Novel real-time prediction of liver graft function during hypothermic oxygenated machine perfusion before liver transplantation

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Abstract: OBJECTIVE: The aim of this study was to determine the predictive value of machine perfusate analysis on graft outcome. BACKGROUND: Ex situ machine perfusion (MP) is gaining increasing interest to potentially repair injured organs and to assess organ function. In the field of liver transplantation, however, no studies exist on reliable prediction of graft function during MP. METHODS: We have used hypothermic oxygenated perfusion (HOPE) for donation after circulatory death (DCD) or extended criteria donation after brain death (DBD) human liver grafts during the last 7 years. Our series includes 100 HOPE-treated liver-transplanted patients with an overall tumor-censored 5-year graft survival of 89%. We monitored 54 livers during HOPE in terms of fluorometric analysis of released mitochondrial flavin (flavin mononucleotide, FMN) in the machine perfusate. RESULTS: Real-time optical measurement of mitochondrial FMN release in machine perfusates of livers disclosed a strong correlation with lactate clearance and coagulation factors at day 1 and 2 after transplantation. Receiver-operating characteristic curve analysis revealed an area under the curve (AUROC) of 0.79 [95% confidence interval (CI), 0.62-0.97] for severe allograft dysfunction and for early graft loss (AUROC 0.93, 95% CI, 0.84-1.0). CONCLUSIONS: Assessment of flavin, a marker of mitochondrial complex I injury, in the perfusate provides a fast prediction of liver graft function and loss during ex situ MP before implantation. This finding may have high clinical relevance, as liver grafts from extended DBD or DCD donors carry considerable risks for recipients. On-line estimation of outcome before implantation would therefore substantially increase safe utilization of liver grafts.

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Novel Real-time Prediction of Liver Graft Function During Hypothermic Oxygenated Machine Perfusion Before Liver Transplantation

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Objective: The aim of this study was to determine the predictive value of machine perfusate analysis on graft outcome.

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Conclusions: Assessment of flavin, a marker of mitochondrial complex I injury, in the perfusate provides a fast prediction of liver graft function and loss during ex situ MP before implantation. This finding may have high clinical relevance, as liver grafts from extended DBD or DCD donors carry considerable risks for recipients. On-line estimation of outcome before implantation would therefore substantially increase safe utilization of liver grafts.

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The assessment of graft quality has always been considered as key confounder for outcome since the early days of liver transplantation (LT).[1] However, still today the assessment of liver quality and the decision to accept or decline a graft before transplantation mostly relies on ''gut feeling,'' in combination with donor demographics and medical history.[2,3]

Machine perfusion (MP) before implantation offers the unique opportunity to develop objective evaluation tools to assess the metabolic status and degree of injury of an organ. Until recently, only a few parameters for metabolic and cellular damage have been quantified in machine liver perfusates including lactate, transaminases, and pH, besides assessing bile production and bile quality.[2,4,5] Two emerging studies looked at the predictive value of novel markers through metabolomics and glyconomics[6–8] suggesting superior clinical relevance of specific metabolic pathways in the liver, compared to the quantification of released cytosolic compounds.[9,10]

Since 2012, hypothermic oxygenated perfusion (HOPE) was routinely used in our center to improve human liver grafts donated after circulatory death (DCD) before implantation.[11] Despite extended functional donor warm ischemia time in our DCD cohort, the outcomes have been excellent, along with significant changes of the mitochondrial metabolism with subsequent recharging of cellular energy indicating mitigated ischemia-reperfusion injury.[12,13]

In this study, we monitored changes in the perfusate of specific mitochondrial metabolisms during HOPE to identify novel markers with robust predictive value for graft function after liver implantation.

METHODS

Patient Cohort and Parameters of Donor Surgery, MP and Graft Implantation

We present here the entire cohort of machine perfused human liver grafts transplanted at our center between 2012 and 2018. A detailed perfusate analysis was performed in the last 54 perfused grafts of this series, e.g. since 2016. Donation after brain death (DBD) livers were procured by the standard retrieval approach with cold in situ flush using Institute-George-Lopez-1 (IGL-1) solution. In contrast, DCD livers were procured using the super rapid retrieval technique, followed by cold storage and transport. HOPE treatment of liver grafts was always performed end-ischemically, that is, after cold storage. Livers were perfused as previously reported through the portal vein only using the Liver Assist device (Organ Assist) with a portal vein pressure limit at 3 mm Hg resulting in low portal flows

(range:150–300 mL/min). Three litres of highly oxygenated (80–100 kPa) University of Wisconsin MP solution (Belzer-MPS) were used to perfuse all livers, and the perfusate was never exchanged during perfusion. HOPE was performed during recipient hepatectomy for at least 1 hour. The routine technique for liver implantation used at our center is the classic cava-replacement technique without the use of veno-venous bypass. Liver reperfusion was done through the portal vein first, followed by subsequent arterial reperfusion. Machine-perfused livers were allocated to candidates with hepatocellular carcinoma (HCC) and subsequent low Model of end-stage liver disease (MELD) score, with an expected long waiting time; the respective donor and recipient characteristics are illustrated in Supplementary Table 1, http://links.lww.com/SLA/B719.

