ORIGINAL ARTICLE

Dual aortic and portal perfusion at procurement prevents ischaemic-type biliary lesions in liver transplantation when using octogenarian donors: a retrospective cohort study

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SUMMARY

Several risk factors for ischaemic-type biliary lesions (ITBL) after liver transplantation (LT) have been identified, but the role of portal vein perfusion at graft procurement is still unclear. This was a prospective study on double aortic and portal perfusion (DP) of liver grafts stratified by donor's decade (<60 yo; 60–69 yo; 70–79 yo and ≥80 yo) versus similar historical cohorts of primary, adult grafts procured with single aortic perfusion (SP) only. The primary study aim was to assess the role of DP on the incidence of ITBL. There was no difference in the incidence of overall biliary complications according to procurement technique for recipients of grafts <80 years. A higher incidence of ITBL was observed for patients receiving grafts ≥80 years and perfused through the aorta only (1.9 vs. 13.4%; $P = 0.008$). When analysing octogenarian grafts, donor male gender (HR = 6.4; $P = 0.001$), haemodynamic instability (HR = 4.9; $P = 0.008$), and type-2 diabetes mellitus (DM2) (HR = 3.0; $P = 0.03$) were all independent risk factors for ITBL, while double perfusion at procurement (HR = 0.1; $P = 0.04$) and longer donor intensive care unit (ICU) stay (HR = 0.7; $P = 0.04$) were protective factors. Dual aortic and portal perfusion has the potential to reduce post-transplant ITBL incidence for recipients of octogenarian donor grafts. Larger series are needed to confirm this preliminary experience.

Transplant International 2019; 32: 193–205

Key words
dual perfusion, graft survival, ischaemic type biliary lesions, octogenarian donors

Received: 15 April 2018; Revision requested: 1 May 2018; Accepted: 5 September 2018; Published online: 15 October 2018

Introduction

The use of old donors in liver transplantation (LT) provides encouraging results and is expanding worldwide [1]. Nevertheless, this practice is not universally implemented [2], due to concerns about a higher risk for primary non-function (PNF), delayed graft function (DGF) [3–5], and worse long-term graft survival [6–8].

Recently, we reported our experience with use of octogenarian deceased liver donors and showed favourable long-term results [9]. However, HCV recurrence and ischaemic-type biliary lesions (ITBL) were the two

doi:10.1111/tri.13342

major reasons for graft loss [9]. While availability of direct antiviral agents will likely reduce to nil the negative impact of donor age on post-transplant HCV recurrence [10], prevention and management of ITBL-related morbidity is pivotal to improve the results of elderly donor LT and donors after cardiac death (DCD) [11,12].

Over the recent years, evidence is accumulating on the role of donor age as an independent risk factor for post-transplant ITBL [9,13], but controversies still exist on the mechanisms leading to ITBL [14]. Ischaemia/reperfusion injury (I/R) and procurement-related injuries to the deep peribiliary vascular plexus have been identified as some of the most relevant factors involved [14,15]. In the setting of octogenarian donors, our experience showed that ITBL is associated with donor age, haemodynamic instability, and type-2 diabetes mellitus (DM2), alongside allocation to higher model for end-stage liver disease (MELD) score recipients [16]. Taken together, these findings support the role of peribiliary microcirculation in the development of ITBL.

On the basis of considerations about the role of microvascular injuries and on retrospective review of incidence of ITBL, in March 2014, we implemented routine dual (aortic and portal) graft perfusion during procurement. The present paper aims at presenting the results achieved with such protocol in terms of post-transplant ITBL in recipient population stratified by donor decades to assess the potential relationship between donor age and procurement technique.

Materials and methods

This was an institutional review board (IRB)-approved, single centre, retrospective study with a historical match at a single centre. All deceased-donor, whole-sise, primary, ABO-compatible, adult LT procedures performed between April 2014 and January 2016 and using grafts procured with dual (aortic and portal) perfusion were considered (DP group). The control group was the historical cohort of consecutive recipients of liver grafts procured using single aortic perfusion in the previous decade (January 2005–March 2014) (single perfusion (SP) group). For the purposes of the current analysis, UNOS status-1 recipients or those with proven post-LT hepatic artery thrombosis (HAT) or stenosis (HAS) were excluded, so were those grafts procured without a full adherence to the new DP procurement protocol. Patients were eventually divided in four sub-groups based on donor age: <60, 60–69, 70–79 and ≥80 years. The aim of the current analysis was to compare the efficacy of DP in the overall population of recipients and according to donor age.

