Original Article

Endothelial glycocalyx damage marker syndecan-1 during hypothermic oxygenated machine perfusion of donor grafts facilitates prediction of early allograft dysfunction after liver transplantation

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Background: Ischemia reperfusion injury (IRI) is a major contributing factor to organ damage in liver transplantation (LT) impacting donor organ quality and patient survival. IRI-inflicted graft injury can be reduced by using hypothermic oxygenated machine perfusion (HOPE) as a preservation strategy instead of static cold storage (SCS). The endothelial glycocalyx is highly sensitive to IRI and its degradation during graft preservation and reperfusion was previously associated with inferior postoperative outcome after LT. Here, we aimed to measure glycocalyx degradation during and after HOPE in order to evaluate its potential for viability-assessment during machine perfusion and outcome prediction in patients undergoing LT.

Methods: Glycocalyx degradation was quantified via enzyme-linked immunoassay (ELISA) for its main component syndecan-1 (Sdc-1) in serum of 40 patients undergoing LT after HOPE. In addition, Sdc-1 was evaluated at multiple time points during HOPE. Patients were followed up for 3.5 years to assess postoperative complications including morbidity, the development of early allograft dysfunction (EAD) and graft survival.

Results: Liver grafts which later developed EAD showed significantly higher Sdc-1 concentrations after 60 min of HOPE compared to grafts exhibiting normal postoperative function (P=0.02). Receiver operating characteristic analysis revealed a strong predictive potential with an area under the curve of 0.73. A cut-off at 808 ng/mL Sdc-1 at 60 min of HOPE allowed identification of a high-risk group with an incidence of EAD of 66.7%. Sdc-1 concentrations increased during all types of HOPE but were significantly higher in HOPE versus dual HOPE (D-HOPE) after 120 min of perfusion (P=0.02).

Conclusions: Sdc-1 evaluated at 60 min during HOPE allows prediction of EAD after LT. Accordingly, Sdc-1 should be considered a potential additional biomarker for viability assessment during HOPE.

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Keywords: Hypothermic oxygenated machine perfusion (HOPE); endothelial glycocalyx; syndecan-1 (Sdc-1); liver transplantation (LT)

Submitted Jan 20, 2024. Accepted for publication Jun 06, 2024. Published online Aug 17, 2024.
doi: 10.21037/hbsn-24-33
View this article at: https://dx.doi.org/10.21037/hbsn-24-33

Introduction

Ischemia-reperfusion injury (IRI) represents a main cause of organ damage during liver transplantation (LT). Typically, upon procurement, donor organs are stored on ice until implantation. This leads to ischemic reprograming of liver tissue and drastic disruption with associated damage after reperfusion (1).

In order to reduce IRI, several attempts of altering the process of organ preservation were made in the past decade, especially in the liver transplant setting (2). Hypothermic oxygenated machine perfusion (HOPE) was shown to reduce non-anastomotic biliary strictures and early allograft dysfunction (EAD) when compared to static cold storage (SCS) (3,4). Further, on a cellular level, HOPE was shown to reduce IRI by replenishing intracellular ATP storages and by reducing the levels of succinate and other molecules accumulated due to anaerobic metabolism (5,6). While positive effects on IRI and patient outcome are well documented for liver grafts subjected to HOPE, available markers for quantification of organ and perfusion quality are scarce at this point.

The endothelial glycocalyx is a dynamic surface layer consisting of a network of glycoproteins, proteoglycans and glycosaminoglycans, covering the luminal side of the vascular endothelium. It is responsible for regulating vascular permeability and for transducing mechanical shear stress, thereby inducing intracellular vascular response pathways. In addition, the glycocalyx maintains a barrier function between endothelium and blood components and further modulates the adhesion of leukocytes (7,8). Of note, the hepatic glycocalyx is especially susceptible to reactive oxygen species (ROS), and its degradation was shown to be mechanistically involved in IRI (9,10). During different stages of organ damage, the endothelial glycocalyx is degraded and its shed components can be used as an indicator to assess the extent of the endothelial damage (11). In this context, syndecan-1 (Sdc-1), a heparan sulfate proteoglycan anchored in the endothelial membrane, was explored as a circulating biomarker for assessing glycocalyx damage after SCS organ preservation and prior to LT (12). Sdc-1 concentrations in graft effluent and recipient serum have been shown to correlate with the degree of liver damage and predicted EAD (13).

The present investigation aimed to evaluate glycocalyx damage during HOPE and perioperatively in liver transplant patients using Sdc-1 concentration measurement. Further, the predictive value of Sdc-1 as a biomarker for outcome after LT using HOPE was explored. We present this article in accordance with the STROBE reporting checklist (available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-24-33/rc).

Highlight box

Key findings

- Syndecan-1 (Sdc-1) concentration in the perfusate allows prediction of early allograft dysfunction after 60 min of hypothermic oxygenated machine perfusion (HOPE).

What is known and what is new?

- Endothelial glycocalyx degradation reflects the extent of graft injury and can be determined by measuring Sdc-1 recipient serum.
- Measuring Sdc-1 during HOPE presents an opportunity for viability assessment prior to liver transplantation.

What is the implication, and what should change now?