Perfusate Analysis During MPHOPE

We measured the following parameters in the perfusate of HOPE treated livers:

1. Lactate and glucose levels in the perfusate were measured real-time during perfusion using the Radiometer ABL800 Flex device. In addition, the concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) perfusate levels was measured (real-time), using the FujiFilm Dri-CHEm 4000i device.
2. Nicotine adenine dinucleotide (NAD), succinic acid, inosine monophosphate (IMP), hypoxanthine, and noncovalently bound mitochondrial flavin (flavin mononucleotide, FMN) were determined by targeted liquid chromatography-mass spectroscopy.
3. FMN was further determined by fluorescence spectroscopy. In detail, a light probe was connected to a halogen light source to emit monochrome light at a wavelength of 450 nm on the circulating perfusate. A spectrometric receiver probe with sufficiently high resolution (eg, 4.6 nm) was used to quantify the proportion of emitted fluorescent light by the FMN molecule. The fluorescence emission maximum of FMN was measured between 500 and 600 nm. Measurement of the fluorescence takes only seconds, and the final value is instantly displayed on a monitor.

Endpoints

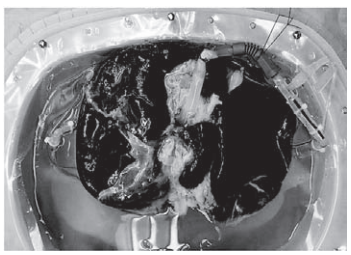
Perfusate measurements were correlated to post-transplant arterial lactate clearance, international normalized ratio, INR, factor V, liver transaminases, hospital stay, and complications using the comprehensive complication index as the novel metric for morbidity until discharge from hospital, and graft loss was recorded until 3 months after LT. Models to assess donor liver quality [donor risk index (DRI) and donor liver index (DLI)] and other composite scores of early allograft function such as early allograft dysfunction (EAD), defined by Olthof et al[14] or the L-Graft score, defined by Agopian et al[15] were correlated with perfusate metabolites and outcomes.

The local ethic committee approved the study protocol (KEK-No. 2015–0200 and KEK No. 2019-01000).

Statistical Analysis

Statistical analysis was performed using the SPSS statistical software package (SPSS, version 20, IBM, Armonk, NY) and Graph Pad Prism7. Continuous variables were summarized as median

Example of DCD HOPE-treatment of a human liver before implantation



Quantification of perfusate parameters during HOPE

Perfusate Parameters (median, IQR)	Overall	DBD	DCD	P value DBD vs. DCD
AST (U)	456 (196-1108)	196 (92-348)	742 (292-1490)	0.0042
ALT (U)	748 (296-1484)	316 (154-740)	1008 (481-1913)	0.0046
LDH (U)	3236 (976-7160)	1124 (332-3284)	5240 (2158-10245)	0.0014
Lactate (mmol/l)	3.05 (2-3.775)	2.7 (1.85-3.45)	3.4 (2.0-5.03)	0.3814
Succinate A.U.	$1.07x10^6$ ($544500-2.31x10^6$)	$2.08x10^6$ ($733250-5.79x10^6$)	$0.99x10^6$ ($23600-2.16x10^6$)	0.3175
FMN 30 min (A.U.)	5889 (4872-7022)	5547 (4260-6111)	6973 (5363-9575)	0.0007
FMN end of HOPE (A.U)	6598 (4886-8684)	4820 (4127-6206)	7889 (6130-10933)	0.0002

