stem cell-derived mesenchymal stromal cells against hepatic ischemia-reperfusion injury in rats. Cytotherapy 2016;18(12):1548–1559. doi:10.1016/j.jcyt.2016.08.002, PMID:27592404.
[109] Anger F, Camara M, Ellinger E, Germer CT, Schlegel N, Otto C, *et al*. Human Mesenchymal Stromal Cell-Derived Extracellular Vesicles Improve Liver Regeneration After Ischemia Reperfusion Injury in Mice. Stem Cells Dev 2019;28(21):1451–1462. doi:10.1089/scd.2019.0085, PMID:31495270.
[110] Dar WA, Sullivan E, Bynon JS, Eltzschig H, Ju C. Ischaemia reperfusion injury in liver transplantation: Cellular and molecular mechanisms. Liver Int 2019;39(5):788–801. doi:10.1111/liv.14091, PMID:30843314.
[111] Nigmet Y, Hata K, Tamaki I, Okamura Y, Tsuruyama T, Miyauchi H, *et al*. Human Atrial Natriuretic Peptide in Cold Storage of Donation After Circulatory Death Rat Livers: An Old but New Agent for Protecting Vascular Endothelia? Transplantation 2019;103(3):512–521. doi:10.1097/TP.0000000000002552, PMID:30461725.
[112] Shimada S, Fukai M, Shibata K, Sakamoto S, Wakayama K, Ishikawa T, *et al*. Heavy Water (D2O) Containing Preservation Solution Reduces Hepatic Cold Preservation and Reperfusion Injury in an Isolated Perfused Rat Liver (IPRL) Model. J Clin Med 2019;8(11):E1818. doi:10.3390/jcm8111818, PMID:31683811.
[113] Alix P, Val-Laillet D, Turlin B, Ben Mosbah I, Burel A, Bobillier E, *et al*. Adding the oxygen carrier M101 to a cold-storage solution could be an alternative to HOPE for liver graft preservation. JHEP Rep 2020;2(4):100119. doi:10.1016/j.jhepr.2020.100119, PMID:32695967.
[114] Gassner JMGV, Nösser M, Moosburner S, Horner R, Tang P, Wegener L, *et al*. Improvement of Normothermic Ex Vivo Machine Perfusion of Rat Liver Grafts by Dialysis and Kupffer Cell Inhibition With Glycine. Liver Transpl 2019;25(2):275–287. doi:10.1002/lt.25360, PMID:30341973.
[115] Boehnert MU, Hilbig H, Armbruster FP. Relaxin as an additional protective substance in preserving and reperfusion solution for liver transplantation, shown in a model of isolated perfused rat liver. Ann N Y Acad Sci 2005;1041:434–440. doi:10.1196/annals.1282.065, PMID:15956742.
[116] Panisello Rosello A, Teixeira da Silva R, Castro C, G Bardallo R, Calvo M, Folch-Puy E, *et al*. Polyethylene Glycol 35 as a Perfusate Additive for Mitochondrial and Glycocalyx Protection in HOPE Liver Preservation. Int J Mol Sci 2020;21(16):E5703. doi:10.3390/ijms21165703, PMID:32784882.
[117] Bardhi E, McDaniels J, Rousselle T, Maluf DG, Mas VR. Nucleic acid biomarkers to assess graft injury after liver transplantation. JHEP Rep 2022;4(3):100439. doi:10.1016/j.jhepr.2022.100439, PMID:35243279.
[118] Watson CJE, Kosmoliaptsis V, Pley C, Randle L, Fear C, Crick K, *et al*. Observations on the ex situ perfusion of livers for transplantation. Am J Transplant 2018;18(8):2005–2020. doi:10.1111/ajt.14687, PMID:29419931.