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Hyperspectral Imaging for Viability Assessment of Human Liver Allografts During Normothermic Machine Perfusion

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Background. Normothermic machine perfusion (NMP) is nowadays frequently utilized in liver transplantation. Despite commonly accepted viability assessment criteria, such as perfusate lactate and perfusate pH, there is a lack of predictive organ evaluation strategies to ensure graft viability. Hyperspectral imaging (HSI)—as an optical imaging modality increasingly applied in the biomedical field—might provide additional useful data regarding allograft viability and performance of liver grafts during NMP. **Methods.** Twenty-five deceased donor liver allografts were included in the study. During NMP, graft viability was assessed conventionally and by means of HSI. Images of liver parenchyma were acquired at 1, 2, and 4 h of NMP, and subsequently analyzed using a specialized HSI acquisition software to compute oxygen saturation, tissue hemoglobin index, near-infrared perfusion index, and tissue water index. To analyze the association between HSI parameters and perfusate lactate as well as perfusate pH, we performed simple linear regression analysis. **Results.** Perfusate lactate at 1, 2, and 4 h NMP was 1.5 [0.3–8.1], 0.9 [0.3–2.8], and 0.9 [0.1–2.2] mmol/L. Perfusate pH at 1, 2, and 4 h NMP was 7.329 [7.013–7.510], 7.318 [7.081–7.472], and 7.265 [6.967–7.462], respectively. Oxygen saturation predicted perfusate lactate at 1 and 2 h NMP (R^2 = 0.1577, P = 0.0493; R^2 = 0.1831, P = 0.0329; respectively). Tissue hemoglobin index predicted perfusate lactate at 1, 2, and 4 h NMP (R^2 = 0.1916, P = 0.0286; R^2 = 0.2900, P = 0.0055; R^2 = 0.2453, P = 0.0139; respectively). **Conclusions.** HSI may serve as a noninvasive tool for viability assessment during NMP. Further evaluation and validation of HSI parameters are warranted in larger sample sizes.

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Liver transplantation (LT) is the only curative therapy for patients with end-stage liver disease. In recent decades, LT is characterized by an increasing waiting list mortality due to the lack of donor organs.[1] The utilization of extended criteria donor (ECD) organs has become frequent in order to overcome the severity of organ scarcity.[2] As ECD organs may be associated with an inferior outcome of LT, risk-minimizing strategies are sought after.[3]

Normothermic machine perfusion (NMP) is a revived preservation technique nowadays frequently utilized in LT. NMP provides advantages over static cold storage (SCS) due to physiological temperature and organ function, inherent to this preservation method. Furthermore, it enables allograft viability testing before LT.[4-6]

Commonly assessed viability parameters during liver graft NMP are perfusate lactate, perfusate pH, glucose metabolism, and production and viscosity of bile, as well as arterial inflow.[7-10] Current literature emphasizes perfusate lactate/lactate clearance as essential parameters assessing liver viability during NMP.[5-8,11] In NMP, hyperlactatemia is predominantly

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caused by relative tissue hypoxia conditioned by impaired liver blood flow and narrowed gluconeogenesis. Hence, visual assessment of liver microperfusion may provide a significant additional insight into graft performance during NMP.

Nasralla et al[6] indicated perfusate pH being valuable as well for assessing allograft viability during NMP; this correlation was confirmed by further trials.[5,8,12-14] Associated with advanced donor liver injury or prolonged ischemia times leading to subsequent tissue hypoxia and anaerobic metabolism, low perfusate pH is invariably observed during NMP.