Donor selection algorithm

The deceased donor evaluation policy in use at our centre has been published previously [9]. Octogenarian donors were preliminary evaluated based on their past medical history and blood tests and declined if one of the following was present: transaminases >250 UI/l; total bilirubin (t-BIL) >3 mg/dl; evidence of chronic liver disease at pre-procurement ultrasound, while no specific cut-off criteria were considered for younger donors. Liver graft biopsy was performed on demand depending on surgical evaluation at procurement. Grafts were discarded in the presence of any of the following conditions: macro-vescicular steatosis ≥30%; necrosis ≥5%; fibrosis ≥2 as per Ishak [17]; severe micro-angiopathy (i.e. arteriolar thickening ≥60%), and macro-angiopathy with arterial anastomosis being technically unfeasible.

Donor variables

Deceased donor data were obtained from clinical charts. Eligibility to liver donation was evaluated as per our institutional policy and according to the Italian National Transplant Agency [Centro Nazionale Trapianti (CNT)] guidelines [18]. The included variables were: age; gender; body weight; height; body mass index (BMI); cause of death; liver function tests at procurement; history of co-morbidities (diabetes mellitus, hypertension, cardiovascular disease, renal disease, haemodialysis and dyslipidemia); use of inotropic agents; history of cardiac arrests or haemodynamic instability; intensive care unit (ICU) stay; location of procurement; serologic status with regard to hepatitis-B virus (HBV) infection, HCV, and cultures when applicable. Donor haemodynamic instability was defined as any donor experiencing a cardiac arrest (documented by a health care provider) or requiring noradrenaline or more than one vasopressor to maintain a mean arterial pressure (MAP) ≥ 60 mmHg in the ICU period or at procurement. Donors were classified as affected by diabetes mellitus, dyslipidemia or hypertension if on medication, irrespective of the onset date. Donors with a history of any cardiac disease or cardiac surgery were considered cardiopathic.

Recipient variables

All LT recipients were evaluated in the pretransplant setting and followed up after transplantation according

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to our institutional policies. Data included in the current analysis were: demographics (age at transplantation; gender; body weight; height; BMI); indication to LT; clinical status at transplantation as per MELD score; donor age × recipient MELD (D-MELD) [19]; post-transplant surgical complications according to the modified Clavien's classification system [20]; type of immunosuppression; graft and patient survival. Patient and graft survival and post-LT complications were censored at time of event or as of February 14, 2017. Graft failure was defined as need for re-transplantation and so considered for the follow up at the time of re-listing at our centre or elsewhere. A severe vascular complication was any post-transplant abnormality in hepatic artery, portal vein or vena cava associated with symptoms or signs and requiring radiological or surgical procedure. Post-transplant biliary complication was any abnormality in the biliary tree associated with symptoms or signs and requiring endoscopic or surgical procedure. ITBL was any donor biliary tract with nonanastomotic stenosis requiring an endoscopic or radiological procedure in the absence of vascular complications. To this regard, any transient cholestasis or any diagnostic test (e.g. T-tube colangiography, MRI, etc.) were not considered as biliary complications if not consistent with biliary tract disease and/or symptoms/signs or not requiring radiological or endoscopic treatment. Immunosuppression was based on calcineurin inhibitors (CNI) in all patients, but protocols varied according to era.

Surgery

Until March 2014, all donors were procured with aortic flush only with Celsior™ solution (Genzyme-Sanofi, Milan, Italy) and *en-bloc* liver and pancreas procurement, as previously described [21]. Aorta was flushed at a pressure of 150 mmHg. Donor bile duct was repeatedly flushed with cold (4 °C) saline solution from the gallbladder immediately after aortic clamping. Grafts were then stored in four sterile plastic bags and 500 g of ice were crushed in the second bag. Grafts were shipped into a cool box filled up with ice and after back-table preparation were stored in a cool box at 4 °C. In addition, grafts were reperfused at the back table with 2 l of cold preservation solution through the portal vein.

