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The Emerging Role of Viability Testing During Liver Machine Perfusion

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The transplant community continues to be challenged by the disparity between the need for liver transplantation and the shortage of suitable donor organs. At the same time, the number of unused donor livers continues to increase, most likely attributed to the worsening quality of these organs. To date, there is no reliable marker of liver graft viability that can predict good posttransplant outcomes. Ex situ machine perfusion offers additional data to assess the viability of donor livers before transplantation. Hence, livers initially considered unsuitable for transplantation can be assessed during machine perfusion in terms of appearance and consistency, hemodynamics, and metabolic and excretory function. In addition, postoperative complications such as primary nonfunction or posttransplant cholangiopathy may be predicted and avoided. A variety of viability criteria have been used in machine perfusion, and to date there is no widely accepted composition of criteria for clinical use. This review discusses potential viability markers for hepatobiliary function during machine perfusion, describes current limitations, and provides future recommendations for the use of viability criteria in clinical liver transplantation.

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Since Thomas Starzl performed the first human liver transplant (LT) in 1963, LT is now fully established as the standard treatment for patients with end-stage liver disease. Yet, the LT community continues to be challenged by the disparity between the need for LT and the shortage of suitable donor organs.[1] In the United States, the percentage of discarded donor livers is expected to increase from 22% in 2010 to 56% in 2030, most likely attributed to worsening organ quality (e.g., older donors, fatty livers; Fig. 1).[2] The decision on whether to accept a donor organ remains partly subjective based on empirical data and clinical experience and by balancing donor, recipient, and liver variables.

Ex situ machine perfusion offers a novel approach in which the function of the donor organ can be assessed before transplantation.[3,4] This way, livers that are initially considered unsuitable for transplantation can be subjected to machine perfusion to test hepatobiliary function and prevent postoperative complications. Normothermic machine perfusion (NMP) is most commonly used to evaluate the metabolic and synthetic functions of the liver and biliary tree.[3] Experience with viability assessment during subnormothermic machine perfusion (SNMP) is still limited. Efforts are made with regards to viability testing during hypothermic machine perfusion (HMP), but this is more challenging because hepatic metabolism is significantly reduced and bile production ceases under hypothermic conditions.[5]

In this review, we provide a brief historical background on viability assessment in LT and discuss potential viability criteria for hepatobiliary function during ex situ machine perfusion. We also describe current limitations and provide future recommendations

Abbreviations: ATP, adenosine triphosphate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DBD, donation after brain death; DCD, donation after circulatory death; EAD, early allograft dysfunction; FMN, flavin mononucleotide; HA, hepatic artery; HMP, hypothermic machine perfusion; ITBL, ischemic-type biliary lesion; LDH, lactate dehydrogenase; LT, liver transplantation; miRNA, micro RNA; NMP, normothermic machine perfusion; PNF, primary nonfunction; PV, portal vein; SNMP, subnormothermic machine perfusion.

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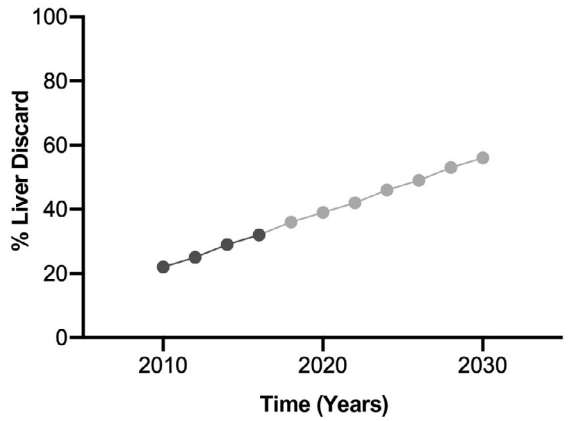

FIG. 1. Expected increase in the donor liver discard rate in the United States between 2010 and 2030. The graph represents the percentage of discarded donor livers based on worsening organ quality and taking into account the increasing national population. Dark gray dots represent actual numbers. Light gray dots represent expected numbers. Figure based on data from Orman et al.[2]

on the use of viability criteria during machine perfusion before LT.

Viability Assessment From a Historical Perspective

The increased proportion of suboptimal donor organs and the evolution of machine perfusion have stimulated a lot of research into viability assessment of donor livers before transplantation. Nevertheless, researchers in the field have thought about viability assessment in LT since the early days. The first report on this topic dates back to 1969 and describes formazan production by liver tissue slices as a rapid method of assessing organ viability before transplantation.[6] Formazan is a colored product formed by the reduction of a salt by dehydrogenase, and the time elapsed until the appearance of the first perceptible color reflected viability of the organ. Towards the end of the 19th century, various studies were published pointing out that the ability of the liver to produce adenosine triphosphate (ATP) may be used to determine viability.[7-9] In the years following, graft viability and injury were mainly assessed by histology, by analyzing several markers in the cold storage solution (transaminases, lactate dehydrogenase [LDH])[10], and by using various imaging techniques, such as magnetic resonance spectroscopy.[11,12] After the introduction of machine perfusion to the clinic around 2010, many efforts have been made to define predictors of hepatobiliary function during ex situ machine perfusion of donor livers.