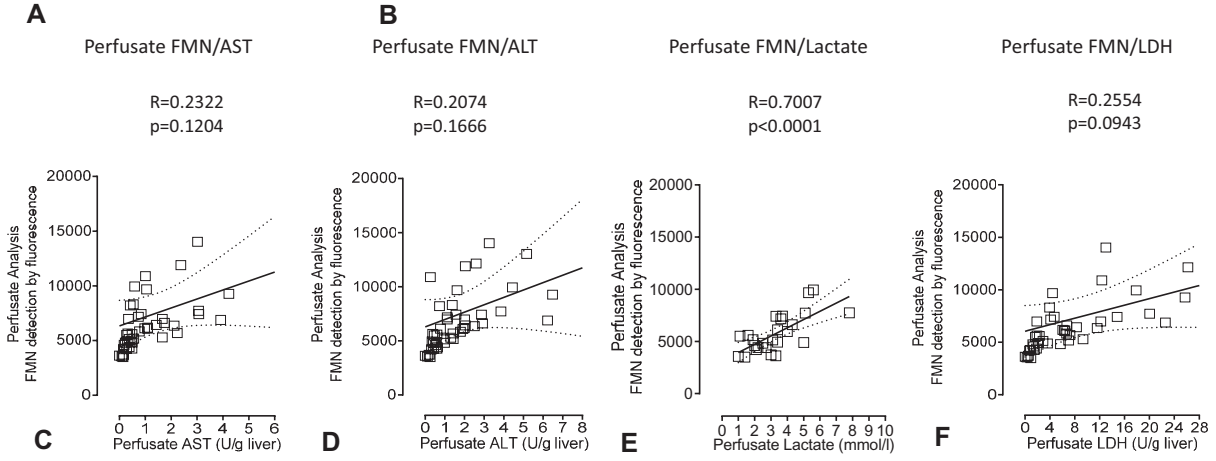


FIGURE 1. Example of HOPE-treatment of human liver (A) and perfusate parameters in 54 HOPE-treated DCD and DBD livers (B). Correlation of perfusate FMN with perfusate AST, ALT, lactate, and LDH (C–F).

values and interquartile ranges, and categorical variables were expressed in percentages. Normality of the distribution of variables was tested using QQ plots and the Kolmogorov–Smirnoff test. Graft failure–free survival and overall patient survival curves were computed using Kaplan–Meier curves. Correlations between fluorimetric perfusate analysis and liver graft function were calculated using Pearson correlation coefficient (R). Reference values for strength of effect size were: "very weak" 0.00–0.19, "weak" 0.20–0.39, "moderate" 0.40–0.59, "strong" 0.60–0.79, and "very strong" 0.80–1.0. Sensitivity and specificity of the predictive value of FMN was tested using receiver-operating characteristic curve (ROC) analysis. Cut-offs for highest specificity and sensitivity were determined by the Youden index. Tests were considered statistically significant at a 2-sided P value of <0.05.

RESULTS

Donor and Recipient Risks in HOPE-Treated Liver Cohort

During the study period, a total of 100 liver grafts (80 DCD, 20 DBD) underwent HOPE for a median of 120 minutes (91–150 minutes) followed by LT. (Supplementary Table 1, http://links.lww.com/SLA/B719). All DCD machine-perfused liver grafts showed a high-risk profile, as depicted by several current risk scores. For example, half of the DCD livers were classified into the futile group of the UK DCD risk score with a median of 10 points.[16] In addition, one-third of our donor livers (DBD and DCD) showed 10% to 30% macrosteatosis in biopsies (Supplementary Figure 1A, http://links.lww.com/SLA/B719). The DLI and DRI were consecutively higher than 2.2 points for DCD livers, and higher than 1.6 for DBD livers, far above recent reported median values in UK cohorts.[17–19] To compensate for inferior graft quality, cold ischemia time (median 4.8 hours) and recipient MELD (median 13 points) were kept as low as possible (HCC recipients). However, in the last 2 years, extended DCD liver grafts were also used for retransplantations and to "rescue," for example, high MELD candidates (Supplementary Figure 1B, http://links.lww.com/SLA/B719). Of note, DBD grafts included in the study were from extended criteria donor, with half being $\geq$60 years' old and 20% presenting a BMI $>30\,kg/m^2$. After HOPE treatment, these grafts were also primarily allocated to recipients with HCC presenting, however, a slightly higher MELD score than the DCD recipients (16 vs 12 points) (Supplementary Table 1, http://links.lww.com/SLA/B719).

Despite an overall high-risk population in our cohort, graft survival, censored for tumor-related death, remained comparable to the benchmark level for an ideal LT, for exampl, 89% at 1 and 5 years.[20] In total, we lost 7 of 100 grafts in the first 3months after transplantation because of hepatic artery thrombosis (1%), ischemic cholangiopathy (1%), primary nonfunction (4%), or sepsis (1%).