Despite these commonly accepted viability criteria, there is still a lack of predictive organ evaluation strategies to ensure graft viability, especially for high-risk liver allografts. In kidney transplantation, hyperspectral imaging (HSI) provided useful data regarding the quality of organ microperfusion and viability.[15] HSI is a noninvasive, contrast-free, optical imaging modality increasingly applied in the biomedical field.[16-18] Based on computational analysis, HSI systems create images of light–tissue interactions by detecting relative reflectance. HSI allows quantification of organic compound indicating oxygen saturation (StO2), tissue hemoglobin index (THI), near-infrared perfusion index (NIR), and tissue water index (TWI) at different depths in a wide large field of view.[19,20] Recently applied for the quantitative analysis of organ perfusion in liver resection, HSI might also be a feasible opportunity of viability assessment in LT, especially for ECD liver allografts.[16,21]

Hence, the objective of the present single-center study was the evaluation of a potential association between HSI parameters and perfusate lactate as well as perfusate pH as ischemia index parameters during NMP of liver allografts.

MATERIALS AND METHODS

This is a single-center, 1-arm exploratory observational study. The study was conducted in accordance with the declaration of Helsinki and approved by the local ethics committee (ID 2021-574-f-S). The requirement for informed consent for the study was waived.

Organ preservation was performed by means of end-ischemic NMP utilizing the OrganOx Metra device (OrganOx Limited, Oxford, United Kingdom). During NMP, viability assessment included hourly measurement of perfusate lactate, perfusate pH, and perfusate glucose levels as well as the assessment of bile production, bile viscosity, and arterial and portal blood flow. A minimum of 6 h of NMP was local protocol to allow the graft to recover from SCS.

Hyperspectral images of liver parenchyma were acquired at 1, 2, and 4 h of NMP. These time points were selected since viability should be proven within 4 h of NMP.[8] For HSI assessment during NMP, all external light sources were turned off, as inhomogeneities of the tissue structure may scatter the incoming light and interfere with HSI measurements.[22] A distance of 50 cm between the liver allograft and the camera with a focal length of 25 mm was utilized for liver parenchyma assessment. All optical values were recorded with the TIVITA Tissue System (Diaspective Vision GmbH, Am Salzhaff, Germany). The analysis software TIVITA Suite Tissue (Diaspective Vision GmbH) provides an RGB image and 4 false color images representing perfusion parameters of the recorded tissue area.[20] These parameters are tissue oxygenation (StO2) of superficial layers (approximately 1 mm penetration depth), NIR of tissue layers in 4–6 mm penetration depth, THI, and tissue water index TWI, displaying the distribution of hemoglobin and water in the observed tissue area, respectively.[19,23]

Data collection and statistical analysis were performed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA) and GraphPad Prism 9 for macOS version 9.3.1 (GraphPad Software, San Diego, CA). Categorical variables are presented as percentages and continuous variables as median [range]. To analyze the association between HSI parameters and perfusate lactate as well as perfusate pH, we performed simple linear regression analysis. A P value ≤0.05 (2-tailed) was regarded as statistically significant.

RESULTS

From February until October 2021, 25 deceased donor liver allografts were included in this study. The recipients were 54 [33–69] y old and 50.0% were male. The recipients' body mass index (BMI) was 26.7 [17.2–57.7] kg/m^2. At the time of organ allocation, the model of end-stage liver disease score was 20 [6–40]. The organ donors were 58 [30–85] y old and 62.5% were male. Donor BMI was 25.0 [17.0–54.7] kg/m^2. Donor risk index was 1.645 [1.089–2.184]. Cold ischemia time was 7.3 [4.2–11.2] h. Duration of NMP was 15.8 [6.9–23.3] h.

Perfusate lactate, perfusate pH, and HSI parameters as assessed during organ preservation are given in Table 1. The results of the linear regression analysis are given in Tables 2 and 3; there is a significant relationship between StO2 and perfusate lactate at 1 and 2 h NMP (Figure 1) as well as between THI and perfusate lactate at 1, 2, and 4 h NMP (Figure 2). Furthermore, StO2 at 1 h NMP and THI at 2 h NMP demonstrated a significant relationship with perfusate pH. At 1 h NMP, we also detected a significant relationship between NIR and perfusate lactate as well as perfusate pH. We did not detect a significant relationship between TWI and perfusate lactate nor between TWI and perfusate pH at any time of measurement.