- The underlying data warrants further investigation of Sdc-1 for viability testing and routine use in hypothermic oxygenated machine perfusion for liver transplantation.

Methods

Patients

Forty consecutive patients undergoing LT were included in this retrospective analysis of a prospectively maintained database and biobank (single center). This study was performed at the General Hospital of Vienna, in accordance with the ethical standards laid down in the declaration of Helsinki (as revised 2013) and approved by the ethics committee of the Medical University of Vienna (Ethikkommission der Medizinischen Universitaet Wien, EK 2209/2018 & 1124/2020). Written informed consent

was signed by all patients prior to participation. The follow-up assessments were conducted through regular control visits, consistent with the clinical routine of our center.

Organ procurement and graft preservation

Organ procurement was carried out according to international standard following cold perfusion with histidine-tryptophan-ketoglutarate-solution (Custodiol® HTK, Koehler; Bensheim, Germany) via the abdominal aorta. No additional portal flush was performed. All liver grafts were preserved in ice-cold HTK after retrieval, until start of HOPE.

For HOPE, cannulas were placed into the portal vein and in case of dual HOPE (D-HOPE) additionally in the hepatic artery, during back table preparation. D-HOPE is the preferred option for machine perfusion of liver grafts in our center and is used whenever possible. The final decision to perform HOPE or D-HOPE was made by discretion of the transplant surgeon in charge, based on anatomic characteristics of the respective liver and logistic details. The graft was primed with Belzer MPS® UW machine perfusion solution, without any supplementation and then connected to the disposable set of the Liver Assist® perfusion device (XVIVO Perfusion AB, Goteborg, Sweden). Grafts were perfused at 10–12 ℃ with continuous portal pressure of 3–5 mmHg and a pulsatile (60 bpm) arterial pressure of 20–25 mmHg. Perfusion was started before skin incision and lasted until the end of hepatectomy.

Sample collection and Sdc-1 measurements

Samples were collected from perfusate during HOPE at 5, 60 and 120 min, as well as from recipient serum preoperatively (pre-OP), postoperatively (post-OP, within 1 hour of reperfusion), and on postoperative days (POD) 1, 3 and 7 after transplantation. After retrieval of blood, samples were centrifuged for 15 min at 3,000 rpm and immediately stored in cryovials at –80 ℃.

Sdc-1 concentrations were determined by enzyme-linked immunoassay (ELISA) via a commercially available kit (Diaclone Research; Besancon, France) according to manufacturer's recommendations.

Concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine kinase, gamma-glutamyl transferase (γGT), alkaline phosphatase (AP), lactate and bilirubin were measured at multiple time points to assess graft injury. Sdc-1 concentrations were tested for association with EAD, as well as graft survival and other clinical parameters, including AST, ALT, γGT, AP, lactate, bilirubin, glomerular filtration rate (GFR), international normalized ratio (INR), activated partial thromboplastin time (aPTT), platelets, hemoglobin and creatinine.

EAD was defined according to clinical standards by reaching at least one of the following laboratory criteria: serum AST or ALT concentration >2,000 IU/L within the first week after transplantation, INR ≥1.6 or serum bilirubin concentration ≥10 mg/dL both on day 7 after transplantation (14).

LT

Patients received a body-to-weight matched and AB0 identical graft. LT was performed with total cava replacement technique without veno-venous bypass and reperfusion was started simultaneously via hepatic artery and portal vein. End-to-end bile duct anastomosis was performed. Neither bile duct, nor intra-abdominal drains were placed routinely. After surgery, all patients received intensive care and local standardized immunosuppression protocol, as described previously (12).

Statistical analysis

Sample size calculation and data analysis methods were based on previous work (13,15). Data analysis was performed with R/RStudio (Vienna, Austria) and SPSS Statistics 25 (IBM, Armonk, NY, USA). For statistical analysis, patients were grouped based on the occurrence of EAD. Sdc-1 concentrations were analyzed using Mann-Whitney *U* test, binary logistic regression and receiver operating characteristics (ROC). In case of multiple testing P values were adjusted following the Benjamini & Hochberg method. Multivariate logistic regression was conducted using stepwise forward exclusion for all parameters with a P value <0.1 upon univariate analysis to account for limited sample size. To test if categorical variables influenced the frequency of EAD, Chi square test was used. Correlation of metric parameters was analyzed by Pearson's correlation, where an R value range 0.00–0.19 is considered "very weak", 0.20–0.39 for "weak", 0.40–0.59 for "moderate", 0.60–0.79 for "strong", and 0.80–1.0 for "very strong". The association between graft survival and Sdc-1 concentration as well as EAD was analyzed with cox proportional hazards model. Missing data were addressed by excluding individual

data points with missing values while retaining cases with complete information for the analysis. This method was chosen to minimize data loss while ensuring the integrity of the analysis.

