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Review Article

Comprehensive Approach to Assessment of Liver Viability During Normothermic Machine Perfusion

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Abstract

Liver transplantation is the most effective treatment of advanced liver disease, and the use of extended criteria donor organs has broadened the source of available livers. Although normothermic machine perfusion (NMP) has become a useful tool in liver transplantation, there are no consistent criteria that can be used to evaluate the viability of livers during NMP. This review summarizes the criteria, indicators, and methods used to evaluate liver viability during NMP. The shape, appearance, and hemodynamics of the liver can be analyzed at a macroscopic level, while markers of liver injury, indicators of liver and bile duct function, and other relevant indicators can be evaluated by biochemical analysis. The liver can also be assessed by tissue biopsy at the microscopic level. Novel methods for assessment of liver viability are introduced. The limitations of evaluating liver viability during NMP are discussed and suggestions for future clinical practice are provided.

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Introduction

Liver transplantation is the best treatment for end-stage liver disease. Improvements in organ preservation, surgical methods, immunosuppressant medication, and other factors have contributed to the success of organ transplantation.[1] Static cold storage is one of the most frequently used methods for preserving and transporting transplanted livers but still has limitations. Although hepatic metabolism is reduced to a minimum during freezing, organ damage cannot be avoided. Furthermore, severe ischemia-reperfusion injury (IRI) is inevitable.[2] Importantly, liver preservation using static cold storage does not allow immediate assessment of liver viability, which is essential for extended criteria donor (ECD) livers. Fortunately, there has been a recent resurgence of interest in normothermic machine perfusion (NMP),[3] which can provide nutrients and oxygen to the liver in a physiological state, minimize organ injury, and limit the damage caused by cold and warm ischemia (Figs. 1, 2).[4,5] Importantly, it can evaluate the organ in real time.[6]

ECD livers are increasingly being used because of an organ shortage. Most ECD livers are donated after circulatory death by elderly donors and by donors with underlying disease.[7] The likelihood of receiving an ECD liver is generally higher, and assessment of ECD livers has become more subjective, which has resulted in potentially usable livers being discarded. Fortunately, NMP allows real-time evaluation of livers in a near-physiological condition (Table 1). However, there is no uniform standard for assessing liver viability under NMP. In this review, we summarize the criteria, indicators, and methods used to assess liver viability for transplantation based on the available literature. We also analyze the current limitations regarding liver transplantation and make recommendations for the future.

Parameters used for assessment of viability

The liver contains cells that store a variety of enzymes used for metabolism of sugar, protein, and urea and for hematopoiesis. The liver also has a role in detoxification and can secrete and discharge bile to regulate digestion.[8,9] Many parameters can be used to measure liver viability (Table 2) but is not clear which are the most appropriate.[10-12,13–19]

The main characteristic used to evaluate the liver is its macroscopic appearance before perfusion. Perfusion parameters can also provide an intuitive understanding of the general condition of the liver during machine perfusion. Liver damage can lead to leakage of enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH). Liver viability can also be assessed in terms of its function, specifically synthesis (bile, urea and coagulation factors) and metabolism

Keywords: Normothermic machine perfusion; Liver viability; Criteria.
Abbreviations: 8-OHdG, 8-hydroxy-2-deoxyguanosine; ADP, adenosine diphosphate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, adenosine triphosphate; CD11b, cluster of differentiation 11β; CD14, cluster of differentiation 14; CIT, cold ischemia time; DAMPs, damage-associated molecular patterns; DBD, donor after brain death; DCD, donation after cardiac death; EAD, early allograft dysfunction; ECD, extended criteria donor; FDG, ^{18}F-fluorodeoxyglucose; Gd-EOB-DTPA, gadolinium-ethoxybenzyl-diethylenetriaminepentaacetic acid; GGT, gamma-glutamyl transpeptidase; HA, hepatic artery; HR-MAS-NMR, high-resolution magic-angle-spinning nuclear magnetic resonance; ICG, indocyanine green; IL10, interleukin-10; IL1β, interleukin-1β; IL6, interleukin-6; IL8, interleukin-8; INR, international normalized ratio; IRI, ischemia-reperfusion injury, LDH, lactate dehydrogenase; LT, liver transplantation; MAP, mean arterial pressure; MAS, macrovesicular steatosis; MRI, magnetic resonance imaging; MSC, mesenchymal stem cell; NAS, non-anastomotic biliary strictures; NMR, nuclear magnetic resonance; NRP, normothermic regional perfusion; PET-CT, positron emission tomography-computer tomography; Pi, phosphate ion; PNF, primary nonfunction; PV, portal vein; ROS, reactive oxygen species; SCS, static cold storage; TNF-α, tumor necrosis factor-alpha; US, ultrasound.

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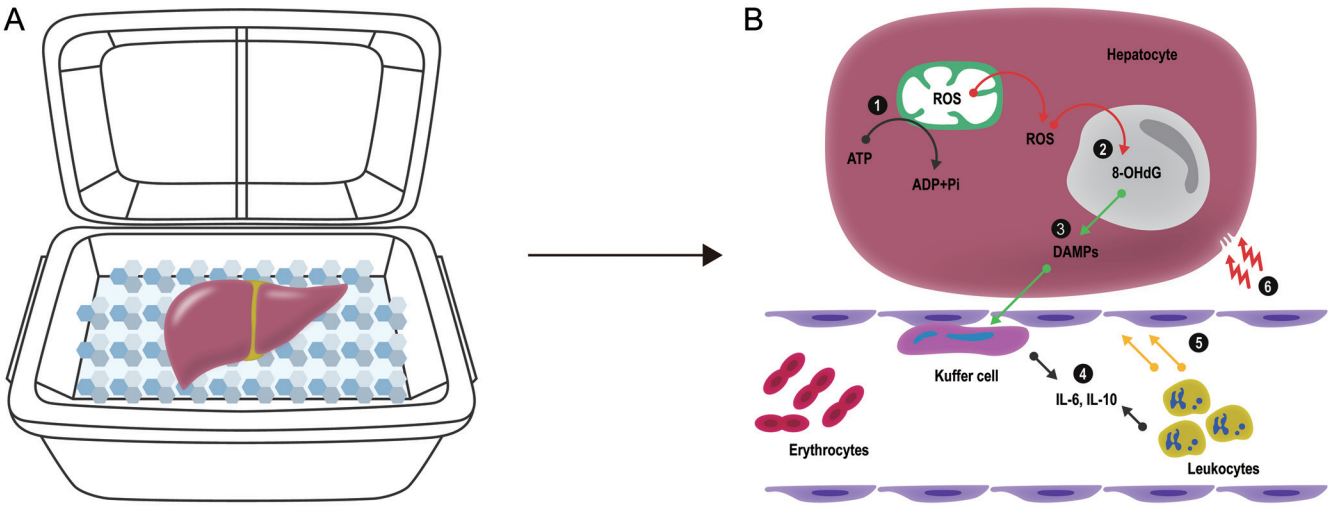

Fig. 1. Mechanisms of ischemia. (A) Ischemic liver. (B) Liver with ischemia characterized by lack of ATP production, decreased energy metabolism,[1] ROS release, DNA damage, activated apoptosis,[2] nuclear release of DAMPs,[3] and further activation of Kupffer cells and infiltration of leukocytes.[5] Activation of Kupffer cells and leukocytes worsen injury with further release of inflammatory cytokines.[4] Various factors lead to destruction of cell membranes.[6] 8-OHdG, 8-hydroxy-2-deoxyguanosine; ADP, adenosine diphosphate; ATP, adenosine triphosphate; DAMPs, damage-associated molecular patterns; IL-10, interleukin-10; IL-6, interleukin-6; Pi, phosphate ion; ROS, reactive oxygen species.

