Endothelial Glycocalyx Shedding Predicts Donor Organ Acceptability and Is Associated With Primary Graft Dysfunction in Lung Transplant Recipients

Timothy M. Sladden, MBBS, BVSc,[1,2] Stephanie Yerkovich, PhD,[1,2] Michelle Grant, RN,[1] Fuming Zhang, PhD,[3,4] Xinyue Liu, PhD,[3,4] Michael Trotter, MBBS,[1,2] Peter Hopkins, MBBS,[1,2] Robert J. Linhardt, PhD,[3,4] and Daniel C. Chambers, MD[1,2]

Background. The endothelial glycocalyx, a sieve-like structure located on the luminal surface of all blood vessels, has been found to be integral to regulation of capillary permeability and mechanotransduction. Given this, we investigated the role of endothelial glycocalyx breakdown products in organ donors and recipients in terms of acceptability for transplant and risk of primary graft dysfunction (PGD). **Methods.** Endothelial glycocalyx breakdown products were measured in the peripheral blood of 135 intended and actual organ donors. Breakdown product levels were tested for association with donor demographic and clinical data, organ acceptability for transplant along with lung recipient outcomes (n = 35). Liquid chromatography mass spectrometry analysis was performed to confirm glycosaminoglycan levels and sulfation patterns on donor samples (n = 15). In transplant recipients (n = 50), levels were measured pretransplant and daily for 4 days posttransplant. Levels were correlated with PGD severity and intubation time. **Results.** Decreased hyaluronan levels in peripheral blood independently predicted organ acceptability in intended and actual donors (odds ratio, 0.96; [95% confidence interval, 0.93–0.99] P = 0.026). Furthermore, high donor syndecan-1 levels were associated with PGD in recipients (3142 [1575–4829] versus 6229 [4009–8093] pg/mL; P = 0.045). In recipient blood, levels of syndecan-1 were correlated with severe (grades 2–3) PGD at 72 hours posttransplant (5982 [3016–17191] versus 3060 [2005–4824] pg/mL; P = 0.01). **Conclusions.** Endothelial glycocalyx breakdown occurs in lung transplant donors and recipients and predicts organ acceptability and development of PGD. Glycocalyx breakdown products may be useful biomarkers in transplantation, and interventions to protect the glycocalyx could improve transplant outcomes.

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INTRODUCTION

In an era where the boundaries of organ procurement are being pushed by advances such as donation after circulatory death (DCD),[1] extended criteria organs,[1] and ex vivo lung perfusion (EVLP),[2,3] additional lung viability markers are required to better determine suitability for transplant. Successful use of "marginal donors" demonstrates that conventional suitability parameters—partial pressure of oxygen in arterial blood (PaO_2), radiographic infiltrate, donor demographic—incompletely characterizes the organ's potential.[4] Novel biomarkers have potential to complement the traditional acceptance criteria potentially expanding the donor pool, while minimizing early

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[1] Queensland Lung Transplant Service, The Prince Charles Hospital, Brisbane, Australia.

[2] Faculty of Medicine, The University of Queensland, Brisbane, Australia.

[3] Departments of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, NY.

[4] Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, NY.

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Correspondence: Daniel Chambers, MBBS, MD, Queensland Lung Transplant Service, Level 1, Administration Building, The Prince Charles Hospital, Rode Rd, Chermside, Queensland 4032, Australia. (daniel.chambers@health.qld.gov.au).

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posttransplant complications, in particular primary graft dysfunction (PGD).

PGD is one of the most serious early posttransplant complications. Increased pulmonary vascular resistance and capillary permeability leads to pulmonary edema and impaired gas exchange.[5-7] The risk of PGD is therefore a significant consideration in the decision to accept an organ for transplant, and although many markers have been examined for donor selection and the prediction of PGD,[8-10] so far none have proven clinical utility. However, few of these biomarkers have specifically examined the vasculature,[11-16] and none have examined the endothelial glycocalyx.

The endothelial glycocalyx is a mesh-like, highly charged layer that projects more than 1 μm into the lumen of all blood vessels. It interacts with overlying albumin and binds circulating plasma components.[17] It is comprised of proteoglycans, namely, syndecan-1 and CD44, that anchor to the underlying endothelium, with branching glycosaminoglycan side chains (namely heparan sulfate, chondroitin sulfate, and hyaluronan) forming a mesh-like barrier to solutes.[17,18] Damage to this structure results in shedding of these components into the bloodstream.[19-21] The role of the endothelial glycocalyx is both diverse and critical as it provides a vascular barrier,[22] transduces sheer stress,[23-26] while permitting leukocyte-endothelial cell interactions[27-29] and macromolecule storage.[30,31] Dysfunction of the endothelial glycocalyx can occur through partial or complete loss of its components and results in impaired vascular regulation and increased vascular permeability.[19,32-34] The glycocalyx is particularly prone to injury induced by ischemia and reperfusion.[27,33] Furthermore, shedding of the pulmonary glycocalyx has been linked to respiratory failure and the development of acute respiratory distress syndrome in mice.[32] The use of heparan sulfate, syndecan-1, and hyaluronan as endothelial glycocalyx breakdown biomarkers in observational human studies have associated increased levels with acute lung injury,[32,35] heart failure,[36] cardiothoracic surgery,[21,37] and major trauma.[38] Blood endothelial glycocalyx biomarkers have been correlated to visual shedding of the pulmonary endothelial glycocalyx in mouse studies[19,39,40]; however this validation and the exclusion of extraendothelial sources remain to be confirmed in human studies.