Results

Donor and recipient characteristics

Patient and donor characteristics of 40 patients included in this study are visualized in *Table 1*. Median age was 54.9 years (IQR, 49.5–61.2 years), and median body mass index (BMI) was evaluated at 27.4 kg/m^2 (IQR, 23.6–30.9 kg/m^2). Median pre-OP model of end-stage liver disease (MELD) score was 18 (IQR, 13–22), which can be considered low and is most likely explained by the high frequency of hepatocellular carcinoma patients in this cohort. In particular, most frequent indications for transplantation were alcoholic cirrhosis (14 patients, 35%), hepatocellular carcinoma (9 patients, 22.5%), primary sclerosing cholangitis (4 patients, 10%), primary biliary cholangitis (4 patients, 10%), metabolic diseases (2 patients, 5%) and acute liver failure (2 patients, 5%) (see *Table 1*).

The grafts transplanted consisted of 38 donations after brain death (DBD) and two donations after circulatory death (DCD). Median Eurotransplant donor risk index (ET-DRI) was 1.635 (IQR, 1.420–1.935). Median cold ischemic time was 294 min (IQR, 233–342 min). For DCD-grafts warm ischemic time was 12 and 23 min. Sixteen grafts were perfused via the portal vein alone and the remaining 24 grafts additionally via the hepatic artery (D-HOPE), the median duration of perfusion was 173 min (IQR, 132–248 min) (see *Table 1*). Importantly, there was no significant difference in duration of perfusion between grafts subjected to HOPE or D-HOPE (P=0.86).

Over the entire hospital stay, 21 patients suffered from complications grade 3b or higher according to Dindo *et al.* (16). Median length of ICU and hospital stay were 9 days (5–12.8 days) and 19 days (16.0–30.5 days) respectively. Fourteen patients (35.0%) developed EAD after LT. Of these fourteen patients, two individuals required retransplantation and displayed early mortality within the first postoperative year. EAD was not associated with an increased incidence of complications grade 3b or higher [10 of 14 (71.4%) *vs.* 11 of 26 (42.3%), P=0.11]. Six patients (15%) deceased within the first year after LT, 8 patients over the entire observational period [median follow-up: 3.5 (3.3–4.0) years] no association with Sdc-1 concentrations could be detected with cox proportional hazards model (P=0.79), however EAD was associated with a significant

Table 1 Baseline characteristics of recipients, donors and hypothermic oxygenated perfusion

Characteristics	Entire cohort (n=40)	No EAD (n=26)	EAD (n=14)	P value
Recipient characteristic				
Female	7 (17.5)	6 (23.1)	1 (7.1)	0.40
Male	33 (82.5)	20 (76.9)	13 (92.9)	
Age (years)	54.9 (49.5–61.2)	53.0 (47.9–63.4)	57.5 (51.2–60.1)	0.43
BMI (kg/m^2)	27.4 (23.6–30.9)	27.4 (23.7–30.4)	27.4 (23.4–31.6)	0.60
MELD score	18 (13–22)	16 (12–22)	19 (15–23)	0.53
Indications for LT				0.20
Alcoholic cirrhosis	14 (35.0)	8 (30.8)	6 (42.9)	
Hepatocellular carcinoma	9 (22.5)	4 (15.4)	5 (35.7)	
Primary sclerosing cholangitis	4 (10.0)	2 (7.7)	2 (14.3)	
Primary biliary cholangitis	4 (10.0)	4 (15.4)	0 (0.0)	
Metabolic diseases	2 (5.0)	2 (7.7)	0 (0.0)	
Acute liver failure	2 (5.0)	1 (3.8)	1 (7.1)	
Other indications	5 (12.5)	5 (19.2)	0 (0.0)	

Table 1 *(continued)*

Table 1 *(continued)*

Characteristics	Entire cohort (n=40)	No EAD (n=26)	EAD (n=14)	P value
Graft and donor characteristics				
Donations after brain death	38 (95.0)	25 (96.2)	13 (92.9)	>0.99
Donations after circulatory death	2 (5.0)	1 (3.8)	1 (7.1)	
Warm ischemic time (min)	17.5	23.0	12.0	–
Female	16 (40.0)	13 (50.0)	3 (21.4)	0.16
Male	24 (60.0)	13 (50.0)	11 (78.6)	
Age (years)	53.0 (45.0–65.0)	56.0 (45.0–65.0)	50.5 (46.5–61.3)	0.79
BMI (kg/m^2)	25.0 (23.0–29.0)	25.0 (23.0–28.0)	27.0 (24.0–29.0)	0.39
Height (cm)	175 (168–180)	170 (166–180)	180 (175–183)	0.02
γGT (U/L)	37.0 (22.5–78.8)	41.5 (26.2–74.8)	25.0 (21.0–73.5)	0.37
DRI	1.596 (1.403–1.961)	1.520 (1.425–1.966)	1.638 (1.402–1.943)	0.87
ET-DRI	1.635 (1.420–1.935)	1.595 (1.413–1.965)	1.665 (1.588–1.888)	0.57
Cold ischemic time (min)	294 (233–342)	268 (189–304)	347 (305–418)	0.007
Cause of death				0.15
Hypoxia	4 (10.0)	2 (7.7)	2 (14.3)	
Cerebrovascular accident	27 (67.5)	20 (76.9)	7 (50.0)	
Trauma	8 (20.0)	3 (11.5)	5 (35.7)	
Not specified	1 (2.5)	1 (3.8)	0 (0.0)	
Perfusion characteristics				
HOPE	16 (40.0)	8 (30.8)	8 (57.1)	0.20
D-HOPE	24 (60.0)	18 (69.2)	6 (42.9)	
Perfusion duration (min)	173 (132–248)	178 (148–240)	155 (68–252)	0.41
Total preservation time (min)	455 (416–574)	439 (407–548)	521 (451–584)	0.08
Portal venous (at 60 min)				
Flow (L/min)	0.30 (0.26–0.38)	0.31 (0.26–0.34)	0.30 (0.24–0.54)	0.86
Pressure (mmHg)	5.0 (3.0–6.0)	4.0 (3.0–5.0)	6.0 (5.0–7.0)	0.02
Vascular resistance (mmHg/L/min)	16.4 (12.4–20.2)	17.0 (11.9–19.4)	14.9 (14.0–22.6)	0.42
Arterial[†] (at 60 min)				
Flow (mL/min)	117.5 (44.8–184.3)	130.5 (47.3–202.8)	94.0 (51.3–127.0)	0.57
Pressure (mmHg)	30.0 (29.0–30.3)	30.0 (29.0–30.0)	30.5 (30.0–31.0)	0.17
Vascular resistance (mmHg/mL/min)	0.25 (0.19–0.55)	0.24 (0.18–0.47)	0.38 (0.24–0.66)	0.67