(lactate, glucose, acid and base). Bile composition (biliary bicarbonate, pH, and glucose) can be used to assess bile duct function.[20,21]

Criteria used for assessment of viability

NMP is the most common method used to evaluate liver viability. However, each transplant center has its own assessment criteria. Eshmuminov *et al*.[10] assessed a series of viability criteria in a 1 week liver machine perfusion experiment. They investigated several indicators of hepatic metabolic function, including lactate and ammonia clearance, maintenance of pH and albumin, and production of blood urea nitrogen. Decreases of markers of liver injury (AST, ALT, LDH) and inflammation [interleukin (IL)-6, IL10] were identified as criteria of extended grafts. Other important predictors were production of bile, synthesis of ATP, and the response to hormones and vasoactive agents. The Birmingham group also developed criteria for determination of liver viability. They investigated a series of major and minor parameters at 120 minutes after the start of perfusion and determined that a viable graft must meet at least one major and two minor criteria. Their major criteria were a lactate level <2.5 mmol/L and production of bile and their minor criteria were a perfusate pH >7.3, stable blood flow >150 mL/min in the hepatic artery and >500 mL/min in the portal vein, and homogenous hepatic perfusion. The criteria were then used in a clinical trial to test viability of the liver for ECD transplantation.[11] The Cambridge group have also published criteria for assessment of the liver by NMP. Their criteria for a viable graft include a maximum bile pH >7.5, a bile glucose concentration <3 mmol/L or >10 mmol/L, but less than that of the perfusate glucose concentration, a perfusate pH >7.2 with ≤30 mL of bicarbonate supplementation, a decrease in glucose lasting beyond 2 h or a perfusate glucose concentration <10 mmol/L, a decrease in peak lactate >4.4 mmol/L/kg/h, and an ALT <6,000 IU/L at 2 h.[12] The Groningen group used the following criteria to measure hepatobiliary function: a lactate level <1.7 mmol/L, a perfusion pH in the range of 7.35–7.45, bile production >10 mL, and a bile duct pH >7.45 within 2.5 h. They also used the difference between the pH of bile and the pH of the perfusate, bicarbonate, and glucose to assess alkalization of the bile and glucose reabsorption by the biliary epithelium (Table 3).[22]

Having now had experience of more than 100 cases of NMP in clinical practice, we have devised our own liver viability criteria, which consist of three components. The first component is the homogeneity of hepatic perfusion, indicated by a liver with a soft and ruddy appearance, a liver surface temperature confirmed by thermal imaging to be in the range of 35–37°C, stable blood flow of >0.1 mL/min/g in the hepatic artery and >0.33 mL/min/g in the portal vein, and a hemodynamic response to vasoactive agents. The second component is liver function, which includes a lactate level <2.5 mmol/L after 3 h perfusion, a perfusate pH >7.3 without constant bicarbonate supplementation, and evidence of bile production. The third component is biliary function, which includes a bile duct pH of >7.5, a biliary glucose concentration <16 mmol/L, and a biliary/perfusion glucose concentration ratio of <0.67. We have used the criteria to evaluate 113 livers that were ischemia-free during NMP. All livers that met our criteria were successfully transplanted with no primary nonfunction detected after transplantation. Although liver viability criteria vary from center to center, there are some similarities. For example, the lactate level, pH of the perfusate, and hemodynamic parameters of liver perfusion are widely considered to be important criteria for assessment of liver viability. Furthermore, with advances in technology, we can now assess not only the functional status of the liver but also indicators of biliary function.

Macroscopic scale of the liver

Shape and appearance

The shape and appearance of the donor liver during machine perfusion is one of the indicators used by surgeons to evaluate its viability.[23] Transplant teams routinely inspect donor livers and make their decision about transplantation accordingly.[24] Surgeons evaluate the organ primarily based on appearance and texture. Yersiz *et al*.[25] found that expe-

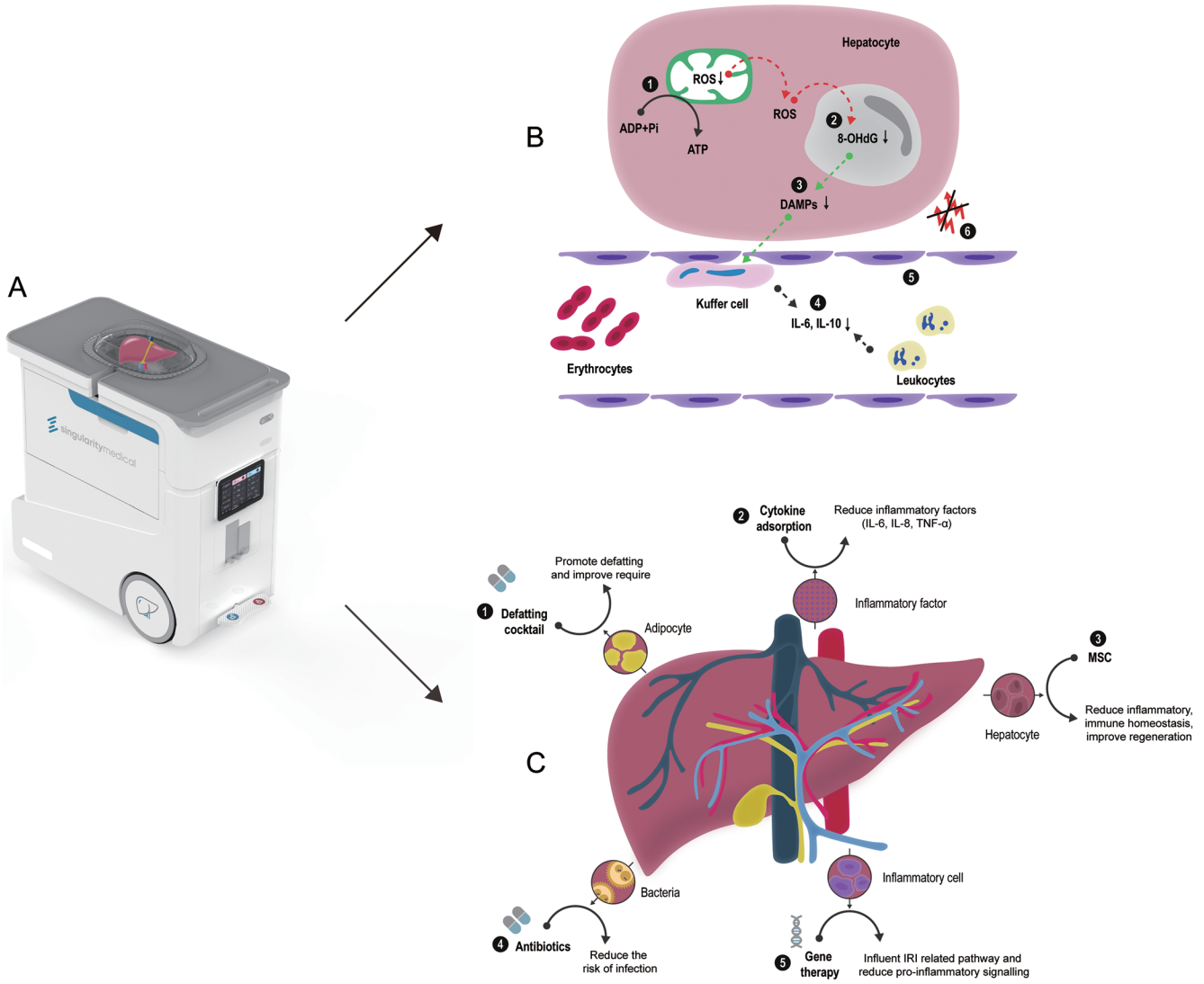

Fig. 2. Effects of NMP in counteracting ischemia. (A) Liver during NMP. (B) Liver during NMP characterized by a good supply of energy and ATP,[1] less necrotic material[2–4] and less leukocyte infiltration.[5] The cell membrane is stabilized.[6] NMP provides physiological conditions for the liver. (C) Various measures can be applied to improve adverse liver conditions during NMP, such as defatting cocktail[1] absorption of cytokines by mesenchymal stem cells (MSC),[3] antibiotics,[4] and gene therapy,[5] each of which has an effect. 8-OHdG, 8-hydroxy-2-deoxyguanosine; ADP, adenosine diphosphate; ATP, adenosine triphosphate; DAMPs, damage-associated molecular patterns; IL-10, interleukin-10; IL-6, interleukin-6; IL-8, interleukin-8; MSC, mesenchymal stem cell; NMP, normothermic machine perfusion; Pi, phosphate ion; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha.

