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Viability criteria assessment during liver machine perfusion

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We read with great interest the publication by Eshmuminov et al. showing ex situ preservation of livers in a functional state for 1 week by normothermic machine perfusion (NMP)[1]. The authors report a thoughtful, stepwise approach for integration of multiple core physiological functions of the liver in their custom-made perfusion system and convincingly show preserved liver function after 7-day perfusion. Although the preserved livers described are functional, we would like to emphasize that their suitability for human transplantation remains unsubstantiated. Here, we discuss emerging research on markers that may provide confidence in the assessment of viability criteria for liver transplantation.

Over the past decade, efforts have been made to define predictors of hepatobiliary viability of the donor liver before transplantation. Viability assessment of extended criteria donor (ECD) grafts may be of particular importance because the quality of these grafts is variable and difficult to predict. The latest advances in machine perfusion have offered possibilities in regard to assessment of organ viability during ex situ preservation.

Normothermic machine perfusion is most commonly used to assess viability, since the organ is maintained in a near-physiological state. Machine perfusate lactate levels and bile production are consistently reported as being among the most important markers of graft viability during NMP (Table 1)[2–12]. Yet it may be questioned whether lactate clearance and bile production are strong markers of liver viability, because both markers have not been able to discriminate viable from nonviable grafts in several studies[5,9]. Likewise, in the study by Eshmuminov et al., both viable and nonviable livers cleared lactate in the perfusate and the majority of grafts produced bile. In their study, viability appears more related to the ability of the liver to respond to vasoactive drugs or endocrine hormones instead. Unresponsive livers showed loss of integrity on histology, as well as the absence of decline in injury and inflammation markers in the perfusate. We believe that future clinical studies may benefit from inclusion of the response to vasoconstrictors and hormones as viability markers for liver function.

In most clinical studies, selection criteria are still based on hepatocellular viability only but increasing attention is given to additional cholangiocyte assessment. The viability of cholangiocytes, or biliary epithelial cells, is important to take into consideration, especially with the incidence of post-transplant cholangiopathy reaching up to 35% in donation after circulatory death livers[13]. Previous studies have shown that livers meeting hepatocellular viability criteria still developed biliary complications after transplantation[9,12]. Therefore, bile composition may be considered an additional parameter of bile duct viability[14]. This is underlined by a study showing low hepatocellular injury during NMP of discarded human livers but high bile duct injury on histology[15]. Based on the aforementioned study, markers for cholangiocyte viability were defined and prospectively applied to a clinical trial on resuscitation, and viability assessment of livers initially declined for transplantation (DHOPE-COR-NMP trial)[5]. Of the 11 transplanted livers in that trial, one recipient developed post-transplant cholangiopathy. The liver that developed cholangiopathy met the viability criteria, but biliary pH, bicarbonate and glucose levels were similar to those in the perfusate, suggesting impaired cholangiocyte function. The group from Groningen[5], therefore, suggest that the ratio or difference between bile and perfusate markers should be used as a marker of viability rather than absolute values. Unfortunately, Eshmuminov et al. measured only biliary bilirubin while biliary pH tends to be the strongest biliary viability criterion[5,9,10]. We suggest that future studies include the differences between perfusate and bile pH, bicarbonate and glucose as markers of cholangiocellular viability.

Another aspect of the study carried out by Eshmuminov et al. is the difficulty in fully assessing coagulation using a heparinized circuit. Perfusate factor V was significantly higher in viable versus nonviable livers at perfusion day 2, but this difference was not sustained thereafter. A study by Karangwa et al.[16] showed that NMP (using a heparinized plasma-based perfusion fluid) activates fibrinolysis, but not coagulation, in liver grafts. Activation of fibrinolysis was most pronounced in livers of poorer quality, and high levels of D-dimer, a fibrin degradation product, correlated with poor liver function.

Recently Muller et al.[17], from the same team of investigators as in the present study, showed, for the first time, viability assessment during hypothermic machine perfusion by evaluation of the release of flavin mononucleotide (FMN) in the perfusate[17]. FMN can be measured in real time and provides a rapid prediction of liver function during machine perfusion. However, two out of the six functioning livers described by Eshmuminov et al. were initially declined for transplantation based on FMN analysis. This underlines the challenge in defining graft viability, and suggests that liver function might not necessarily imply viability after transplantation.

Ideal viability markers should be reliable, easy to collect and rapid to process. Therefore, viability assessment based on metabolic profiling[18] or detection of microRNAs[19], for example, is interesting, but clinical application remains limited because of time constraints. Technical developments might improve these techniques in the future, allowing for more rapid assessment and utilization in the clinic.