Data are presented as n (%) or median (interquartile range), unless otherwise specified. [†], parameters of arterial perfusion were only evaluated for grafts subjected to D-HOPE. EAD, early allograft dysfunction; BMI, body mass index; MELD, model for end stage liver disease; LT, liver transplantation; γGT, gamma-glutamyl transferase; DRI, donor risk index; ET-DRI, Eurotransplant donor risk index; HOPE, hypothermic oxygenated perfusion; D-HOPE, dual HOPE.

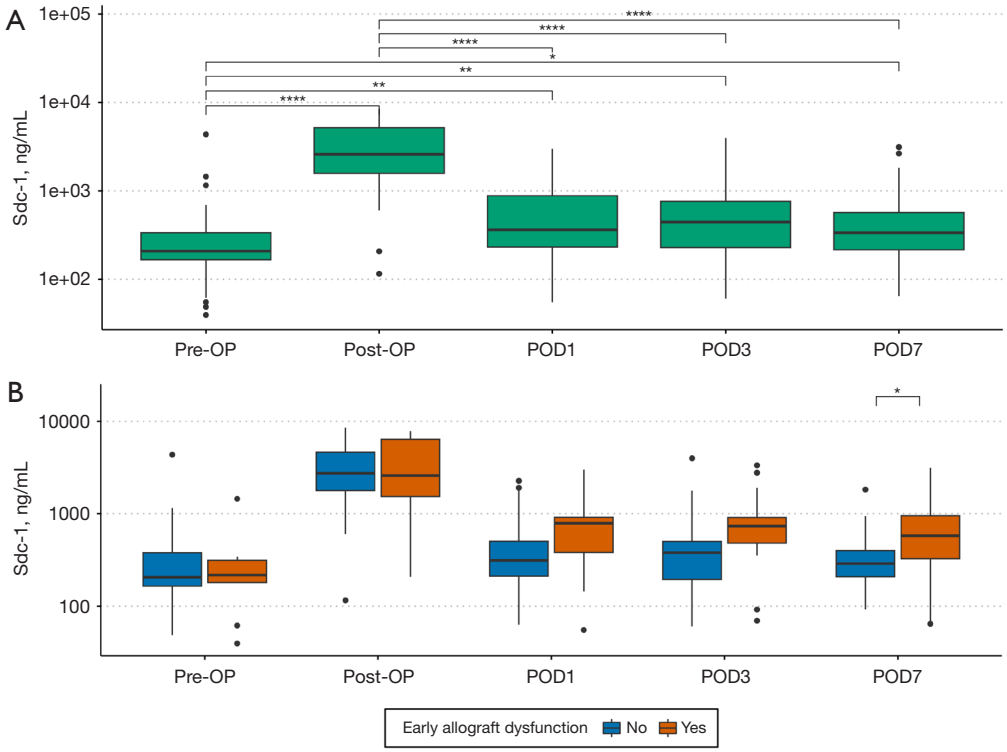

Figure 1 Sdc-1 concentration in serum of recipients after HOPE. (A) All patients. (B) Patients regarding the occurrence of EAD. Asterisks are indicating P values of Mann-Whitney *U* testing: *, P≤0.05; **, P≤0.01; ***, P≤0.001; ****, P≤0.0001. Number of observations: pre-OP, n=40; post-OP, n=40; POD1, n=38, POD3, n=39; POD7, n=38. Sdc-1, syndeacan-1; HOPE, hypothermic oxygenated machine perfusion; EAD, early allograft dysfunction; OP, operative; POD, postoperative day.

reduction in 1-year survival (recipient death: EAD 4 of 14 *vs.* no EAD 2 of 26; see Figure S1). Biliary complications were observed in 13 patients (32.5%), 8 of which were EAD patients (P=0.03).

