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The use of organ donor blood in liver transplantation

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Abbreviations

AMR: antibody-mediated rejection

BPCR: biopsy-proven chronic rejection

BPTAR: biopsy-proven treated acute rejection

CIT: cold ischaemia time

CMV: cytomegalovirus

DB: donor blood

DCD: donation after circulatory death

DRI: donor risk index

DST: donor-specific transfusion

HTK: histidine-tryptophan-ketoglutarate

INR: international normalised ratio

LT: liver transplantation

MELD: model for end-stage liver disease

NDB: no donor blood

pRBC: packed red blood cells

UW: University of Wisconsin

VLTU: Victorian Liver Transplant Unit

ABSTRACT

Background

Blood removed from organs during deceased donor organ procurement is routinely discarded but is a potential resource for donor-specific transfusion (DST) in subsequent liver transplantation (LT). This study retrospectively analyses the impact of DST on intraoperative bank blood product usage, long-term graft and patient survival, as well as frequency of rejection post-LT.

Methods

A total of 992 adult LT performed from 1993 to 2018 in a single quaternary centre were included. Intraoperative blood product usage, patient and graft survival, as well as acute and chronic rejection were assessed in patients who received blood retrieved from the organ donor, the 'donor blood' (DB) group (n = 437) and patients who did not, the 'no donor blood' (NDB) group (n = 555).

Results

Processing of DB ensured safe levels of potassium, magnesium and insulin. There were fewer units of bank red blood cells transfusion required in the DB group compared to NDB group (2 vs 4 units, P =

0.01). Graft survival was significantly superior in the DB group (10-year survival 75% vs 69%, respectively, P = 0.04) but DST was not an independent predictor of graft survival. There was no significant difference in patient survival or rejection between the groups. There was no difference in treated, biopsy-proven rejection between the two groups.

Conclusions

This is the first large-cohort study assessing long-term outcomes of intraoperative DST in LT. The collection of organ donor blood and subsequent use in LT recipients appeared feasible with appropriate quality checks ensuring safety. DST resulted in a reduction in the use of packed red blood cells. There was no difference in the rate of rejection or graft or patient survival.

INTRODUCTION

Liver transplantation (LT) can be associated with significant intraoperative blood loss. This is related to coagulopathy, thrombocytopenia, portal hypertension and the complexity of LT surgery(1, 2). Patient blood management includes preoperative haemoglobin optimisation, careful haemostasis in surgery and the use of cell saver technology to reduce the need for blood transfusion.

Blood removed from the organs during the deceased donor organ procurement is usually discarded. Organ donor blood could instead be transfused in LT recipients with potential benefits in reduction in bank blood product utilisation and reduced rejection rates. Donor-specific transfusion (DST) has been shown to have a positive impact on graft survival and reducing incidence of rejection episodes, particularly in kidney transplantation(3). A study by Sollinger et al(4) of DST in living-unrelated

donor-recipient kidney transplantations demonstrated graft survival equivalent to that achieved with human leukocyte antigen-identical donors. Experimental and clinical studies of LT have demonstrated the potential benefit of DST in reducing acute rejection by possibly inducing immunological tolerance to the liver allografts(5-10). A clinical study by Sato et al(7) of intraportal DST in recipients during LT demonstrated a decrease in the dosage of immunosuppression compared with LT recipients who did not receive intraportal DST.

While DST in kidney transplantation has been shown to be effective, studies in LT are limited and involve a small sample of recipients. The Victorian Liver Transplant Unit (VLTU), one of six LT units in Australia and New Zealand, is the only provider of LT in Victoria and Tasmania. The transfusion of organ donor blood during adult LT was introduced in the VLTU in 1993. The working hypotheses of the program were that DST is safe and that LT recipients who received DST will have reduced intraoperative bank packed red blood cells (pRBC) usage with potential immunological benefits. This retrospective study aims to analyse our experience of DST in LT recipients from 1993 to 2018 with the hypothesis that DB can reduce intraoperative pRBC transfusion without an adverse impact on rejection, patient or graft survival. This study received ethics approval from the institution's Human Research Ethics Committee, LNR/18/Austin/353. This is the first large-scale study analysing the long-term outcomes of DST in LT.

MATERIAL AND METHODS

Study population.

Between April 1993 and December 2018, 1,211 transplants were performed by VLTU. After exclusion of paediatric LT (n= 210), 1,001 adult patients who had LT were identified as the cohort of the current study. Nine cases were omitted due to missing data on RBC transfusion, yielding a total of 992 recipients.

Safety considerations.