Pretransplant viability testing has become an important tool in an era in which we are pushing the boundaries of organ transplanta-

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Table 1 | Viability assessment criteria during machine perfusion of human livers

Reference	Liver transplantation	Viability criteria
Hypothermic machine perfusion		
Muller et al.[17]	Yes	Within 30 min of hypothermic machine perfusion <10,000 units FMN in perfusate
Subnormothermic machine perfusion		
Bruinsma et al.[18]	No	Metabolic profiling
NMP		
Sutton et al. (2014)[20]	No	Cumulative bile production ≥30 g during 6 h of NMP
Mergental et al.[2]	No	Within 3 h of NMP: (1) perfusate lactate must be <2.5 mmol l⁻¹ or the liver must produce bile; (2) other criteria: (i) perfusate pH >7.30; (ii) hepatic artery flow >150 ml min⁻¹ and portal vein flow >500 ml min⁻¹; (iii) homogenous graft perfusion with soft consistency of parenchyma
Karangwa et al.[16]	No	Within 6 h of NMP: D-dimer ≤3,500 ng ml⁻¹ in perfusate
Watson et al.[9]	Yes	(1) Changes in lactate, glucose and transaminase concentrations (2) Maintaining pH without supplemental bicarbonate
Watson et al.[10]	Yes	(1) Changes in lactate, glucose and transaminase concentrations (2) Maintaining pH without supplemental bicarbonate
Mergental et al.[3]	No	Major: lactate <2.5 mmol l⁻¹ and bile production; minor: perfusate pH >7.30, hepatic artery flow >150 ml min⁻¹ and portal vein flow >500 ml min⁻¹; homogenous graft perfusion with soft consistency of parenchyma
Matton et al.[5]	Yes	After 2.5 h of NMP: (1) biliary bicarbonate >18 mmol l⁻¹; (2) biliary pH >7.48; (3) biliary glucose <16 mmol l⁻¹; (4) bile/perfusate glucose ratio <0.67; (5) biliary LDH <3,689 U l⁻¹
Ciria et al.[7]	Yes	(1) Adequate flows without increased pressure; (2) decreasing lactate levels
Pavel et al.[6]	No	After 6 h of NMP: (1) hemodynamic stability (absolute); (2) maximum acceptable AST/ALT peak <3,000 IU l⁻¹ (absolute); (3) perfusate pH >7.25 (absolute); (4) lactate <40 mg dl⁻¹ (absolute); (5) bile production (relative); (6) bile flow >10 ml h⁻¹ (relative)
Bral et al. (2019)[21]	No	Clearance of liver transaminases
De Vries et al.[8]	Yes	After 2.5 h of NMP: (1) lactate 0.5–1.7 mmol l⁻¹; (2) perfusate pH 7.35–7.45; (3) cumulative bile production ≥10 ml; (4) biliary pH >7.45
Van Leeuwen et al.[5]	Yes	After 2.5 h of NMP: (1) lactate ≤1.7 mmol l⁻¹; (2) perfusate pH 7.35–7.45; (3) bile production >10 ml; (4) biliary pH ≥7.45
Zhang et al.[11]	Yes	Within 4 h of NMP: (1) perfusate lactate ≤2.5 mmol l⁻¹; (2) bile production; (3) perfusate pH ≥7.30; (4) stable hepatic artery flow >150 ml min⁻¹ and portal vein flow >500 ml min⁻¹
Matton et al.[19]	No	After 30 min of NMP: release of HDmiR-122 and CDmiR-122 in perfusate
Cardini et al.[4]	Yes	(1) Rapid decrease and maintenance of lactate levels; (2) bile output and biliary pH; (3) maintenance of physiological pH; (4) warning signals: exceptionally high or sharp incline of AST, ALT, LDH
Mergental et al.[12]	Yes	Within 4 h of NMP: lactate <2.5 mmol l⁻¹ and two or more of the following within 4 h of starting perfusion: (1) evidence of bile production; (2) pH >7.30; (3) metabolism of glucose; (4) hepatic artery flow >150 ml min⁻¹ and portal vein flow >500 ml min⁻¹; (5) homogenous perfusion

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CDmiR, cholangiocyte-derived microRNA; HDmiR, hepatocyte-derived microRNA; LDH, lactate dehydrogenase

tion with ECD grafts and longer preservation times. Many viability markers have been used and, to date, there is no widely accepted composition for markers used in clinical studies. Livers declined for transplantation based on certain viability criteria are not transplanted and, therefore, have no follow-up. Definitive validation of viability would require well-powered multicenter randomized controlled trials. Most probably it will be necessary to analyze a combination of several viability parameters together. In this regard, machine learning/artificial intelligence has the potential to play an important role.

Viability assessment during machine perfusion has been refined over the years with successful outcomes, but ongoing clinical trials and transparent data sharing remain critical to further optimization of viability criteria to predict postoperative outcomes.