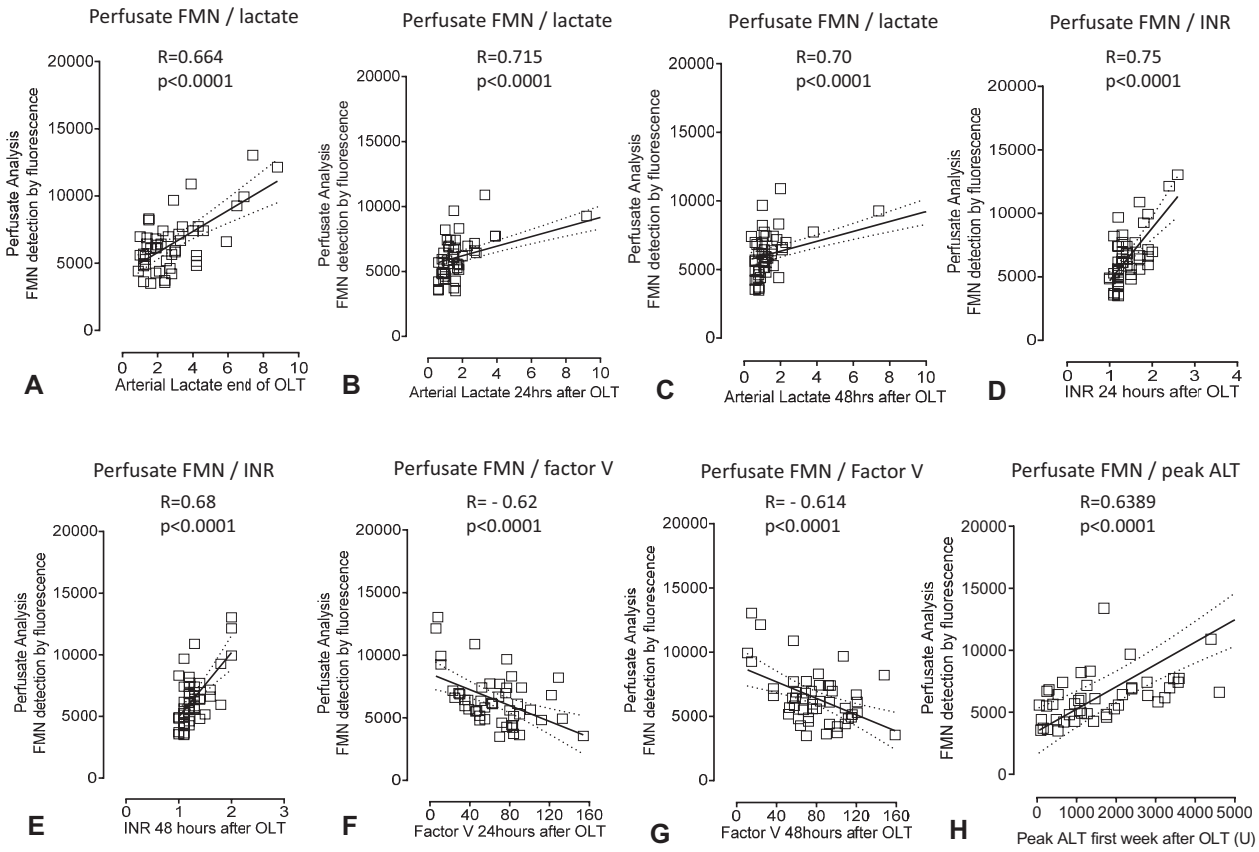


FIGURE 2. Correlation of perfusate FMN with post-transplant lab parameters of liver function and injury, such as arterial lactate (A–C), INR (D and E), and factor V (F and G), peak ALT (H).