Organ donor blood has been routinely collected from brain-deceased donors during procurement operations undertaken by the VLTU since 1993 and transfused into recipients. Donation after circulatory death (DCD) donors were excluded because of the time critical nature of organ perfusion in these cases. Up to 2004, both ABO compatible and identical donor recipient pairs were transfused with organ donor blood after cross-matching. Processing of organ donor blood with the cell saver removed plasma ABO antibodies in all cases. From 2005 onwards, to enhance safety and improve quality of care, the protocol for selection of appropriate organ donors for DST included the donor and recipient being ABO identical, not rhesus positive-to-negative and not cytomegalovirus (CMV) positive-to-negative.

During the donor workup, detailed medical and social interviews with relatives of the deceased were undertaken by donor coordinators. These included questioning regarding infective risks such as sexual history, use of intravenous drugs and incarceration. In addition, viral serology and nucleic acid testing (routinely used since 2002) for human immunodeficiency virus (HIV), hepatitis B and C were conducted. Blood and body fluid cultures taken during the period of hospitalisation of the donor that were also available at the time of organ offer were documented and communicated. If a donor culture became positive after transplantation, the unit was notified and appropriate antibiotic cover was given. Donor families consented to donation and transfusion of organ donor blood for transfusion and the recipients also consented to transfusion of organ donor blood during transplantation. The 'donor blood' (DB) group comprised LT recipients who were transfused with blood retrieved during their deceased liver donor procurement procedure and the 'no donor blood' (NDB) group comprised LT recipients who did not receive organ donor blood.

Collection of organ donor blood.

Donors received broad-spectrum intravenous antibiotics at the commencement of the procurement operation followed by 25,000 units of intravenous heparin prior to cannulation. The abdominal aorta was cannulated with a 22 French arterial cannula (Medtronic, Dublin, Ireland). Blood was retrieved in a sterile closed system from the donor's inferior vena cava at the time of deceased donor liver procurement. A 32 French catheter with side holes (Portex Limited, Hythe, Kent, UK) was connected to a cell saver reservoir (Cell Saver Elite, Haemonetics, Braintree, Massachusetts, USA) through a length of tubing, which was clamped prior to insertion. The inferior vena cava was ligated superior to the confluence of the common iliac veins and was cannulated with the Portex catheter, with the tip of the catheter being placed at the level of the insertion of the hepatic veins into the inferior vena cava. Suction was applied to the reservoir. Commencement of organ perfusion occurred immediately after the release of the clamp on the tubing connected to the inferior vena cava catheter and at the time of cross-clamping of the supracoeliac aorta.

Donor perfusion.

Aortic and portal perfusion was used from 1993 to 2005 and aortic-only thereafter. The majority of cases used solutions such as Soltran (Soltran, Baxter Healthcare Ltd, Thetford, Norfolk) or hypertonic citrate solution (Ross solution, Orion Laboratories, Balcatta, WA, Australia) as a low viscosity initial flush. This was followed by either histidine-tryptophan-ketoglutarate (HTK) solution (Custodiol, Dr Franz Kohler Chemie Gmbh, Alsbach-Hähnlein, Germany) or Belzer University of Wisconsin (UW) solution (Viaspan, DuPont Merck Pharmaceutical Co., Netherlands or Bridge to Life Ltd., Columbia, South Carolina).

Processing and transfusion of donor organ blood.

The reservoir with donor organ blood was transported with the liver in a temperature-validated shipper. Blood was washed and concentrated in a cell saver (Cell Saver Elite, Haemonetics, Braintree, Massachusetts, USA). The concentrated red cells were resuspended in normal saline and cross-matched before transfusion. Once the result of cross-matching was received, the LT recipient was transfused with the blood derived from the donor of the liver they were to receive through routine systemic venous access. The decision to transfuse was at the discretion of the anaesthetist using standard transfusion triggers. Blood from the operative field was collected through the reservoir that was previously used to collect organ donor blood and returned to the patient as an autologous transfusion. All recipients received broad-spectrum antibiotics at commencement of the operation and for 24 hours postoperatively.

Assessing safety.

The concentrations of potentially harmful components derived from the perfusion fluid, including insulin, potassium and magnesium in the untreated retrieved organ donor blood and in the cell suspension after processing were measured from 1993 to 2004. During this time period, the cell suspension was also cultured to assess potential contamination. Measurements of components and culturing organ donor blood were halted once safety was established.

Assessing efficacy.

The volume and haemoglobin concentration of organ donor blood concentrate transfused were measured to assess efficacy of the transfusion. The VLTU database and anaesthetic records were analysed to compare intraoperative blood product usage, biopsy-proven treated acute rejection

(BPTAR), biopsy-proven chronic rejection (BPCR), antibody-mediated rejection (AMR), early allograft dysfunction (as defined by Olthoff et al.(11)), patient survival, graft survival and death-censored graft survival in the DB and NDB group. Patient survival was censored at retransplantation whereas graft survival was not.

Statistical analysis.