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Viability Assessment During NMP

NMP is most commonly used to assess liver viability because the organ is maintained in a near-physiological state being continuously perfused with an oxygenated solution at a temperature of around 37°C.[3] During NMP, the following 2 types of viability criteria can be used: those focusing on the liver (hepatocellular criteria) and those focusing on the bile duct (cholangiocellular criteria).[13] In the future, there will also likely be vascular criteria of viability to identify grafts with a high risk of microcirculatory collapse or vascular thrombosis. Liver function can be assessed in terms of metabolic function (lactate and ammonia clearance, pH maintenance, urea production) and excretory function (bile production, drug clearance, and production of coagulation factors).[14] In addition, graft appearance, consistency, and hemodynamics (flow, pressure, and resistance) can be evaluated as markers of liver quality. Function of the biliary epithelium can be assessed by analyzing bile composition (biliary levels of glucose, pH, and bicarbonate[15]; Fig. 2).

Hepatocellular Viability Criteria

LACTATE METABOLISM

The liver is the primary organ involved in lactate clearance in vivo. After hepatic uptake, lactate is

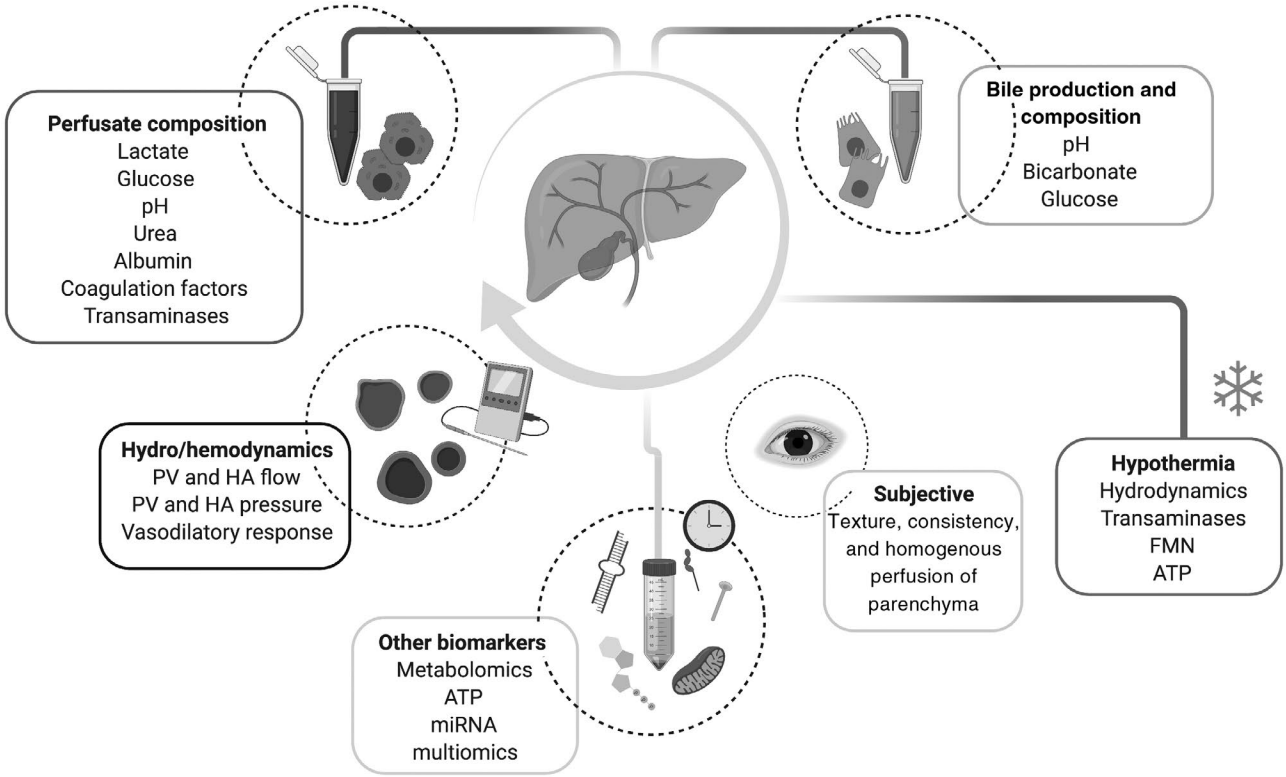

FIG. 2. Viability markers during liver machine perfusion. Liver function (hepatocellular) can be assessed by evaluating perfusate composition, hydrodynamics/hemodynamics, and other biomarkers or assessment techniques. Bile duct function (cholangiocellular) can be assessed by evaluating bile composition. Created with BioRender.com (Biorender, Toronto).