As early predictability of nonviability during NMP and implementation of possible future treatment options during NMP early on are of considerable interest, the relationship between significant early HSI parameters and late perfusion markers was analyzed. For StO2 at 1 h NMP, there is a significant relationship with pH at 4 h NMP (R^2 = 0.1649, P = 0.0440). The other HSI parameters are not significantly associated with pH at 4 h NMP (THI at 1 h NMP: R^2 = 0.0573, P = 0.2491; NIR at 1 h NMP: R^2 = 0.0675, P = 0.2203). None of the HSI parameters at 1 h NMP are significantly associated

TABLE 1.

Established and potential viability parameters assessed during organ preservation

	1 h NMP	2 h NMP	4 h NMP
Perfusate lactate (mmol/L)	1.4 [0.3–4.0]	0.9 [0.3–2.8]	0.9 [0.1–2.2]
Perfusate pH	7.329 [7.013–7.510]	7.318 [7.018–7.472]	7.265 [6.967–7.462]
StO2 (%)	49.8 [29.4–72.4]	54.2 [32.4–71.0]	56.6 [36.5–65.7]
THI	65.5 [44.5–86.8]	61.7 [44.3–85.3]	65.1 [48.3–83.7]
NIR	22.6 [0.6–68.2]	29.1 [8.5–74.0]	33.9 [4.1–65.6]
TWI	24.4 [16.3–31.3]	25.8 [17.5–32.8]	24.8 [5.8–32.0]

NIR, near-infrared perfusion index; NMP, normothermic machine perfusion; StO2, oxygen saturation; THI, tissue hemoglobin index; TWI, tissue water index.

TABLE 2.

Association between HSI parameters and perfusate lactate

	R^2	*P*
StO2		
1 h NMP	0.1577	**0.0493**
2 h NMP	0.1831	**0.0329**
4 h NMP	0.0571	0.2501
THI		
1 h NMP	0.1916	**0.0286**
2 h NMP	0.2900	**0.0055**
4 h NMP	0.2453	**0.0139**
NIR		
1 h NMP	0.1685	**0.0464**
2 h NMP	0.0041	0.7611
4 h NMP	0.0492	0.3213
TWI		
1 h NMP	0.0185	0.5169
2 h NMP	0.0288	0.4177
4 h NMP	0.1102	0.1218

HSI, hyperspectral imaging; NIR, near-infrared perfusion index; NMP, normothermic machine perfusion; StO2, oxygen saturation; THI, tissue hemoglobin index; TWI, tissue water index.

TABLE 3.

Association between HSI parameters and perfusate pH

	R^2	*P*
StO2		
1 h NMP	0.2855	**0.0059**
2 h NMP	0.0849	0.1575
4 h NMP	0.0665	0.2132
THI		
1 h NMP	0.0531	0.2678
2 h NMP	0.2201	**0.0180**
4 h NMP	0.0051	0.7395
NIR		
1 h NMP	0.1807	**0.0384**
2 h NMP	0.0474	0.2958
4 h NMP	0.0846	0.1891
TWI		
1 h NMP	0.0619	0.2303
2 h NMP	0.0235	0.4647
4 h NMP	0.0149	0.5699

Bold indicates statistically significant of *P* values.
HSI, hyperspectral imaging; NIR, near-infrared perfusion index; NMP, normothermic machine perfusion; StO2, oxygen saturation; THI, tissue hemoglobin index; TWI, tissue water index.

