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Heme Oxygenase-1 as a Predictor of Early Allograft Dysfunction in Liver Transplantation: A Prospective Observational Pilot Study

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Background. Early allograft dysfunction (EAD), defined as hepatic insufficiency within a week of orthotopic liver transplantation (OLT), affects up to 25% of recipients and is associated with morbidity and mortality. Ischemia–reperfusion injury (IRI), the primary driver of EAD, reflects immune and cellular dysregulation triggered by transient tissue oxygen deprivation. Heme oxygenase-1 (HO-1), a cytoprotective enzyme, plays a central role in mitigating hepatic IRI. We hypothesized a distinct perioperative HO-1 signature correlates with EAD. **Methods.** We conducted a prospective observational pilot study of 43 primary adult, deceased donor OLT recipients at Emory University Hospital between August 2023 and July 2024. EAD was defined by the Olthoff criteria, using serum bilirubin, international normalized ratio (INR), and aminotransferases. Perioperative arterial serum was assayed preimplantation at anesthesia induction (timepoint, T1), and 2 (T2), and 48 h (T3) postimplantation to measure HO-1 using multiplex immunoassay and compared between recipients with and without EAD. Group comparisons (EAD versus non-EAD) used Mann-Whitney U for continuous variables (reported as medians) and chi-square or Fisher exact test for categorical variables. HO-1 changes over time were assessed with the Friedman test and post hoc comparisons. The Spearman correlation evaluated associations between HO-1 and clinical parameters. **Results.** EAD occurred in 9 of 43 patients (20.9%). Recipients who developed EAD exhibited significantly higher HO-1 levels 2 h postimplantation (T2) (22,665 pg/mL [interquartile range (IQR): 17 881, 25 361] versus 10,765 pg/mL [IQR: 8060, 18 903], $P = 0.0284$) and a significantly greater peri-implantation rise in HO-1 levels (T2-T1) (21,050 pg/mL[IQR: 17 645, 24 765] versus 9,264 pg/mL [IQR: 6876,17514], $P = 0.0358$), compared with those without EAD. Receiver operating characteristic curve analysis revealed moderate predictive ability of the peri-implantation rise in HO-1 levels (T2-T1) (area under the curve = 0.7157). **Conclusions.** Peri-implantation HO-1 kinetics may represent a novel biomarker of EAD. Larger validation studies and mechanistic investigations are warranted to refine risk stratification and guide targeted interventions to mitigate EAD.

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The study was approved by the Emory University Institutional Review Board under protocol number 5728. Written, informed consent was obtained confidentially in accordance with the Declaration of Helsinki and Istanbul and participation did not affect medical care.

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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INTRODUCTION

Approximately 20%–25% of the >10 000 deceased donor orthotopic liver transplantations (OLTs) performed annually in the United States (Organ Procurement and Transplantation Network 2024) are complicated by impaired allograft function within the first postoperative week, termed early allograft dysfunction (EAD).[1,2] EAD is associated with adverse transplant outcomes including allograft failure, persistent coagulopathy, metabolic acidosis, multisystem organ failure, sepsis, and mortality.[3,4] Established risk factors include advanced donor age, donation after circulatory death (DCD), prolonged ischemia time, hepatic steatosis, and recipient-related factors such as age, body mass index (BMI), and severity of illness (eg, Model for End-Stage Liver Disease [MELD] score).[1,2] Although EAD arises from multiple donor, recipient, and surgical factors, ischemia–reperfusion injury (IRI) remains its central pathogenic driver.[5-7] Established risk factors likely exert their effects by modulating the balance between cytoprotective and proinflammatory IRI signaling pathways, thereby influencing the severity of allograft injury.[8] IRI is a complex self-perpetuating cascade in which temporary oxygen deprivation (ischemia) followed by reoxygenation (reperfusion) triggers dysregulated immune and cellular responses within the allograft.[5-7] Resultant oxidative stress and inflammation lead to hepatocellular damage[9] and hemodynamic instability, clinically manifesting as ischemia reperfusion syndrome.

In response to ongoing organ shortages and long waitlists, the deceased donor pool has expanded to include more high-risk extended criteria donors and marginal allografts, including those with advanced age, electrolyte disturbances, high vasoactives needs, hepatitis C virus infection, DCD, hepatic steatosis, and prolonged ischemia time. Extended criteria and marginal allografts are particularly vulnerable to IRI,[9] which may contribute to higher rates of EAD and pose a challenge with further expansion of the donor pool.[10] Identifying markers of IRI that predict EAD would therefore be beneficial to enable early intervention and potentially mitigate associated risks.

Heme oxygenase-1 (HO-1) is a crucial cytoprotective enzyme within the Kelch-like ECH-associated protein 1 (Keap1)/Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway in Kupffer cells and hepatocytes. It plays a pivotal role in hepatic IRI, as demonstrated in murine models, with supporting clinical evidence from human liver tissue studies.[11-13] Under basal conditions, Keap1 binds Nrf2 in the cytoplasm, targeting it for proteasomal degradation. Upon oxidative stress, Nrf2 evades Keap1-mediated degradation, translocates to the nucleus,[14] and activates antioxidant response elements, inducing genes such as *HMOX1* that encodes HO-1.[11] HO-1 enzymatically degrades pro-oxidant heme into carbon monoxide, biliverdin, and free iron, molecules that collectively exert anti-apoptotic, anti-inflammatory, and antioxidant effects.[14] Furthermore, HO-1 activation triggers negative feedback mechanisms that inhibit Toll-like receptor 4/nuclear factor kappa B–mediated proinflammatory signaling, thereby attenuating the production of reactive oxygen species and recruitment and activation of inflammatory cells during hepatic IRI.[12]

Preclinically, elevated HO-1 is associated with reduced inflammatory cell infiltration, attenuated hepatocellular injury, and improved allograft function after OLT.[13,15-17] Conversely, impaired HO-1 induction correlates with heightened inflammatory responses, increased hepatocellular injury, and worse transplant outcomes.[13,15-17] These findings underscore both the therapeutic potential of targeting HO-1 pathways to mitigate hepatic IRI and improve allograft viability, and the value of predictive serum biomarkers such as HO-1 for early identification of patients at risk for EAD, enabling timely interventions to improve OLT outcomes. To our knowledge, this is the first study to evaluate serum HO-1 as a predictor of EAD, building on prior preclinical findings in IRI. We tested the hypothesis HO-1 levels predict EAD development, and, in combination with other analytes, contribute to a distinct serum signature associated with EAD after OLT.