rienced surgeons made their macroscopic evaluation of steatosis based on parenchymal texture criteria (i.e., degree of yellowness, degree of firmness, round liver edges, and scratch marks) before receiving the results of liver biopsy. Their study demonstrated that an organ with macrosteatosis can be identified to some extent based on macroscopic evaluation. Furthermore, macroscopic appearance during machine perfusion can also be an indicator of viability. Mergental *et al*.[11] included macroscopic appearance among their criteria for liver viability and noted that a well-perfused liver with optimal macroscopic appearance was a good indicator of viability. Unfortunately, assessment of the shape and appearance of the liver is highly subjective. Friend *et al*.[26] found that these features became less sensitive as the degree of steatosis decreased. Organs with significant damage, malignancy, or ischemia are easily detected by experienced surgeons.[24] Although some of the liver transplantation literature has included macroscopic appearance among the criteria for acceptance or rejection of donor livers, subjective judgments alone are highly variable and may lead to mistakes. For example, surgeons often consider a liver with mild or toxic steatosis to be normal. The appearance of the liver is the most basic indicator of its viability, particularly under static cold storage conditions, and should still be assessed. However, with the advent of machine perfusion, we can now evaluate liver viability using more objective indicators. For example, we use thermal imaging to assess the temperature of the liver to determine whether hepatic perfusion is homogenous (Fig. 3A, B).

Hemodynamic analysis

Vascular resistance and perfusion pressure in the liver are

Table 1. Overview of parameters used for assessment of donor liver quality

	Parameter	Detection method	Advantages	Disadvantages
Macroscopic Scale	Appearance	Visual observation	Convenient, easy, cheap, non-invasive, do it at any time	Inaccurate, no clear criteria, rely on personal judgment
	Hemodynamic analysis (HA and PV flow)	Shown on the perfusion machine	Real-time, convenient, easy	Cellular function cannot be assessed
Parameter of liver injury	Liver transaminases (ALT and AST)	Biochemical analysis	Non-invasive, rapid, assess the extent of cell damage	Lack of specificity, cannot assess liver function
Liver function	Lactate	Blood gas analysis	Non-invasive, widely use	Different criteria
	Bile production	Measuring by graduated cylinder	Non-invasive, easy	Value is not high, cannot assess bile duct viability,
	Urea	Routine laboratory analysis	Non-invasive, new, easy,	Not widely used
	Coagulation factors	Routine laboratory analysis	Non-invasive, new, easy	Susceptible to additives, not widely used
	Glucose metabolism	Blood gas analysis	Associated with IRI	Not widely used
Bile duct function	Bicarbonate	Routine laboratory analysis	Non-invasive	Susceptible to additives, not widely used
	biliary pH	Routine laboratory analysis		
	biliary glucose	Routine laboratory analysis		
Microscopic Expression	Histology	Biopsy	Accurately assess liver condition	Invasive, requires long time, not being able to evaluate in real time

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HA, hepatic artery; PV, portal vein.

major factors affecting blood flow in the hepatic artery and portal vein. When the liver is exposed to cold ischemia, its endothelial cells are particularly vulnerable to IRI, leading to impairment of the hepatic circulation,[27] which, in turn results in poor perfusion of the liver and may worsen ischemia.[28] Increased vascular resistance during perfusion is an indicator of probable liver injury; if high, the resistance may cause organ impairment. After investigating 12 discarded human livers, the Birmingham group proposed that indicators of a graft that is feasible for transplantation should include a blood flow of >150 mL/min in the hepatic artery (Fig. 4A) and >500 mL/min in the portal vein (Fig. 4B). This parameter is used in most clinical and animal studies.[12,29,30] For example, in a study of long-term liver perfusion by Eshmuminov *et al*.,[10] blood in the hepatic artery flowed in a pulsating manner at an elevated mean arterial pressure of 65 mmHg and the portal vein received blood at a low pressure of 5–10 mmHg.

When blood flow is impaired and there is high resistance, histological examination usually shows lesions.[13,31] Decreased blood flow may also be associated with cell damage, such as cirrhosis and fibrosis.[10] Furthermore, increased vascular resistance is associated with impaired liver function.[32,33] For example, severe macrosteatosis may result in sinusoidal stenosis and reduced perfusion.[34] Some studies have found that the perfusion blood flow was higher than the standard and remained stable, which does not indicate homogenous hepatic perfusion. Some livers with blood flow that met the standard were still rejected for transplantation because of poor quality.[11,31,35] We consider that hemodynamic parameters are indicators that can be monitored and adjusted in real time during machine perfusion. However, given that donor livers differ in weight, correction of the flow per unit liver weight may give a better indication of liver viability.

Hepatic injury and functional assessment of the liver during NMP

Liver transaminases

Ischemic liver injury mainly causes damage to hepatocytes and sinusoidal endothelial cells.[36] When hepatic cells are damaged, intracellular liver enzymes may flow out into the perfusate (Fig. 5). AST and ALT are two important transaminases present in high concentrations in hepatocytes. An *in vivo* study confirmed that the natural half-life of ALT as 47±10 h and that of AST was 17±5 h,[37] which suggests that both these enzymes could be used as relatively stable indicators of liver injury. AST is one a clinically accepted biomarkers of long-term graft and patient survival.[12,38] However, ALT level may be a more representative indicator of damage to hepatocytes because ALT is more liver-specific,[6] whereas changes in the AST level may result from hemolysis. Indeed, Nasralla *et al*.[39] reported a rapid increase in ALT during preservation of injured livers.[14] It has been suggested that transaminases should be normalized by liver weight if they are used as markers.

Several studies have shown that ALT and AST concentrations usually plateau after 2 h of NMP,[12,15,40] possibly because most enzymes are washed out by that time. It has been confirmed that the ALT level at 2 h is correlated with the

Table 2. Experience with transplantation of liver grafts subjected to normothermic *ex situ* machine perfusion