metabolized within the hepatocytes by oxidation or as a substrate for gluconeogenesis.[16] Lactate production may arise from anaerobic glycolysis, but a certain amount also originates from erythrocytes in the perfusate. During end-ischemic NMP, the following 3 lactate phases are generally observed in a functioning liver: an initial lactate peak up to 1 hour after NMP initiation, then a rapid drop within 2 hours, followed by a steady low state for the remaining perfusion time[17] (Fig. 3A). When NMP is initiated in the donor hospital by using normothermic regional perfusion or ischemia-free LT, the lactate peak tends to be much lower.

Many machine perfusion studies have shown an association between lactate metabolism and postoperative liver function.[18-22] Nonfalling perfusate lactate levels are considered an adverse sign in most clinical viability assessment studies to date[18,21-28] (Table 1). The Birmingham group has performed a preclinical study in which they compared lactate-clearing versus non-lactate–clearing livers.[19] Livers in the lactate-clearing group were more likely to maintain a physiological perfusate pH, stabilize perfusate hematocrit, and had higher bile production compared with the non-lactate–clearing group. It becomes evident from the studies presented in Table 1 that the majority of livers are able to clear lactate during machine perfusion. If livers were unable to sufficiently clear lactate, this was most commonly attributed to poor perfusion after vascular reconstruction (i.e., anatomical variant), marked steatosis, or traumatic lesions to the liver parenchyma.[21,23,24,26] Some livers that cleared lactate during NMP still showed signs of early allograft dysfunction (EAD) after transplantation or were even lost as a result of primary nonfunction (PNF).[23,25,27] In some cases, the criteria for lactate clearance are initially met, but the liver deteriorated thereafter with increasing lactate.[18,28] The clinical significance of this remains uncertain.

Lactate can be determined using a point-of-care blood gas analyzer, making it an easy and rapid marker to measure. However, controversy exists on whether