Hence, we hypothesized that injury to the endothelial glycocalyx determined by increased circulating endothelial glycocalyx breakdown products in the organ donation process is associated with poor organ function, reduced acceptability of donor lungs for transplant and ultimately PGD in transplant recipients. Specifically, the aims of the study were to (1) correlate circulating endothelial glycocalyx breakdown products in peripheral blood of potential lung donors to organ function and acceptability for transplant and (2) to assess glycocalyx breakdown products in peripheral blood of lung transplant recipients and investigate any association with PGD.

MATERIALS AND METHODS

Ethics

This study was approved by The Prince Charles Hospital Human Research and Ethics Committee (HREC/13/QPCH/154). Families of patients considered for organ donation were approached for consent for research as a part of the normal organ donation process. Lung transplant recipients were approached for consent pretransplant, and only those who provided written, informed consent were included in the study.

Organ Donors

Donor demographic and clinical data were collected from charts held with the Organ and Tissue Authority, Brisbane, Australia. Organs were either acceptable for transplant or not acceptable as outlined in Figure 1. To remove interphysician bias, the organ classification made at the time of transplant was reviewed by an independent, blinded, transplant physician who reclassified the organs as either acceptable for organ transplant or unacceptable as per current clinical protocols. When there was a discrepancy, a third blinded transplant physician reviewed the notes. Those deemed not acceptable for transplant were further classified as not medically suitable for transplant due to poor PaO_2 (collected at 100% FiO_2, 5 cm H_2O positive end expiratory pressure), chest infiltrates on radiograph and aspiration pneumonia or other medical reasons such as donor age, history of cancer, failure to progress to DCD within the time frame or medical conditions unrelated to the lungs.

Lung Transplant Recipients

Between January 2013 and December 2015, 65 lung transplants were performed with 50 (77%) of these recipients providing consent and having blood taken at the time of listing and then at 12 hours posttransplant and daily

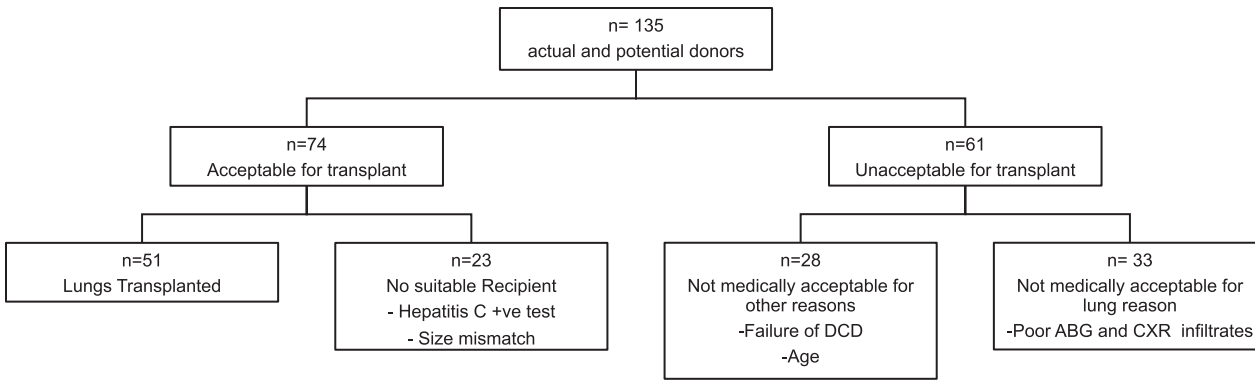

FIGURE 1. Organ donor outcomes. CXR, chest x-ray; DCD, donation after circulatory death.

for 4 days. All transplants were bilateral sequential lung transplants performed while on cardiopulmonary bypass. Blood samples were immediately centrifuged (1000 *g* for 10 min) and plasma was stored at −80°C for later batch analysis. Demographic and clinical data, including PGD grades (as defined by International society of Heart Lung Transplantation guidelines[41]), time in the intensive care unit (ICU), time incubated, and 30-day mortality were accrued. PGD was assessed initially at 24 hours and then at 72 hours as this time point is recognized to best predict subsequent outcomes.[9] Severe PGD was defined as PGD grade 2 or PGD grade 3, as these have been associated with inferior short- and long-term mortality.[42,43]

ELISA Measurement of Endothelial Glycocalyx Breakdown Products

Endothelial glycocalyx breakdown products were measured using commercially available hyaluronan (R&D Systems Inc., Minneapolis, MN), syndecan-1(R&D Systems Inc., Minneapolis, MN), CD44 (R&D Systems Inc., Minneapolis, MN), and heparan sulfate (Cusabio Biotech Co Ltd, Wuhan, Hebei, China) enzyme-linked immunosorbent assays (ELISAs) following manufacturer's instructions.