References

1. Eshmuminov, D., Becker, D., Bautista Borrego, L., Hefti, M., Schuler, M. J. & Hagedorn, C. et al. An integrated perfusion machine preserves injured human livers for 1 week. *Nat. Biotechnol.* **38**, 189–198 (2020).
2. Mergental, H., Perera, M. T. P. R., Laing, R. W., Muiesan, P., Isaac, J. R. & Smith, A. et al. Transplantation of declined liver allografts following normothermic *ex-situ* evaluation. *Am. J. Transplant.* **16**, 3235–3245 (2016).
3. Mergental, H., Stephenson, B. T. F., Laing, R. W., Kirkham, A. J., Neil, D. A. H. & Wallace, L. L. et al. Development of clinical criteria for functional assessment to predict primary nonfunction of high-risk livers using normothermic machine perfusion. *Liver Transpl.* **24**, 1453–1469 (2018).
4. Cardini, B. et al. Clinical implementation of prolonged liver preservation and monitoring through normothermic machine perfusion in liver transplantation. *Transplantation* **104**, 1917–1928 (2020).
5. van Leeuwen, O. B., de Vries, Y., Fujiyoshi, M., Nijsten, M. W. N., Ubbink, R. & Pelgrim, G. J. et al. Transplantation of high-risk donor livers after ex situ resuscitation and assessment using combined hypo- and normothermic machine perfusion: a prospective clinical trial. *Ann. Surg.* **270**, 906–914 (2019).
6. Pavel, M.-C., 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.* **235**, 383–394 (2019).
7. Ciria, R., Ayllon-Teran, M. D., González-Rubio, S., Gómez-Luque, I., Ferrín, G. & Moreno, A. et al. Rescue of discarded grafts for liver transplantation by ex vivo subnormothermic and normothermic oxygenated machine perfusion: first experience in Spain. *Transpl. Proc.* **51**, 20–24 (2019).
8. de Vries, Y., Matton, A. P. M., Nijsten, M. W. N., Werner, M. J. M., Berg, A. P. & Boer, M. T. et al. Pretransplant sequential hypo- and normothermic machine perfusion of suboptimal livers donated after circulatory death using a hemoglobin-based oxygen carrier perfusion solution. *Am. J. Transplant.* **19**, 1202–1211 (2019).
9. Watson, C. J. E., Kosmoliaptsis, V., Randle, L. V., Gimson, A. E., Brais, R. & Klinck, J. R. et al. Normothermic perfusion in the assessment and preservation of declined livers before transplantation: hyperoxia and vasoplegia-important lessons from the first 12 cases. *Transplantation* **101**, 1084–1098 (2017).
10. Watson, C. J. E., 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.* **18**, 2005–2020 (2018).
11. Zhang, Z., Ju, W., Tang, Y., Wang, L., Zhu, C. & Gao, N. et al. First preliminary experience with preservation of liver grafts from extended-criteria donors by normothermic machine perfusion in Asia. *Ann. Transplant.* **25**, e921529 (2020).
12. Mergental, H. et al. Transplantation of discarded livers following viability testing with normothermic machine perfusion. *Nat Commun.* **11**, 2939 (2020).
13. de Vries, Y., von Meijenfeldt, F. A. & Porte, R. J. Post-transplant cholangiopathy: classification, pathogenesis, and preventive strategies. *Biochim. Biophys. Acta Mol. Basis Dis.* **1864**, 1507–1515 (2018).
14. Brüggenwirth, I.M.A., Porte, R.J. & Martins, P.N. Bile composition as a diagnostic and prognostic tool in liver transplantation. *Liver Transplant.* **9**, 1177–1187 (2020).
15. Matton, A. P. M., de Vries, Y., Burlage, L. C., van Rijn, R., Fujiyoshi, M. & de Meijer, V. E. et al. Biliary bicarbonate, pH, and glucose are suitable biomarkers of biliary viability during ex situ normothermic machine perfusion of human donor livers. *Transplantation* **103**, 1405–1413 (2019).
16. Karangwa, S. A., Burlage, L. C., Adelmeijer, J., Karimian, N., Westerkamp, A. C. & Matton, A. P. et al. Activation of fibrinolysis, but not coagulation, during end-ischemic ex situ normothermic machine perfusion of human donor livers. *Transplantation* **101**, e42–e48 (2017).
17. 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.* **270**, 783–790 (2019).
18. Bruinsma, B. G., Sridharan, G. V., Weeder, P. D., Avruch, J. H., Saeidi, N. & Özer, S. et al. Metabolic profiling during ex vivo machine perfusion of the human liver. *Sci. Rep.* **6**, 22415 (2016).
19. Matton, A.P.M. et al. Cell-free microRNAs as early predictors of graft viability during ex vivo normothermic machine perfusion of human donor livers. *Clin Transplant.* **34**, e13790 (2020).
20. Sutton, M. E. et al. Criteria for viability assessment of discarded human donor livers during ex vivo normothermic machine perfusion. *PLoS One* **9**, e110642 (2014).
21. Bral, M. Clearance of transaminases during normothermic ex situ liver perfusion. *PLoS One* **14**, e0215619 (2019).

Data availability

Author contributions

Competing interests

Additional information