MATERIALS AND METHODS

Design and Eligibility Criteria

This pilot, prospective, single-center, observational, cohort study was conducted at Emory University Hospital, a large academic medical center in Atlanta, GA, between August 2023 and July 2024. The study was approved by the Emory University Institutional Review Board under protocol number 5728. Detailed methods have been described previously.[18] Eligible patients were adults (age ≥ 18 y) undergoing primary OLT from deceased donors, including both donation after brain death and DCD. Exclusion criteria were living donor donation, repeat OLT within a week of transplantation, multiple organ transplantation, and pregnancy or lactation. Patients receiving allografts from DCD donors who underwent normothermic regional perfusion (NRP), a technique similar to extracorporeal membrane oxygenation that mitigates donor warm ischemia time by restoring oxygenated circulation to the donor organs following circulatory death, were included. Recipients of allografts maintained on normothermic machine perfusion (NMP), which provides oxygenated perfusion at physiologic temperature before implantation, were also included. Recipients of allografts maintained on hypothermic machine perfusion were not included because of lack of institutional availability.

Outcomes

The primary outcome was the development of EAD, defined by the Olthoff criteria as the presence of 1 or more of the following serum laboratory values: total bilirubin level ≥10 mg/dL on postoperative day 7, international normalized ratio level ≥1.6 on postoperative day 7, and peak aspartate transaminase (AST) or alanine transaminase level >2000 IU/L within the first 7 postoperative days.[1] Secondary outcomes include 7-d peak AST level, incidence of postoperative acute kidney injury (defined by Kidney Disease Improving Global Outcomes criteria), estimated blood loss (EBL; quantified via intraoperative cell saver data and anesthesiologist approximation), extubation in the operating room, intensive care unit (ICU) length of stay (LOS), hospital LOS, and 30-d all-cause mortality. HO-1 was selected as a candidate biomarker of EAD based on its established cytoprotective role in hepatic IRI and previous experimental evidence.[11-13,15-17,19]

Human Subject Enrollment and Recruitment

As previously described,[18] a post hoc power analysis using effect size estimates from Friedman et al indicated a sample size of ≥20 participants per group (≥40 total) would provide 70% power (with $\alpha = 0.10$) to detect a difference between

EAD and non-EAD groups.[20] Eligibility was determined by manual electronic medical record review, and patients were recruited in the ward, ICU, or preoperative holding area without demographic quotas. Written, informed consent was obtained confidentially in accordance with the Declaration of Helsinki and Istanbul and participation did not affect medical care.

Procedures

The protocol for HO-1 measurement and collection of standard-of-care (SOC) laboratory tests has been described previously.[18] Serum was collected via a preoperatively placed arterial line at 3 timepoints (T): (1) preimplantation after the induction of general anesthesia (baseline, T1), (2) 2 h postimplantation (T2), and (3) 48 h postimplantation (T3). At each timepoint, approximately 12 mL was drawn into 2 edetic acid tubes (about 6 mL each), processed within 30 min by centrifugation at 2000*g* for 20 min at 4 °C, and plasma aliquots were stored at −80 °C for batch analysis under institutional biorepository protocols. HO-1 concentrations were quantified using Meso Scale Discovery electrochemiluminescence immunoassays (Meso Scale Diagnostics LLC, Rockville, MD), following manufacturer instructions. Serum HO-1 levels were compared between recipients with and without EAD. Patients were followed for 30 d postoperatively, with weekly chart review for relevant exposures, covariates, and outcomes, including established EAD risk factors, perioperative immunosuppression, and the use of NRP and/or NMP.

Statistical Analysis

Descriptive statistics were used to summarize baseline demographic and clinical characteristics. Continuous variables were reported as mean ± SD or median with interquartile range (IQR) based on normality assessed by the Shapiro-Wilk test. Categorical variables were expressed as frequencies and percentages. Comparisons between groups, EAD versus non-EAD, were performed using Student *t*-test or Mann-Whitney U test for continuous variables, and Chi-square or Fisher exact test for categorical variables, as appropriate. Changes in serum HO-1 levels across the 3 timepoints were analyzed using repeated measures ANOVA or Friedman test with post hoc pairwise comparisons. Correlation analyses between serum HO-1 concentrations and clinical/laboratory parameters were conducted using Pearson or Spearman correlation coefficients. Case exclusion was used for analyses with missing data. Due to the small sample size and exploratory design, multivariable adjustment for covariates was not performed to avoid overfitting and preserve interpretability. A two-sided P value <0.05 was considered statistically significant. Statistical analyses were performed using the R project for statistical computing.

RESULTS

Patient Characteristics

A total of 135 patients were eligible for enrollment. Twelve were excluded, 11 for multiple organ transplantation and 1 for retransplantation within a week of OLT. Enrollment was nonconsecutive and based on study team availability, with patients enrolled at varying times throughout the day and night. All eligible patients who were approached consented to participate in the study. A total of 44 patients were initially enrolled. One patient was excluded from analysis because of aborted surgery before allograft implantation, leaving 43 patients for analysis. Among these, 1 (2.3%) patient received an allograft procured with NRP, which later underwent static cold storage (SCS) preservation before implantation. Overall, 24 (55.8%) patients received allografts preserved by SCS, and 19 (44.2%) patients received allografts preserved by NMP. Samples were successfully collected from all patients at T1 and T2 (**Figure S1, SDC,** https://links.lww.com/TXD/A829). At T3, 13 of 43 samples (30.2%) were missing because of the loss of arterial line access per SOC.