Data are presented as number and percentage or median and interquartile range (25%-75%). Categorical data were compared using the Chi-squared test and continuous data were compared using the Mann-Whitney U test for baseline characteristics. Paired data assessing safety parameters were compared using the Wilcoxon signed rank test. All statistical analyses are reported using 2-tail tests. Patient survival, graft survival and death-censored graft survival were assessed using Kaplan-Meier analysis and log-rank. Risk factors for graft survival were initially analysed using univariate Cox proportional hazards regression. Variables with a P value less than 0.1 on univariate analysis were entered into multivariate backward stepwise Cox proportional hazards regression analysis. P values less than 0.05 were considered significant. Statistical analysis was undertaken using IBM SPSS Statistics Version 26.

RESULTS

A total of 437 LT and 555 LT were in the DB and NBD group respectively. Out of the 437 LT that involved intraoperative DST, 121 received DST only. A total of 671 LT cases involved pRBC transfusion, with 265 in DB group and 406 in the NDB group respectively. The DB group had 315 cases that involved banked blood products whereas the NDB group had 439.

Safety.

Processing of organ donor blood resulted in a significant reduction in potassium, magnesium and insulin concentration to levels that were safe for transfusion into LT recipients (Table 1). Haemoglobin concentration increased from a median of 43 g/L to 164.5 g/L and leukocyte count from 3.9 x 10^9/L to 8.2 x 10^9/L (Table 1).

Baseline characteristics.

Comparison of donor, patient and transplant characteristics between the two groups is summarised in Table 2. Donor characteristics that were not different between the two groups include donor age (P = 0.68), reduced/split graft (P = 0.38) and rhesus status (P = 0.78). The donor risk index (DRI) was significantly lower in the DB group compared to the NDB group (1.43 vs 1.57, P <0.001). There were no donation after circulatory death (DCD) donors in the DB group (0 vs 6.9% in NDB group, P <0.001). There were more local donors in the DB group compared to the NDB group (80.1% vs 57.1%, P <0.001) and fewer national donors (shipped livers) in the DB group compared to the NDB group (6.2% vs 31.5%, P <0.001). There was a statistically significant difference between the groups in terms of donor cause of death (P = 0.016); anoxia (14.0% in DB vs 20.2% in NDB), trauma (18.5% vs 21.6%) and other causes of death (9.4% vs 9.9%) were less frequent, while stroke (57.9% vs 48.3%) was more frequent in the DB group compared to the NDB group. There were more type O blood group and fewer type A blood group donors in the DB than the NDB group (54.7% vs 43.4% and 34.6% vs 42.7% respectively, P <0.001). More donors in the DB group were CMV negative (43.5% vs 28.5%, P <0.001).

There was no significant difference in the DB and NDB group in terms of recipient age (P = 0.35), sex (P = 0.23), model for end-stage liver disease (MELD) score (P = 0.17), bilirubin level at time of

transplant (P= 0.50), international normalised ratio level at time of transplant (P = 0.61) and the primary disease process (P = 0.14). Creatinine level at time of transplant was significantly lower in the DB group than the NDB group (74 vs 87 μmol/L, P <0.001). There were significantly more recipients who were blood type O in the DB group compared with the NDB group (45.1% vs 32.4%) and fewer who were blood type A (39.1% vs 44.7%, P <0.001). More DB group recipients were CMV seropositive (83.7% vs 70.3%, P <0.001).

There was a trend to shorter cold ischaemia time (CIT) in the DB group (402 vs 423 minutes, P = 0.06) but no difference between the groups in the proportion of cases with a CIT > 12 hours, a cut-off above which initial graft function and long-term outcome have been shown to be inferior(12). The distance between the donor and recipient hospitals was less in the DB group (12 vs 21 km, P < 0.001). There were more ABO identical transplants in the DB group (88.3% vs 75.9%, P <0.001). A larger percentage of transplants in the NDB group were CMV positive-to-negative as well as rhesus positive-to-negative (23.3% vs 4.7%, P <0.001 and 16.1% vs 4.2%, P <0.001 respectively). There was a statistically significant difference in the perfusion technique, with patients in DB less likely to receive aortic-only perfused grafts than recipients in the NDB group (68.9% vs 85.4%, P <0.001). Recipients in the DB group were more likely to receive grafts perfused with UW solution than those in the NDB group (82.4% vs 75.9%, P = 0.013).

Intraoperative blood product usage.

Patients in the DB group received a median of 4 units of DST (Table 3). The median intraoperative pRBC requirement was halved in the DB group compared with the NDB group (2 vs 4 units, P <0.001). Recipients in the DB group were transfused with more autologous blood compared to recipients in the NDB group during LT (5 vs 4 units, P <0.001). There was no statistically significant difference in the usage of fresh frozen plasma, platelets or cryoprecipitate between the two groups.