Perioperative Sdc-1 levels in recipient serum are regenerating slower in patients with EAD

To assess the perioperative course of glycocalyx degradation, Sdc-1 was measured in serum of patients who received LT after HOPE. The perioperative dynamics of Sdc-1 concentration are visualized in *Figure 1A*. A significant increase of Sdc-1 from baseline was observed in the immediate postoperative period (median pre-OP 198 ng/mL *vs.* median post-OP 3,070 ng/mL, P<0.001), which was followed by a sudden decrease on POD1 (median POD1 =741 ng/mL, P<0.001).

Levels remained high in the subsequent postoperative period, when compared to baseline and returned to almost pre-OP levels on POD7 (median POD7 =336 ng/mL, P=0.05). Moreover, perioperative Sdc-1 levels were compared between patients who did or did not develop EAD. Here, no difference in Sdc-1 levels was observed pre-OP, post-OP and on POD1. On POD3 patients without EAD showed a trend towards lower Sdc-1 concentration (median POD3 no EAD: 379 ng/mL *vs.* median POD3 EAD: 734 ng/mL, P=0.054), whereas lower Sdc-1 concentrations were seen in patients without EAD on POD7 (median POD7 no EAD: 288 ng/mL *vs.* median POD7 EAD: 577 ng/mL, P=0.04) as seen in *Figure 1B*.

Sdc-1 concentration during HOPE is elevated in EAD grafts

Consequently, we aimed to evaluate Sdc-1 levels at different time points during HOPE (*Figure 2A*). Sdc-1 concentration increased after 60 min (median 451 ng/mL) of HOPE, compared to 5 min (median 252 ng/mL, P<0.001). Sdc-1 levels remained high until 120 min of HOPE (median 625 ng/mL, P<0.0001), while no additional significant increase was observed between 60 and 120 min