EAD occurred in 9 of 43 total patients (20.9%), consistent with other studies.[1,2] In the SCS subgroup, 8 of 24 (33.3%) patients developed EAD, compared with 1 of 19 (5.3%) patients in the NMP subgroup. Patient characteristics by outcome group, EAD or non-EAD, separated by preservation method (SCS versus NMP), are presented in Tables 1 and 2. Patient characteristics by preservation method, SCS or NMP, can be found in **Table S1** (**SDC,** https://links.lww.com/TXD/A829). Overall, 41.9% of patients were female. The top causes of end-stage liver disease (ESLD) were metabolic-associated steatohepatitis (MASH) (n = 16, 37.2%) and alcoholic cirrhosis (n = 10, 23.3%). Seven of 43 patients (16.3%) had hepatocellular carcinoma as a primary or secondary cause of ESLD and 3 of 43 (7.0%) patients had acute liver failure. Median donor and recipient ages were 51 y (IQR: 35.5, 59.8 y) and 57 y (IQR: 43.3, 62 y), respectively; median recipient BMI was 31.5 kg/m^2 (IQR: 25, 36 kg/m^2) and MELD score was 23.5 (IQR: 17.3, 32.5). In the SCS subgroup, patients who developed EAD were predominately male (62%) and had a median donor age of 55.5 y (IQR: 38, 61.5 y), recipient age of 59 y (IQR: 51.5, 63.5 y), recipient BMI of 34 kg/m^2 (IQR: 32.5, 36 kg/m^2), MELD score of 28 (IQR: 27, 35), and postoperative peak 7-d AST level of 3215 U/L (IQR: 2,413, 6,318 U/L). In the NMP subgroup, there was only 1 case of EAD. This patient was female with a donor age of 51 y, recipient age of 37 y, recipient BMI of 34 kg/m^2, MELD score of 28, and postoperative peak 7-d AST level of 2265 U/L.

Serum HO-1 levels at each timepoint are summarized in Table 3. Boxplots illustrating HO-1 levels across timepoints, as well as the change from preimplantation (baseline) to 2 h postimplantation (T2-T1), termed peri-implantation, are presented in Figure 1A–D.

There was no significant association between 2 h post-implantation HO-1 levels and the timing of methylprednisolone administration (before versus after T2). The mean (SD) HO-1 level was 13 657.02 pg/mL (17 349.74) among patients who received methylprednisolone before T2, compared with 10 277.58 pg/mL (15 786.99) among those who received it after T2. The distribution of Wilcoxon scores for HO-1 levels by timing of methylprednisolone exposure is shown in **Figure S2** (**SDC,** https://links.lww.com/TXD/A829). Furthermore, methylprednisolone administration timing (before versus after T2) did not significantly differ between groups by EAD status (P = 0.7348) or preservation method (P = 0.7590).

HO-1 Levels and the Development of EAD

At baseline, measured preimplantation after anesthesia induction (T1), EAD patients exhibited a near-significant decrease in serum HO-1 levels compared with non-EAD patients (1008 pg/mL [IQR: 732, 1373] versus 1215 pg/mL

TABLE 1.

Baseline characteristics of SCS patients by EAD status, non-EAD and EAD.

Variable	Subset	Non-EAD (n = 16)	EAD (n = 8)
Donor age (y)	Median (IQR)	41.5 (30.5, 52)	55.5 (38, 61.5)
Donor mode of death, N (%)	DCD	2 (13)	1 (13)
	ECD	2 (13)	1 (13)
	SCD	12 (74)	6 (74)
Recipient age (y)	Median (IQR)	58 (43, 64)	59 (51.5, 63.5)
Recipient sex, N (%)	Female	8 (50)	3 (38)
	Male	8 (50)	6 (62)
BMI (kg/m^2)	Median (IQR)	25.5 (23.5, 30)	34 (32.5, 36)
MELD score	Median (IQR)	24.5 (18, 35)	28 (27, 35)
Primary ESLD etiology, N (%)	Acute liver failure	2 (12)	1 (12)
	Alcoholic cirrhosis	5 (32)	0
	Autoimmune	0 (0)	1 (12)
	Graft failure	1 (6)	0
	HCC	1 (6)	0
	HCV	0	3 (38)
	MASH	4 (25)	3 (38)
	PBC	1 (6)	0
	PSC	2 (12)	0
Comorbidities, N (%)			
Hepatic encephalopathy	No	2 (12)	2 (25)
	Yes	14 (88)	6 (75)
Hepatopulmonary syndrome	No	12 (75)	7 (88)
	Yes	4 (25)	1 (12)
Nonobstructive CAD	No	11 (69)	5 (63)
	Yes	5 (31)	3 (37)
Obstructive CAD	No	16 (100)	7 (88)
	Yes	0	1 (12)
Hepatorenal syndrome	No	10 (63)	5 (63)
	Yes	6 (37)	3 (37)
Esophageal varices	No	5 (31)	4 (50)
	Yes	11 (69)	4 (50)
Ascites	No	4 (25)	3 (38)
	Yes	12 (75)	5 (62)
Hypertension	No	0	0
	Yes	16 (100)	8 (100)
Preoperative laboratory values			
Creatinine (mg/dL)	Median (IQR)	0.95 (0.72, 1.33)	1.11 (0.84, 1.53)
INR	Median (IQR)	1.72 (1.48, 2.76)	1.36 (1.1, 2.25)
Hemoglobin (g/dL)	Median (IQR)	9.45 (8.9, 12.25)	10.9 (8.7, 13.05)
Platelets (10^9/L)	Median (IQR)	82 (62, 118)	88 (62.5, 127.5)
Fibrinogen (mg/dL)	Median (IQR)	169 (137, 138)	204.5 (122.5, 301.5)
Total ischemia time (h)	Median (IQR)	6.42 (5.17, 7.18)	6.17 (4.64, 8.07)
Cold ischemia (h)	Median (IQR)	5.81 (4.59, 6.69)	5.71 (3.9, 7.49)
Warm ischemia (min)	Median (IQR)	32.5 (29, 37.5)	35 (28, 48)
Inferior vena cava clamp type, N (%)	Piggyback	16 (100%)	6 (75)
	Total cross clamp	0	2 (25)
Day 7 INR	Median (IQR)	1.03 (0.94, 1.1)	1.17 (1.05, 1.47)
Day 7 Total Bilirubin (mg/dL)	Median (IQR)	2.05 (0.85, 4.25)	2.55 (2.1, 5.1)
Peak 7-d AST (U/L)	Median (IQR)	997 (609, 1509.5)	3215 (2413, 6318)
Peak 7-d ALT (U/L)	Median (IQR)	482 (230, 957.5)	1304 (543, 1948)
Methylprednisolone exposure (Relative to T2), No. (%)	Before	6 (38)	3 (38)
	After	10 (62)	5 (62)