There were significantly more recipients in the DB group who were transfused no pRBC during transplantation compared to the NDB group (39.4% vs 26.8% P <0.001). Additionally, recipients in the DB group were more likely to not require no bank blood products at all in comparison to the NDB group (27.8% vs 20.9%, P = 0.01).

Rejection episodes.

There was no significant difference in BPTAR (DB group 26.3% vs NDB group 21.4%, P = 0.07), AMR or BPCR between the groups (Table 4).

Early allograft dysfunction

There was no significant difference in early allograft dysfunction (DB group 22.9% vs NDB group 25.3%, P = 0.404).

Patient and graft survival.

The median patient survival for the entire study cohort was 20.4 years whereas the median graft survival was 17.9 years. The median graft survival was 17.9 years and 18.1 years in the DB and NDB group respectively. Graft survival was significant better in the DB group (P = 0.044, figure 1). The 1-, 5-, 10- and 20-year graft survival in the DB and NDB groups was 91%, 83%, 75% and 48% vs 86%, 77%, 69% and 44%, respectively. The median patient survival was 20.7 and 19.3 years for the DB and NDB group respectively. There was no significant difference in patient survival (P = 0.133). The 1-, 5-, 10- and 20-year patient survival in the DB and NDB groups was 94%, 87%, 80% and 52% vs 91%, 83%, 75% and 49% respectively. The median death-censored graft survival was not reached for

both groups. There was no significant difference in death-censored graft survival (P = 0.139). The 1-, 5-, 10- and 20-year death-censored graft survival in the DB and NDB groups was 97%, 96%, 94% and 92% vs 95%, 93%, 92% and 90%, respectively.

Univariate analysis indicated that DST was associated with increased graft survival (OR 0.889, 95% CI 0.793-0.997, P = 0.04, Table 5). Donor factors that were associated with decreased graft survival in the univariate analysis were DCD (OR 2.281, 95% CI 1.326-3.925, P = 0.003), DRI (OR 1.098, 95% CI 0.988-1.219, P = 0.08), national sharing (OR 1.242, 95% CI 1.019-1.515, P = 0.03), anoxia as cause of death (OR 2.455, 95%CI 1.638-3.678, P <0.001) and other causes of death besides stroke, trauma and anoxia (OR 1.572, 95% CI 1.040-2.375, P = 0.03). Recipient factors including recipient age (OR 1.023, 95% CI 1.012-1.035, P <0.001), recipient creatinine (OR 1.002, 95% CI 1.000-1.003, P = 0.01) and certain primary diseases were associated with decreased graft survival. Primary sclerosing cholangitis is the reference category and is significantly lower risk (P <0.001). The main primary diseases in this association were fulminant hepatic failure (OR 3.423, 95% CI 1.722-6.805, P <0.001), hepatitis B (1.973, 95% CI 0.929-4.190, P = 0.09), hepatitis C (OR 1.993, 95% CI 1.059-3.750, P = 0.033), malignancy (OR 3.659, 95% CI 1.833-7.305, P <0.001), nonalcoholic steatohepatitis (OR 4.695, 95% CI 1.936-11.385, P <0.001) and other causes not listed (OR 2.446, 95% CI 1.162-5.151, P = 0.019). Transplant factors that were associated with decreased graft survival include CIT (1.001, 95% CI 1.000-1.002, P = 0.006) and graft number (1.479, 95% CI 1.001-2.183, P = 0.049).

Multivariate analysis of reduced graft survival revealed that the following variables were independently associated with reduced graft survival: DRI (OR 1.410, 95% CI 1.101-1.804, P = 0.006), DCD (OR 2.023, 95% CI 1.160-3.528, P = 0.01), recipient's age at time of LT (OR 1.023, 95% CI 1.009-1.036, P <0.001), recipient's creatinine level at time of LT (OR 1.002, 95% CI 1.000-1.003, P = 0.026) and CIT (OR 1.001, 95% CI 1.000-1.002, P = 0.03). DST was not independently

associated with increased graft survival (OR 0.953, 95% CI 0.831-1.093, P = 0.5). The OR and 95% CI for all factors in univariate and multivariate analyses are summarised in Table 5.

DISCUSSION

This is the first report of the long-term results of a large-scale study of routine DST in LT. The routine collection of deceased organ donor blood is a feasible procedure that can be incorporated into the organ procurement procedure, including remote donors (the donor hospital was ≥ 1,000 km distance in 5% of DB cases). The technician-perfusionist who routinely attends organ donor procedures also facilitates the collection of organ donor blood. The reservoir used to collect the blood is reused in the LT for collection of autologous blood from the operative field, while the cell saver is used for preparation of the organ donor blood and of the blood salvaged at the operation. Therefore, the process of collecting organ donor blood is not demanding in terms of time, resources, feasibility or cost.

