Title: Flavin Mononucleotide as A Biomarker of Organ Quality – A Pilot Study

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The authors declare no conflicts of interests.

Abbreviations:

ECD, extended-criteiria donor

DCD, donation after circulatory death

NMP, normothermic machine perfusion

NRP, normothermic regional perfusion

FMN, flavin mononucleotide

IRI, ischaemia-reperfusion injury

C-I, complex I

NIHR, National Institute for Health Research

BTRU, Blood and Transplant Research Unit

AU, artificial unit

QAS, quality assessment score

MEAF, Model for Early Allograft Function

ALT, alanine aminotransferase

INR, international normalised ratio

IQR, interquartile range

ROC, receiver-operating characteristic curve

CI, confidence interval

DGF, delayed graft function

PNF, primary non-function

OR, odds ratio

NHSBT, National Health Service Blood and Transplant

Abstract

Background: Flavin mononucleotide (FMN), released from damaged mitochondrial complex-I during hypothermic liver perfusion, has been shown to be predictive of 90-day graft loss. Normothermic machine perfusion (NMP) and normothermic regional perfusion (NRP) are used for organ reconditioning and quality assessment prior to transplantation. This pilot study aimed to investigate the changes of FMN levels during normothermic reperfusion of kidneys, livers and lungs, and examine whether FMN could serve as a biomarker to predict post-transplant allograft quality.

Methods: FMN concentrations in perfusates collected during NMP of kidneys, abdominal NRP, and *ex-vivo* lung perfusion, were measured using fluorescence spectrometry, and correlated to the available perfusion parameters and clinical outcomes.

Results: Among seven transplanted kidneys out of the 11 kidneys that underwent NMP, FMN levels at 60 minutes of NMP were significantly higher in the allografts that developed delayed graft function and primary non-function (p=0.02). 15 livers from 23 circulatory death donors that underwent NRP were deemed suitable for transplantation. Their FMN levels at 30 minutes of NRP were significantly lower than those not procured for transplantation (p=0.004). In contrast, little FMN was released during the 8 lung perfusions.

Conclusions: This proof of concept study suggested that FMN in the perfusates of kidney NMP has the potential to predict post-transplant renal function, while FMN at 30 minutes of NRP predicts whether a liver would be accepted for transplantation.

More work is required to validate the role of FMN as a putative biomarker to facilitate

safe and reliable decision making prior to embarking on transplantation.

Introduction

For all solid organ transplantation, there is a discrepancy between number of patients on the waitling lists and level of transplant activity. Despite this organ shortage, a significant number of the available donor organs are not accepted for transplantation. For example, from April 2018 to March 2019, only 84% of the kidneys, 62% of livers, 25% of pancreases, 26% of hearts and 18% of lungs, offered for donation in the United Kingdom, were transplanted into recipients[1]. Organs from extended-criteria donors (ECD), such as donors of older age and donation after circulatory death (DCD), have been increasingly considered for transplant. However, these organs are known to be associated with higher risks of poor post-transplant outcomes. In order to increase organ utilisation rates, alternative methods of organ preservation and assessment have been explored in the last decade, including both, *ex-situ* normothermic machine perfusion (NMP) and normothermic regional perfusion (NRP) in DCD donors. NMP revives donor organs prior to transplantation, providing an opportunity to assess ECD organs[2-4]. NRP aims to reverse warm ischaemic injury and so improve allograft quality in the DCD setting[5].

Comprehensive evaluation of allograft quality is of vital importance to minimise unnecessary organ discard and optimise donor-recipient matching. However, as of yet, there has been no successfully validated scoring system or biomarker that is in widespread clinical use to facilitate transplant teams' decision making process prior to implantation[6-9]. The lack of objective measurement leaves transplant surgeons to accept organs based on their clinical experience. There is a need for robust and reliable assessment techniques, with rapidly obtainable results in a routine setting.

Muller *et al*. used the release of flavin mononucleotide (FMN) from liver into the hypothermic oxygenated perfusion solution as a surrogate marker for ischaemia-reperfusion injury (IRI). They demonstrated that a real-time fluorometric reading of FMN levels in the acellular perfusate after 30 minutes of hypothermic perfusion had a strong correlation with post-transplant hospital stay and cumulative complications, and was predictive of 90-day graft loss[6].