Author	N	DBD or DCD	Year, Site	Perfusion time	CIT	Assessment during normothermic machine perfusion
Eshmuminov *et al*.[10]	10	NR	2020, Switzerland	7 days	NR	AST, ALT, LDH, IL-6, IL-10, ATP, lactate, ammonia clearance, pH, albumin, blood urea, bile production, the respond to hormones and vasoactive drugs
Mergental *et al*.[11]	12	Both	2018, UK	6 hours	483 min	HA and PV flow, lactate, bile production, perfusate pH, homogenous hepatic perfusion
Watson *et al*.[12]	47	Both	2018, UK	4 hours	NR	ALT, lactate, bile pH, bile glucose, perfusate pH, glucose
Vries *et al*.[13]	7	DCD	2018, Netherlands	2.5 hours	289 min	lactate, bile production, perfusate pH, bile pH
Nasralla *et al*.[14]	120	Both	2018, Europe	6.125 hours	1,206 min	HA and PV flow, ALT, AST, LDH, GGT, arterial pressure, pH, lactate, bile production, perfusate, blood gas parameters
Waston *et al*.[15]	12	Both	2017, UK	4.73 hours	427 min	HA and PV flow, lactate, glucose, ALT, perfusate pH,
Weissenbacher *et al*.[16]	55	Both	2021, Austria	15.1 hours	384 min	AST, ALT, GGT, LDH, lactate, bile production, perfusion hemodynamics, pH
Sutton *et al*.[17]	12	Both	2014, Netherlands	6 hours	6.5 hours	ALP, ALT, GGT, LDH, ATP, bile, biliary bicarbonate, glucose, blood gas and biochemical parameters, oxygen consumption, total bilirubin, albumin, and biopsies
Linares-Cervantes *et al*.[18]	17	Both	2021, Canada	4 hours	30 min; 70 min; 120 min	HA and PV flows, HA, and PV resistance, AST, ALT, LDH, lactate perfusate pH, urea, albumin, oxygen consumption and perfusate glucose, bile phospholipids,, bile cholesterol, bile acids, bile bilirubin, bile production, bile pH, bile lactate, bile HCO3-, bile glucose, bile Na+, bile K+
Boteon *et al*.[19]	10	Both	2019, UK	12 hours	737 min	HA and PV flow, ALT, AST, GGT, ATP, lactate, bile pH, urea and oxygen uptake, 4-HNE, CD11b, CD14, TNF-α, IL10, IL-1β, 8-HOdG, biopsies

4-HNE, 4-hydroxynonenal; 8-HOdG, 8-hydroxy-2-deoxyguanosine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, adenosine triphosphate; CD11b, cluster of differentiation 11β; CD14, cluster of differentiation 14; CIT, cold ischemia time; DBD, donor after brain death; GGT, gamma-glutamyl transpeptidase; HA, hepatic artery; IL-10, interleukin-10; IL-1β, interleukin-1 beta; IL-6, interleukin-6; LDH, lactate dehydrogenase; PV, portal vein; TNF-α, necrosis factor-alpha.

peak ALT in the first 7 days after transplant.[12] Other studies have demonstrated that peak perfusate transaminase levels are poor predictors of graft survival and post-transplant function.[41–43] However, transaminase levels can act as an aid to assessment of liver viability during NMP. Some investigators have considered a peak ALT >6,000 IU/L (Fig. 4C) and a peak AST >1,000 IU/L (Fig. 4D) to be markers of severe liver injury.[14] Considering that absolute enzyme

Table 3. Clinical studies of viability assessment before liver transplantation

Reference	Viability Criteria
The First Affiliated Hospital, Sun Yat-sen University	Viability assessed within 3 h of perfusion: Appearance of liver is soft and ruddy, Temperature of liver 35 to 37°C, hemodynamic response to vasoactive substances, Stable HA flow >0.1 mL/min/g, Stable PV flow >0.33 mL/min/g, Lactate level <2.5mmol /L, Perfusate pH >7.3 without constant bicarbonate supplementation Biliary pH >7.5, Biliary glucose concentration <16 mmol/L, Biliary/perfusion glucose concentration <0.67
The Birmingham group (2018)[11]	Viability is assessed within 2 h of perfusion: Lactate level <2.5mmol /L, The presence bile generation, Perfusate pH >7.3, Stable HA flow >150 mL/min, Stable PV flow >500 mL/min, Homogenous hepatic perfusion
The Cambridge group (2018)[12]	Viability is assessed within 2 h of perfusion: Bile pH >7.5, Bile glucose concentration <3 mmol/L or >10 mmol but <perfusate glucose, Perfusate pH >7.2 without more than 30 mL bicarbonate supplementation, Falling glucose beyond 2 h or perfusate glucose <10 mmol/L, Peak lactate fall >4.4 mmol/L/kg/h, ALT <6,000 IU/L at 2 h
The Groningen group (2022)[22]	Viability is assessed within 2 h of perfusion: Lactate <1.7 mmol/L, Perfusate pH 7.35 to 7.45, Bile production >10 mL, Biliary pH >7.45 ΔpH >0.10 ΔHCO3− >5.0 mmol/L ΔGlucose < −5.0 mmol/L

ALT, alanine aminotransferase; HA, hepatic artery; PV, portal vein.

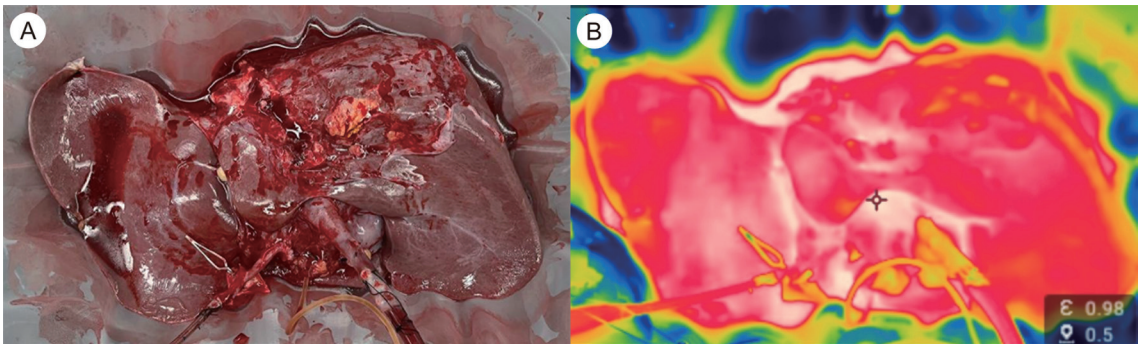

Fig. 3. (A) Appearance of the liver and (B) thermal imaging of the liver during perfusion.

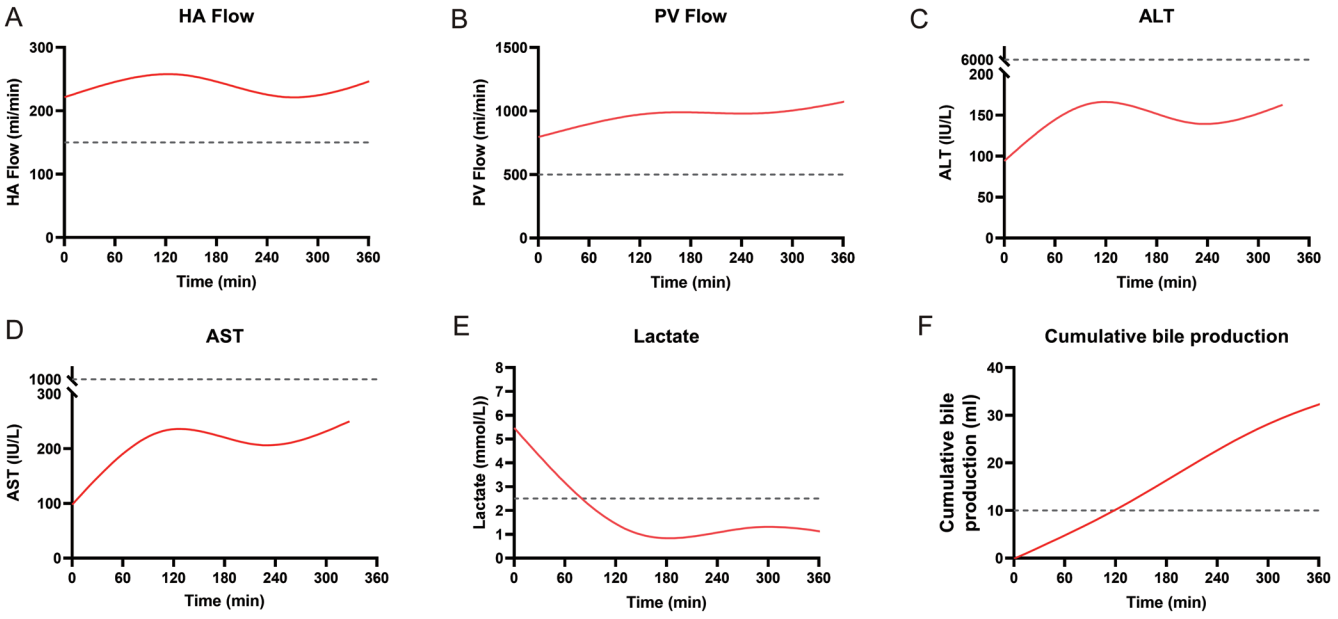

Fig. 4. Hepatic characteristics in viable donor livers during NMP. Dotted lines indicate cutoff values used as viability criteria in clinical machine perfusion studies. (A) HA flow, (B) PV flow, (C) ALT, (D) AST, (E) lactate, (F) cumulative bile production. Figures are based on 40 ischemia-free liver transplants performed at The First Affiliated Hospital, Sun Yat-sen University. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HA, hepatic artery; NMP, normothermic machine perfusion; PV, portal vein.