No missing values.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAD, coronary artery disease; DCD, donation after circulatory death; ECD, extended criteria donor (via donation after brain death); ESLD, End-stage liver disease; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalized ratio; IQR, interquartile range; MASH, metabolic dysfunction-associated steatohepatitis; MELD, Model for End-Stage Liver Disease; NMP, normothermic machine perfusion; PBC, Primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SCD, standard criteria donor (via donation after brain death); SCS, static cold storage.

TABLE 2.

Baseline characteristics of NMP patients by EAD status, non-EAD and EAD

Variable	Subset	Non-EAD (n = 18)	EAD (n = 1)
Donor age (y)	Median (IQR)	56.5 (43, 62)	51 (51, 51)
Donor mode of death, N (%)	DCD	11 (61)	1 (100)
	ECD	1 (6)	0
	SCD	6 (33)	0
Recipient age (y)	Median (IQR)	57 (43, 61)	37 (37, 37)
Recipient gender, N (%)	Female	6 (33)	1 (100)
	Male	12 (67)	
BMI (kg/m^2)	Median (IQR)	33 (27, 37)	34 (34, 34)
MELD score	Median (IQR)	18.5 (16, 27)	28 (28, 28)
Primary ESLD etiology, N (%)	Cryptogenic Cirrhosis	1 (6)	0
	Alcoholic Cirrhosis	4 (22)	1 (100)
	Autoimmune	0	0
	Graft failure	2 (11)	0
	HCC	0	0
	HCV	0	0
	MASH	9 (50)	0
	Metabolic disease	1 (6)	0
	Other	1 (6)	0
Comorbidities, N (%):			
Hepatic encephalopathy	No	2 (11)	0
	Yes	16 (89)	1 (100)
Hepatopulmonary syndrome	No	15 (83)	0
	Yes	3 (17)	1 (100)
Nonobstructive CAD	No	13 (72)	1 (100)
	Yes	5 (28)	0
Obstructive CAD	No	17 (94)	1 (100)
	Yes	1 (6)	0
Hepatorenal syndrome	No	14 (78)	0
	Yes	4 (22)	1 (100)
Esophageal varies	No	2 (11)	1 (100)
	Yes	16 (89)	0
Ascites	No	2 (11)	0
	Yes	16 (89)	1 (100)
Hypertension	No	18 (100)	1 (100)
	Yes	0	0
Preoperative laboratory values			
Creatinine (mg/dL)	Median (IQR)	0.92 (0.84, 1.13)	0.82 (0.82, 0.82)
INR	Median (IQR)	1.55 (1.32, 1.8)	3 (3.0, 3.0)
Hemoglobin (g/dL)	Median (IQR)	10.45 (9.5, 12.9)	7.7 (7.7, 7.7)
Platelets (10^9/L)	Median (IQR)	71.5 (54, 99)	40 (40, 40)
Fibrinogen (mg/dL)	Median (IQR)	171.5 (144, 221)	89 (89, 89)
Total pump time (h)	Median (IQR)	15.77 (14.73, 19.92)	15.58 (15.58, 15.58)
Recipient warm ischemia (min)	Median (IQR)	36.5 (33, 40)	37 (37, 37)
Day 7 INR	Median (IQR)	0.9 (0.9, 0.99)	Not available
Day 7 total bilirubin	Median (IQR)	1.95 (1.1, 2.9)	3.2 (3.2, 3.2)
Peak 7-d AST	Median (IQR)	690 (395, 1089)	2265 (2265, 2265)
Peak 7-d ALT	Median (IQR)	285.5 (190, 399)	468 (468, 468)
Methylprednisolone exposure (relative to T2), N (%)	Before	7 (39)	1 (100)
	After	11(61)	0

No missing values.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAD, coronary artery disease; DCD, donation after circulatory death; ECD, extended criteria donor (via donation after brain death); ESLD, end-stage liver disease; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalized ratio; IQR, interquartile range; MASH, metabolic dysfunction-associated steatohepatitis; MELD, Model for End-Stage Liver Disease; NMP, normothermic machine perfusion; PBC, Primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SCD, standard criteria donor (via donation after brain death); SCS, static cold storage; T2, timepoint 2, 2 h postimplantation.

[IQR: 1109, 1797], $P = 0.0554$). Two hours postimplantation (T2), EAD patients had significantly higher serum HO-1 levels than those without EAD (22 665 pg/mL [IQR: 17 881, 25 361] versus 10 765 pg/mL [IQR: 8060, 18 903], $P = 0.0284$). Forty-eight hours postimplantation (T3), EAD patients exhibited a near-significant increase in serum HO-1 levels compared with non-EAD patients (11 927 pg/mL [IQR: 10 135, 14 625] verses 4278 pg/mL [IQR: 3647, 7915], $P = 0.0554$). EAD

TABLE 3.