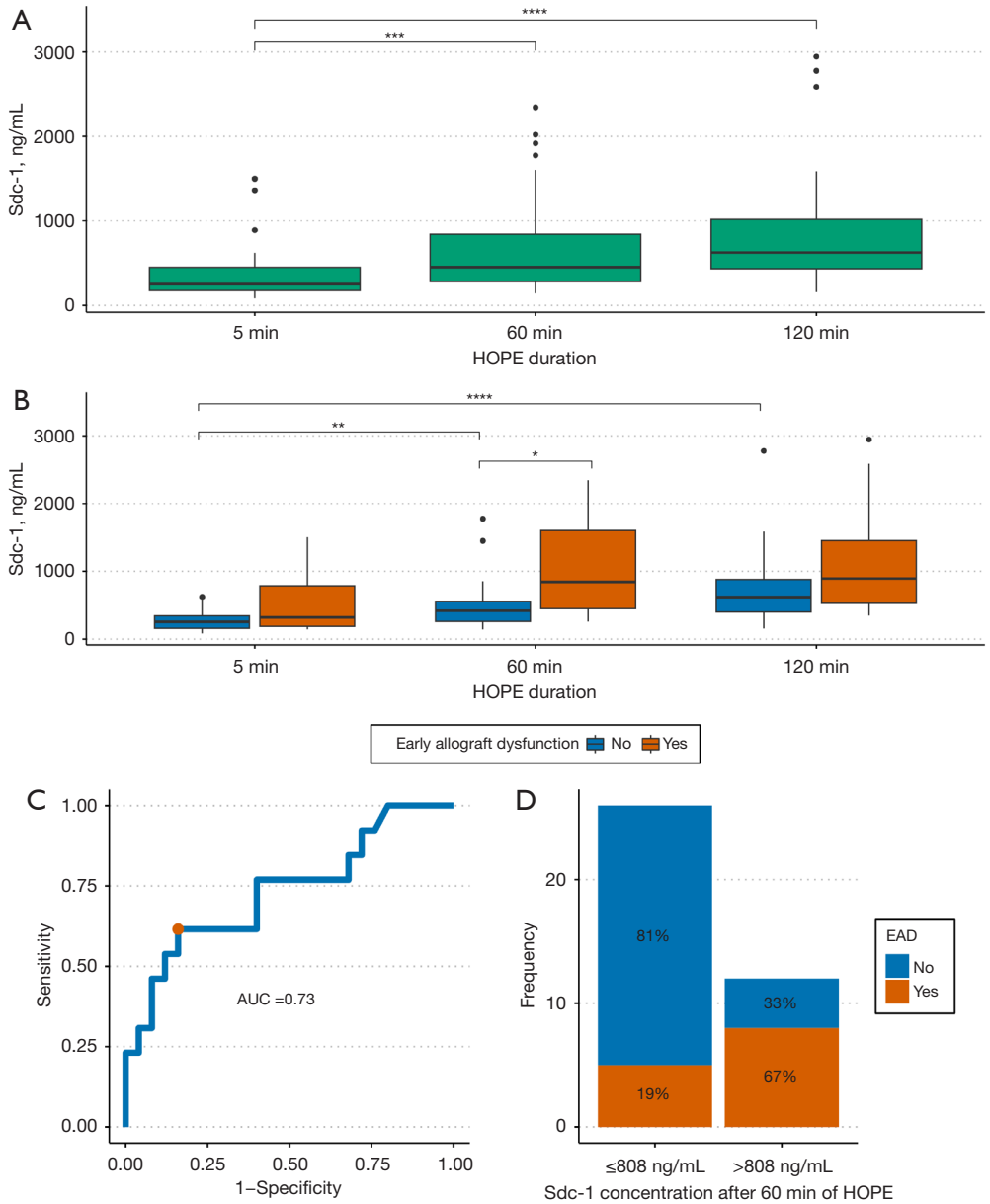

Figure 2 Sdc-1 concentration in perfusate during HOPE. (A) All HOPE patients. (B) HOPE patients regarding the occurrence of EAD. (C) ROC of EAD prediction by Sdc-1 concentration in perfusate after 60 min of HOPE. AUC of 73% (P=0.02, cut-off value of >808 ng/mL indicated by orange point, sensitivity 66.7% and specificity 84.6%). (D) Incidences of EAD in respective subgroups according to Sdc-1 cut-off after 60 min of HOPE. Asterisks are indicating P values of Mann-Whitney *U* testing: *, P≤0.05; **, P≤0.01; ***, P≤0.001; ****, P≤0.0001. Number of observations: 5 min, n=39; 60 min, n=38; 120 min, n=31. Sdc-1, syndeacan-1; HOPE, hypothermic oxygenated machine perfusion; EAD, early allograft dysfunction; ROC, receiver operating characteristic; AUC, area under the curve.

(P=0.12). When comparing HOPE and D-HOPE, Sdc-1 concentrations were comparable within the first 60 min of perfusion, whereas after 120 min D-HOPE perfused grafts showed significantly lower Sdc-1 levels (median 120 min D-HOPE: 558 ng/mL, median 120 min HOPE: 1,048 ng/mL, P=0.02, see Figure S2). Sdc-1 concentrations at 60 min showed a weak correlation with cold ischemic time (R=0.36) and donor size (R=0.33) as shown in Table S1.

When comparing Sdc-1 concentrations in perfusate of grafts that did or did not develop EAD (*Figure 2B*) similar

Table 2 Prediction of EAD with Sdc-1 at 808 ng/dL

Statistical measures	Value (95% CI)
Specificity (%)	84.0 (63.9–95.5)
Sensitivity (%)	61.5 (31.5–86.1)
Negative predictive value (%)	84.0 (63.9–95.5)
Positive predictive value (%)	66.7 (42.5–84.4)

EAD, early allograft dysfunction; Sdc-1, syndecan-1.

values were observed after 5 min of HOPE (median 5 min no EAD: 252 ng/mL, median 5 min EAD: 320 ng/mL, P=0.18). However, after 60 min of perfusion, the Sdc-1 concentration was significantly higher in the perfusate of grafts which later developed EAD (median 60 min no EAD: 417 ng/mL, median 60 min EAD: 843 ng/mL, P=0.02) compared to graft exhibiting normal postoperative function. Interestingly, levels of Sdc-1 in perfusate were not different between the two evaluated subgroups at 120 min of HOPE (median 120 min no EAD: 621 ng/mL, median 120 min EAD: 894 ng/mL, P=0.10).

Sdc-1 after 60 min of HOPE allows robust prediction of EAD

In order to evaluate predictive potential of 60-min Sdc-1 concentrations for EAD, ROC analysis was carried out. Strikingly, a good discriminatory potential with an area under the curve (AUC) of 0.73 was observed (*Figure 2C*). A cut-off was evaluated at 808 ng/mL and obtained a sensitivity of 61.5% with a specificity of 84.0%, as well as a positive predictive value of 66.7% and a negative predictive value of 84.0% for the prediction of EAD (*Table 2*). Using this cut-off, a high-risk group with an incidence of 66.7% EAD could be identified (67% *vs.* 19%, P=0.01, *Figure 2D*). Intriguingly, there was no correlation of Sdc-1 with other dynamic biomarkers routinely evaluated during HOPE (*Table 3*). However, Sdc-1 at 60 min was found to correlate with recipient transaminases on POD1 and with peak transaminases (Table S1). Primary outcome parameter, EAD, was evaluated for all 40 recipients. Primary variable of interest, Sdc-1 concentration at 60 min, had two missing values.

Discussion

The present investigation explored dynamics of Sdc-1 as a marker for glycocalyx degradation during HOPE and within the perioperative period in patients undergoing LT. Sdc-1 was found to increase within the perfusate during HOPE and in patient sera during the immediate postoperative period, indicating that glycocalyx damage occurs during HOPE and can still be observed in LT recipients shortly after the operation. Strikingly, Sdc-1 at 60 min during HOPE is significantly increased in patients who will develop EAD. This difference allows prediction of EAD, which renders Sdc-1 a vital biomarker during HOPE.