FMN, a small molecule bound to the mitochondrial complex I (C-I), oxidises NADH and generates electrons for ubiquinone reduction[10]. During ischaemia, the non-covalent bond between the reduced FMN and mitochondrial C-I becomes weaker[11]. Upon reperfusion, succinate-supported reverse electron transfer drives FMN dissociation and generates reactive oxygen species[12]. The loss of FMN results in a decline of C-I activity and thus mitochondrial dysfunction[11,12].

During NMP and NRP, the restoration of perfusion to ischaemic organs inevitably causes IRI and FMN release from the damaged mitochondria. Perfusion approaches provide a unique opportunity to objectively evaluate potential organs prior to implantation. This study retrospectively analysed FMN levels in perfusate samples collected during NMP (kidneys and lungs) and abdominal NRP (livers) by the research groups in the network of the National Institute for Health Research (NIHR) Blood and Transplant Research Unit (BTRU) in Organ Donation and Transplantation between Cambridge and Newcastle Universities. We aimed to investigate the changes of FMN levels during normothermic reperfusion of kidneys, livers and lungs, and examine

whether FMN level in perfusates collected during reperfusion could serve as a biomarker to predict post-transplant allograft quality.

Materials and Methods

FMN measurement calibration

Riboflavin 5'-monophosphate sodium salt hydrate (Sigma-Aldrick, United States) was dissolved in 0.9% sodium chloride to make a series of FMN solutions with concentrations ranging from 12.5 ng/ml to 625.0 ng/ml. 200μl of each solution in triplicates were loaded into a black non-binding 96-well microplate (Greiner Bio-one, Austria) at room temperature under dimmed light. The microplate was analysed by fluorescence spectroscopy (Infinite$^{\circledR}$ 200 PRO, Switzerland). A monochrome light with excitation wavelength of 450nm was used and the fluorescence with wavelength of 525nm emitted by the FMN was recorded with 100% gain. The fluorescence readings in artificial unit (AU) were displayed in an Excel spreadsheet produced by the spectrometer. This experiment was performed three times. The average fluorescence readings of the FMN solutions were plotted against their known concentrations to generate a standard curve. The same steps were repeated for the FMN solutions, that were stored at 4°C for 1 day and 7 days and at -80°C for 28 days, to assess whether cold storage alters FMN concnentration.

Organ perfusion studies

A series of organ perfusion studies were carried out at Cambridge and Newcastle Universities under the research governance infrastructure established by the BTRU. During these studies, as detailed below, perfusate samples were prospectively

collected from NMP for kidneys and lungs, and NRP for livers in DCD donors. All of the perfusate samples taken were centrifuged immediately after collection. The supernatants were stored at -80°C until analysis.

Normothermic kidney perfusion

11 single kidneys that were turned down by all UK transplant centres were recruited for the NMP project by the Cambridge group. Apart from 1 pair which were declined by all of the UK transplant centres because of old donor age, the others were all turned down due to poor *in situ* perfusion. These kidneys were perfused with oxygenated red blood cell-based solution on the NMP circuit for 60 minutes at 35.0-36.0°C, according to the previously established protocol[2]. Perfusate samples were collected just prior to and after 60 minutes of NMP, and the perfusion related parameters, including lactate, potassium, venous pH and urine output, were recorded. The quality assessment score (QAS), based on macroscopic appearance, mean renal blood flow and total urine output, was performed at the end of NMP[2]. Seven of these kidneys were implanted into recipients. The post-transplant outcomes, such as complications, hospital stay and follow-up creatinine, were collected from the hospital electronic database. The Remuzzi score of these kidneys was assessed by a consultant pathologist using wedge biopsies taken at the end of cold storage.

Abdominal normothermic regional perfusion

Abdominal NRP was undertaken in 23 DCD donors by the Cambridge group. Following verification of death at least 5 mnutes after asystole, a normothermic circulation of oxygenated donor blood was restored to the abdominal organs using an extra-corporeal membrane oxygenation circuit. During the 2-hour NRP, abdominal

organs were monitored closely and blood samples were taken every 30 minutes for blood gas and biochemical analysis, as per the protocol published previously[5]. A total of 15 livers were accepted for clinical transplantation. FMN levels in the blood samples collected prior to and at 30 minutes, 60 minutes, 90 minutes and 120 minutes of NRP were measured. The recipients' characteristics and post-transplant biochemical results, complications and outcomes were retrieved. The model for early allograft function (MEAF) score was calculated based on the maximum alanine aminotransferase (ALT) and international normalised ratio (INR) within the first 3 days post-transplant and bilirubin level on the 3rd day post-transplant[13].