Liquid Chromatography Mass Spectrometry Analysis of Endothelial Glycocalyx Breakdown Products

To gain insight into the mechanism(s) leading to glycocalyx shedding, we selected a random small subgroup of donors (n = 15), who had sufficient sample for liquid chromatography mass spectrometry (LCMS). The aim of LCMS was to determine the sulfation patterns of heparan sulfate, which can point to the mode(s) of glycocalyx shedding. Second, we aimed to measure low–molecular weight hyaluronan levels, a potent proinflammatory molecule. Because the commercially available hyaluronan ELISA cannot detect hyaluronan fragments <35 kDa, assessment of levels of individual disaccharides (400 Da) was dependent on LCMS. See **Materials and Methods (SDC,** http://links.lww.com/TP/B658) for in-depth LCMS methodology.

Statistical Analysis

Results are presented as mean (± standard deviation [SD]), unless otherwise indicated. All statistical analyses were performed using STATA 11 (StataCorp, TX). Group differences were assessed by *t* test, Kruskal-Wallis test, Mann-Whitney *U* test, Pearson Chi-square, or Fisher exact test, as appropriate. Paired samples were compared by Wilcoxon signed-rank test. Logistic regression was used to identify factors associated with organ utilization. Covariates included donor demographic factors, clinical factors, and endothelial glycocalyx breakdown products. Variables were included in a multivariate model if $P < 0.1$, and the model retained covariates where predictors were statistically significant at $\alpha = 0.05$.

RESULTS

Donor Demographics

In our jurisdiction between 2011 and 2014, there were 350 listed organ donors, including actual organ donors and intended donors, those which were not medically suitable for transplant or due to positive serological tests or family/coronial limitations or DCD donors in which death failed to occur within time frame for retrieval. Of these, 135 where consent for research had been given had plasma samples available, collected at the time of donor notification. Organ donor classification into acceptable (n = 74) and unacceptable (n = 61) organs for transplant along with those organs which were transplanted is provided in Figure 1. As would be expected, lungs deemed acceptable for transplant had reduced donor aspiration events and a higher PaO_2 (Table 1).

Endothelial Glycocalyx Breakdown Products and Donor Characteristics

Donor peripheral blood levels of glycocalyx breakdown products are shown in Figure 2. Circulating hyaluronan levels were lower in donors whose lungs were acceptable for transplant compared with those whose lungs were deemed not acceptable. Hyaluronan levels were also lower in donors whose organs were actually transplanted (59.9 [26.2–91.5] versus 74.4 [40–163] ng/mL; $P = 0.03$). Higher hyaluronan levels were also associated with cigarette or marijuana use (49 [26–80] versus 90 [34–135] ng/mL; $P = 0.03$). Both syndecan-1 and hyaluronan levels were increased in donors with a traumatic cause of death (eg, motor vehicle accidents) compared with atraumatic causes (eg, intracranial hemorrhage) (3564 [1865–6561] versus 5685 [3140–8454] pg/mL; $P = 0.01$ and 54 [28–111] versus 89 [60–240] ng/mL; $P = 0.007$). Elevated syndecan-1 levels were also seen in donors with unstable blood pressure (3727 [1989–6565] versus 5215 [3402–9717] pg/mL; $P = 0.01$) and those with a history of lung disease (3487 [1865–6965] versus 5042 [3738–9275] pg/mL; $P = 0.007$). Heparan sulfate levels were not related to any donor factors (data not shown). Donation type, PaO_2, intubation period, and aspiration events were not associated with peripheral blood levels of any endothelial glycocalyx breakdown products. Glycocalyx product levels were correlated with each other: heparan sulfate levels were significantly associated with hyaluronan ($r = 0.24$; $P = 0.01$), syndecan-1 ($r = 0.21$; $P = 0.03$), and CD44 ($r = 0.52$; $P = <0.001$), while hyaluronan was associated with syndecan-1 ($r = 0.4$; $P = <0.001$) but not CD44 ($r = 0.17$; $P = 0.16$).

Low Hyaluronan Levels Are Independently Associated With Lung Acceptability in Organ Donors

We next used logistic regression modeling to understand what factors were independently associated with acceptability of lungs for transplant (Table 2). Univariate analysis showed that a donor history of aspiration events, having a pre-existing lung condition, the presence of diabetes and smoking >20 pack years were all associated with decreased acceptability. In contrast, low hyaluronan levels and an increased PaO_2 were associated with acceptability of lungs for transplant. No other donor or clinical factors were associated. Multivariate analysis identified that only a higher PaO_2 and decreased hyaluronan levels were associated with the organs being acceptable for transplant. Indeed, for each 10 ng/mL decrease in hyaluronan, there was a 4% increase in the likelihood of the organ being acceptable for transplant.

TABLE 1.