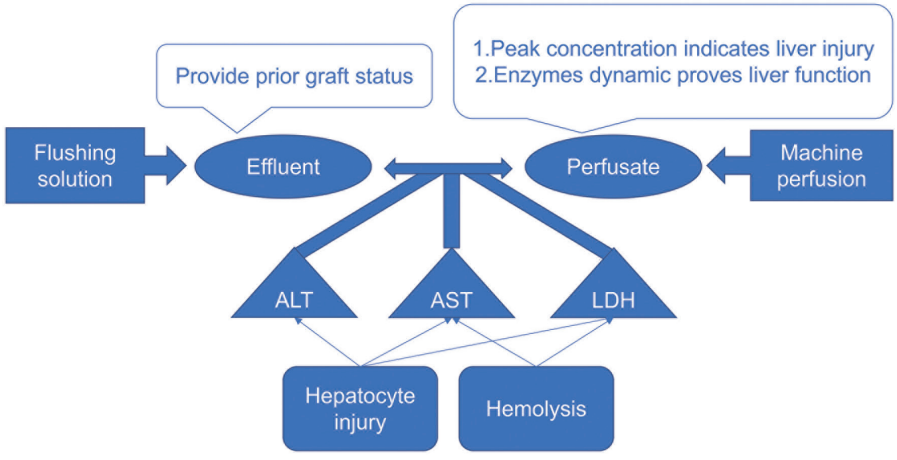

Fig. 5. Production process of AST, ALT, and LDH and their dynamic changes process during machine perfusion. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

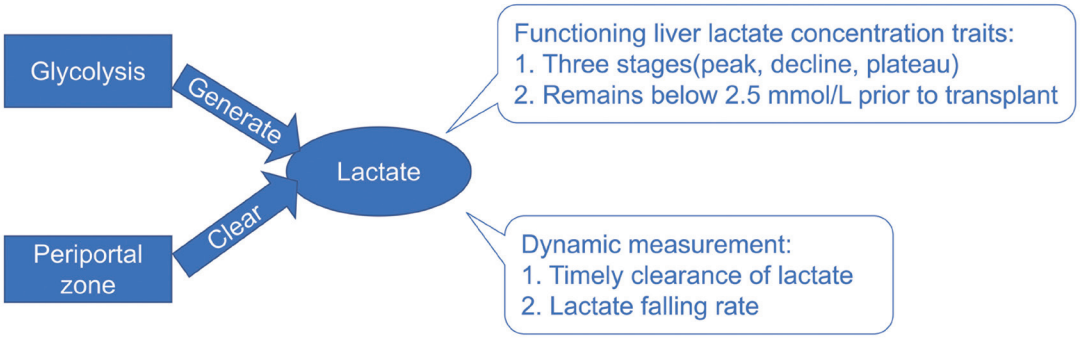

Fig. 6. Production process of lactate and their dynamic changes process during machine perfusion.

levels depend on liver mass and perfusate volume and may not be good markers of liver viability, it may be preferable to focus on the dynamics of these enzymes. A sudden rapid increase in transaminases during NMP may be a signal of severe preservation injury, and maintenance of steady concentrations after reaching a plateau may suggest no ongoing damage.[12] Machine perfusion may cause "wash-out," or lower transaminase levels in the liver post-transplant as a result of release of transaminases that have accumulated in the NMP circuit.[16,39,44] Clearance of the transaminases may provide additional information about graft function. Previous studies had shown that transaminases can be taken up by sinusoidal cells in the liver and cleared by Kupffer cells.[45] Eshmuminov *et al*.[10] used the decline in transaminase levels as an indicator of good liver viability. Bral *et al*.[44] also reported that a healthy porcine liver perfused in an NMP circuit cleared high levels of transaminases injected into the perfusate. The findings indicate that increased transaminase levels do not necessarily reflect the functional status of a liver but that clearance of exogenous or endogenous transaminases during NMP may indicate good liver viability.

Measurement of transaminase levels in the perfusate collected from the flushing solution immediately before perfusion may also provide useful information. Pacheco *et al*.[46] reported a correlation between effluent and post-transplant transaminase levels. Furthermore, Lange *et al*.[47] suggested that low transaminase levels in the effluent may exclude the risk of graft failure as a result of donor problems. A high transaminase level in the effluent suggests significant liver injury during retrieval and cold storage.[48] Some authors have suggested that measurement of effluent transaminase levels could be an early indicator of a need for further assessment of a liver during NMP.[12] We believe that dynamic changes in transaminase levels during perfusion can help in monitoring of liver injury and its mitigation during machine perfusion.

Lactate

Lactate is one of the main precursors in gluconeogenesis, which is an oxygen-dependent process that takes place primarily in the liver. Hepatocytes can use lactate as a substrate to generate glucose via gluconeogenesis. Lactate in the perfusate comes primarily from anaerobic glycolysis in hepatocytes. Perfusate lactate levels in livers that function well during NMP and are chosen for transplant go through sequential stages of peaking, declining, and plateauing, which suggests that well-functioning livers are able to clear lactate (Fig. 6).[14] Lactate clearance is used clinically as a marker of liver function immediately post-transplant and is also one of the most widely accepted indicators of liver function during NMP. Many studies have used lactate clearance as an indicator of liver viability,[14] and failure of the lactate level to decrease indicates liver injury. A decline in the perfusate lactate level to ≤2.5 mmol/L (Fig. 4E) within a few hours of NMP is an important indicator of viability.[49] The rate of decline in lactate is also included in some criteria, and a decrease of >4.4 mmol/L/kg/h may predict a well-functioning graft. Reiling *et al*.[50] set the perfusion lactate threshold within the first 2 hours of NMP at 2.0 mmol/L. However, Leeuwen *et al*.[51] recommended a lactate threshold of 1.7 mmol/L during NMP. Livers that cannot clear lactate or cannot clear it rapidly may be discarded. Some believe that the lactate level should remain at <2.5 mmol/L for a period of time before any transplant decision is made. As with transaminases, it has been suggested that lactate be normalized per unit liver weight.[12]

However, there are still some problems when using lactate level as a criterion for judgment of liver viability. Some grafts may develop early allograft dysfunction or primary nonfunction after transplantation even if they successfully clear most of the lactate during NMP,[12] possibly because part of the liver can already clear perfusate lactate. Therefore, clearance of perfusate lactate may not predict good function of the whole liver. It has been reported that the lactate is mainly cleared in the periportal zone, which can provide adequate oxygen and energy for gluconeogenesis.[52] Hence, injury to the periportal zone, usually involving pan-lobular injury, may affect lactate clearance, whereas damage to other zones may not have the same effect. Although a decrease in perfusate lactate may not directly predict a viable liver, no change in lactate is still a sign of liver injury. Addition of exogenous lactate in the NMP circuit via the flushing solution could improve our ability to analyze the rate of decrease in lactate.[12] Exactly when lactate should be measured remains unclear. However, the Birmingham team obtained measurements at 2.5, 3, and 4 h when determining liver viability in their studies.[11,49,53] Lactate level continues to be regarded as a strong indicator of liver viability. However, lactate fails to decrease beyond a certain level in some eligible livers during perfusion. Therefore, we suggest that the change in the lactate level after perfusion and the slope of curve of the decrease in lactate be used as the criteria.