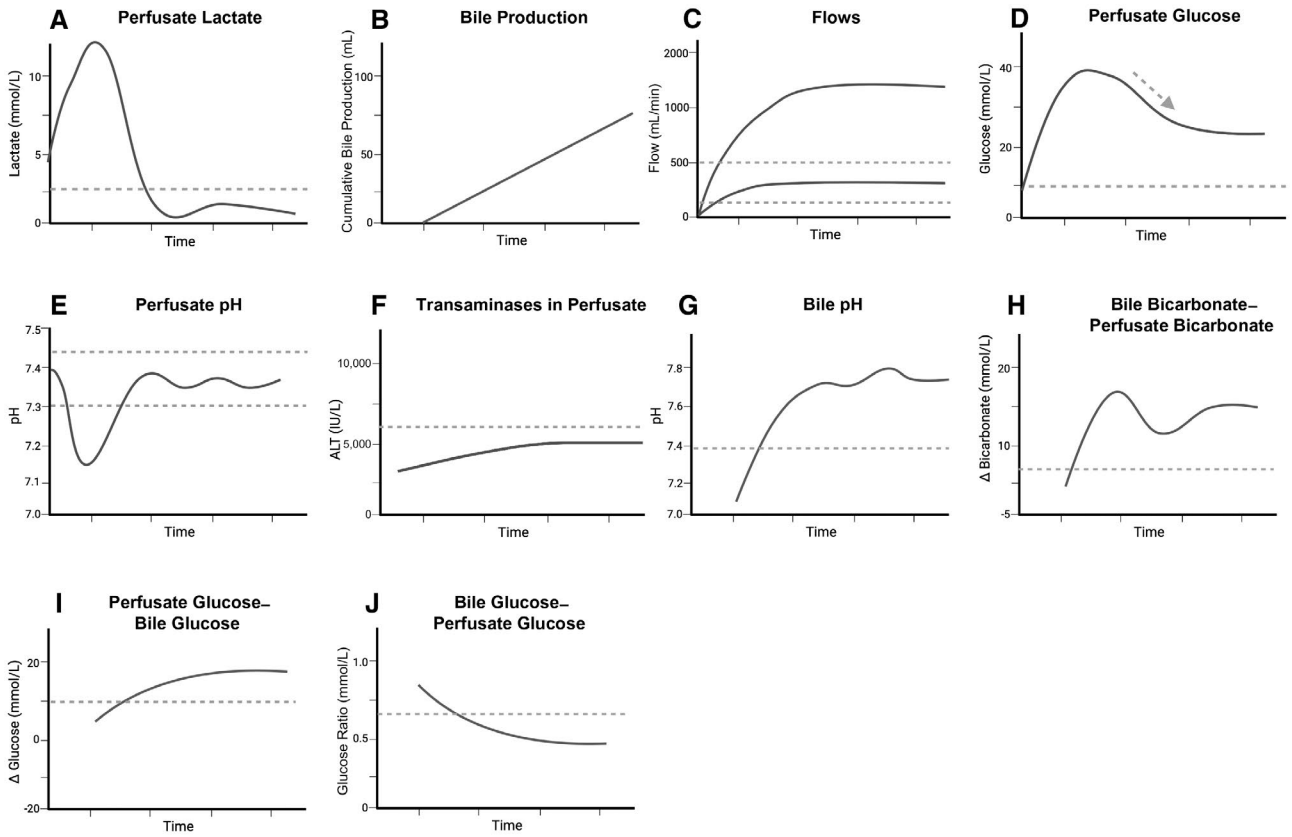

FIG. 3. Perfusate and bile characteristics of viable donor livers during ex situ machine perfusion. Dotted lines indicate cutoff values used as viability criteria in clinical machine perfusion studies. Figures are based on the studies included in Table 1 and are adapted from de Meijer et al.[3] and Watson et al.[27]: (A) perfusate lactate, (B) bile production, (C) flows, (D) perfusate glucose, (E) perfusate pH, (F) transaminases in perfusate, (G) bile pH, (H) bile bicarbonate – perfusate bicarbonate, (I) perfusate glucose – bile glucose, and (J) bile glucose – perfusate glucose. Created with BioRender.com.

lactate clearance is an accurate marker of liver viability. Although nonfalling lactate levels are considered an adverse sign, falling lactate levels do not necessarily imply viability. Even if only a small part of the liver is functioning, the relatively small amount of perfusate used during machine perfusion can be cleared from lactate. Table 1 shows that different lactate thresholds are used by different groups and at different time points during NMP. We suggest that mere lactate clearance may be used to distinguish between "good" and "very bad" livers, but should otherwise be used in combination with other criteria. Besides using absolute lactate values, the slope of the curve or delta from peak to steady state have been suggested as surrogates for liver function. Another, perhaps more accurate, option would be to measure lactate clearance corrected per unit of liver weight.

BILE PRODUCTION

Bile production is a unique function of the liver and requires the integrity of multiple cellular and metabolic components, including sufficient ATP content. Bile is produced by hepatocytes, and modification is done by the cholangiocytes lining the bile ducts.[29]

The majority of clinical machine perfusion series with viability assessments have included bile production as a criterion for LT (Table 1).[18,21,22,24-26,28] Most studies do not report a specific cut-off value for bile production, but the transplant group from Groningen defined >10 mL of bile production within 2.5 hours of NMP as a minimum (Fig. 3B).[21,22] Regardless, evidence from recent studies suggests that the predictive value of bile production for liver function may have been overestimated. Diminished outcomes in livers

TABLE 1. Clinical Studies Using Viability Assessment Before LT

Reference	Viability Criteria	LT/Tested	DCD/DBD	Outcome (Percentage and/or Number of Cases)
Mergental et al.[28] (2020)	• Perfusate lactate ≤2.5 mmol/L • Bile production • Perfusate pH ≥7.30 • Metabolism of glucose • Stable HA flow ≥150 mL/minute and PV flow ≥500 mL/minute • Homogenous graft perfusion with soft consistency of the parenchyma	22/31	17/14	100% 90-day graft survival, 7 EAD, 4 ITBL
Cardini et al.[26] (2020)	• Rapid decrease and maintaining lactate levels • Bile output and biliary pH • Maintaining a physiological pH • Warning signals: exceptionally high or sharp incline of AST, ALT, LDH	25/39	4/35	88% graft and patient survival at 20 months; no ITBL
Zhang et al.[24] (2020)	• Perfusate lactate ≤2.5 mmol/L • Bile production • Perfusate pH >7.30 • Stable HA flow >150 mL/minute and PV flow >500 mL/minute	4/4	3/1	100% graft and patient survival at 6 months; 1 EAD, 1 ITBL
Bral et al.[25] (2019)	• Starting lactate level • Lactate clearance • Bile production • Necessity of bicarbonate pH correction	43/46	13/33	100% graft survival at 3 months; no ITBL
van Leeuwen et al.[22] (2019)	• Perfusate lactate ≤1.7 mmol/L • Bile production ≥10 mL in 2.5 hours • Perfusate pH 7.35-7.45 • Bile pH >7.45	11/16	16/0	100% graft and patient survival at 6 months; 1 ITBL
Watson et al.[27] (2018)	• Changes in lactate, glucose, and transaminase concentrations • Maintaining pH without supplemental bicarbonate	22/47	35/12	1 PNF, 1 EAD, 4 ITBL at a median follow-up of 50 months