Characteristics of 135 organ donors by acceptability for transplant who had endothelial glycocalyx breakdown products measured in a peripheral blood sample at the time of listing for organ donation

Organ donor cohort characteristics			
Variable	**Acceptable (74)**	**Unacceptable (61)**	***P***
Age (y)	43.5 (15.3)	43.3 (18)	0.941
Female sex, n (%)	38 (51.4)	29 (47.5)	0.730
Smoking history >20 PkYrs, n (%)	27 (54)	17 (44.7)	0.519
Marijuana use, n (%)	16 (21.6)	17 (44.7)	0.687
Pre-existing lung condition, n (%)	13 (17.8)	20 (32.8)	0.069
Diabetes (yes), n (%)	4 (5.4)	6 (9.8)	0.347
Intubation time (h)	88.66 (77.3)	91.02 (65.4)	0.861
Cause of death (nontraumatic) n (%)	57 (77)	41 (67.2)	0.246
Aspiration event (yes), n (%)	22 (31.9)	32 (55.2)	0.011[a]
Chest trauma (yes), n (%)	4 (6.8)	4 (5.5)	0.999
Unstable blood pressure n (%)	14 (18.92)	14(22.95)	0.671
Donor arterial blood gas at offer			
pH	7.37 (0.08)	7.37 (0.09)	0.994
PaO_2 (mm Hg)	410 (87)	304 (144)	<0.001[a]
$PaCO_2$ (mm Hg)	40.7 (9.0)	40.6 (8.3)	0.939
Bicarbonate	23.50 (3.18)	23.32 (5.90)	0.816
Base excess	−1.42 (3.66)	−0.49 (4.90)	0.217
Lungs transplanted, n (%)	51 (68.9)	0 (0)	–
Unacceptable for medical reasons, n (%)	0 (0)	33 (54.1)	–
Donor type: DCD, n (%)	15 (20.3)	22 (36.7)	0.051

A total of 74 (55%) were deemed suitable for transplantation and 61 (41%) were actually transplanted. Unstable blood pressure—donors requiring prolonged ionotropic support or medical management for hypertensive crisis. Mean (SD) is shown unless otherwise specified.
[a]Indicates $P < 0.05$.
DCD, donation after circulatory death; PkYrs, pack years; SD, standard deviation.

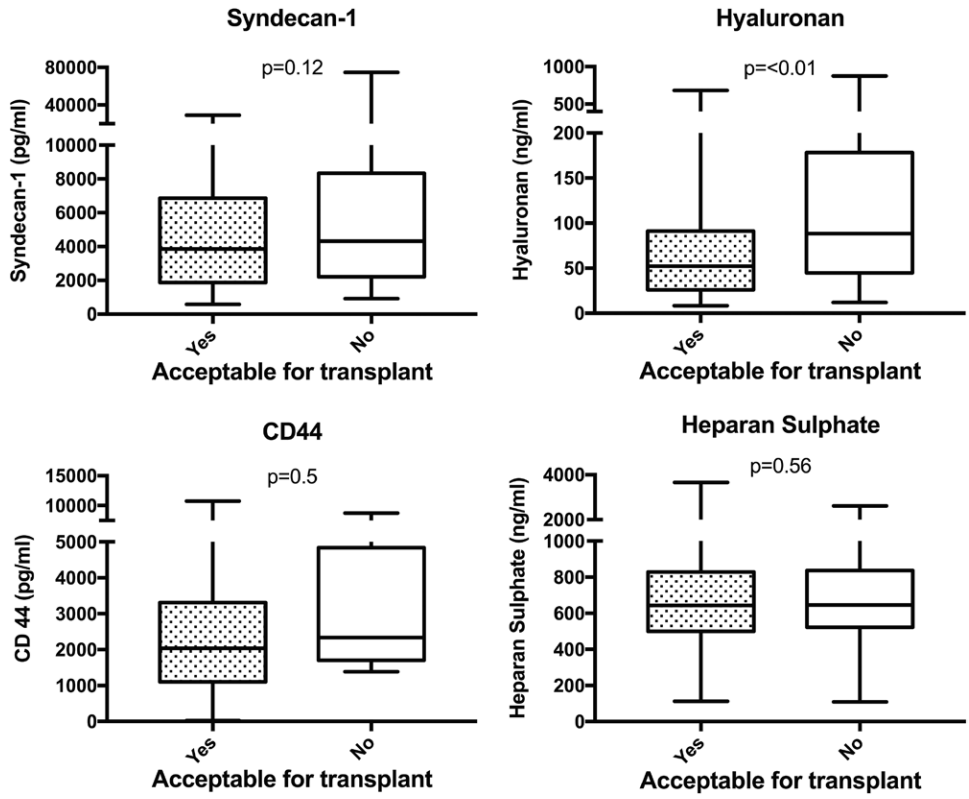

FIGURE 2. Endothelial glycocalyx breakdown products in peripheral blood of organ donors measured at the time of listing for organ transplant compared between those which lungs were acceptable for transplant (actually transplanted and those with no suitable recipient) and those unacceptable for transplant (poor lung function, age, failure of DCD progression). *$P < 0.05$. DCD, donation after circulatory death.