Production of bile

Bile production is one of the important functions of the liver and requires an intact network of sinusoidal cells, hepatocytes, bile duct cells, and energy.[17] It is widely recognized as a predictor of liver viability during machine perfusion and to correlate with secretion of bilirubin, the concentration of bicarbonate in bile, and the lactate level.[17,54] Bile salts are an important driver of bile production. Experiments in a porcine model suggested that levels of bile salts are not depleted

until after 10 h of NMP, suggesting that there is no need to add bile salts if the duration of perfusion is to be shorter than 10 h.[17] Furthermore, another study found no significant difference in donor characteristics between livers with high bile production and those with low bile production during machine perfusion.[15] In terms of a cutoff value, the Groningen group proposed a cumulative bile secretion of >20 g in 6 h or bile secretion >10 mL in 2.5 h as a marker of a well-functioning liver (Fig. 4F).[55,56] There may be two patterns of bile accumulation. One is a steady increase during perfusion, and the other is an initial increase followed by a gradual decrease. However, some evidence shows that the amount of bile secreted may not be related to duration of ischemia.[18]

Bile production also has some limitations as a marker. First, it is primarily driven by hepatocytes, so it cannot be used as a marker of bile duct viability. Even livers with a sufficiently high bile secretion rate may develop biliary complications.[57–59] Second, technical problems with bile duct drainage tubes, such as incorrect positioning and leakage, may give the illusion of insufficient bile production. Finally, in some cases, the level of bile production is not related to the stability of arterial blood flow.[6] In our opinion, bile production during perfusion indicates that the liver is viable. However, it is necessary to check that the bile present is not the original residual bile of the donor liver. Bile may not be produced during perfusion by some livers that qualify for transplantation. Therefore, comprehensive judgment is required.

Urea

Urea is synthesized in the liver and is a relatively new marker of liver viability. It has been demonstrated that livers with a long duration of ischemia have significantly lower urea levels within 4 h of NMP, whereas live donor livers with a short duration of ischemia have a significantly higher multiplicative increase in urea levels after 2–4 h consistent with the degree of ischemia.[18] Previous studies using non-transformed or non-transplanted models have reported conflicting data on urea levels during NMP, reflecting either cell injury or liver viability.[60,61] However, in some studies, urea synthesis was consistently used as a predictor of liver viability during NMP and was correlated with liver viability after transplantation. The cutoff value is considered to be a ≥0.5-fold increase in the urea level.[18] Urea is usually measured routinely by laboratory analysis and requires slightly more time to process. It is not clear whether an elevated urea level is harmful to the perfused liver or if it has an adverse impact on the transplant outcome, but excess urea can be removed by dialysis.[6] The role of the urea level during machine perfusion remains to be clarified.

Coagulation factors

Most coagulation factors are synthesized in the liver.[62] Hepatocytes produce coagulation factors FV/FVII/FIX/FX and antithrombin and hepatic sinusoidal cells produce FVIII.[63,64] Although heparin is added to the perfusate as an anticoagulant during NMP, production of coagulation factors can still be used to assess liver viability. Eshmuminov *et al*.[10] reported that the level of coagulation factor V was significantly higher in well-functioning livers than in non-functioning livers after 48 h of perfusion but not thereafter. Furthermore, it has been shown that a decrease in the international normalized ratio after several hours of machine perfusion indicated recovery of liver viability and production of coagulation factors.[65–67] Activation of fibrinolysis appears to be more pronounced in livers of poorer quality and is associated with IRI in hepatocytes.[68] It has also been demonstrated that NMP at end-ischemia leads to activation of fibrinolysis and that a high D-dimer concentration in perfusate can be considered an indicator of severe ischemic injury and unfavorable liver viability.[68]

As mentioned above, liver viability can be assessed by markers such as the international normalized ratio and fibrinolysis, but some coagulation factors cannot be used as markers because addition of heparin as part of the common perfusion protocols interferes with their measurement.[65] It has also been shown that the rates of increase in fibrinogen, FV, and FIX do not correlate with peak AST after transplantation. Accumulation of anticoagulant factors is common, but the apparent concentration and accumulation rate are low in severely injured livers.[63] We believe that coagulation factors can be used as indicators of liver viability, but it is difficult to define specific absolute values. At present, coagulation factors cannot be used accurately as predictors in clinical medicine.

Glucose metabolism

The liver has an important role in glucose metabolism and is a key organ involved in maintaining a stable blood glucose level. The liver participates in the synthesis and decomposition of glycogen, glycolysis, gluconeogenesis, and other metabolic pathways to maintain glucose homeostasis in the internal environment.[69] Static cold storage of a liver results in decomposition of glycogen in hepatocytes and anaerobic metabolism due to hypoxia, ischemia, and a deficiency of ATP. That is one of the reasons for the increased glucose concentration in the perfusate during the initial stage of NMP.[70] However, if liver injury is severe, the glucose level will still be low at the start of NMP. Glucose stimulation tests can be used to fully assess the viability of this type of liver. In this test, glucose is added to the perfusate, and if the liver is active enough, there is a decrease in the glucose concentration in the perfusate.[10,15] Furthermore, use of pancreatic hormones is another application of glucose metabolism in the liver and can also reflect liver viability.[10]

Acid-base balance of the perfusate

The liver is an important regulator of acid-base balance and achieves homeostasis mainly by metabolism of lactate, synthesis of albumin, ketogenesis, and production of urea.[71] However, liver injury, such as IRI, usually results in the liver perfusate having a low pH during machine perfusion. Therefore, most transplant centers maintain the pH of liver perfusate at a physiological level (7.3–7.45) during machine perfusion to increase the likelihood of liver viability (Fig. 7A).[10,30]

The composition of the perfusate, addition of alkaline substances during machine perfusion, and the partial pressure of carbon dioxide may affect the pH of the perfusate. For example, bicarbonate, although used as a marker of liver viability, is routinely added to the perfusate during NMP to maintain pH. A bolus of bicarbonate is usually added at the start of NMP to maintain a bicarbonate concentration of 10–40 mmol/L depending on the pH of the perfusate.[72] The pH is often combined with other parameters when evaluating liver viability because of multiple confounding factors. Nevertheless, a perfusate with too high or too low pH may be an indication of poor liver viability. We suspect that measuring perfusate pH alone may not be an accurate way of determining liver viability. The partial pressure of carbon dioxide, bicarbonate concentration, and lactate level are also important factors affecting the pH of the perfusate. Therefore, liver viability can only be evaluated accurately by considering all of these factors.