Mergental et al.[18] (2016)	• Perfusate lactate ≤2.5 mmol/L or bile production
	  ○ Perfusate pH >7.30
	  ○ Stable HA flow >150 mL/minute and PV flow >500 mL/minute
	  ○ Homogenous graft perfusion with soft consistency of the parenchyma
		5/6	4/2	100% graft and patient survival at 6 to 19 months; no ITBL

with good bile production have been observed and vice versa.[14,21,23,25] Because bile production is mainly hepatocyte driven, it should be used as a marker of hepatocyte viability, but not cholangiocyte viability. This is underlined by studies showing that the volume of bile production is not correlated with the risk of posttransplant cholangiopathy.[22,23,27] In a clinical viability study including 12 high-risk donor livers, for example, 2 livers with some of the highest bile production rates showed evidence of ischemic cholangiopathy 2 months after LT.[23] Complete absence of bile flow is most likely a technical artifact attributed to malpositioning of the biliary drain or leakage along the drain.[30] This may explain why successful transplantations have been reported of livers that did not produce bile during NMP, yet, apparently were viable.

HYDRODYNAMIC/HEMODYNAMIC STABILITY

During machine perfusion, arterial and portal flows are dependent on perfusion pressure and resistance within the vasculature. Longer periods of ischemia can have a deleterious effect on the hepatic microcirculation, leading to increased vascular pressure and reduced liver perfusion.[31] This effect is mainly brought about by downregulation of vasoprotective pathways and dysfunction of endothelial cells. Reduced flows are often seen in steatotic livers characterized by narrowed hepatic sinusoids relative to lean livers, which can lead to aggravated reperfusion injury.[32]

Increased vascular resistance during machine perfusion has generally been considered a sign of poor

liver function and the association seems to be more pronounced in warm rather than cold machine perfusion.[33] Consequently, most clinical NMP studies have included certain flow thresholds (>150 mL/minute for hepatic artery [HA] and >500 mL/minute for portal vein [PV]) as a key parameter of liver function (Fig. 3C).[18,24,28] Nonetheless, all livers included in these studies reached the flow thresholds. Other studies also failed to find differences in flow between transplanted versus nontransplanted livers.[18,21,22,27] Correcting flow per unit liver weight (mL/minute/mmHg) could be more discriminative compared with mL/minute only. Another approach might be to evaluate the ability of the liver to respond to vasoactive drugs, such as epinephrine.[14] Livers unresponsive to vasoactive drugs ("vasoplegia") have been associated with a loss of integrity on histology as well as with higher perfusate levels of injury and inflammation markers compared with livers that were responding to vasoactive agents.[14]

GLUCOSE METABOLISM

The liver has a major role in controlling glucose homeostasis by controlling various pathways of glucose metabolism, including glycogenesis, glycolysis, and gluconeogenesis.[34]

Glycogenolysis is activated during static cold storage when ATP levels are low because of a lack of oxygen, which partly explains the initial raise in glucose levels after initiating machine perfusion.[35] In viable livers, high glucose levels should block glycogenolysis and stimulate glycogenesis, leading to a fall in glucose levels (Fig. 3D).[27] In some perfusions, perfusate glucose levels may not rise, indicating glycogen exhaustion and/or extensive lobular damage. To rule out severe lobular injury, a glucose challenge may be given in which functioning livers should metabolize glucose leading to a drop in concentration.[23] In a study by Eshmuminov et al. demonstrating maintained liver function after 7 days of ex situ perfusion, glucose substitution was needed to maintain physiological levels for livers that were later considered not viable for transplantation.[14] Viable livers released glucose by gluconeogenesis in response to insulin application, which could be further investigated as a marker for liver metabolic function. Glucose can be determined using a point-of-care blood gas analyzer, making it an easy and rapid marker to measure.