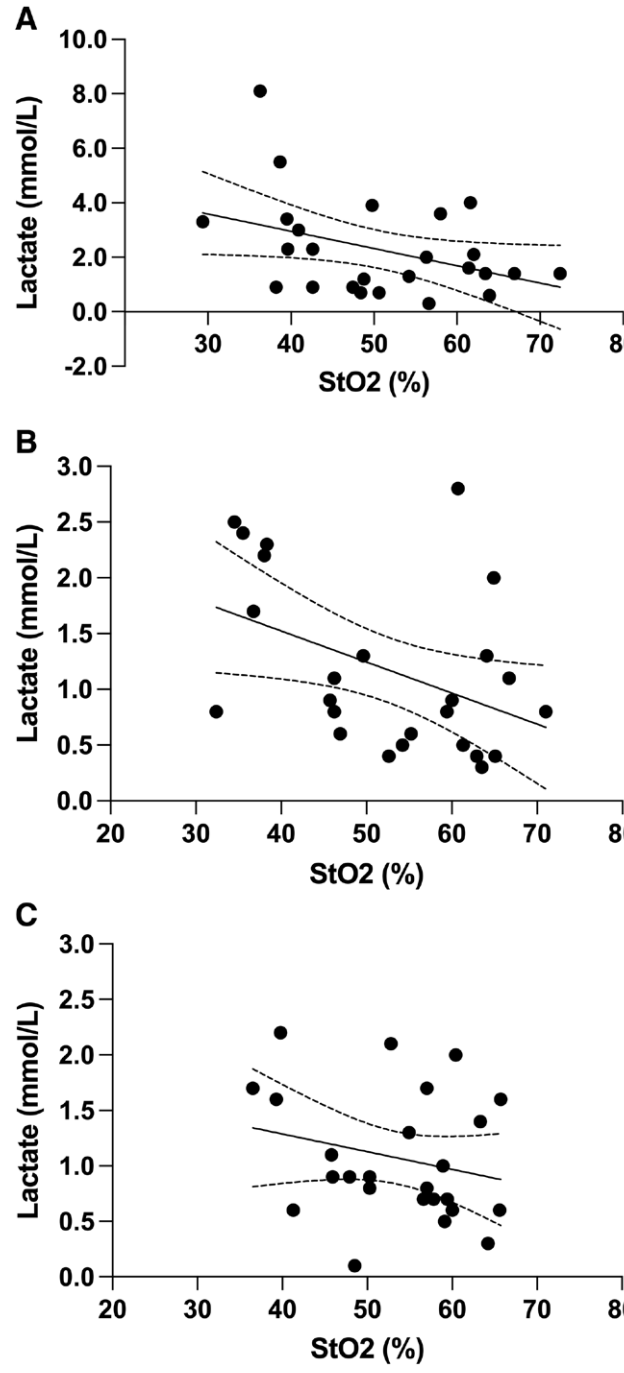

FIGURE 1. Relationship between StO2 and perfusate lactate at 1 h NMP (A), 2 h NMP (B) and 4 h NMP (C). NMP, normothermic machine perfusion; StO2, oxygen saturation.

with lactate at 4 h NMP (StO2: $R^2 = 0.1005$, $P = 0.1226$; THI: $R^2 = 0.1382$, $P = 0.0673$; NIR $R^2 = 0.0155$, $P = 0.5619$).

HSI parameters at any assessed time points did not demonstrate any significant relationship with perfusate glucose levels, quantity and quality of bile production, nor with arterial or portal blood flow.

One liver allograft was discarded due to missed viability criteria during NMP. The criteria were initially met; however, metabolic function thereafter deteriorated with increasing lactate within the first 4 h. Donor criteria of the discarded liver allograft were comparable to those of the transplanted allografts (age 61 y, male sex, BMI 27.2 kg/m^2, donor risk index 1.697). The discarded graft displayed decreased and stagnant StO2 levels (StO2 at 1, 2, and 4 h NMP: 38.7, 35.5, and 36.5, respectively), whereas the transplanted grafts demonstrated higher levels and an increase in StO2 over time. Furthermore, elevated and increasing THI levels were present in the discarded graft (THI at 1, 2, and 4 h NMP: 71.5, 77.3, and 79.0, respectively).