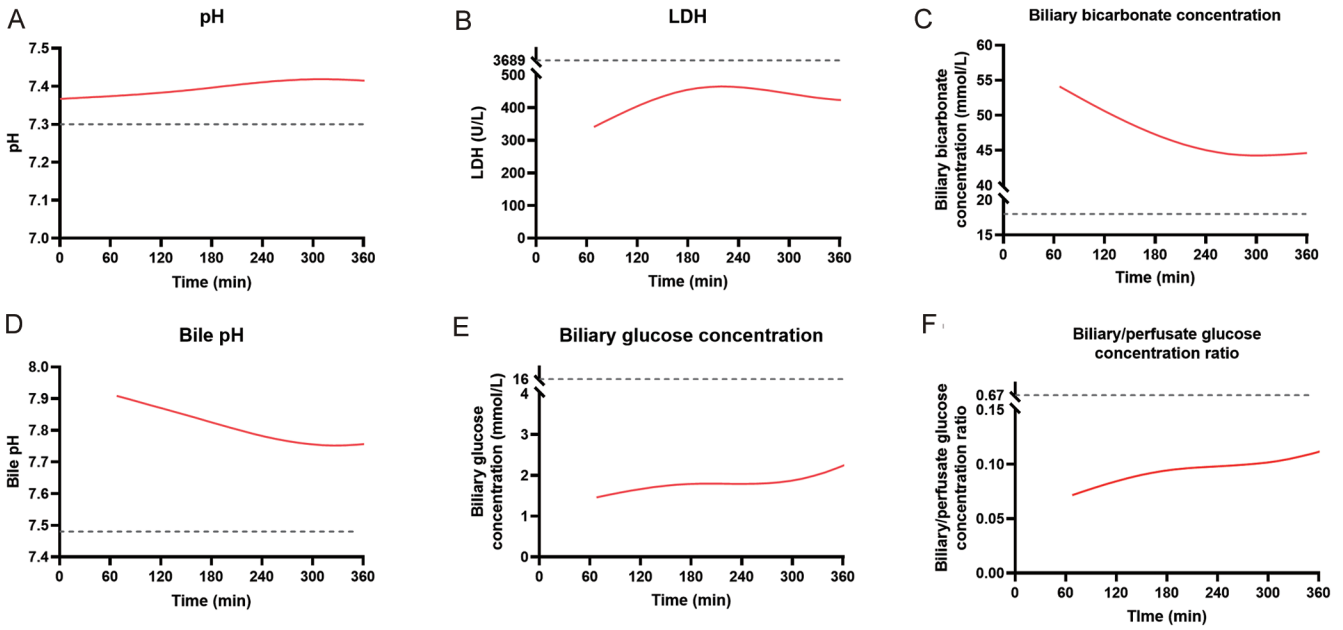

Fig. 7. Perfusate and bile duct characteristics of viable donor livers during NMP. Dotted lines indicate cutoff values used as viability criteria in clinical machine perfusion studies. (A) perfusate pH, (B) LDH, (C) biliary bicarbonate concentration, (D) bile pH, (E) biliary glucose concentration, (F) biliary/perfusion glucose concentration ratio. Figures are based on 40 ischemia-free liver transplantation of The First Affiliated Hospital, Sun Yat-sen University. LDH, lactate dehydrogenase; NMP, normothermic machine perfusion.

Assessment of bile duct viability

Many clinical studies of machine perfusion have used liver viability criteria based on hepatocellular viability.[73] Other indicators, such as those related to the bile duct, often have supporting roles. However, IRI in bile duct cells is a direct cause of biliary complications after liver transplantation.[74] The bile duct contains delicate cells with a high oxygen requirement and low tolerance to hypoxia which are more vulnerable to IRI than hepatocytes and are prone to injury. Clinical experiments had demonstrated that livers meeting the criteria for hepatocyte viability may still develop post-transplant biliary complications, including non-anastomotic biliary strictures. Therefore, there is now increasing interest in identifying a biomarker of bile duct viability.

LDH is a marker of biliary epithelial cell injury.[75] When hepatocytes and bile duct cells become necrotic or the osmotic pressure increases, LDH is released into the interstitial fluid, resulting in an increased LDH level in blood or bile. Therefore, bile duct injury can be detected by measuring the LDH concentration during machine perfusion (Fig. 3). Furthermore, biliary function is also an important indicator of liver viability. The main functions of bile duct cells are to secrete bicarbonate, absorb glucose from the bile, and make bile more alkaline. Thus, bicarbonate, glucose, and the pH of bile are suitable parameters for assessment of bile duct viability.[73]

Sutton *et al*.[17] found that transplants with a high bile output also secreted more bicarbonate and suggested that the bicarbonate level in bile could be used as an indicator of the function of bile duct cells during NMP. Matton *et al*.[73] subsequently demonstrated that biliary bicarbonate, pH, and glucose were accurate biomarkers of bile duct injury in NMP and suitable for assessing bile duct viability before transplantation. They also found LDH to be a suitable indicator. In their study, they tested for these biomarkers in 23 high-risk livers that were histologically graded on a scale of 0–7 for bile duct injury. They found that a biliary bicarbonate concentration of >18 mmol/L (Fig. 7C), a biliary pH of >7.48 (Fig. 7D), a biliary glucose concentration of <16 mmol/L (Fig. 7E), a biliary/perfusion glucose concentration ratio of <0.67 (Fig. 7F), and an LDH concentration of <3,689 U/L were strongly associated with low histological evidence of bile duct injury during the first 2.5 h of NMP. At present, our center includes bile duct viability as a criterion when evaluating liver viability. We believe that good biliary function can effectively reduce the incidence of postoperative biliary complications.

Liver microscopic expression/liver biopsy

The donor liver can also be examined by biopsy. In some machine perfusion experiments, the specimens were fixed with formalin and embedded in paraffin. Hematoxylin-eosin (HE) staining is usually used to observe necrosis and apoptosis[76,77] and myeloperoxidase can be used to stain neutrophils.[78] Intelligent use of immunohistochemical stains often adds to the medically relevant information that can be obtained from liver biopsies.[79] Pathological variables such as steatosis, monomorphonuclear infiltrate, and necrosis are significantly associated with an increased risk of mortality.[76] Afford *et al*.[19] performed a study in which they classified the degree of macrovesicular steatosis into four categories, namely, none (<5%), mild (5–30%), moderate (30–60%), and severe (>60%), and noted that steatosis was one of the main reasons for surgeons rejecting livers for transplantation. Furthermore, fat, cholestasis, and ballooning detected in early post-transplant biopsies are widely attributed to IRI.[80] However, biopsy cannot monitor the liver in real time and can only be used for the purposes of retrospective analysis. We believe that although microscopic analysis of the liver during machine perfusion may not reflect liver viability in real time, techniques such as frozen section may be able to detect damage to a liver and

whether it may be suitable for transplantation as soon as possible.

New ways to measure liver viability

Recent technological advances have provided an increasing number of methods to evaluate liver viability.

Imaging

Magnetic resonance imaging (MRI) uses a radiofrequency pulse to the human body in a static magnetic field that activates hydrogen protons. When the pulse is stopped, the protons generate a magnetic resonance signal during the relaxation process.[81–83] Yang *et al*.[84] used a nuclear magnetic resonance (NMR) relaxation analyzer-based assay to assess the viability of *ex vivo* porcine livers obtained after circulatory death. In their study, NMP was used as a platform for viability testing of the porcine livers. During NMP, the liver-targeting contrast agent gadolinium-ethoxybenzyl-diethylenetriaminepentaacetic acid (Gd-EOB-DTPA), which is a hepatocyte-specific MRI contrast agent that shortens the longitudinal relaxation time (T1) in the liver parenchyma,[85–88] was injected into the perfusate. Uptake and metabolism of Gd-EOB-DTPA reflected the function of hepatocytes. In the normal liver, approximately 50% of Gd-EOB-DTPA is absorbed by hepatocytes and excreted into the bile 20 m after a single dose. In that way, a quantitative comparison of the signal intensity through measuring the T1 relaxation time before and after contrast enhancement over a certain period of time can be used to evaluate the hepatocyte function. The longitudinal relaxation rate of bile (R1=1/T1) is also used as an indicator. Delayed elevation of biliary R1 in the early stage of NMP suggests impaired function of a liver graft during warm and cold ischemic injury, which may be associated with an increase in ALT after 4 h of NMP. This study showed that NMP and MRI can be combined for dynamic evaluation of the viability of porcine livers.