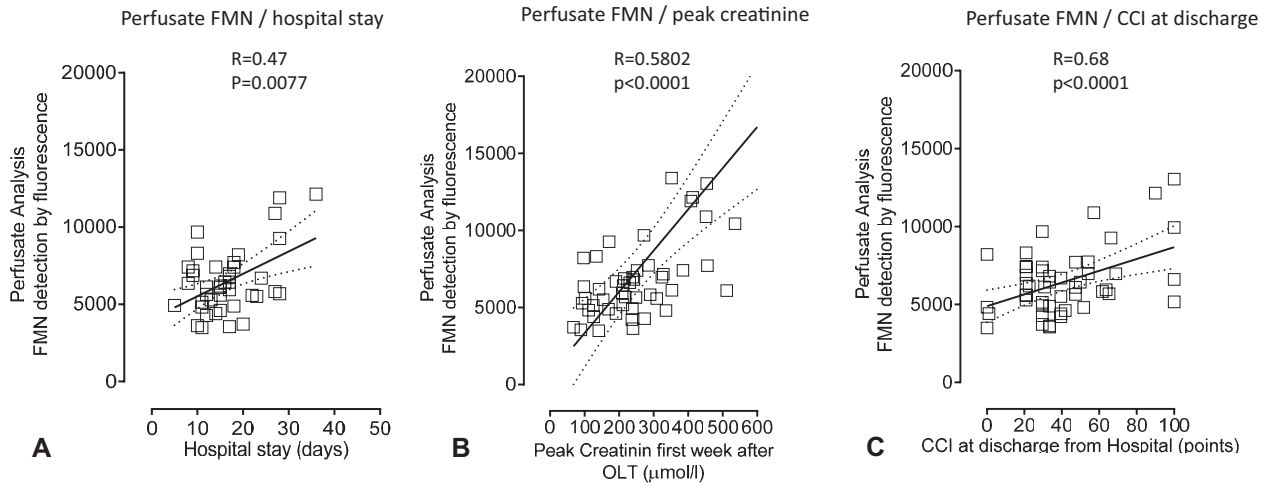
Prediction of Graft Function

We performed a detailed perfusate analysis in 54 HOPE-treated livers (19 DBD, 35 DCD livers). Real-time fluorometric measurements during MP were validated with mass spectroscopy (Supplementary Figure 1B, http://links.lww.com/SLA/B719). The results show, first, clear differences between DBD and DCD livers in terms of perfusate AST, ALT, LDH levels, and FMN, in contrast to perfusate succinate and lactate (Fig. 1B). Of note, the release of liver transaminases from DCD livers into the perfusate during HOPE was significantly lower (median AST 742 U/L, median ALT U/L 1008) (Fig. 1B), when compared to previous published values from DCD livers during end-ischemic normothermic perfusion.[2,21] Second, the release of mitochondrial flavin, for example, FMN, occurred independently from the release of cytosolic hepatocellular enzymes, for example, AST, ALT, or LDH, consistent with a mitochondrial origin of FMN (Fig. 1C, D, F). Third, we found a strong correlation of FMN release with coagulation factors and peak transaminases after LT ($R > 0.6$) (Fig. 2A–H), whereas conventional perfusate parameters, including AST and ALT, correlated with peak transaminases after LT, but failed to predict liver graft function (Supplementary Figure 2, http://links.lww.com/SLA/B719, Supplementary Table 2, http://links. lww.com/SLA/B719).

Furthermore, perfusate FMN appeared clinically relevant, as we found a correlation with hospital stay and more importantly with cumulative complications after LT, in contrast to other perfusate values (Fig. 3A–C). FMN analysis in machine perfusate was also predictive for both, early allograft dysfunction [c statistic 0.72, 95% confidence interval (CI), 0.57–0.86], defined by Olthof et al, and for high-risk grafts, defined by the L-Graft score (c statistic 0.79, 95% CI, 0.62–0.97) (Supplementary Figure 3, http://links.lww.com/SLA/B719). We consecutively grouped machine-perfused livers according to their FMN release within the first 30 minutes of HOPE in 2 risk groups, for example, low and high FMN perfusate. Perfusate mass spectroscopy revealed that high FMN release correlated significantly with higher perfusate concentrations of electron donors, for example, NAD, and purine metabolites, for example, IMP and hypoxanthine. In contrast, perfusate succinate decreased with higher FMN release (Fig. 4A–E). This inverse relationship of perfusate succinate and purine metabolites points to inhibition of mitochondrial electron transfer between complex I and II, and allows ''on line'' recognition of mitochondrial injury, based on the amount of FMN, released into cold perfusate.

Prediction of Early Graft Loss

High perfusate FMN correlated also with graft loss within 3 months after LT, and revealed higher predictability (area under the ROC curve 0.93, 95% CI, 0.84–1.0) than any other available scores, such as DRI, DLI, and L-Graft (Fig. 5A–D). The threshold for highest accuracy in terms of early graft loss was identified at 10000 A.U, e.g. 100 ng FMN/ml perfusate. Of note, perfusate AST and ALT also showed high c-statistics, whereas perfusate lactate und glucose failed to predict graft failure (Fig. 5E).



Correlation of Perfusate Parameters with outcome	R	P value
FMN 30 min and Hospital Stay	0.47	0.0077
FMN 30 min and Peak Creatinine	0.5802	<0.0001
FMN 30 min and CCI during hospital stay	0.68	<0.0001
AST and Hospital Stay	0.1065	0.4966
AST and Peak Creatinine	0.1299	0.4064
AST and CCI during hospital stay	0.2469	0.0982
ALT and Hospital Stay	- 0.1339	0.4100
ALT and Peak Creatinine	0.08471	0.6033
ALT and CCI during hospital stay	0.2719	0.0777
Lactate and Hospital Stay	0.05980	0.7624
Lactate and Peak Creatinine	0.3212	0.1023
Lactate and CCI during hospital stay	0.1743	0.3485

D

FIGURE 3. Correlation of perfusate FMN, AST, ALT, and lactate with hospital stay, kidney function, and overall complications (comprehensive complication index).