Overall length of hospital stay was 20 [9–83] d. Two recipients underwent liver retransplantation, due to portal vein thrombosis at postoperative day (POD) 18, and due to ischemic-type biliary lesions at POD 77. Four patients died at POD 41, 53, 69, and 83, respectively, including both patients who had required liver retransplantation. The cause of death was septic multiorgan failure in all 4 recipients. Perfusion parameters of NMP did not differ between surviving patients

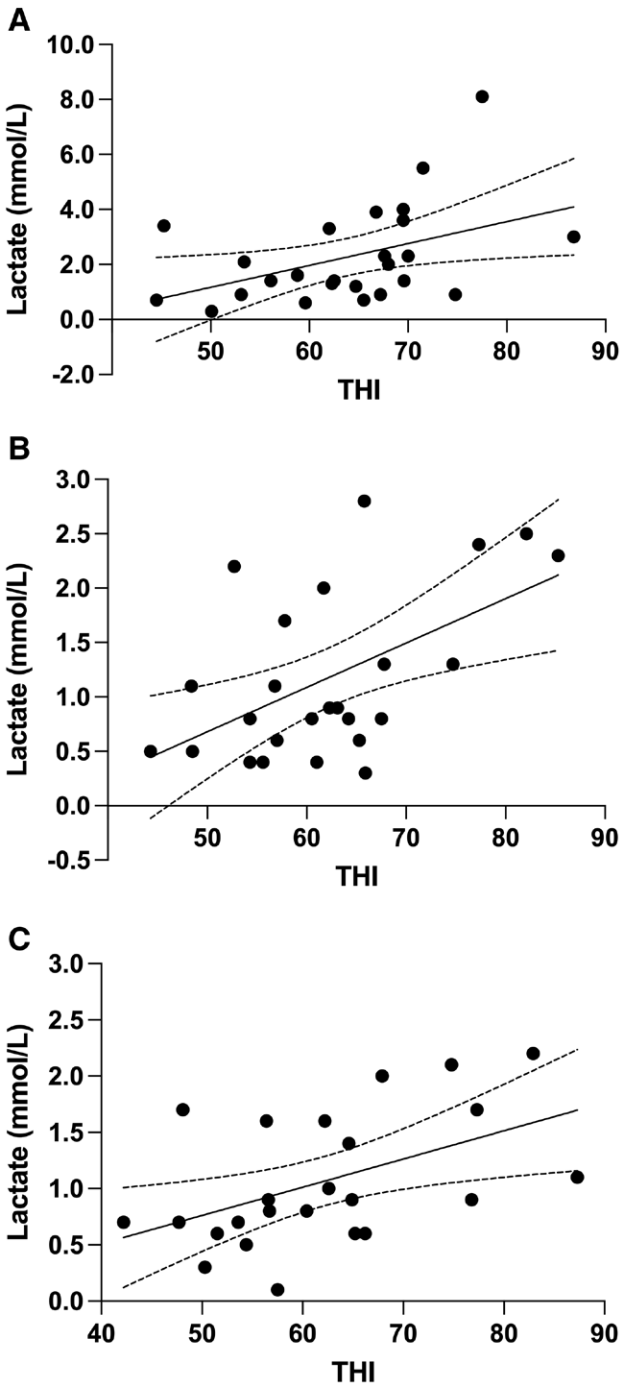

FIGURE 2. Relationship between THI and perfusate lactate at 1 h NMP (A), 2 h NMP (B), and 4 h NMP (C). NMP, normothermic machine perfusion; THI, tissue hemoglobin index.

and deceased patients. Nor did HSI parameters StO2, THI, and TWI differ between surviving patients and deceased patients. For NIR, there is a trend toward lower values at all assessed time points in deceased patients; however, this did not reach statistical significance in this small study cohort. Delta-NIR did not differ according to recipients' outcome (Figure 3). Early allograft dysfunction[24] was present in 37.5% of recipients. In organs developing early allograft dysfunction, there is a trend toward lower NIR values and less improvement in NIR during NMP; however, this did not reach statistical significance (Figure 4).