ACID-BASE BALANCE, ALBUMIN, AND UREA

The liver has been recognized as an important regulator of acid-base homeostasis, including 4 main components. First, and most important, is the glutamine axis. Glutamine is hydrolyzed by the liver to form ammonia, which then enters the urea cycle to form urea.[36] Urea production consumes the base bicarbonate, which plays an important role in acid-base regulation. Urea production is considered a sign of liver function, and its levels in the perfusion fluid increase during NMP.[14,37] It is currently unclear whether increasing urea levels are harmful for the perfused liver, but dialysis may be used to remove excess levels. In addition, interruption of ammonia metabolism will result in worsening perfusate acidosis, and the requirement for bicarbonate supplementation can be used as a surrogate marker for liver viability. Second, and as described previously, the liver has the capacity to metabolize lactate and avoid metabolic acidosis. Third, albumin (or gelofusine) in the perfusate is synthesized by hepatocytes and behaves as a weak acid in the physiological pH range. Maintenance of albumin levels may be used to assess hepatic synthetic function.[14] Studies have shown limited albumin production during NMP in livers subjected to long warm ischemia compared with increasing perfusate albumin levels in fresh controls.[38] Fourth, hepatic ketogenesis is involved, but the effect is negligible. In general, a near physiological perfusate pH between 7.30 and 7.45 is considered a viability marker by most groups (Fig. 3E). However, composition of the perfusion solution, addition of alkali, and perfusate partial pressures of carbon dioxide may all affect pH levels. The measurement of pH levels can be performed using a point-of-care blood gas analyzer, but albumin and urea are commonly determined by routine laboratory analysis requiring slightly more time to process.

PRODUCTION OF COAGULATION FACTORS

The liver is the primary site of synthesis of nearly all anticoagulation and procoagulation factors along with several components of the fibrinolytic system.[39] Although most NMP protocols use a heparinized circuit, the production of coagulation factors may be used to assess liver viability. Declining international normalized ratios during perfusion have been observed

after several hours of machine perfusion, suggesting recovery of liver function and production of coagulation factors.[40] In the recent study by Eshmuminov et al., perfusate coagulation factor V was significantly higher in functioning versus nonfunctioning livers after 48 hours of perfusion, but this difference was not sustained thereafter.[14] Activation of fibrinolysis seems to be more pronounced in livers of poorer quality and correlates with hepatocellular ischemia/reperfusion injury.[41]

LIVER TRANSAMINASES

Liver transaminases synthesize and break down amino acids and convert energy storage molecules. When a liver is injured, cell membranes become more permeable and transaminases may leak into the perfusate. Transaminases are in general poor markers of liver injury in hepatitis, liver resection, and LT. There is a poor correlation between peak transaminases and histological changes. Both peak transaminases in the liver donor and after transplant have not correlated with outcomes.[42]

Previous studies have shown diminished outcomes in livers that had high perfusate transaminase levels during machine perfusion.[22,25,27] In a study by Watson et al., 1 liver with alanine aminotransferase (ALT) levels reaching >9000 iU/L in the perfusate suffered from PNF after transplantation.[27] Similarly, Bral et al. found a liver with perfusate ALT levels >9000 iU/L to require retransplantation at 3 months.[25] Other studies have shown perfusate ALT levels >6000 iU/L in livers that were discarded based on other viability criteria (Fig. 3F).[22,25] Some suggest that the role of transaminases as an injury marker in machine perfusion studies (as opposed to static cold stored livers) is poorly defined. In machine perfusion studies, a so-called "wash-out" effect may occur because transaminases are flushed from the liver and diluted by several liters of perfusion solution.[43] Transaminases can also be cleared by the liver itself, further complicating the analysis.[44] In addition, reperfusion and oxygenation during machine perfusion lead to the release of accumulated transaminases in the organ to the perfusion solution. If perfusate transaminase levels are used as a marker for liver injury, they should be corrected for liver weight and perfusate volume to generalize cut-off values. In addition, plateauing transaminase concentrations suggest no ongoing hepatocellular injury during machine perfusion, and an exceptionally sharp increase in transaminases can be used as a warning sign for severe liver injury and, as such, as an indirect marker for poor outcome.[26,27] In the NMP trial by Nasralla et al., the ALT levels of livers with severe preservation injury increased more rapidly during NMP compared with livers with less injury.[17] ALT levels are preferred over aspartate aminotransferase (AST) because they are more liver specific, and AST levels may also rise from hemolysis on the circuit. Increased hemolysis has been associated with severe preservation injury,[17] but sheer-stress–induced hemolysis from the perfusion pump and circuit tubing during longer perfusion periods also contributes to severe preservation injury. Instead of absolute transaminase levels, the clearance of endogenous or exogenous transaminases during NMP may be used to test liver function.[44]

Cholangiocellular Viability Criteria

BILE COMPOSITION

Cholangiocytes are mainly responsible for changes in bile composition. They absorb useful solutes such as glucose and render bile more alkalotic by bicarbonate secretion. Therefore, low levels of biliary glucose, high levels of bicarbonate, and an alkalotic pH are considered markers of cholangiocyte viability.[29]

In most clinical machine perfusion studies to date, viability criteria were based on hepatocellular viability only, but increasing attention is given to additional cholangiocyte assessment. Viability of cholangiocytes, or biliary epithelial cells, is important to take into consideration, especially with the incidence of posttransplant ischemic cholangiopathy reaching up to 35% in livers donated after circulatory death.[45] Clinical studies have shown that livers that meet hepatocellular viability criteria can still be at risk to develop biliary complications after LT.[22,23,28] Instead, an analysis of the composition of bile produced during NMP seems to be a promising approach to predict bile duct viability and biliary complications after LT.[15]