Serum HO-1 levels at each timepoint in all patients (SCS and NMP)

Cohort	Timepoint No. (description)	Sample (n)	Non-EAD (n = 33)	EAD (n = 9)	*P*
All patients	T1, preimplantation	43	1215 (1109, 1797)	1008 (732, 1373)	0.0554
	T2, 2 h postimplantation	43	10 765 (8060, 18 903)	22 665 (17 881, 25 361)	0.0284
	T3, 48 h postimplantation	30	4278 (3647, 7915)	11927 (10 135, 14 625)	0.0554
	Change between T1 and T2, periimplantation	43	9264 (6876, 17 514)	21 050 (17 645, 24 765)	0.0358

Values are reported as median (IQR). The units for HO-1 levels are pg/mL.
EAD indicates early allograft dysfunction; HO-1, heme oxygenase-1; IQR, interquartile range; NMP, normothermic machine perfusion; SCS, static cold storage; T, timepoint.

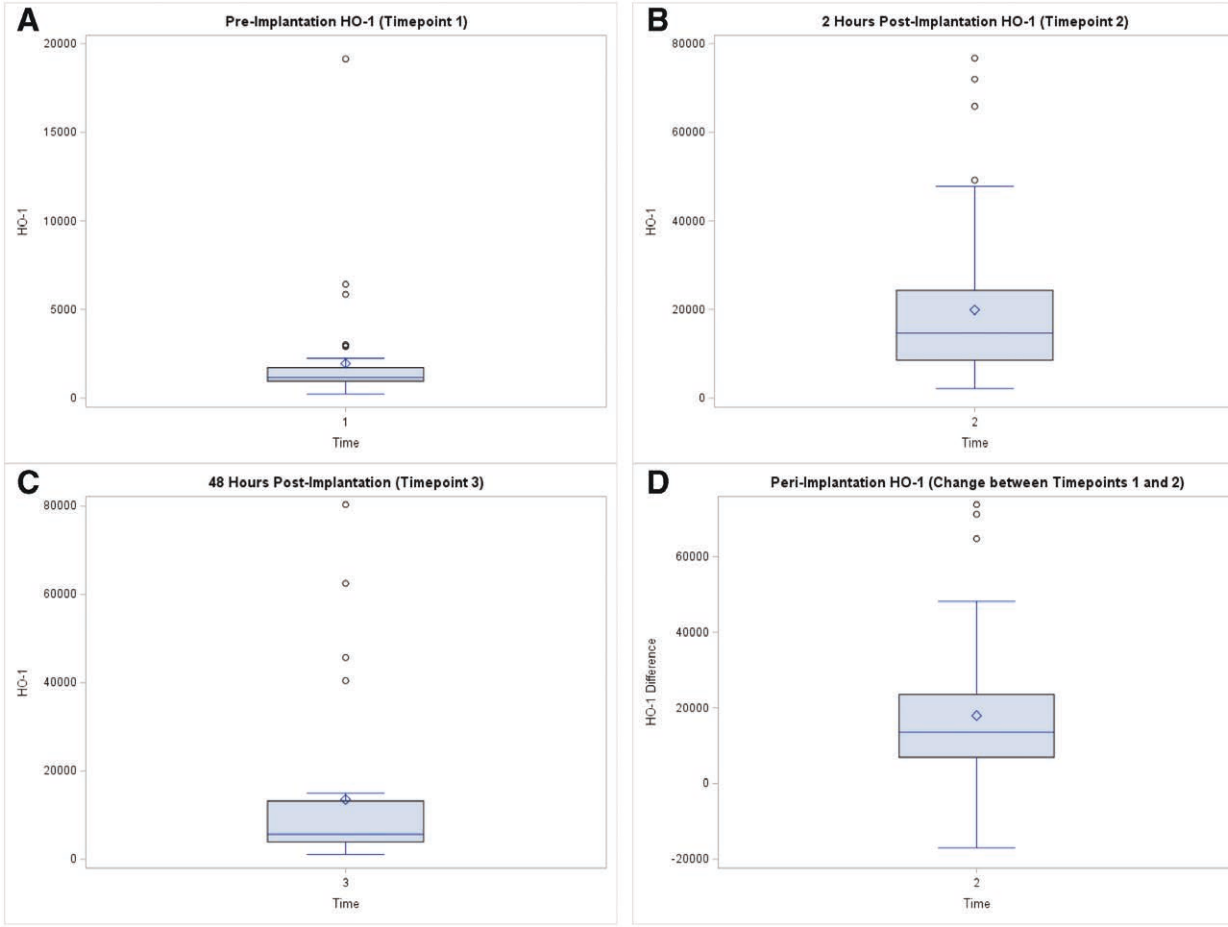

FIGURE 1. Boxplots illustrating HO-1 levels at each timepoint and peri-implantation, between timepoints 1 and 2. A, Serum HO-1 levels pre-implantation at T1. B, Serum HO-1 levels 2 h postimplantation at T2. C, Serum HO-1 levels 48 h postimplantation at T3. D, Peri-implantation change in the serum HO-1 level between T1 and T2. HO-1, heme oxygenase-1.

patients also exhibited a significantly greater peri-implantation increase in serum HO-1 levels compared with non-EAD patients, as measured by the change in level between T1 and T2 (21 050 pg/mL [IQR: 17 645, 24 765] versus 9,264 pg/mL [IQR: 6876, 17 514], $P = 0.0358$).

When analyzed independently, the SCS subgroup demonstrated similar findings. Two hours postimplantation (T2), serum HO-1 levels were significantly higher in EAD patients compared with non-EAD patients (20 849 pg/mL [IQR: 17 734, 36 488] versus 10 520 pg/mL [IQR: 7397, 17 923], $p = 0.0275$). EAD patients also demonstrated a markedly greater increase in serum HO-1 levels peri-implantation compared with non-EAD patients (19 355 pg/mL [IQR: 17 190, 35 466] versus 8512 pg/mL [IQR: 5571, 17 324], $P = 0.0321$). Within the SCS subgroup, neither preimplantation (baseline) nor 48-h postimplantation serum HO-1 levels differed significantly between EAD and non-EAD patients (Table 4). A separate NMP subgroup analysis was not performed because only 1 patient developed EAD.