TABLE 2.

Factors associated with the acceptability of donor lungs

Univariate	Odds ratio (95% CI)	*P*
Age (per 5 y)	0.95 (0.88-1.02)	0.17
Female sex	0.97 (0.61-1.57)	0.92
Traumatic cause of death	0.74 (0.43-1.28)	0.28
DCD vs DBD	0.65 (0.39-1.10)	0.11
Donor intubation time (per day)	1.00 (0.92-1.09)	0.96
Unstable donor blood pressure	0.80 (0.46-1.41)	0.44
Donor aspiration event	0.40 (0.24-0.67)	<0.001[a]
Donor chest trauma	1.12 (0.56-2.21)	0.31
Offer pH	2.34 (0.13-42.37)	0.57
Offer PaO_2 >300 mm Hg	6.91 (3.67-13.01)	<0.001[a]
Offer $PaCO_2$ (per 100 mm Hg)	0.44 (0.10-1.96)	0.28
Offer base excess	0.99 (0.95-1.05)	0.88
Offer bicarbonate	1.03 (0.97-1.08)	0.33
History of cancer	0.64 (0.28-1.47)	0.29
Pre-existing lung condition	0.35 (0.21-0.60)	<0.001[a]
Smoking >20 pack years	0.59 (0.33-1.05)	0.069
Marijuana usage	0.85 (0.49-1.48)	0.57
Donor diabetes	0.34 (0.13-0.91)	0.03[a]
Syndecan-1 (ng/mL)	0.98 (0.93-1.02)	0.34
Heparan sulfate (ng/mL)	1.03 (0.47-2.25)	0.93
Hyaluronan (per 10 ng/mL)	0.96 (0.94-0.99)	0.02[a]
Multivariate		
Offer PaO_2 > 300 mm Hg	11.95 (4.15-34.36)	<0.001
Hyaluronan (per 10 ng/mL)	0.96 (0.93-0.99)	0.026

Variables with $P < 0.1$ were included in multivariate analysis.
DBD, donation after brain death; DCD, donation after circulatory death; CI, confidence interval.

TABLE 3.

Characteristics of 50 transplant recipients, and their organ donors, by PGD grade at 72 h who had endothelial glycocalyx breakdown products measured in the peripheral blood pretransplant and then daily posttransplant

Donor and recipient characteristics by PGD grade at 72 h			
Variable	**PGD 0–1 (37)**	**PGD ≥ 2 (13)**	***P***
Donor			
Female, n (%)	19 (51)	8 (61)	0.38
Age	42 (31–56)	48 (37–55)	0.34
BMI	28.2 (23–31)	22.9 (21–25)	0.016[a]
Smoking history, n (%)	26 (78)	7(53)	0.094
Recipient			
Female, n (%)	18 (49)	11 (69)	0.15
Age	48.7 (37–59)	52.8 (41–61)	0.55
BMI	22.9 (21–26)	21.8 (20–26)	0.67
Diagnosis			
COPD, n (%)	15 (83)	3 (17)	
Pulmonary fibrosis and other, n (%)	9 (60)	6 (40)	
Cystic fibrosis, n (%)	13 (76)	4 (24)	
Ischemic time (min)	235 (194–320)	254 (225–343)	0.82
Intubation time (h)	24.6 (12–45)	138.6 (34–330)	<0.001[a]
ICU time (h)	77.7 (54–127)	234.8 (128–400)	<0.001[a]

Medians (IQR) shown unless otherwise specified.
BMI, body mass index (weight [kg]/height [m^2]; COPD, chronic obstructive pulmonary disorder; ICU, intensive care unit; IQR, interquartile range; PGD, primary graft dysfunction.

Donor Syndecan-1 Is Associated With Recipient PGD Development

Given that low donor hyaluronan levels were associated with an increased likelihood of organs being acceptable for transplant, we assessed whether circulating glycocalyx breakdown products were associated with the development of PGD in the recipient. Of the 135 organ donors, 51 proceeded to transplant with 35 performed at our institution and for whom we had follow-up data available. At 24 and 72 hours, 10 (29%) and 6 (17%) of recipients, respectively, had PGD grade ≥ 2. Higher donor syndecan-1 levels were seen in recipients who developed PGD grade ≥ 2 (3142 [1575–4829] versus 6229 [4009–8093] pg/mL; $P = 0.0453$) at 24 hours; however, this was not significant at 72 hours. No significant associations were seen for heparan sulfate or hyaluronan (**Figure S1, SDC,** http://links.lww.com/TP/B658).