Watson et al. confirmed the usefulness of biliary pH as a marker for bile duct viability.[27] In their series on viability assessment of 47 high-risk livers, they found that 3 of the transplanted livers unable to achieve a biliary pH >7.4 developed cholangiopathy after LT (Fig. 3G). In addition, livers that developed cholangiopathy were associated with lower biliary bicarbonate and had glucose levels similar

to the perfusate. The Groningen group added biliary pH to their viability criteria for LT (besides bile production, perfusate lactate, and perfusate pH), and it turned out to be the most frequent reason for discard in their series on 7 initially declined livers.[21] Both livers that were unable to produce bile with a pH >7.45 showed signs of substantial histological injury on bile duct biopsies. No cases of ischemic cholangiopathy were observed in the transplanted livers that had produced bile with a pH >7.45 during NMP. The same group applied these viability criteria in the prospective dual hypothermic oxygenated machine perfusion - controlled oxygenated rewarming - NMP (DHOPE-COR-NMP) trial for the viability assessment of initially declined donor livers.[22] Of the 11 transplanted livers in this trial, 1 recipient developed nonanastomotic biliary strictures. This liver met the viability criteria, but in hindsight, biliary pH, bicarbonate, and glucose levels were similar to levels in the perfusate, suggesting impaired cholangiocyte function. Therefore, it is suggested that the ratio or difference between bile and perfusate markers should be used instead of absolute values (Fig. 3H-J). Bile composition can be analyzed by using a point-of-care blood gas analyzer.

Viability Assessment During SNMP

Viability testing during SNMP could serve an intermediate role, benefiting from a lower metabolic demand compared with NMP while maintaining sufficient metabolism for viability testing.

To date, there are only a few reports describing viability markers during SNMP, which come predominantly from the group from Boston, MA. Although metabolism is reduced during SNMP, lactate is cleared from the perfusate and there is urea production, albumin secretion, and bile production.[46-49] Cholangiocytes are able to secrete bicarbonate into bile, increasing pH up to 7.6, which is comparable to bile produced during NMP. In addition, metabolomic profiling during SNMP was able to cluster livers with similar metabolic function, such as donation after circulatory death (DCD) or steatotic livers.[48] Metabolomics is currently a time-consuming analysis, but future applications with, for example, real-time metabolomic profiling could help distinguish viable from nonviable donor organs before transplantation.

Viability testing during SNMP is still in its infancy, but it seems that all the viability parameters used during NMP can also be assessed during perfusion at slightly lower temperatures. Data from clinical studies using SNMP before transplantation will be needed to correlate viability markers with postoperative outcomes.

Viability Assessment During HMP

During hypothermia, most aspects of cellular activity are reduced to minimal levels making real-time assessment of metabolism, inflammation, and function more challenging.

HYDRODYNAMIC STABILITY

Flow and resistance values during HMP have been analyzed as a marker of liver viability, but the predictive power seems low. In a study from Italy using hypothermic oxygenated perfusion for donation after brain death (DBD) livers, flow and resistance showed no correlation with outcome.[50] Grafts with EAD, however, did show a less steep decrease of HA resistance throughout the perfusion compared with grafts with immediate function. The vascular resistance slope may be explored as a potential viability marker by other groups. Different ways to analyze flow, such as by using magnetic resonance imaging, might be able to provide a more in-depth analysis and perhaps a better way to assess viability in the future.[51]

LIVER TRANSAMINASES

Several studies have reported a correlation between perfusate transaminase levels during HMP and outcome after LT, especially with regard to peak postoperative transaminase levels.[52] The recent oxygenated hypothermic perfusion in preservation of hepatic grafts study from France showed that 4 patients with EAD after transplantation had significantly higher transaminase levels in the perfusate compared with patients with immediate liver function.[53] A cutoff value of 800 UI/L was defined for AST and ALT with corresponding area under the curves of 0.92 and 0.91, respectively. The group from Turin, Italy, performed an extensive study on perfusate parameters during 50 HMP procedures including DBD livers only.[54] They analyzed perfusate ALT, AST, LDH, lactate, glucose, and pH in correlation to postoperative outcomes. All the parameters except lactate correlated with EAD, but ALT showed the highest predictive power with a cutoff at 537 IU/L. Nonetheless, the only factor remaining independently associated with EAD after multivariable analyses

was macrovesicular steatosis. The first results of the DHOPE-COR-NMP trial from Groningen revealed that 2/7 livers that were declined for transplantation after viability assessment had ALT levels >2000 UI/L during HMP.[21] The 5 livers that were transplanted had ALT levels ranging between 200 and 1300 UI/L. None of these grafts experienced EAD. Therefore, very high transaminase levels (>1000) should be considered a warning sign for substantial injury, but larger numbers are probably needed to define more robust cutoffs.