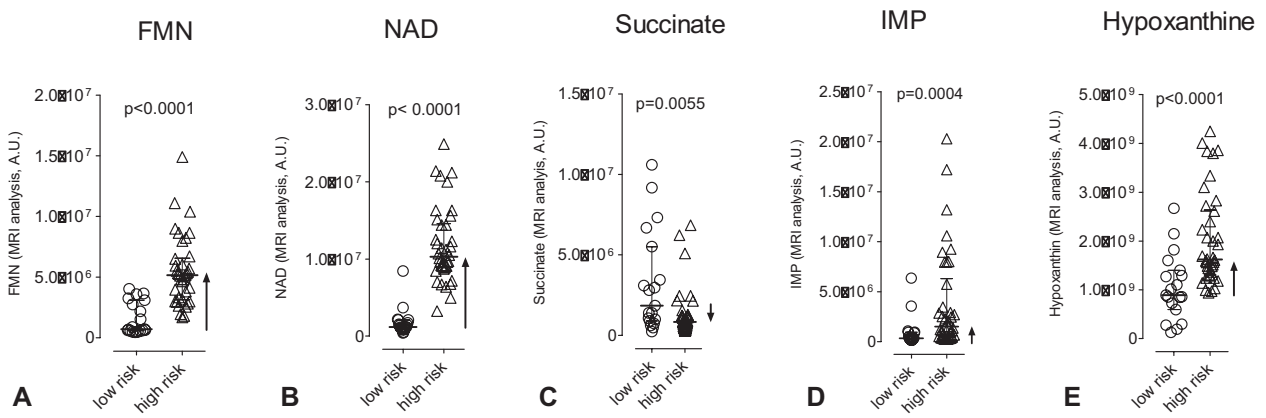
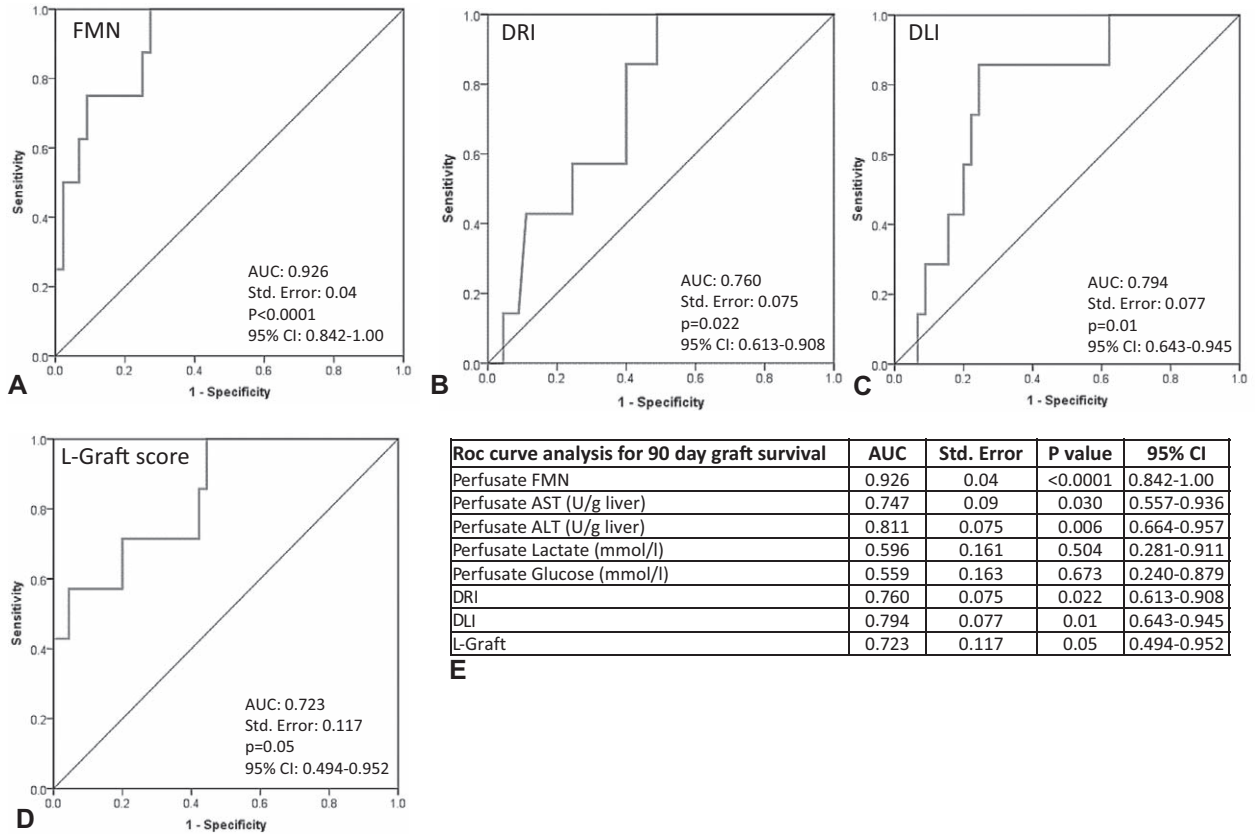


FIGURE 4. Analysis of perfusate FMN, NAD, succinate, IMP, and hypoxanthin in high-risk patients classified by perfusate FMN>10000 AU.

DISCUSSION

To our knowledge, this is the first report on prediction of graft function and early graft loss by perfusate analysis during cold perfusion of human livers. We show an accurate and readily available prediction of liver viability during ex situ MP before implantation, based on the level of mitochondrial complex I injury through quantification of FMN in perfusate. This marker disclosed superiority compared to conventional parameters for liver viability. We expect a high clinical relevance of these results, as liver grafts from marginal DBD or extended DCD donors carry considerable risks for the recipients. Reliable on-line estimation of outcome before implantation would therefore substantially increase the safe utilization of liver grafts.