Serum HO-1 as a Predictive Biomarker of EAD

Serial receiver operating characteristic curve analysis demonstrated baseline serum HO-1 levels had a moderate discriminatory ability for predicting EAD (area under the curve = 0.6993; Figure 2A). When combined with established EAD risk factors, such as donor age, total ischemia time, and recipient BMI and MELD score, lower baseline HO-1 may improve EAD prediction. Furthermore, the peri-implantation rise in

TABLE 4.

Serum HO-1 levels at each timepoint in SCS cases

Cohort	Timepoint No. (Description)	Sample (n)	Non-EAD (n = 33)	EAD (n = 9)	*P*
Only SCS cases	T1, preimplantation	24	1253 (1123, 2497)	1021 (792.6, 1494)	0.2207
	T2, 2 h postimplantation	24	10 520 (7397, 17 923)	20 849 (17 734, 36 488)	0.0275
	T3, 48 h postimplantation	17	4147 (3255, 13 158)	12 315 (10 531, 14 983)	0.2207
	Change between T1 and T2, periimplantation	24	8512 (5571, 17 324)	19 355 (17 190.2, 35 466.9)	0.0321

Values are reported as median (IQR). The units for HO-1 levels are pg/mL.
EAD indicates early allograft dysfunction; HO-1, heme oxygenase-1; IQR, interquartile range; SCS, static cold storage; T, timepoint.

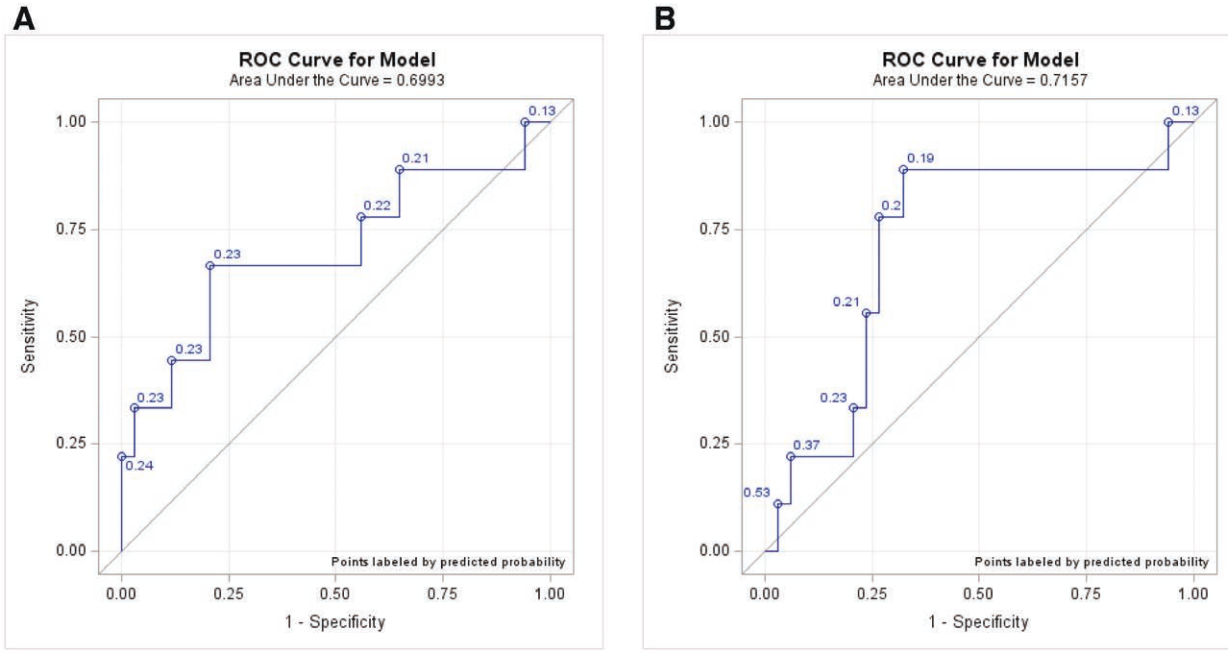

FIGURE 2. Boxplots illustrating HO-1 levels at each timepoint and peri-implantation, between timepoints 1 and 2. A, Preimplantation (baseline) serum HO-1 levels have a moderate discriminatory ability for predicting EAD ($r = 0.6993$). B, The peri-implantation rise in serum HO-1 shows strong discriminatory ability ($r = 0.7157$). EAD, early allograft dysfunction; HO-1, heme oxygenase-1.

serum HO-1 also showed moderate discriminatory ability (area under the curve = 0.7157; Figure 2B). When considered alongside established risk factors, a greater peri-implantation increase in HO-1 may also enhance prediction of EAD.

No Significant Difference in HO-1 Between Preservation Methods

Direct comparison of SCS and NMP subgroups revealed no significant differences in absolute serum HO-1 levels at any timepoint or in the peri-implantation rate of rise between baseline and 2 h postimplantation (T2-T1) (Table 5).

Correlation Between Serum HO-1 and Secondary Outcomes

The peri-implantation rise in HO-1 level, from baseline to 2 h postimplantation, was significantly associated with peak 7-d AST levels (Spearman $r = 0.64391$, $P < 0.0001$). AST is an established surrogate of postoperative allograft function. The peri-implantation rise in HO-1 was not associated with other secondary outcomes, including postoperative acute kidney injury, EBL, extubation in the operating room, ICU LOS, hospital LOS, or 30-d all-cause mortality (Table 6).

TABLE 5.

Serum HO-1 levels at each timepoint in all patients (EAD and non-EAD)

Cohort	Timepoint N. (description)	Sample (n)	SCS (n = 24)	NMP (n = 18)	*P*
All patients	T1, preimplantation	43	1198 (1002, 1841)	1140 (936.2, 1766)	0.9415
	T2, 2 h postimplantation	43	14 646 (8317, 20 849)	13 624 (9571.4, 27 464.7)	0.7411
	T3, 48 h postimplantation	30	10 531 (3934.7, 14 268.4)	4196 (3858, 7581)	0.2674
	Change between T1 and T2, peri-implantation	43	13 235 (6295, 19 355)	12 514 (7607, 26 528)	0.7030

Values are reported as median (IQR). The units for HO-1 levels are pg/mL.
EAD indicates early allograft dysfunction; HO-1, heme oxygenase-1; IQR, interquartile range; NMP, normothermic machine perfusion; SCS, static cold storage; T, timepoint.