From March 2014, deceased donors were procured with double aortic and portal flush with Celsior™ solution. Aorta was flushed at a pressure of 150 mmHg while no additional pressure was used for the portal vein, which was flushed through the inferior mesenteric vein. Similarly, to the SP group, aorta was flushed with a minimum of 5 l or until livers were free of blood, while two additional litres were used for portal vein flush in the DP group. Portal perfusion was performed simultaneously with aortic perfusion at procurement; cases in which portal perfusion was performed after aortic one or on the back-table only, were not considered as DP. Donor bile duct was flushed with room temperature saline solution from the gallbladder immediately before and with cold (4 °C) saline after aortic clamping. To be considered for the study, time from donor cross-clamping to get the liver in the ice should have been shorter than 45 min. To minimise ice contact-related injuries, grafts were stored in four sterile plastic bags and 500 g of ice were crushed in the second one. Grafts were shipped in an empty metallic box, which was placed inside a cool box filled up with ice. Grafts were then reperfused at the back table with 2 l of cold preservation solution through the portal vein. After back table preparation, grafts were again placed in four sterile plastic bags into the metallic box and then stored in a cool box at 4 °C until surgery. Shipped organs were considered for this study only if the new procurement protocol was strictly observed.

All transplant procedures were performed using conventional technique with vena cava replacement and veno-venous bypass; grafts were simultaneously reperfused through the portal vein and hepatic artery in the recipient. A T-tube was routinely used for duct-to-duct biliary anastomosis. Variables included in the current analysis were: cold ischaemia time (CIT); duration of the anhepatic phase and intra-operative complications. Anhepatic phase was the time from portal cross-clamp in the recipient to graft reperfusion. The T-tube was removed 3 months after transplantation.

Graft allocation policy

Starting 2005, a MELD-based allocation algorithm for adult transplantation was implemented in our region. Exceptions to MELD scores were graded according to international guidelines published elsewhere [22]. Donor grafts >80 years were not allocated to recipients with biochemical MELD score >24.

Data management and statistical analysis

According to variables and their level of distribution, descriptive statistics are reported as medians, interquartile ranges (IQR), and frequencies, as appropriate. After testing the continuous variables using the

Kolmogorov–Smirnov test, we used the Student's *t* test in case of normal distribution and the Mann–Whitney *U* test in case of skewed distribution. In case of categorical variables, a two-tailed Fisher's exact test was used to minimise biases related to the small number of events. The cause specific competing-risk-adjusted cumulative incidence of ITBL at 1 year was obtained considering graft loss and/or patient death (if unrelated to biliary complications) as competing risk. Patient and graft survival was according to Kaplan–Meier and differences across groups were analysed using the log-rank test. Inferential analyses were performed with the intent to identify the risk factors for the development of post-transplant ITBL and graft loss. Initially, two univariate Cox proportional hazard models were performed, in both the cases testing 20 different variables. All the variables presenting a $P < 0.2$ were then introduced in the multivariable models. A backward conditional method was adopted for the construction of the final models: the significance level used for the backward elimination was 0.1. All the variables presenting a $P < 0.05$ was considered statistically significant. All statistical calculations were performed with the SPSS 23.0 software (Chicago, IL, USA).

Results

Overall results

Donors, recipients and surgery

Between April 1st, 2014 and December 31st, 2015, 207 LT were performed at our centre. Seven (3.4%) patients were excluded from current analysis: three re-transplantations; three primary transplants on UNOS-1 patients, and one split liver procedure. Forty (19.3%) patients were excluded due to nonadherence with the procurement protocol or to arterial complications, as shown in Fig. 1. Finally, 160 primary LT recipients were considered: 34 (21.3%) who received a graft <60 years; 22 (13.8%) who received a graft 60–69 years; 51 (31.8%) who received a graft 70–79 years, and 53 (33.1%) transplanted with a graft ≥80 years.