Recipient Endothelial Glycocalyx Breakdown Products and PGD

We next assessed circulating endothelial glycocalyx levels in lung transplant recipients (n = 50) and their association with PGD, intubation time, and ICU length of stay (Table 3). Pretransplant samples were collected on average 65 days (SD 93 days) before transplant at the time of listing. At 24 and 72 hours, 15 (30%) and 13 (26%) of patients, respectively, had PGD grade ≥2. Severe (≥2) PGD was associated with a lower donor body mass index, longer recipient intubation time, and increased recipient ICU length of stay (Table 3). Lung transplant recipients who had PGD grade ≥ 2 at 72 hours had a sustained elevation in syndecan-1 levels ($P = 0.01$; Figure 3) compared with those who had PGD grade 0–1 (Figure 3). There was no difference in circulating recipient hyaluronan or heparan sulfate levels by PGD grade.

At all posttransplant time points, peripheral blood syndecan-1 was positively correlated with intubation time and ICU length of stay (Table 4). Hyaluronan levels also showed a positive correlation with intubation time and ICU length of stay; however, this was not consistent across all time points. Interestingly, pretransplant recipient hyaluronan levels predicted intubation time and ICU length of stay posttransplant (Table 4). There was no correlation between recipient endothelial glycocalyx breakdown products and total (warm + cold) ischemic time at any time posttransplant time point (data not shown).

Liquid Chromatography Mass Spectrometry

LCMS was performed on plasma from a subset of 15 organ donors (n = 10 acceptable, all of which were transplanted and n = 5 unacceptable for transplant, all for lung reasons). Levels of hyaluronan measured by LCMS were on average 8 times higher than those measured by ELISA (391 [265–476] versus 54 [23–88]; $P \leq 0.001$). This increase likely represents increased proinflammatory low–molecular weight hyaluronan, <35kDa hyaluronan fragments, which are not detected by ELISA.[44] There were no between-group differences in sulfation pattern for any of the glycocalyx products (data not shown). For heparan sulfate, a predominance of unsulfated disaccharides (0S) was seen with a relative absence of trisulpahted (TriS) and disulfated (NS6S and NS2S) heparan sulfate (Figure 4).

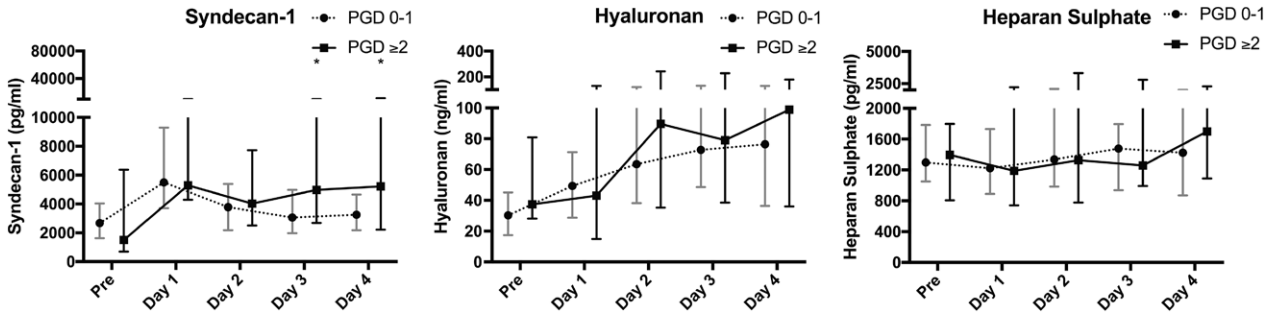

FIGURE 3. Transplant recipient endothelial glycocalyx breakdown product levels over time with a comparison between those recipients who developed PGD grades 0–1 and PGD grade ≥ 2 at the 72-h time point. *$P < 0.05$ (median and IQR displayed). IQR, interquartile range; PGD, primary graft dysfunction.

TABLE 4.

Correlations between recipient intubation time and total ICU length of stay and glycocalyx breakdown product levels in blood pretransplantation and day 1, 2, 3, and 4 after transplantation

	Pretransplant	Day 1	Day 2	Day 3	Day 4
Intubation time					
Hyaluronan	$r = 0.356$, $P = 0.013$[a]	$r = 0.242$, $P = 0.083$	$r = 0.308$, $P = 0.025$[a]	$r = 0.342$ $P = 0.013$[a]	$r = 0.235$, $P = 0.099$
Syndecan-1	$r = 0.248$, $P = 0.09$	$r = 0.396$, $P = 0.004$[a]	$r = 0.43$, $P = 0.001$[a]	$r = 0.400$, $P = 0.003$[a]	$r = 0.511$, $P = 0.001$[a]
Heparan sulfate	$r = 0.23$, $P = 0.116$	$r = 0.277$, $P = 0.05$	$r = 0.236$, $P = 0.089$	$r = 0.25$, $P = 0.073$	$r = 0.27$, $P = 0.062$
ICU length of stay					
Hyaluronan	$r = 0.306$, $P = 0.035$[a]	$r = 0.356$, $P = 0.01$[a]	$r = 0.340$, $P = 0.013$[a]	$r = 0.282$, $P = 0.043$[a]	$r = 0.268$, $P = 0.06$
Syndecan-1	$r = 0.572$, $P = 0.083$	$r = 0.363$, $P = 0.01$[a]	$r = 0.426$, $P = 0.01$[a]	$r = 0.406$, $P = 0.003$[a]	$r = 0.500$, $P = 0.001$[a]
Heparan sulfate	$r = 0.047$, $P = 0.75$	$r = 0.277$, $P = 0.047$[a]	$r = 0.236$, $P = 0.089$	$r = 0.250$, $P = 0.073$	$r = 0.266$, $P = 0.062$

[a] indicates $P < 0.05$.
ICU, intensive care unit.