TABLE 6.

Relationships between peri-implantation HO-1 and secondary outcomes

Study measure (variable)	Continuous variable	Spearman's P	*P*
Peri-implantation serum HO-1 level (change between T1 and T2)	Peak 7-d AST	0.64391	<0.0001
	ICU LOS	0.10984	0.4832
	Hospital LOS	0.04512	0.7739
	EBL	0.104	0.5075
	Categorical variable	Wilcoxon Z	*P*
	Postoperative AKI	0.1579	0.4373
	Extubating OR	0.0397	0.4843
	30-d all-cause mortality	0.0000	0.5000

Peri-implantation HO-1 levels refers to the change in level between T1 and T2. The corresponding statistical test used is listed.
AKI, acute kidney injury; AST, aspartate aminotransferase; EAD, early allograft dysfunction; EBL, estimated blood loss; HO-1, heme oxygenase-1; ICU, intensive care unit; LOS, length of stay; NMP, normothermic machine perfusion; OR, operating room; SCS, static cold storage; T, timepoint.

DISCUSSION

HO-1 is a well-established cytoprotective enzyme that mitigates oxidative stress by degrading pro-oxidant heme and generating antioxidant biliverdin and carbon monoxide.[11-13,19] Importantly, HO-1 expression is highly inducible and can increase substantially in various disease states, including cardiovascular disease, critical illness, and inflammatory conditions.[21-24] Interpretation of HO-1 levels thus requires consideration of both the clinical context and assay employed.

As expected, a distinct serum HO-1 pattern was associated with EAD. Notably, both the significant elevation in HO-1 levels at 2 h postimplantation (T2) and the peri-implantation rise (T2-T1) correlated with the development of EAD. These data may reflect a compensatory response to oxidative stress, a direct contributor to hepatocellular injury, or increased HO-1 release into the circulation secondary to cell lysis in the setting of EAD. However, the underlying mechanism remains unknown, and we can only hypothesize at this stage. Although moderate induction of HO-1 is cytoprotective, excessive activity may precipitate ferroptosis, a regulated form of cell death increasingly implicated in hepatic IRI and potentially EAD, through accumulation of free iron and subsequent lipid peroxidation.[25-27] It is plausible both insufficient and excessively elevated levels of HO-1 may be detrimental. Although mechanistic insights are beyond the scope of this study (our data support associations rather than causal inferences), the ability to identify patients at elevated perioperative risk for EAD remains clinically valuable. If validated in larger cohorts, this signal could inform the design of preemptive interventional trials aimed at mitigating EAD in high-risk patients. Nonetheless, the potential dual role of HO-1 in EAD underscores the exploratory nature of our study and highlights the need for further investigation.

Interestingly, lower pre-implantation HO-1 levels (T1) and elevated HO-1 levels 48 h postimplantation (T3) were not significantly associated with EAD. In the context of prior murine data, lower preimplantation HO-1 levels may reflect reduced cytoprotective capacity, potentially predisposing patients to EAD. Higher HO-1 levels 48 h postimplantation are consistent with our other findings. These associations did not reach statistical significance, and the limited sample size may have constrained statistical power.

Alternatively, our near-significant findings may be because of the variability in HO-1 levels across patients. Prior studies report mean serum HO-1 levels of ~1953 ± 612 pg/mL in healthy controls.[23] In cirrhosis, baseline HO-1 is elevated, particularly in advanced disease and in etiologies such as viral hepatitis and MASH.[28,29] In our study overall, 6 of 9 (66.7%) patients who developed EAD had preimplantation (baseline) HO-1 levels below the normal range. All patients with EAD demonstrated markedly elevated HO-1 levels 2- and 48-h postimplantation. In our cohort, the etiology of cirrhosis among the 9 patients who developed EAD was variable, including MASH (44.4%), hepatocellular carcinoma (44.4%), hepatitis C virus (33.3%), autoimmune (11.1%), alcohol-related (11.1%), and acute liver failure following right hepatectomy for a metastatic neuroendocrine tumor (11.1%), and several patients had multiple etiologies. Cardiovascular comorbidities, including hypertension and both obstructive and nonobstructive coronary artery disease, were common, present in 6 of 9 (66.7%) patients with EAD, potentially impacting HO-1 levels. To account for this high degree of variability in baseline HO-1 because of cirrhosis etiology and comorbidity, we examined the peri-implantation change in HO-1 levels between T1 and T2.

We initially had reservations about combining the SCS and NMP subgroups for the EAD analysis, as NMP introduces oxygen earlier, reduces total ischemia time, and may exert immunomodulatory effects. However, no significant differences in absolute HO-1 levels or peri-implantation rise were observed between groups. This suggests any immunomodulatory effects of NMP may not substantially influence circulating HO-1, though our study was underpowered to detect subtle differences. Theoretically, NMP could reduce oxidative injury and limit HO-1 upregulation or release, whereas SCS may comparatively promote greater hepatocellular injury and HO-1 cellular leakage. Nevertheless, HO-1 levels were comparable across preservation methods, perhaps reflecting recipient-driven responses rather than preservation-specific effects. These interpretations are speculative and highlight the exploratory nature of our study and the need for mechanistic investigations.

Interestingly, the peri-implantation rise in HO-1 from T1 to T2 correlated with peak 7-d AST levels, an established early surrogate marker of allograft injury after OLT that is associated with increased risk of early allograft loss and poor patient outcomes.[30-33] While correlation does not imply causation and these data cannot not be interpreted mechanistically as HO-1 driving the increase in AST, this finding suggests serum HO-1 levels increase in parallel with hepatocellular injury.

This again emphasizes the exploratory, hypothesis-generating nature of our study.