Normothermic lung perfusion

Eight single lungs, turned down for transplantation, were assessed using *ex situ* normothermic perfusion by the Newcastle group. Along with the physiological monitoring, the acellular perfusate samples were collected every 30 mintues during perfusion. The samples taken before and at 30 minutes, 60 minutes and 90 minutes of the perfusion were used for FMN measurement. As this project was designed for research purposes only, none of these lungs were transplanted.

Ethics

All of the projects had ethical approval granted by regional or national research ethic committees (16/NE/0230 and 15/EE/0175).

FMN concentration measurement

After thawing, 200μl of each samples in triplicates were loaded into the microplate and analysed by the fluorescence spectrometry using the same settings detailed above.

The fluorescence readings in AU were correlated with the perfusion parameters and clinical data when appropriate.

<u>**Statistical analysis**</u>

Shapiro-Wilk test of normality was performed for each set of data. Continuous variables with normal distribution were reported as mean $\pm$ standard deviation and non-Gaussian data as median and interquatile range (IQR). To compare the means of continuous variables, two-sided paired and independent t-tests were used when appropriate. Correlation of the fluorescence readings and other perfusion parameters with post-transplant outcomes were calculated using the Pearson correlation coefficient (r). Sensitivity and specificity of the predictive value of FMN was analysed using receiver-operating characteristic curve (ROC) analysis. Binary logistic regression was used to assess the predictive value of FMN for mortality. *P*-values of less than 0.05 were considered as statistically significant. All statistical analyses were undertaken using the GraphPad Prism 8.0 and IBM SPSS Statistics version 24 64-bit edition.

Results

<u>**Fluorescence spectrometry measurement of FMN**</u>

The fluorescence readings had a linear relationship with the FMN concentrations within the range from 12.5 ng/ml to 625.0 ng/ml (Figure 1a). These readings did not change significantly after the samples had been stored at 4°C for up to 7 days and -80°C for up to 28 days (Figure 1b). Therefore, FMN levels in the perfusate solutions remain stable during -80°C storage. It is feasible and reliable to measure FMN levels in the perfusate samples collected previously and stored in -80°C freezer.

Normothermic kidney perfusion

The 11 kidneys recruited were from 7 DCD donors (4 males and 3 females) with age of 51.9±18.2 years (Table 1). The mean terminal creatinine level was 68.1±20.4 μmol/L. These kidneys were procured in the standard fashion with cold flush after explantation and preserved with static cold storage. The warm ischaemic time and cold ischaemic time prior to the onset of NMP were 14.4±2.7 minutes and 11.1±5.6 hours respectively. After 1 hour of NMP, the FMN levels in the perfusion circuits increased significantly from 3359±2309 AU to 7815±3386 AU (P = 0.005, 95% confidence interval (CI): 1683 to 7231 AU) (Figure 2a). However, it had no correlation with QAS assessed at the end of NMP (r=-0.23, P=0.5) (Figure 2b). The perfusion parameters and QAS of these 11 kidneys were also summarised in Table 1.

Seven out of the 11 kidneys were deemed suitable for clinical transplantation. All of them had both QAS and Remuzzi socres less than 3. The 7 recipients (6 males and 1 female) were 47.4±11.5 years old (Table 2). After transplantation, one (14.3%) of these 7 kidneys developed delayed graft function (DGF) and two (28.6%) developed primary non-function (PNF). These 3 kidneys had significantly higher FMN levels at 60 minutes of NMP than the other 4 which had initial graft function (11029±3315 AU vs. 5201±1474 AU, P=0.02, 95% CI: -1141 to -10515 AU) (Figure 2c). None of the recipients developed acute rejection. One patient passed away with a functioning allograft on post-transplant day 10, because of myocardial infarction. All of the other patients were followed up for at least 3 months by the transplant team. Among the parameters that were measured at the end of NMP, FMN, potassium level and urine output were correlated with the recipients' hospital stay; and FMN, venous pH and

urine output were strongly correlated with their creatinine on 14 days, 1 month and 3 months post-transplant (Table 3).