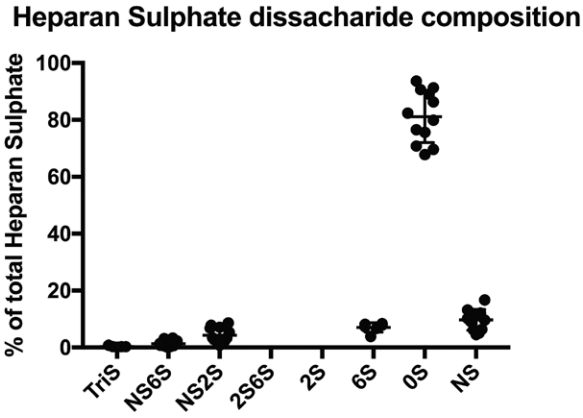

FIGURE 4. Sulfation pattern of heparan sulfate in peripheral blood of organ donors (n = 15) (liquid chromatography mass spectrometry). Heparan sulfate was isolated from donor plasma and digested into its constituent disaccharides to allow for individual disaccharide-specific measurement for each donor and hence determination of sulfation pattern. Data are displayed as percentage contribution of individual disaccharide units, separated according to level of sulfation and sulfation site (trisulfated = TriS; disulfated = NS6S, NS2S, 2S6S; monosulfated = NS, 2S, 6S; and unsulfated = OS), of the total glycosaminoglycan amount.

This sulfation pattern is consistent with a nonheparanase-mediated cause for heparan sulfate shedding.

DISCUSSION

Given the importance of the endothelial glycocalyx in maintaining vascular integrity, its fragility and susceptibility to damage and shedding during ischemia-reperfusion, and pulmonary glycocalyx shedding being central to the pathogenesis of acute respiratory distress syndrome, we hypothesized that endothelial glycocalyx shedding would be associated with pulmonary dysfunction in lung donors and recipients. We first confirmed that endothelial glycocalyx products were detectable in blood from organ donors and recipients, suggesting glycocalyx shedding throughout the transplantation process. In donors, elevated blood hyaluronan levels were independently associated with reduced donor lung acceptability for transplantation. In recipients, elevated pretransplant blood hyaluronan levels were associated with the duration of intubation postoperatively, syndecan-1 levels at multiple time points were associated with higher PGD grades, and both products measured postoperatively at multiple time points were associated with prolonged intubation times. Taken together these findings are consistent with the idea that glycocalyx disruption contributes to poor pulmonary function in both donors and recipients, suggesting that glycocalyx shedding plays a role in lung ischemia/reperfusion injury pathogenesis and may be important in PGD pathogenesis.

Poor organ utilization rates and PGD are two of the most important clinical problems in lung transplantation, with no previous studies examining the role the endothelial glycocalyx plays in either of these. Our data suggest that damage to the glycocalyx is common in donors and recipients, and that the extent of glycocalyx damage predicts lung dysfunction—manifesting as nonacceptance in the potential donor and poor organ function and PGD in the recipient. The rationale for the differences seen between products and outcomes is likely related to the different

location and function of individual components within the endothelial glycocalyx and the different pathological processes involved. Syndecans and CD44 are proteoglycans firmly anchored to the cell surface by transmembrane domains, while hyaluronan, chondroitin sulfate, and heparan sulfate chains branch from those anchored proteoglycans into the vasculature and comprise the bulk of endothelial glycocalyx mass.[30]

Although not fully elucidated, mechanisms of glycocalyx damage differ, as hyaluronan and other glycoaminoglycans have been shown to be cleaved by reactive oxygen species[28,45,46] and sheddases, such as heparanase,[47] while syndecan-1 requires matrix metalloprotease activation.[48,49] An in-depth analysis of mechanisms behind this breakdown was not the focus of this study; however, the LCMS heparan sulfate pattern suggests that a sheddase (eg, heparanase)-mediated mechanism of shedding is unlikely.[35] We hypothesized a reactive oxygen species–mediated mechanism given the high amounts of low–molecular weight hyaluronan disaccharides, detected on LCMS, which can be caused by reactive oxygen species and ischemic stress fragmenting high–molecular weight hyaluronan.[50]

The glycocalyx has been shown to be shed in response to injuries including sepsis,[32] trauma,[51] and hemorrhagic shock.[20] Given that these processes can occur in organ donors, it is not surprising we demonstrated higher levels of endothelial glycocalyx breakdown products in donors who underwent trauma, had unstable blood pressures, and had lower blood pH levels. The lack of association between glycocalyx breakdown products and PaO_2 may be due to the fact these breakdown products represent injury to the endothelium only and not the alveolar epithelium, which also plays a role in lung fluid homeostasis.[52] Alternatively, given the findings in EVLP that PaO_2 is not an accurate indicator of lung injury,[4] PaO_2 in organ donors may not be accurately representing lung acceptability for transplant in the EVLP era.[53,54]