Faitot *et al*.[89] used HR-MAS-NMR for extemporaneously untargeted metabolic profiling and found a strong correlation between lactate, choline-derived metabolites of the graft, and liver function. They found HR-MAS-NMR to be a useful technique for evaluation of liver viability with potential for assessment of the efficiency of liver resuscitation on machine perfusion. However, it is difficult to use MRI during NMP because the machine contains metal, which is a contraindication to MRI.

Positron emission tomography-computed tomography (PET-CT) can be used to evaluate liver viability. ^{18}F-fluorodeoxyglucose (FDG) PET-CT reflects the metabolic and functional status of genes and molecules in lesions. It uses positron-labeled glucose and other metabolites as imaging agents, uptake of which provides information on various disease states.[90,91] Orita *et al*.[92] used FDG PET-CT to assess two human livers and six porcine livers after 1 week of *ex vitro* machine perfusion. They injected FDG through the portal vein and then continued machine perfusion for 55 m. The organ was then cooled by the machine to prevent necrosis and subsequently transferred for PET-CT. That study demonstrated the feasibility of FDG PET-CT in evaluation of liver viability after NMP. Although the study did not assess liver viability during NMP, it confirmed that PET-CT can be used to assess liver viability and could be used to evaluate livers during NMP in the future.

Ultrasonography uses the penetrability of sound waves to assess the function and anatomy of organs and has many advantages over radiographic imaging, including better safety, real-time display, universality, and less discomfort.[93] Ultrasonography can be used to detect hepatic fibrosis, hepatic cirrhosis, and fatty degeneration of the liver. It can also be used to observe blood flow, vascular permeability, and angiogenesis.[94] Dynamic contrast-enhanced ultrasound imaging can quantify blood flow parameters, including velocity of blood flow and relative vessel volume.[95] Contrast-enhanced ultrasound can detect liver steatosis using parameters such as the degree and rates of portal vein perfusion and total hepatic perfusion, which are decreased in patients with liver steatosis.[96] Although ultrasound is rarely used to assess machine perfusion, it has potential for assessment of liver viability. In the future, we plan to assess the value of ultrasonography in evaluation of liver viability in the transplant setting.

Indocyanine green (ICG) is a diagnostic dye that can be used to assess liver function and effective blood flow in the liver. After intravenous injection into the liver, ICG immediately combines with plasma protein and distributes rapidly in blood vessels throughout the liver. It is efficiently and selectively absorbed by hepatocytes after intravenous injection and is then excreted into bile in free form. The ICG clearance test is a useful quantitative evaluation of liver function before transplantation.[97,98] Tang *et al*.[98] found that the donor ICG retention rate at 15 m (ICGR15) before procurement was independently associated with survival at 3 months post-transplantation. That study also reported that the optimal donor ICGR15 cutoff was 11.0%/m and that it could be used as an early indicator of liver quality. Dondossola *et al*.[99] investigated ICG clearance as an indicator of liver function during NMP in a porcine model. They measured ICG and performed 805 nm spectrophotometry in samples of perfusate and bile, which revealed a decline in ICG in the perfusate and an increase in bile, indicating good liver viability. They also noted that the ICG clearance test was a suitable indicator of liver function and transplantability. In an experimental porcine study by Quero *et al*.,[100] computer-assisted dynamic analysis of the fluorescence signal from ICG discriminated arterial and venous bowel ischemia. As yet, the ICG clearance test has not been used in any human liver NMP trials. However, it has potential for assessment of liver viability during NMP in the future because it is safe and easy to perform.

Automated liver assessment

Computer software and artificial intelligence are methods that can help to obtain standardized organ analyses with no interobserver or intraobserver differences, classification bias, or technical limitations.[101,102] In general, the presence of hepatic steatosis increases the risk of graft dysfunction. The degree of hepatic steatosis is an important consideration when deciding whether to transplant a liver. Pathological examination remains the best method for evaluation of hepatic steatosis. However, Cesarettiey *et al*.[103] developed a noninvasive method for assessment of liver steatosis that involves fully automated texture analysis of the liver graft by machine-learning algorithms using photographs obtained by a smartphone and has an accuracy rate of up to 89%. Other researchers developed an automated machine-learning algorithm that was able to detect and discriminate microsteatosis and macrosteatosis on images of HE-stained liver biopsy specimens.[104] The test could be performed in 0.72 s on average and reached an accuracy of 97%. Fernando *et al*.[105] also developed a machine-learning algorithm that could detect macrosteatosis and determine its extent in pretransplant liver biopsies. However, instead of HE-stained specimens, they used Sudan-stained frozen sections because they have the advantages of being more rapid to perform and are fat-specific. In the era of big data, use of artificial intelligence rather than humans for evalu-

ation of liver viability is likely to become commonplace in the future.

Raman spectrometry

Spectroscopic methodologies are widely used in medical diagnostics. Raman spectroscopy is one such method and can be used to assess samples at the macro, micro, and cellular levels.[106] Raman spectroscopy has been shown to acquire biochemical and structural information rapidly and non-invasively with high spatial resolution by generating point spectra or spectral images.[107,108] Ember *et al*.[109] assessed the left median and right lateral lobes in a porcine liver using a handheld Raman spectrometer before circulatory arrest, after 45 m of warm ischemia, and after 2 h of normothermic regional perfusion. They found that the total signal obtained in the liver after 45 m of warm ischemia was significantly weaker than that obtained before circulatory arrest. They also found that the correlation between congestion and the observed Raman signal was reflected by a change in the bulk optical properties of the liver when measured *in situ*. Their findings suggest that Raman spectroscopy is an effective tool for detecting microvascular injury and can help determine whether to transplant an ECD liver. It can also be used to measure liver-related indicators during NMP. All of the above techniques have the potential to help us evaluate liver function more effectively. They are simple, easy to use, and provide more rapid and comprehensive assessment of liver function than would be possible using laboratory data alone.

Conclusions

Limitations and future perspectives

The advent of dynamic organ perfusion techniques has made it possible to assess the viability of parenchymal cells during preservation and to transplant marginal organs. Measurement of liver viability during machine perfusion can make it easier to identify livers suitable for transplantation and reduce the risk of post-transplant complications. However, those methods still have some limitations. The first is the continuing lack of a unified evaluation standard for liver viability during NMP. For example, although lactate levels and bile production have been consistently reported to be important indicators of liver viability during NMP, several studies have found that the two parameters do not distinguish between viable and nonviable grafts.[38] The second limitation is the variation in the current machine perfusion protocols, to which an increasing number of variables have been added, such as temperature, composition of the perfusate, and additives, including bile salts, insulin, bicarbonate, and deoxycholic acid, resulting in some measurements that do not accurately reflect liver viability. Third, some biomarkers, such as ATP, metabolomics indicators, and microscopic biopsy findings, cannot provide real-time feedback on the current status of the liver and are often used retrospectively. However, advances in laboratory technology may allow more widespread use of these methods in the future. Despite current limitations, dynamic preservation by NMP allows livers initially deemed unsuitable for transplantation to be reassessed for viability. Although there is no universal standard, each research center has a comprehensive set of criteria for assessment of liver viability.

Clinical trials should continue and data should be shared among all centers. Combined data from multiple centers and more postoperative events will be critical for optimizing indicators of survival and for creating viability scores that can reliably predict postoperative complications. Novel and simpler methods for evaluation of liver viability should be developed and used. Furthermore, using big data, a scoring system could be devised for evaluation of liver viability. The ability to transplant a liver that reaches a certain score would be a huge breakthrough in transplant medicine.

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Conflict of interest

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Author contributions

Designed the research, reviewed the literature, wrote the manuscript, and participated in preparation of the figures and tables (QZ, JL, HL, JZ, YL). All authors read and approved the manuscript for publication.

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