Abdominal normothermic regional perfusion

23 circulatory death donors (17 males and 6 females) of ages 50.2±13.9 years underwent NRP, after a median of 13 minutes (IQR 10-22 minutes) agonal phase and 15.3±2.9 minutes of asystolic duration (Table 4). During 2 hours of abdominal NRP, FMN levels in the perfusion circuits first peaked at 30 minutes, then reduced at 60 minutes and stabilised afterwards (Figure 3a). The FMN fluorescence reading at 30 minutes of NRP was significantly higher than that of the readings at baseline (27504±1516 AU vs. 20797±10759 AU, P=0.008, 95% CI: -1738 to -10069 AU). For the 15 livers which were accepted for clinical transplantation, their FMN fluorescence readings at 30 minutes were significantly lower than those of the 8 livers declined (19628±7456 AU vs. 33368±12798 AU, P=0.004, 95% CI: 4802 to 22677 AU) (Figure 3b). Higher FMN level at 30 minutes of NRP was predictive of livers being declined for transplantation (area under the ROC curve (AUC) was 0.85, 95% CI: 0.67 to 1.00).

Table 5 listed the characteristics and post-transplant biochemical results, comoplications and hospital stay. One patient died on post-transplant day 34 because of graft-versus-host disease. No other severe complications, graft loss or mortality were reported. FMN reading at 30 minutes of NRP was not correlated with neither lactate (r=-0.16, P=0.6) or INR at 24 hours (r=0.17, P=0.6), or peak ALT within 7 days post-transplant (r=-0.08, P=0.8), nor MEAF score (r=-0.18, P=0.5). In this cohort, neither FMN reading (Odds Ratio (OR) 1.00, P=0.4) nor MEAF score (OR 1.96, P=0.4) was predictive of mortality.

Normothermic lung perfusion

Eight single lungs from 5 donors were included in this study. The donor ages ranged from 22 to 63 years (median 52 years, IQR 40-56 years). Although they represented a heterogenous group of donors with their lungs declined for different reasons, they uniformly release little FMN into the perfusate. The perfusate FMN fluorescence reading was 1149 ± 35 AU prior to perfusion and gradually increased as the perfusion went on. The FMN fluorescence reading was 1197 ± 37 AU at 30 minutes, 1208 ± 49 AU at 60 minutes, and 1241 ± 53 AU at 90 minutes of normothermic perfusion. Although the FMN levels at 60 minutes and 90 minutes were significantly higher than the baseline level (P values of 0.003 and 0.02 respectively), the differences in the readings were very small.

Discussion

This novel study is the first to examine FMN fluorescence readings in the perfusate samples collected during the normothermic machine perfusion and regional perfusion projects of three different types of solid organs. The findings demonstrated that FMN release during post-ischaemic reperfusion was consistent with clinical judgement of liver allograft quality and associated with post-transplant renal function in kidney transplantation. FMN measurement is simple and quick to perform and fluorescence spectrometry is widely available in the biochemistry laboratories in most secondary healthcare facilites. In addition, We have shown that it is a robust molecule whose concentration does not alter after cold storage. If proven and validated as a reliable marker of allograft quality in larger cohorts, it would provide an objective measure to

inform decisions about whether or not to transplant an ECD organ during machine perfusion or NRP.

NMP technology has been adopted by many transplant centres to assess potential ECD kidneys, which would otherwise be discarded[2,3,14]. For example, in the NMP project done by the Cambridge group, the 7 kidneys would not have been transplanted, if they had not been assessed during NMP. However, ECD kidneys are associated with poorer post-transplant outcomes; as in this project, 3 out of the 7 transplanted kidneys did not have initial function. FMN fluorescence readings in the perfusates at 60 minutes of NMP, albeit a small cohort size, seemed to be able to differentiate kidneys that had initial function from those that did not. Higher release of FMN into perfusates during NMP suggests more extensive mitochondrial dysfunction. Kidney, as the organ with the second highest mitochondrial content, relies on energy produced by adequate amount of functioning mitochondria. Therefore, these kidneys that lost more FMN were expected to have reduced initial function post-transplant. Although the FMN level had no correlation with QAS, in this cohort of kidneys with low QAS, FMN was demonstrated to have better predictive value for post-transplant renal function than QAS. More work is required to validate the predictive power of FMN in larger cohorts, but FMN is a promising biomarker with potential to improve decision making during NMP.