DISCUSSION

The decision whether to utilize liver allografts for transplantation is frequently based on donor history and subjective assessment by the transplanting surgeon. Mergental et al demonstrated that objective assessment of liver function during NMP might salvage a high proportion of liver allografts that are currently discarded.[8] Lactate clearance, pH regulation, and glucose evolution during NMP seem to be promising measures of viability.[5] Nevertheless, formal viability parameters in NMP are yet to be defined and validated.

The core ability of HSI is the assessment of tissue microperfusion.[21] Recently, HSI was examined for the quantitative analysis of liver perfusion.[16,21] More importantly, HSI was lately applied in porcine kidneys and discarded human liver grafts in experimental settings of NMP.[25,26] Nevertheless, HSI data analysis is restricted by the low penetration depth of the propagating light and the analyzed tissue. Hence, tissue damage in subjacent regions is not detectable. Thus, combining biochemical assessment methods of NMP with HSI technology seems to be beneficial for viability assessment of human liver allografts for transplantation.

Accordingly, the objective of our pilot study was the evaluation of a potential association between HSI parameters and current perfusate viability parameters during NMP. During NMP, sufficient delivery of oxygen at the tissue level is the pioneering amendment sustaining allograft viability and furthering aerobic metabolism.[27] Oxygen deprivation is the point of origin that by implication affects parenchymal damage, which in the case of SCS of donor organs is deteriorated by ischemia–reperfusion injury, once blood circulation is reestablished.[28,29] To define suitable perfusate viability parameters for investigating a correlation to HSI indices, we therefore focused on perfusate lactate and perfusate pH as ischemia index parameters. Notwithstanding, other recommended liver NMP viability parameters such as perfusate glucose levels and (high) quality bile production, constituting indirect markers for impaired microperfusion, might be appropriate likewise.[8-10,30]

We chose the early phase of NMP, that is, up to 4 h, for the assessment of the established and the new potential viability parameters, since the final decision whether to utilize a potential donor liver is usually based on achieving a sufficient lactate clearance by 4 h NMP at the latest. Therefore, additional

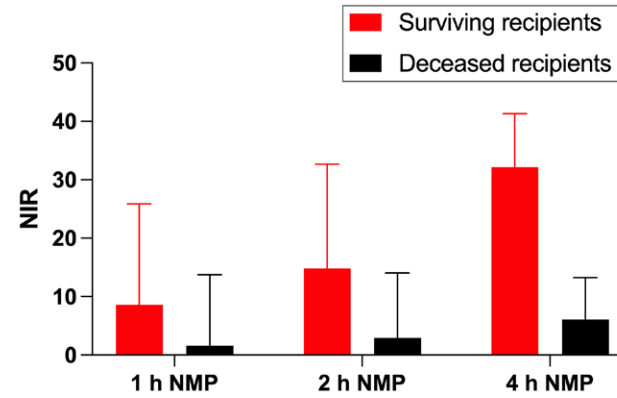

FIGURE 3. NIR with regard to patient survival (data are presented as median and interquartile range; NMP 1 h: $P = 0.2338$, NMP 2 h: $P = 0.1573$, NMP 4 h: $P = 0.0784$). NIR, near-infrared perfusion index; NMP, normothermic machine perfusion.