MP strategies receive increasing attention in the field of transplantation, as they offer great advantages for assessment of



Roc curve analysis for 90 day graft survival	AUC	Std. Error	P value	95% CI
Perfusate FMN	0.926	0.04	<0.0001	0.842-1.00
Perfusate AST (U/g liver)	0.747	0.09	0.030	0.557-0.936
Perfusate ALT (U/g liver)	0.811	0.075	0.006	0.664-0.957
Perfusate Lactate (mmol/l)	0.596	0.161	0.504	0.281-0.911
Perfusate Glucose (mmol/l)	0.559	0.163	0.673	0.240-0.879
DRI	0.760	0.075	0.022	0.613-0.908
DLI	0.794	0.077	0.01	0.643-0.945
L-Graft	0.723	0.117	0.05	0.494-0.952

E

FIGURE 5. ROC analysis of perfusate FMN, DRI, DLI, L-Graft score, perfusate AST, ALT, glucose, and lactate on 90-day graft loss.

graft quality before implantation.[22] However, current viability tests during perfusion target mostly lactate clearance, release of hepatocellular enzymes, or bile production, which all have been found to be weak parameters predicting later liver function.[2,3] In contrast, we demonstrate that monitoring of mitochondrial injury during cold oxygenated perfusion is not only feasible, but serves as reliable parameter for functional recovery of livers during implantation.

The strength of a cold oxygenated perfusion approach appears 2-fold. First, because of the lower demand of energetic substances in the cold, oxygen carriers are not needed during hypothermic liver perfusion.[11,23,24] Acelluar perfusates with dissolved oxygen appear therefore sufficient for maintaining mitochondrial function during hypothermia or sub-normothermia, with the simultaneous major advantage for implementation of novel optical measurements or even treatment during perfusion, for example by eradication of hepatitis C by short wave ultraviolet light.[25] Second, reactive oxygen species are significantly less produced during cold oxygenation of ischemic liver cells, as compared to normothermic conditions. Consistently, AST/ALT release in the perfusate during HOPE of high-risk DCD grafts was less than the release observed during normothermic perfusion.[26,27] Hypothermic oxygenated perfusion offers therefore not only protection from reperfusion injury with subsequently improved liver function, but may serve as prediction tool for early graft function in the recipient.

Our results based on the new finding of a small compound, FMN, released from the first electron transferring mitochondrial protein during oxygenated perfusion. Complex I is a sophisticated mitochondrial protein, containing 8 iron–sulphur clusters and non-covalently bound FMN.[28] This complex uses 2 substrates, NADH and ubiquinone, and couples the energy of oxidoreduction to proton translocation by an array of proton pumps. While under physiologic conditions, FMN is very tightly bound, it dissociates from complex I when the respiratory chain is halted and the ubiquinone pool is highly reduced.[28] Of note, the affinity of FMN in its pocket is dependent on structural alterations around the FMN site, for example, the redox potential of cluster N1a is likewise one of the determinants of the affinity of FMN for complex I. Recent data obtained from ischemic brain mitochondria have suggested that the FMN site of mitochondrial complex I is primarily responsible for the production of superoxide anions under normothermic conditions, together with the simultaneous release of mitochondrial FMN.[29]

The novel finding of this study is that mitochondrial FMN is also released in ischemic livers subjected to cold re-oxygenation, and is easily and readily detectable in acellular machine perfusates owing to the natural fluorometric properties of FMN. Therefore, FMN release serves as surrogate marker for impaired cellular energy production, as high FMN release correlates with ATP-breakdown, detectable by increased purine metabolites in machine perfusates (Fig. 5). These results contrast with the low predictive value of conventional perfusate parameters, such as transaminases or lactate levels, which failed to recognize impaired liver function after implantation. Instead of solely focusing on the release of cytosolic compounds, future perfusate analysis should target on real-time monitoring of mitochondrial metabolism to enable an accurate prediction of oxidative stress and downstream activation of the hepatic inflammasome upon transplantation. The combination of several key mitochondrial metabolites including FMN, succinate, xanthine, and hypoxanthine may allow future detailed assessment of mitochondrial function of liver grafts.

The limitations of this study include the need for further validation in other machine perfused liver cohorts. In addition, current thresholds to refuse or accept livers remain subjective, but a high accuracy based on ROC analysis could be identified in this study suggesting a threshold at 10000 A.U. to refuse livers regardless of the use of a DCD or DBD liver. Whether similar thresholds can be confirmed in other cohorts such as steatotic livers remains to be determined.

In summary, this study suggests that assessment of liver graft quality is feasible and reliable during cold oxygenated perfusion. Cold MP approaches have been frequently challenged in terms of their presumed weakness in prediction of graft function, compared to normothermic perfusion. This shortcoming is now circumvented by the ability to monitor mitochondrial metabolism by perfusate analysis during HOPE, and thereby enabling proper decision about acceptance or not of high-risk grafts.