Results demonstrated that after processing, components such as potassium, magnesium and insulin levels are safe for organ donor blood to be transfused in LT recipients. The haemoglobin and leukocyte counts are increased due to concentration of organ donor blood after processing.

Patients who received organ donor blood required a smaller volume of blood bank pRBC during the intraoperative period. More recipients in the DB group required no transfusion than recipients in the NDB group. Although DST in LT may not be independently associated with improved patient and graft survival, potential benefits include minimization of exposure of recipients to third party antigens and freeing up the valuable resource of bank blood for other patients who need bank blood products.

In the current study, there was no significant difference in patient survival between the DB and NDB group. Kaplan-Meier analysis showed that recipients who received DST at transplantation had reduced graft loss compared to recipients who did not. However, it is acknowledged that the groups differ with respect to important variables that are likely to impact on graft survival. In particular, in comparison to the NDB group, the DB group had a lower DRI donors, no DCD cases, more local donors, fewer donors who died of anoxia and more who died of stroke, more blood group O and fewer blood group A and AB donors, fewer CMV positive donors, lower recipient creatinine, more blood group O recipients and fewer of the other blood groups, more rhesus positive recipients, more CMV positive recipients, more aortic plus portal perfusion, more use of UW perfusion solution, earlier year of transplantation, a trend to shorter CIT, a greater distance between donor and recipient hospitals, more ABO identical transplants and fewer CMV D+/R- and rhesus D+/R- transplants. In order to account for the impacts of these differences on graft survival in the groups, multivariate analysis which included these factors as well as whether or not the patients received organ donor blood was undertaken. Interestingly, multivariate analysis demonstrated that DST is not a variable independently influencing graft survival. The key variables shown to affect liver graft survival found in the study were DRI, DCD, recipient age, recipient creatinine and CIT, which are well known in the literature(13-17).

The lack of significant difference in BPTAR is incongruent with previous animal studies and small clinical studies that found that DST reduces the frequency of acute rejection(5-10). However, this is in keeping with Sato's(18) study which involved a small sample of LT patients receiving postoperative DST through the portal vein. They demonstrated no difference in total frequency of acute cellular rejection episodes compared to the group who did not receive DST. A possible future research direction involves the assessment of the use of organ donor blood versus bank blood in normothermic *ex vivo* perfusion.

Establishing microchimerism involving donor and recipient cells is considered a necessary precondition for tolerance as it results in changes to donor lymphocytes and cell repopulation post-transplantation(19). Various animal studies have demonstrated improvement in hepatic allografts of rats receiving DST before transplantation, possibly due to induction of immune tolerance(5-10). Proposed mechanisms of inducing immune tolerance after DST include creating an environment favouring T-helper 2 cytokines, as well as changes in migration of donor dendritic cells and passenger leukocytes in the lymphoid system(20-22).

Limitations of the study include its retrospective nature, the change in selection of cases over time and the fact that in addition to organ donor blood, some patients in the DB group received bank blood either intraoperatively or in the postoperative period, which may have effects on the outcomes of LT, particularly with regard to rejection.

There have been marked improvements in haemovigilance and blood product safety in transfusion medicine since the introduction of the DST program by the VLTU. This includes rigorous donor screening and testing, routine leucodepletion and transfusion-related acute lung injury mitigation practices. It is important to maximise the safety of donor blood transfusion and review of this practice is being undertaken. Current safety protocols included informed consent of LT recipients, detailed medical and social interviews with relatives of donors by donor coordinators, viral serology and nucleic acid testing, availability of microbiological cultures of the donor during the hospitalisation, administration of broad-spectrum antibiotics to donors and recipients, processing, cross-matching and microbiological culture of organ donor blood, communication between the organ procurement organisation and transplant units of any significant results or clinical events after transplantation, regular clinical meetings and auditing processes. Given the lack of benefit in terms of preventing rejection, consideration is being given to using a leukocyte filter when administering organ donor blood in order to further enhance the safety of DST.

CONCLUSIONS

This is the first large-scale study of the use of intraoperative unrelated DST in LT with long-term outcomes. Collection of deceased organ donor blood and its subsequent transfusion in LT recipients is feasible and safe. In contrast to previous animal model studies, there was no difference in the rate of rejection. Patient survival was equivalent in patients who received or did not receive DST. Although graft survival was superior in the DB group, DST was not independently associated with improved graft survival. Patients who received DST required less blood bank pRBC transfusion and were more likely to require no red cell or blood product transfusion at all. This has significant implications on overall demand placed on blood donors and blood bank inventory, though the reduction of bank blood usage needs to be weighed against the ability to provide a product of equivalent quality and safety.