The majority of biomarkers during machine perfusion prior to LT in clinical use today, are so far only explored during normothermic machine perfusion (17-20). Here, quantification of organ function is essential, as the primary use of normothermic machine perfusion at this point is viability testing for marginal liver grafts. In contrast, HOPE is believed to improve graft quality by reducing IRI. However, the availability of quantitative parameters for the prediction of organ viability in the setting of HOPE is limited. To our knowledge, the only biomarker used for viability assessment during HOPE today, is flavin mononucleotide (FMN) (21,22). FMN is a prosthetic group of respiratory chain complex 1, and is released upon mitochondrial damage. Although reported outcomes of studies in which FMN was used for viability assessment were satisfactory, it has yet to be validated in a bigger patient cohort or a multicenter trial. While viability assessment based on mitochondrial damage seems very promising, additional markers reflecting on other pathways are needed in order to add further insight and improve prediction of outcome after LT. The endothelial glycocalyx as a mechanistic site of IRI could potentially address this need. Interestingly, perfusate Sdc-1 did not correlate with other routinely used markers evaluated during HOPE. In particular, we could not observe an association with transaminases, lactate or FMN in perfusate. This pattern further underlines the importance of Sdc-1 evaluation during HOPE, as this biomarker adds information to the prediction of postoperative outcome independently of currently used parameters. Several of these biomarkers obtained comparable AUCs in ROC analysis, however their discriminatory potential regarding EAD in logistic regression analysis was inferior to Sdc-1 (Figure S3, Table S2).

During ischemia, the absence of oxygen stalls the mitochondrial electron transfer chain, leading to a lack of cellular ATP and accumulation of succinate, lactate and calcium in the cytosol, causing damage at intracellular membranes and enzymes ultimately leading to apoptotic and

Table 3 Correlation of perfusate variables with Sdc-1 after 5, 60 and 120 min of hypothermic oxygenated perfusion

Variables	Sdc-1			P value (EAD yes *vs*. no)
	5 min	60 min	120 min	
Sdc-1				
5 min	1.00			0.17
60 min	0.87****	1.00		0.02
120 min	0.80****	0.93****	1.00	0.10
AST				
5 min	0.03	−0.04	−0.09	0.30
60 min	0.11	0.10	0.02	0.01
120 min	0.17	0.12	0.19	0.058
ALT				
5 min	0.02	−0.04	−0.07	0.33
60 min	0.09	0.07	0.00	0.008
120 min	0.16	0.13	0.17	0.047
LDH				
5 min	0.02	−0.04	−0.07	0.42
60 min	0.04	0.07	−0.01	0.008
120 min	0.12	0.09	0.17	0.07
Lactate				
5 min	0.10	0.04	−0.15	0.45
60 min	0.09	0.04	−0.05	0.18
120 min	−0.05	−0.03	0.09	0.94
Glucose				
5 min	0.23	0.11	−0.07	0.74
60 min	0.28	0.26	0.01	0.58
120 min	−0.02	0.03	0.06	0.58
FMN				
5 min	−0.06	−0.09	−0.16	0.005
60 min	−0.06	−0.03	−0.07	0.03
120 min	−0.03	−0.07	−0.02	0.13
Portal venous flow				
5 min	0.14	0.06	−0.02	0.55
60 min	0.12	0.00	−0.12	0.86
120 min	0.09	−0.14	−0.17	0.88

Table 3 *(continued)*

Table 3 *(continued)*

Variables	Sdc-1			P value (EAD yes *vs*. no)
	5 min	60 min	120 min	
Portal venous pressure				
5 min	0.26	0.23	−0.18	0.02
60 min	0.29	0.26	−0.12	0.02
120 min	0.02	−0.16	−0.19	0.11
Portal venous vascular resistance				
5 min	0.03	0.11	−0.05	0.33
60 min	0.01	0.12	0.07	0.67
120 min	−0.04	0.09	0.07	0.47
Arterial flow				
5 min	0.43*	0.42*	0.54*	0.29
60 min	0.40	0.37	0.49*	0.57
120 min	0.51*	0.57*	0.51*	0.63
Arterial pressure				
5 min	0.07	0.05	−0.10	0.10
60 min	0.11	0.09	−0.11	0.17
120 min	−0.31	−0.08	−0.11	0.76
Arterial vascular resistance				
5 min	0.34	0.3	−0.24	0.28
60 min	0.3	0.25	−0.22	0.42
120 min	−0.26	−0.27	−0.25	0.63

*, P≤0.05; ****, P≤0.0001 of Pearson correlation. Sdc-1, syndecan-1; EAD, early allograft dysfunction; AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactate dehydrogenase; FMN, flavin mononucleotide.

necrotic cell death (23). After reperfusion, excessive amounts of ROS are produced, due to electrolyte-imbalances and mitochondrial damage, inducing damage to cellular membranes, proteins and DNA. Ultimately, this process culminates in necrosis and activation of apoptosis which further promotes non-pathogen triggered inflammatory responses via release of damage-associated molecular patterns and cytokines (23-25). During HOPE, the graft is perfused with oxygenated Belzer-MPS®-UW-solution at 10–12 ℃, via cannulas in the portal vein and optionally also via the hepatic artery. HOPE restores oxygen supply and therefore allows the organ to regenerate its ATP-storage in a controlled setting. HOPE has been shown to reduce IRI, leading to better graft function and lower complication rates (3,4,26). Glycocalyx oxidative stress during IRI is mediated by xanthine oxidase and NADPH oxidase 2. The release of ROS activates metalloproteases which cleave the glycocalyx meshwork-components and additionally stalls protease inhibitors further aggravating glycocalyx degradation (10). As the amount of ROS produced is depending on the duration of ischemia, shortening cold ischemic time by HOPE will benefit the grafts endothelial glycocalyx compared to SCS alone with comparable overall conservation time.