Muller *et al.* demonstrated that with hypothermic oxygenated perfusion after cold storage that livers from DCD donors can be safely implanted in selected recipients with short-term and median-term graft survivals similar to that of the standard critieria livers[6]. This work reflects an isolated organ perfusion system.

A period of *in-situ* normothermic perfusion in DCD donors before organ extraction has also been suggested to reverse the deleterious effect of warm ischaemia on solid organs[5]. During NRP, retrieval surgeons have the opportunity to fully inspect and assess the organs and prepare for procurement without undue pressure to minimise warm ischaemic time. The findings that the livers declined for transplantation had higher FMN fluorescence readings at 30 minutes of NRP suggested that more FMN was released from livers perceived as poor quality based on surgeons' clinical experience. However, during NRP, other abdominal organs also contribute FMN to the perfusion circuit. This might be the reason that the FMN level at 30 minutes of NRP was not associated with post-transplant liver function, in contrast to the findings reported in the isolated circuit by Muller *et al.*[6]. Interestingly, the FMN levels in the NRP circuit reduced after reaching a peak at about 30 minutes. One possible explanation was that NRP for longer than 30 minutes partially repaired the IRI induced mitochondrial damage and thus reduced mitochondrial dysfunction in the donor organs. Further investigation is required to explore this speculation.

In contrast to kidneys and livers, lungs did not release much FMN during NMP and the amounts released were consistent despite the drastic difference in donor characteristics. Lungs have strikingly low metabolic activity, reflected in a far smaller mitochondrial mass, compared with metabolically very active organs such as kidneys and livers. In addition, the presence of oxygen in the partially inflated lungs may ameliorate or even abolish any true ischaemia during static cold storage.

This study is limited by the small sample sizes, its retrospective nature and the number of available perfusion and post-transplant parameters. In the future, the exact

concentration of FMN in perfusate samples, assessed by the point-of-care fluorescence spectrometry, will be confirmed using high performance liquid chromatography-mass spectrometry. It is also unclear how the pre-existing FMN in the donor's blood and the packed red blood cells used for blood-based perfusion influence the release of FMN from damaged mitochondrial C-I. In future organ reperfusion projects, one could consider the use of haemoglobin-based oxygen carriers or other acellular perfusates to minimise the background FMN level.

In conclusion, FMN analysis is quick to perform, widely accessible and consistent in spite of cold storage. Its concentration during kidney NMP, as a point of care measurement, has the potential to predict post-transplant renal function, and facilitate safe and reliable assessment of kidneys prior to transplantation. A more detailed examination of the association between FMN levels in the perfusates and post-transplant outcomes in larger cohorts is required to confirm and validate the role of FMN as a putative biomarker of allograft quatliy.

Acknowledgements

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Figure Legends

Figure 1. a) Concentration curves of FMN generated by fluorescence spectrometry. b) Comparison of the FMN fluorescence readings of fresh samples, samples stored at 4°C for 1 day and 7 days, and at -80°C for 28 days (n=3).

Figure 2. a) The FMN fluorescence readings of the perfusates taken prior to and at 60 minutes of normothermic machine perfusion of kidneys (n=11). b) Correlation of the FMN fluorescence readings in the perfusates at 60 minutes of NMP with QAS (n=11). c) Comparison of the FMN levels at 60 mintues of NMP in the kidneys that had initial function (n=4) to those of the ones that did not (n=3).

Figure 3. a) The changes in the FMN fluorescence readings during the 2 hours of abdominal normothermic regional perfusion. b) Comparison of the FMN levels at 30 mintues of NRP in the livers that were accepted for transplantation (n=15) to those of the ones that were declined (n=8). c) ROC analysis of FMN levels at 30 minutes of NRP on prediction of livers being accepted for transplantation after NRP.

Table 1. Donor characteristics and perfusion-related details of the normothermic

kidney perfusion project

Donor characteristics	N = 7
Age (years)[#]	51.9 ± 18.2
Sex ratio (M:F)	4:3
BMI (kg/m^2)[#]	30.1 ± 7.6
Creatinine at retrieval (µmol/L)[#]	68.1 ± 20.4
Cause of death	
Hypoxic brain injury	3
Intracranial haemorrhage	2
Trauma	1
Cardiac arrest	1
Perfusion related details	**N = 11**
Cold ischaemic time prior to NMP (hours)[#]	14.4 ± 2.7
Warm ischaemic time prior to NMP (minutes)[#]	11.1 ± 5.6
Perfusion parameters at the end of NMP	
FMN (AU)[#]	7815 ± 3386
Venous pH[#]	7.38 ± 0.09
Potassium (mmol/L)[#]	11.72 ± 2.32
Lactate (mmol/L)[#]	8.2 ± 2.4
Total urine output (ml)[#]	178 ± 192
Quality assessment score*	2 (1-3)