We consider our findings robust as study compliance was optimal with no missing samples at the pre-implantation and 2-h postimplantation timepoints. Missing values 48 h postimplantation were primarily attributable to arterial access removal per the SOC. We contend these missing data have minimal impact on our findings, as the most notable changes in HO-1 occurred during the pre- and narrow peri-implantation periods. The pre-implantation and 2 hours postimplantation timepoints also facilitate earlier detection of evolving allograft dysfunction, potentially enabling more timely interventions, pending further validation of our findings.

Study Limitations

Our study has several limitations related to sample size, heterogeneity, and variability. Our study was not powered to detect definitive differences in serum HO-1 levels between EAD and non-EAD groups and allograft preservation strategies varied within the cohort. We also note some values were missing at the 48-h postimplantation timepoint (T3), which limits the analyses that include this measurement. Our findings should therefore be cautiously interpreted and must be validated in a larger cohort.

In addition, the use of serum HO-1 as a biomarker presents unique challenges because of inherent variability in cirrhosis etiology and comorbidities. HO-1 is also primarily an intracellular enzyme, typically confined to the cytoplasm under normal conditions. Nevertheless, HO-1 can enter the bloodstream through passive leakage from damaged or dying cells, as occurs during ESLD and hepatic IRI. Consequently, serum HO-1 may serve as an indirect marker of ongoing tissue-level processes, such as hepatocellular apoptosis and necrosis, rather than reflecting baseline enzymatic activity. HO-1 has been successfully used as a biomarker in other diseases, such as respiratory distress syndromes, interstitial lung disease, and COVID-19.[34-37] Similarly, serum HO-1 is clinically meaningful in the context of hepatic IRI and EAD.[13]

As a pilot study, our primary goal was to generate preliminary, hypothesis-generating data. The study was not powered to detect definitive differences in serum HO-1 levels between EAD and non-EAD groups. Accordingly, these findings should be regarded as an initial basis for scientific inquiry rather than conclusive evidence. Interpreted within a Bayesian framework, the results imply the probability HO-1 is plausibly involved in EAD after OLT, rather than the expected frequency of this outcome.

Because of financial constraints, patients were enrolled nonconsecutively based on study team availability, with enrollment occurring across all times of day and night. We do not have readily accessible data confirming the 43 enrolled patients were representative of the 123 eligible patients, potentially introducing bias. However, because no specific enrollment strategy was used (eg, restricting to certain times of day), the cohort represents a random sample of eligible patients.

Serum HO-1 levels may also have been influenced by variable exposure to the perioperative administration of immunosuppressive agents. At Emory University Hospital, a single dose of methylprednisolone is administered intraoperatively during the neohepatic phase once hemostasis is achieved. This is followed by a short postoperative course starting on the first night of surgery. Mycophenolate mofetil is typically initiated on the first night of surgery, while tacrolimus may be started on postoperative day 1 or 2, depending on the patient's baseline renal function. For patients with a creatinine clearance <60 mL/min, basiliximab is given intraoperatively after methylprednisolone to delay the initiation of reduced-dose tacrolimus and mitigate perioperative kidney injury. T1 was obtained before the administration of any immunosuppressive agents. T2, obtained 2 h postimplantation, often reflects exposure to intraoperative methylprednisolone and, in some cases, basiliximab, depending on bleeding, resuscitation needs, and baseline renal function. T3, obtained 48 h postimplantation, reflects exposure to methylprednisolone, mycophenolate mofetil, and tacrolimus in most patients. This variability would most likely impact T3, the levels of which did not achieve statistical significance.

Although variable exposure to immunosuppression may influence HO-1 levels, we speculate the timing of sample collection likely precedes the onset of measurable immunosuppressive effects, reducing the impact of this confounding. Methylprednisolone can alter cytokine levels as early as 4 h postadministration.[38,39] Specifically, intravenous methylprednisolone can suppress proinflammatory cytokines such as tumor necrosis factor-α and interleukin (IL)-8 and increase anti-inflammatory cytokines like IL-10.[38,39] Although several studies suggest methylprednisolone can upregulate HO-1 expression,[40,41] evidence indicates glucocorticoids may antagonize HO-1 induction in the liver.[42] Other immunosuppressants show variable effects. Basiliximab, a monoclonal antibody targeting the IL-2 receptor on activated T cells, has not been linked to HO-1 modulation. Mycophenolate mofetil can increase HO-1 expression in murine models of acute liver injury,[43] but, like tacrolimus, was administered after the first 2 sampling timepoints in our study. Tacrolimus has context-dependent effects, with upregulation observed in murine liver models.[44-46]

Given the timing of sample collection and our findings, methylprednisolone is the most relevant immunosuppressant likely to influence HO-1 levels. In our cohort, the timing of methylprednisolone administration, whether before versus after T2, was similar between EAD and non-EAD groups, as well as between SCS and NMP-preserved allograft subgroups. Furthermore, there was no significant association between 2-h postimplantation HO-1 levels (T2), the timepoint which demonstrated the most statistical significance, and the timing of methylprednisolone administration. Moreover, even when administered beforehand, T2 was typically collected within an hour of methylprednisolone dosing, minimizing but not eliminating potential confounding. Collectively, this suggests that methylprednisolone exposure may have had minimal impact on peri-implantation HO-1 expression in our study.

Finally, our results may have been influenced by large-volume blood product resuscitation during OLT. Patient blood turnover with transfusion of donated blood products could have diluted or "washed out" serum HO-1 levels. However, this effect would be expected to lower serum HO-1 levels at T2 and T3, which was not observed. Despite high-volume blood product resuscitation in some cases, serum HO-1 levels demonstrated statistically significant changes over the course of the study, suggesting these measurements provide meaningful insight into the relationship between serum HO-1 levels and the development of EAD. Furthermore, there was no correlation between the peri-implantation rise in HO-1 (T2-T1) and EBL.

CONCLUSIONS

In summary, we observed an elevated rise in peri-implantation HO-1 levels correlated with the development of EAD. Our data support this may be a potential predictive biomarker of EAD and, if validated in a larger cohort, may identify patients for preemptive approaches to avert this severe complication.

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