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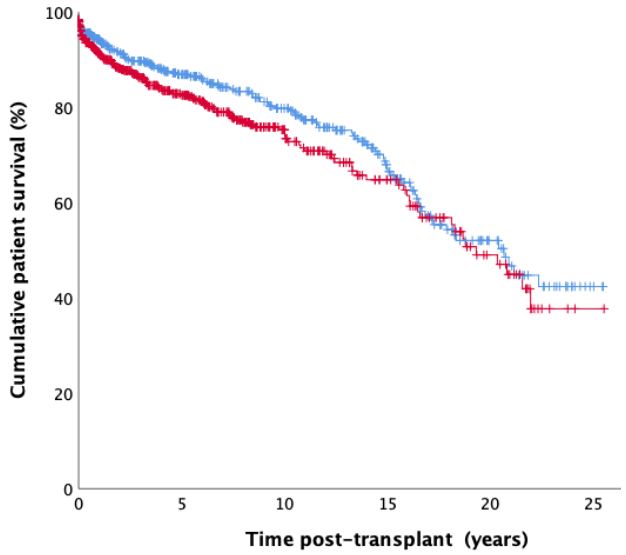
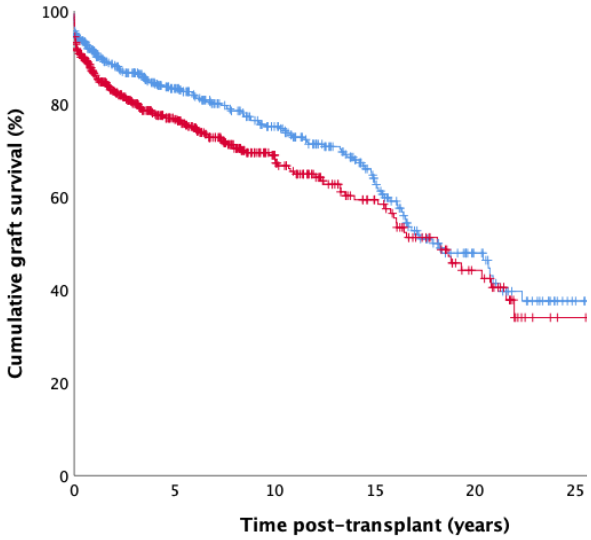
FIGURE LEGENDS

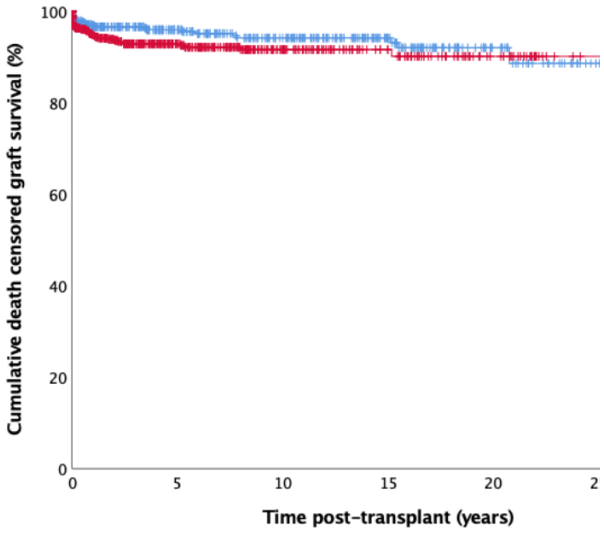
Figure 1: (a) Kaplan-Meier graph of cumulative post-transplant patient survival in the 'donor blood' group and 'no donor blood' group (P= 0.134). (b) Kaplan-Meier graph of cumulative post-transplant graft survival in the 'donor blood' group and 'no donor blood' group (P = 0.045). (c) Kaplan-Meier graph of cumulative death-censored graft survival in the 'donor blood' and 'no donor blood' group (P = 0.139).

a)



b)





TABLES

Table 1. Potassium, magnesium, insulin and haemoglobin concentration and leukocyte count in retrieved organ donor blood prior to processing and in cell suspension after processing.

Component	Donor blood		Cell suspension		P value
	Median	IQR	Median	IQR	
Potassium (mmol/L)	19.7	11.8-33.3	3.1	2.0-4.7	< 0.0001
Magnesium (mmol/L)	5.71	3.6-7.6	0.47	0.23-0.77	< 0.0001
Insulin (µU/mL)	42.8	8.5-240.0	4.8	3.0-26.7	< 0.0001
Haemoglobin (g/L)	43	18-117	165	145-175	0.006
Leukocyte count (x 10^9/L)	3.9	1.0-8.3	8.2	4.9-13.6	0.005

Abbreviations: IQR, interquartile range.

Table 2. Comparison of baseline characteristics between 'donor blood' and 'no donor blood' group for donor, recipient and graft factors. Data are shown as N (%) or median (interquartile range).