Values are presented as [#]mean ± standard deviation and *median (interquartile range)

Table 2. Recipient characteristics and post-transplant complications and renal function

of the normothermic kidney perfusion project

Recipient characteristics	N = 7
Age (years)[#]	47.4 ± 11.5
Sex ratio (M:F)	6:1
Creatinine prior to transplant (μmol/L)[#]	597.4 ± 140.1
Hospital stay (days)*	9.1 ± 3.8
Primary non-function	2
Delayed graft function	1
Acute rejection	0
Follow-up creatinine (μmol/L)	
1st day[#]	471.3 ± 138.3
5th day[#]	480.0 ± 213.6
14th day[#]	301.7 ± 175.0
1st month[#]	249.5 ± 166.4
3rd month[#]	275.8 ± 223.0
Death	1

Values are presented as [#]mean ± standard deviation and *median (interquartile range)

Table 3. Correlation of the perfusion parameters at the end of normothermic kidney

perfusion with the recipients' hospital stay and renal function post-transplant

| | Creatinine Post-transplant | | | | | Hospital Stay |
	1 day	5 days	14 days	1 month	3 months	
FMN	r = -0.28 *P* = 0.5	r = 0.32 *P* = 0.5	r = 0.95 *P* = 0.003	r = 0.91 *P* = 0.01	r = 0.88 *P* = 0.02	r = 0.77 *P* = 0.04
Lactate	r = -0.04 *P* = 0.9	r = 0.66 *P* = 0.1	r = 0.62 *P* = 0.2	r = 0.51 *P* = 0.3	r = 0.46 *P* = 0.4	r = 0.38 *P* = 0.4
Potassium	r = -0.22 *P* = 0.6	r = 0.47 *P* = 0.3	r = 0.85 *P* = 0.03	r = 0.81 *P* = 0.06	r = 0.78 *P* = 0.07	r = 0.91 *P* = 0.004
Venous pH	r = 0.03 *P* = 0.9	r = -0.19 *P* = 0.7	r = 0.83 *P* = 0.04	r = 0.84 *P* = 0.04	r = 0.85 *P* = 0.03	r = 0.60 *P* = 0.2
Urine output	r = 0.06 *P* = 0.9	r = -0.27 *P* = 0.6	r = -0.95 *P* = 0.003	r = -0.94 *P* = 0.004	r = -0.92 *P* = 0.01	r = -0.76 *P* = 0.04
QAS	r = -0.06 *P* = 0.9	r = 0.29 *P* = 0.5	r = -0.07 *P* = 0.9	r = -0.11 *P* = 0.8	r = -0.15 *P* = 0.8	r = 0.12 *P* = 0.8

Table 4. Donor characteristics and procurement details of the abdominal normothermic regional perfusion project

Donor characteristics	N = 23
Age (years)[#]	50.2 ± 13.9
Sex ratio (M:F)	17:6
BMI (kg/m^2)[#]	26.8 ± 5.1
Cause of death	
Hypoxic brain injury	6
Intracranial haemorrhage	13
Trauma	4
Cardiac arrest	0
Agonal phase duration (minutes)*	13 (10-22)
Asystolic duration (minutes)[#]	15.3 ± 2.9

Values are presented as [#]mean ± standard deviation and *median (interquartile range)

Table 5. Recipient characteristics and post-transplant outcomes of the abdominal normothermic regional perfusion project

Recipient characteristics	N = 15
Age (years)[#]	53.6 ± 12.5
Sex ratio (M:F)	9:6
Lactate at 24 hours post-transplant[#]	1.5 ± 0.7
INR at 24 hours post-transplant[#]	1.8 ± 0.4
Peak ALT within 7 days post-transplant*	513 (412-1102)
MEAF score[#]	4.7 ± 2.5
Post-transplant complications	
Anastomotic stricture	0
Biliary leak	0
Cholangiopathy	0
Hospital stay (days)[#]	20.4 ± 13.2
Death	1

Values are presented as [#]mean ± standard deviation and *median (interquartile range)