Experimentally, it has been demonstrated that the glycocalyx is shed in response to ischemia/reperfusion injury.[33,45,55-57] Human kidney transplant studies, utilizing direct vascular visualization, have confirmed that the glycocalyx is shed immediately upon reperfusion, correlating with a rise in circulating syndecan-1, which rapidly normalizes.[34] Casanova, in a porcine lung autotransplant model, also demonstrated a significant increase in circulating syndecan-1 postreperfusion.[58] The sustained increase in syndecan-1 that we saw posttransplant suggests ongoing endothelial glycocalyx injury. Shed, circulating syndecan-1 can also act as a chemokine, contributing to trans-endothelial flux of neutrophils into the lung.[59] Furthermore, circulating low–molecular weight hyaluronan fragments, as detected with LCMS, are potent proinflammatory-signaling molecules which bind to toll-like receptors on macrophages, resulting in inflammatory cytokine production.[60,61] Together, our work and that of previous investigators are consistent with the idea that ischemia-reperfusion leads to rapid and profound shedding, with these endothelial glycocalyx breakdown products likely playing a role in perpetuating pulmonary injury.

The lack of association of heparan sulfate, measured by ELISA, with donor outcomes and the weak association with other breakdown products may have been a specificity limitation of the ELISA test. The antiheparan sulfate antibodies utilized in the ELISA only detect specific heparan sulfate epitopes, which are determined by their unique sulfation patterns, and as such may not have detect the unsulfated heparan sulfate (0S) that predominated in our donor blood samples.[62] The heparan sulfation pattern of organ donors was similar to that demonstrated by Schmidt et al[35] in ICU patients with respiratory failure secondary to direct lung injury, namely pneumonia.

An unexpected finding was that pretransplant hyaluronan levels in transplant recipients, usually collected at the time of listing, were positively correlated to intubation time (see Table 4). This finding may simply relate to systemic illness in the recipient pretransplant predisposing to prolonged recovery times; however, an alternate, intriguing possibility, is that an inherent defect in glycocalyx function (eg, mutations in the superoxide dismutase gene)[63] may both lead to disease requiring transplantation (eg, pulmonary hypertension) and predispose to impaired posttransplant graft function. Furthermore, previous investigators have shown that higher hyaluronan levels in the plasma of lung transplant recipients are associated with acute rejection and diffuse alveolar damage[64] as well as long-term development of bronchiolitis obliterans syndrome.[65]

This study expands the number of potential biomarkers for PGD. Previously studied markers for endothelial injury include intracellular adhesion molecule-1,[66,67] endothelin-1,[15] vascular endothelial growth factor,[12,13] and angiopoeitin-2.[68] These putative biomarkers have been variably sensitive and specific in predicting PGD, individually reflecting the multifactorial nature of PGD, but together this group of studies highlights vascular injury in donors and recipients synergistically contributes to PGD pathogenesis. Given that the hallmarks of PGD are an increase in inflammatory activation, pulmonary vascular resistance, and capillary permeability,[5,7] and the loss of endothelial glycocalyx components experimentally results in impaired mechanotransduction[69] and endothelial hyperpermeability,[32] it seems likely that the breakdown of the glycocalyx contributes to PGD pathogenesis and warrants further study.

Our study has some unavoidable limitations. Although we present a substantial body of circumstantial evidence for endothelial glycocalyx breakdown peritransplant, we have not been able to directly demonstrate pulmonary endothelial glycocalyx shedding. The intrathoracic location and the presence of air in the lung preclude the use of real-time imaging techniques, which is the gold standard of glycocalyx dysfunction studies.[17] Although we are unable to exclude a contribution from the extracellular matrix to the glycocalyx breakdown products measured in peripheral blood, many studies have demonstrated that these blood biomarkers correlate well with directly observed glycocalyx loss,[19-21] and in animal models, the shedding of pulmonary glycocalyx components is not only detectable in peripheral blood but also associated with increased pulmonary permeability and inflammation.[32,70] We further acknowledge that we are unable to completely exclude a contribution to the blood pool of glycocalyx fragments from the synthesis of new glycocalyx products in response to injury. However, because the glycocalyx is produced

locally by the endothelium, rather than systemically, such a contribution is likely to be small.

CONCLUSION

In conclusion, we have shown that glycocalyx shedding is ubiquitous in organ donors and recipients and that the degree of shedding, as assessed by levels of hyaluronan and syndecan-1 in peripheral blood, correlates with organ acceptability and the severity of subsequent PGD, respectively. Further studies will be needed to validate these findings and determine if measurement of endothelial glycocalyx products may be a useful adjunct to existing organ selection processes and further, whether interventions designed to protect and repair the glycocalyx can reduce the incidence of PGD and improve transplant outcomes.

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