MITOCHONDRIAL FUNCTION

Recently, the Zurich group presented, for the first time, viability assessment during HMP by measuring flavin mononucleotide (FMN), which is released upon injury to mitochondrial complex I.[55] FMN can be measured in real time with a light probe and fluorescence spectroscopy, enabling the rapid prediction of liver function during machine perfusion. The authors showed a strong correlation between FMN release with coagulation factors and peak transaminases after LT. Perfusate FMN was the most accurate predictor of 3-month graft survival (C statistic of 0.93) when compared with other risk scores, such as the donor risk index and the L-Graft score. The prediction of perfusate FMN analysis on ischemic-type biliary lesions (ITBL) specifically could not be addressed because there were only a few events. Also, some livers that were initially declined for transplantation based on FMN analysis later turned out to be functioning in the preclinical series of 1-week liver preservation by NMP performed by the same group of investigators.[14] This illustrates the challenges in defining viability criteria in preclinical studies, that is, that liver function during machine perfusion does not necessarily imply viability after transplantation. In a pilot study by Wang et al., FMN levels during liver normothermic regional perfusion were significantly higher in livers that were not procured for transplantation. There was no correlation between FMN and postoperative biochemical markers.[56] Besides measuring FMN, mitochondrial function may be assessed by oxygen consumption, carbon dioxide production, or ATP synthesis.[57]

Current Limitations and Future Perspectives

There are several limitations to study liver graft viability. One is that there is no broadly accepted definition of graft viability. Liver graft viability in a broad sense is the ability to sustain life for some time (absence of PNF), but this time frame is not defined. A liver that will develop massive ischemic cholangiopathy or fibrosis leading to graft loss or patient death within a few months after transplantation should not be considered "viable" before transplant. It would be more important to develop graft risk scores incorporating perfusion parameters and biomarkers to correlate with long-term graft survival instead of simply distinguishing between "viable" and "nonviable." Incidental cases of PNF or ischemic cholangiopathy provide valuable information and give new clues to determine cutoff values for certain parameters. However, these events are rare and require a large number of patients. Large randomized clinical trials would be needed to proof the predictive power of viability criteria.[58] However, until viability criteria markers are validated, studies with extremely marginal organs (outside current acceptance criteria by the transplant center) should preferably not be performed for ethical reasons (e.g., transplanting a liver that does not meet the predetermined criteria with a high risk of PNF or posttransplant cholangiopathy). It is important to point out that a discarded organ (also called "orphan" organ) is an organ that is declined by all other centers, but this does not necessarily mean that it is nonviable and that machine perfusion is the only way to transplant it without risks. Several studies reported acceptable outcomes when using discarded livers without prior viability assessment.[59,60]

Another complicating factor in the search of suitable viability markers is the broad variety in machine perfusion protocols. Machine perfusion variables such as temperature, perfusion duration, type of perfusate, active oxygenation, perfusion volume, and perfusate additives such as bile salts, insulin, and bicarbonate make it hard to generalize data. Preclinical studies defining viability markers without validation in a transplantation model should be interpreted with caution and might be of less value. Furthermore, many studies have focused on short-term outcomes, but biliary complications often arise later at a median of 3 to 4 months after LT. Histological analysis of bile ducts from discarded livers has been used as a surrogate marker, but longer follow-up is desired to find viability criteria associated with biliary complications after LT. Finally, some potential biomarkers such as the detection of micro RNAs, ATP production, metabolic profiling, or multiomics have limited clinical applicability because they are not rapidly processed or would delay transplantation. Real-time assessments of biomarkers are crucial to make timely clinical decisions to accept an organ for transplant. Technical advances in laboratory techniques might improve the use of these biomarkers in the future.

Despite the current limitations, dynamic preservation by machine perfusion has great potential to assess the viability of donor livers that are initially labeled not suitable for transplantation. Several markers can be easily and rapidly measured using point-of-care blood gas analysis, such as lactate and glucose in the perfusate and biliary pH, glucose, and bicarbonate. Other markers, such as coagulation factors or transaminases in the perfusate, can be determined using routine laboratory analysis and are obtained relatively quickly. Viability assessment during machine perfusion has been refined over the years, and the results of ongoing clinical trials and transparent data sharing will further optimize viability criteria to predict postoperative outcomes. Combining data from several centers with more postoperative events will be key to optimize cutoff values for viability markers. Artificial intelligence and machine-learning analysis of all biomarkers obtained during perfusion could be used to create a viability score to reliably predict postoperative complications. Viability testing of initially declined livers and therapeutic strategies to optimize organs during machine perfusion seems one of the most promising strategies to increase organ utilization rates.

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