Characteristic	Donor blood group	No donor blood group	P value
N	437	555	
Donor factors			
DRI	1.425 (1.19-	1.57 (0- 7.441)	<0.001
Age (years)	45 (28-54.75)	44.5 (6-78)	0.681
DCD	0 (0)	38 (6.9)	<0.001
Split graft	60 (13.8)	66 (11.9)	0.381
Sharing*			<0.001
Local	350 (80.1)	317 (57.1)	
Regional	60 (13.7)	63 (11.4)	
National	27 (6.2)	175 (31.5)	
Cause of death			0.016
Anoxia	61 (14.0)	112 (20.2)	
Stroke	254 (57.9)	268 (48.3)	
Trauma	41 (18.5)	55 (21.6)	
Other	81 (9.4)	120 (9.9)	
Blood group			0.001
A	151 (34.6)	237 (42.7)	
AB	6 (1.4)	21 (3.8)	

B	41 (9.4)	56 (10.1)	
O	239 (54.7)	241(43.4)	
Rhesus status**			0.782
Negative	57 (13.0)	66 (11.9)	
Positive	374 (85.6)	483 (87.0)	
CMV status			<0.001
Negative	190 (43.5)	158 (28.5)	
Positive	169 (38.7)	286 (51.5)	
Indeterminant	0 (0)	1 (0.2)	
Recipient factors			
Sex			0.225
Male	301 (68.9)	362 (65.2)	
Female	136 (31.1)	193 (34.8)	
Age (years)	53 (45-59)	54 (18-72)	0.348
Disease			0.135
Biliary atresia	4 (0.9)	2 (0.4)	
Autoimmune	17 (7.3)	22 (4.3)	
Alcohol	61 (14.0)	64 (11.5)	
Cryptogenic	32 (7.3)	24 (4.3)	
Metabolic	16 (3.7)	18 (3.2)	

Fulminant hepatic failure	25 (5.7)	55 (9.9)	
Malignancy	59 (13.5)	84 (15.1)	
Hepatitis B	33 (7.6)	27 (4.9)	
Hepatitis C	86 (19.7)	107 (19.3)	
NASH	20 (4.6)	23 (4.1)	
Primary sclerosing cholangitis	45 (10.3)	65 (11.7)	
Primary biliary cirrhosis	18 (4.1)	27 (4.9)	
Other	21 (4.8)	37 (6.7)	
MELD score	19 (14-26)	20 (6-54)	0.172
Bilirubin (µmol/L)	84.5 (32-277.	90 (0-1055)	0.496
Creatinine (µmol/L)	74 (24.24-112	87 (0-905)	<0.001
INR	1.6 (1.3-2.1)	1.6 (0-10.5)	0.607
Blood group			0.001
A	171 (39.1)	248 (44.7)	
AB	14 (3.2)	30 (5.4)	
B	55 (12.6)	97 (17.5)	
O	197 (45.1)	180 (32.4)	
Rhesus status			<0.001
Positive	371 (93.7)	420 (81.6)	
Negative	25 (6.3)	95 (18.4)	

CMV status			<0.001
Positive	103 (83.7)	156 (70.3)	
Negative	20 (16.3)	66 (29.7)	
Transplant factors			
Perfusion			<0.001
Aortic and portal	136 (31.1)	81 (14.6)	
Aortic only	301 (68.9)	474 (85.4)	
Perfusion solution***			
UW	360 (82.4)	421 (75.9)	0.013
HTK	48 (11.0)	62 (11.2)	0.926
Graft number			0.450
1	412 (94.3)	512 (92.3)	
2	23 (5.3)	40 (7.2)	
3	2 (0.5)	3 (0.5)	
Year of transplant	2008 (2001-201	2012 (2006-2016)	<0.001
CIT (minutes)	402 (319.5-509	423 (335-423)	0.058
CIT****			0.503
> 12 hours	21 (4.8)	36 (6.5)	
≤ 12 hours	412 (94.3)	515 (92.8)	
Distance (km)*****	12 (10-44)	21 (10-679)	<0.001

Distance			<0.001
< 1,000 km	416 (95.2%)	454 (82.2%)	
≥ 1,000 km	21 (4.8%)	98 (17.8%)	
ABO compatibility******			<0.001
Identical	386 (88.3)	421 (75.9)	
Compatible	46 (10.5)	94 (16.9)	
Incompatible*******	1 (0.2)	34 (6.1)	
CMV D+/R-	20 (4.7)	105 (23.3)	<0.001
Rhesus D+/R-	17 (4.2)	83 (16.1)	<0.001

*Local= Melbourne metropolitan area, regional = rural Victoria and Tasmania, national = other states and New Zealand