Our results indicate that glycocalyx is degraded during SCS and reoxygenation, as Sdc-1 concentration increases after start of HOPE until 60 min. Afterwards the increase in concentration slows down until 120 min indicating a plateau phase. Therefore, we hypothesize that the majority of Sdc-1 is released and/or shaded due to ischemic damage and

reoxygenation and glycocalyx degradation is stalled after the initial phase of HOPE. The association between Sdc-1 at 60 min and the duration of cold ischemic time further underlines this hypothesis.

For perfusions lasting longer than 60 min D-HOPE seems favorable in terms of glycocalyx degradation, whereas no difference for the first 60 min could be observed. This finding is indicative of a diverging sensitivity of arterial and portal venous endothelial glycocalyx during HOPE. Higher levels of Sdc-1 at late time points during HOPE might be attributed to insufficient supply of oxygen to arterial endothelium and subsequent increased degradation of its glycocalyx as an indicator of stress. Intriguingly, Sdc-1 concentrations in D-HOPE grafts showed moderate correlation with arterial flow rates, whereas no correlation with portal venous flow rates could be detected. Accordingly, an increase in arterial flow might ultimately also be associated with augmented glycocalyx degradation due to higher shear stress. However, the data included in the present investigation only allows for exploratory evaluation of underlying pathomechanisms. Thus, further mechanistical studies specifically focusing on differences in glycocalyx degradation between HOPE and D-HOPE are strongly encouraged. Especially explored differences regarding arterial flow and the dynamic of glycocalyx degradation need to be addressed in further translational investigations in order to improve the understanding of relevant mechanisms and to reveal potential clinical consequences.

Consequently, the optimal discriminatory potential of Sdc-1 between grafts which are more or less likely to develop EAD is met at 60 min of HOPE. In fact, Sdc-1 at 60 min reaches an AUC of 0.73 upon ROC analysis, indicating a good discriminatory potential for EAD. This further translates into a high-risk group of liver grafts with an incidence of EAD as high as 66.7%. Importantly, 80.7% of the grafts that were beneath the cut-off did not develop EAD. No association between Sdc-1 and survival could be detected, however all cases of recipient death within the first 6 months had developed EAD. Therefore, viability assessment with Sdc-1 could help to reduce the risk for early recipient death. Interestingly, the presently included cohort of donor organs can be considered low risk, as reflected by an ET-DRI of 1.635. Nonetheless, use of Sdc-1 allowed the identification of high-risk organs in this study. Accordingly, the present manuscript underlines the need for improved biomarkers for donor evaluation as well as during machine perfusion.

While our investigation provides novel insights into glycocalyx degradation during HOPE and liver graft viability assessment, several questions need to be addressed in future research. In fact, the limited number of patients included in the present investigation does not allow general application of the results on a broad spectrum of diverse donors and recipients. Accordingly, the presented results need to be validated in larger patient cohorts in order to consolidate the role of Sdc-1 as a marker for EAD development after HOPE. Further, there is no analytical method available which allows real-time estimation of Sdc-1 at this point. Accordingly, methodological improvements for Sdc-1 analysis need to be made in order to include it as a standard parameter during HOPE. Ultimately, mechanisms underlying IRI inflicted glycocalyx degradation and their implications for graft viability and transplantation outcome need to be studied in further detail.

Conclusions

The results of this study provide new insights into the degradation of the endothelial glycocalyx in the context of LT. Our findings suggest that Sdc-1 allows objective evaluation of liver graft quality and prediction of patient outcome at 60 min of HOPE. The presented data suggests that endothelial glycocalyx degradation may substantially contribute to pathophysiological aspects of IRI which is underrepresented by currently used liver assessment. The use of Sdc-1 assessment has the potential to ultimately improve overall outcomes after LT.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-24-33/rc

Data Sharing Statement: Available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-24-33/dss

Peer Review File: Available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-24-33/prf

Funding: This work was supported by Start Up Grant

2021 – Research Platform Transplantation of the Medical University of Vienna.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://hbsn.amegroups.com/article/view/10.21037/hbsn-24-33/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was performed at the General Hospital of Vienna, in accordance with the ethical standards laid down in the declaration of Helsinki (as revised 2013) and approved by the ethics committee of the Medical University of Vienna (Ethikkommission der Medizinischen Universitaet Wien, EK 2209/2018 & 1124/2020). Written informed consent was signed by all patients prior to participation.

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Cite this article as: Rauter L, Kollmann D, Schiefer J, Spasic M, Raeven P, Dingfelder J, Pereyra D, Baron DM, Pompouridou E, Soliman T, Berlakovich G, Györi G. Endothelial glycocalyx damage marker syndecan-1 during hypothermic oxygenated machine perfusion of donor grafts facilitates prediction of early allograft dysfunction after liver transplantation. HepatoBiliary Surg Nutr 2025;14(2):233-245. doi: 10.21037/hbsn-24-33