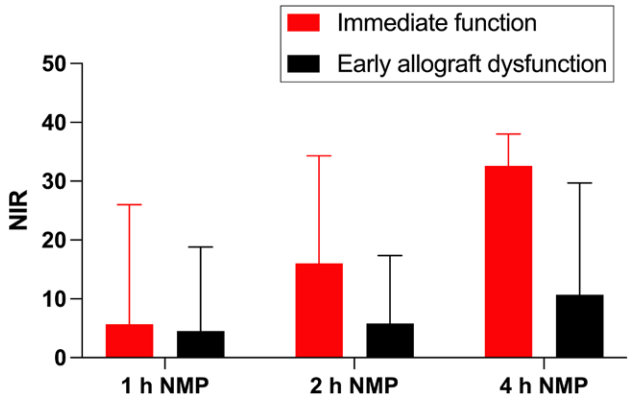

FIGURE 4. NIR with regard to initial graft function (data are presented as median and interquartile range; NMP 1 h: $P = 0.8725$, NMP 2 h: $P = 0.1135$, NMP 4 h: $P = 0.1865$). NIR, near-infrared perfusion index; NMP, normothermic machine perfusion.

viability assessment by HSI in this crucial phase of NMP is of particular interest.

In a porcine model of hepatic artery occlusion causing ischemia–reperfusion injury, a significant correlation of HSI indices and capillary lactate was recently demonstrated, underlining the potential of HSI to predict liver viability. Hereby, correlation of capillary lactate was significant and higher for StO2 compared with NIR. However, investigating the ischemic and reperfusion phase, StO2 correlated better with the reperfusion phase and NIR revealed a higher correlation for the ischemic phase.[31]

Recently, Urade et al[21] presented a significant correlation between StO2, NIR, and TWI with local lactate discriminating ischemic and nonischemic areas in a porcine model of left vascular inflow occlusion during anatomical left hepatectomy. Nonetheless, a correlation of THI and TWI with local lactate was not detected.[21] Another study utilizing HSI for evaluating skin microcirculatory patterns in septic patients showed a predictive validity of palm and fingertip THI forecasting 28-d mortality. Therefore, THI emerged to be indicative for a seriously impaired microcirculatory situation.[32]

We detected a significant relationship between perfusate lactate and StO2 as well as THI during NMP of human liver allografts. An impaired microperfusion of liver allografts became evident in a decreased lactate clearance as well as an increased tissue hemoglobin concentration combined with a low StO2. Similar HSI patterns were obtained from skin measurements in septic patients and might likewise be interpreted as red blood cell pooling, indicative for a disturbed perfusion or venous congestion in the investigated area.[32]

Increased TWI might also illustrate venous congestion; however, we did not observe a correlation of TWI and perfusate markers of viability. Probably, differences in TWI leading to perfusate lactatemia might only be noticeable in truly damaged liver allografts. Applying NMP, merely 1 liver allograft in our study was discarded due to missed performance criteria. This graft showed insufficient lactate clearance. Besides, StO2 was decreased, and THI was elevated in this graft during the entire observation period, in comparison to the subsequently transplanted grafts. A detailed subgroup analysis of discarded and transplanted grafts was not possible in our study. The small proportion of discarded organs might be determined by sophisticated and rigorous donor evaluation.

Our study has several limitations. First, it is a single-center approach comprising a small sample size with an elevated mortality rate. To date, no final conclusions toward a routine clinical application of HSI for viability assessment during NMP may be drawn. Secondly, both perfusion-related and HSI-based viability assessments only indicate a snapshot of liver condition focusing on allograft function during the early posttransplant period. To date, the impact of NMP viability parameters providing information on long-term graft survival is uncharted. For HSI parameters, we detected a trend toward remarkably higher NIR values at every time of data assessment in surviving recipients. As the deceased recipients in our study all died due to septic complications that might possibly have arisen or at least worsened in the setting of a mediocre graft function, this finding is of important notice, despite the lack of its statistical significance in this small study cohort. The implementation of the identified HSI index parameters in larger clinical trials of NMP is required to confirm their utility and safety.

In summary, this study demonstrates a significant relationship of HSI indices and current NMP viability assessment criteria, highlighting the incorporation of these innovative technologies as a viable future concept in solid organ transplantation, providing valuable information on allograft perfusion quality and viability prior to transplantation.

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