**Donor rhesus status missing data: donor blood group 6 (1.4%), no donor blood group 6 (1.1%)

***Perfusion solution missing data: donor blood group 29 (6.6%), no donor blood group 72 (13.0%)

****CIT missing data: door blood group 4 (0.9%), no donor blood group 3 (0.5%)

*****Distance between donor and recipient hospitals using great circle calculation

******ABO compatibility missing data: donor blood group 4 (0.9%), no donor blood group 3 (0.5%)

******Incompatible = blood group A, non-A1 to O

Abbreviations: CAH, chronic active hepatitis; CIT, cold ischemic time; CMV, cytomegalovirus; D+/R-, donor positive to recipient negative; DCD, donation after circulatory death; DRI, donor risk index; HTK, histidine-tryptophan-ketoglutarate; INR, international normalised ratio; MELD, model for end-stage liver disease; NASH, nonalcoholic steatohepatitis; UW, University of Wisconsin.

Table 3. Comparison of 'donor blood and 'no donor blood' group in terms of intraoperative blood product usage.

Blood product (units)	Donor blood group		No donor blood group		P value
	Median	IQR	Median	IQR	
Donor blood	4	2-4	0	0	< 0.001
Bank packed red cells	2	0-5	4	0-6	< 0.001
Autologous blood	5	2-12	4	2-9	< 0.001
Fresh frozen plasma	2	0-4	2	0-4	0.172
Platelets	0	0-3	1	0-2	0.246
Cryoprecipitate	0	0-5	0	0-6	0.132

Abbreviations: IQR, interquartile range.

Table 4. Comparison between 'donor blood' and 'no donor blood' group in terms of rejection.

Rejection	'Donor Blood' group	'No donor blood' group	P value
BPTAR	115 (26.3)	119 (21.4)	0.073
AMR	2 (0.5)	1 (0.2)	0.429
BPCR	10 (2.3)	22 (4.0)	0.138

Abbreviations: AMR, antibody-mediated rejection; BPCR, biopsy-proven chronic rejection; BPTAR, biopsy-proven treated acute rejection.

Table 5. Univariate and multivariate analysis (Cox proportional hazards regression) of risk factors for graft loss.

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	P-value	OR	95% CI	P-value
Donor blood transfusion	0.889	0.793-0.997	**0.04**			
DRI	1.098	0.988-1.219	0.08	1.410	1.101-1.804	**0.006**
DCD	2.281	1.326-3.925	**0.003**	2.023	1.160-3.528	**0.01**
Donor age	1.004	0.997-1.011	0.22			
Split/Reduced	0.958	0.713-1.288	0.78			
Cause of Death						
Trauma	Reference		0.88			
Anoxia	2.455	1.638-3.678	**<0.001**			
Stroke	1.244	0.936-1.652	0.13			
Other	1.572	1.040-2.375	**0.03**			
Sharing						
Regional	Reference		0.10			
Local	1.034	0.867-1.234	0.71			
National	1.242	1.019-1.515	**0.03**			
Recipient MELD	1.002	0.987-1.018	0.77			
Recipient creatinine	1.002	1.000-1.003	**0.011**	1.002	1.000-1.003	0.026
Recipient age	1.023	1.012-1.035	**<0.001**	1.023	1.009-1.036	<0.001

Recipient INR	0.993	0.861-1.146	0.92
Recipient bilirubin	1.000	0.999-1.000	0.38
Disease category			
PSC	Reference		**<0.001**
Alcohol	1.345	0.706-2.562	0.37
Autoimmune	1.335	0.598-2.981	0.48
Biliary atresia	1.164	0.259-5.240	0.84
Cryptogenic	1.377	0.678-2.799	0.38
FHF	3.423	1.722-6.805	**<0.001**
Hepatitis B	1.973	0.929-4.190	0.08
Hepatitis C	1.993	1.059-3.750	**0.03**
Malignancy	3.659	1.833-7.305	**<0.001**
Metabolic	1.401	0.587-3.345	0.45
NASH	4.695	1.936-11.385	**<0.001**
PBC	1.954	0.996-3.832	0.051
Other	2.446	1.162-5.151	**0.019**
ABO compatibility			
Identical	Reference		0.56
Compatible	0.963	0.753-1.231	0.76
Incompatible	0.942	0.771-1.150	0.81

CMV D+/R-	0.832	0.558-1.242	0.37			
Rhesus D+/R-	1.017	0.688-1.505	0.93			
CIT	1.001	1.000-1.002	**0.006**	1.001	1.000-1.002	**0.029**
Year of transplant	1.002	0.983-1.021	0.86			
Graft number	1.479	1.001-2.183	**0.049**			

Abbreviations: CIT, cold ischemic time; CMV, cytomegalovirus; DCD, donation after circulatory death; DRI, donor risk index; FHF; fulminant hepatic failure; INR, international normalised ratio; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