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DISCUSSANTS

Peter Schemmer (Graz, Austria):

There is growing significance in improved graft function after MP for the preservation of livers; however, reliable predictors of the grafts' function after LT are still pending. In this study, the real-time optical measurement of mitochondrial FMN release in MP perfusates has been nicely correlated with both graft function and early graft loss. Most of the studies focusing on parameters to characterize liver function during MP are performed under normothermic or subnormothermic conditions. To my knowledge, this is indeed the first time that a real-time judgment of liver function is feasible under hypothermic conditions. Interestingly, your results correlate well with published data on ATP levels in liver tissue, and therefore, it might be a promising tool for a multimodal approach to further characterize and predict the fate of the transplanted livers during MP. With the combination of both improved preservation and the prediction of the function, HOPE has developed a conclusive strategy with substantial benefits for the recipients' safety after LT; however, it is still unclear which livers profit the most from this.

You mentioned that DCD and DBD livers behave differently during HOPE. Is this finding per se a discriminator for the use of FMN analysis during MP? What is the optimal time point to determine whether a graft is suitable for LT or not? Are there predictive cutoff values of FMN analysis even before HOPE? The authors focus on their findings after 30 minutes of HOPE. Has the amount of FMN changed significantly when livers where perfused for a longer time period? Is there a correlation between the duration of MP and graft survival? Are there minimum and maximum timespans of HOPE for best graft assessment?

Despite these remaining questions, this well-conducted study is of high importance and provides better insights into the pathophysiological processes during HOPE, and thus, enlightens the mystery of MP, to optimize outcome after LT by reliable ex vivo graft evaluation.

Response From Philipp Dutkowski (Zurich, Switzerland):

Thank you for these interesting questions. First, I think that we should differentiate between assessment during MP, and repair of injured organs. While in principle, every liver graft would profit from a reliable and quick assessment, repair is only needed in poor-quality livers. For example, I believe that DBD livers without relevant steatosis would rather not benefit from any MP. But, we are sometimes surprised to see DCD livers with immediate good function and DBD livers, which unexpectedly perform poorly. Based on this, assessment of livers before implantation would definitely increase safety. Therefore, at this stage, I would advise to place every graft on a perfusion machine for a short period of time, to assess quality.

Regarding the optimum timeframe for mitochondrial FMN measurement, key processes of ischemia reperfusion injury occur within the first minutes. Based on this, I believe that the first 30 minutes of cold oxygenated perfusion would be a reliable timeframe for measuring machine perfusates, as FMN does not continue to increase in most liver perfusates after the initial 0.5 hour of perfusion. With respect to the cases with high values of FMN after 30 minutes, I would recommend to repeat this analysis after 60 minutes. In my opinion, FMN measurements before oxygenated MP, such as during cold storage, makes less sense because there is no significant release of mitochondrial FMN without the action of oxygen.

The maximum required time period of cold oxygenated MP relates to the metabolic situation of the perfused livers. Maximum benefit is reached when all accumulated succinate and NADH are metabolized, and ATP pools are uploaded. Therefore, in principal, a longer perfusion approach is required for more injured grafts, but these are preliminary data.

Stefan Schneeberger (Innsbruck, Austria):

Congratulations on a very elegantly presented study. You did 2 things: you assessed a new methodology, and used new endpoints to assess the validity and quality of these new parameters, which is a good thing. I believe in your endpoints. However, did you also check the more traditional endpoints of delayed graft function, including all of the parameters that are traditionally used to assess this; and, does it still have a predictive value?

My second question relates to bile duct interest. Did you do a long-term assessment to observe any changes in MRCP and whether there's any predictive value of your assessment with respect to bile duct viability?

Response From Philipp Dutkowski (Zurich, Switzerland):

Thank you, Dr. Schneeberger, for these very good questions. We have checked the predictive value of perfusate analysis for FMN on early allograft dysfunction, defined by Olthoff et al, and on the L-Graft risk score. Both parameters showed a high correlation with perfusate FMN, in contrast to perfusate liver enzymes. Your second question targets biliary injury. Currently, we cannot address the prediction of perfusate FMN analysis on irreversible biliary injury, as we only have a few cases with irreversible cholangiopathy in our series.

Antonio D. Pinna (Abu Dhabi, United Arab Emirates):

Thank you for this great presentation. I have 1 question. Are we going to move the transplant to after the measurement of

riboflavin, or will we continue performing HOPE during the hepatectomy?

Response From Philipp Dutkowski (Zurich, Switzerland):

That is a very important point. When we introduced the HOPE principle 7 years ago, we already started the recipient hepatectomy during the MP, before perfusate analysis. Currently, however, we avoid this practice, as the result of the perfusate analysis can be unexpectedly high. Therefore, we clearly recommend waiting for the perfusate analysis before starting the recipient hepatectomy.

Norbert Senninger (Münster, Germany):

Why did you choose to only perform portal perfusion, rather than performing both portal and arterial perfusion?

Response From Philipp Dutkowski (Zurich, Switzerland):

This is related to several previous experimental studies in rat, pig, and human livers, which confirmed an excellent perfusion of the entire liver, including the extrahepatic bile duct, by single portal vein perfusion. Based on this, we do not believe that an additional arterial perfusion is needed in a cold environment, if portal perfusate is highly oxygenated.