Between 1 January 2005 and 31 March 2014, a total of 963 LT procedures were performed. After exclusion of 19 (2.0%) ABO-incompatible transplants, 21 (2.2%) UNOS-1 status recipients, 36 (3.7%) re-transplantations and 28 (2.9%) patients with post-transplant arterial complications, a total of 859 primary transplants were considered: 305 (35.5%) patients received a graft <60 years; 181 (21.1%) patients received a graft from donors 60–69 years; 256 (29.8%) patients received a graft from donors 70–79 years, and 117 (13.6%) patients received a graft ≥80 years. Reasons for patients' exclusion are detailed in Fig. 1.

The donor and recipient characteristics of interest of the study population are shown in Tables 1 and 2. Donor characteristics show a higher incidence of comorbidities (dyslipidemia, hypertension, diabetes mellitus, cardiopathy), a longer ICU stay (median 3 vs. 2 days; $P = 0.003$) and a lower rate of extra-regional procurements (1.9% vs. 12.8%; $P = 0.02$) in the DP group (Table 1). Recipient and transplant variables were comparable between the two groups, except for a higher median recipient age in the DP group (55.0 vs. 53.0; $P < 0.001$), higher D-MELD in the younger decades receiving DP grafts (602 vs. 517; $P = 0.05$) and higher incidence of HCC in the 70–79 decades receiving SP grafts (48.8% vs. 31.4%; $P = 0.03$; Table 2).

Biliary complications

The overall incidence of biliary complications was higher in the SP group (16.1% vs. 8.1%; $P = 0.01$). When stratified by donor decade, SP group showed a higher incidence of biliary complications only in the recipients of octogenarian grafts (23.9% vs. 9.4%; $P = 0.04$); nevertheless, difference loses significance if papillary dysfunction (which cannot be related to the procurement technique) is not considered (23.1% vs. 9.4%; $P = 0.058$).

The incidence of ITBL was statistically higher only in recipients of SP grafts from donors ≥80 years (15.4% vs. 1.9%; $P = 0.008$) and so was the 1-year competing-risk-adjusted incidence of ITBL (16.0% vs. 2.0%; $P = 0.02$; Table 3).

Octogenarian grafts analysis

Recipients survival

A total of 170 (16.8%) patients were transplanted with graft of octogenarian donors. Median post-transplant follow-up time was 3.7 years (IQR = 1.8–7.3). The overall 1-, 3- and 5-year patient survival for recipients of octogenarian grafts was 90.6%, 86.8% and 77.4% respectively. According to type of perfusion at procurement, the 1- and 3-year patient and graft survival was 89.0% and 83.9% for the SP group (median follow-up 5.1 years; IQR = 3.5–7.7), versus 94.3% and 94.3% for the DP group (median follow-up 1.84 years; IQR = 1.5–2.4; $P = 0.09$; Fig. 2).

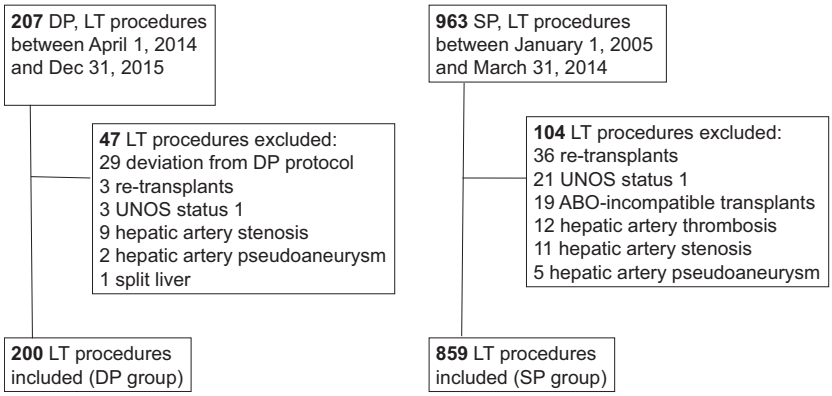

Figure 1 Patients' disposition algorithm.

Three patients (5.7%) died in the DP Group (one intraoperative death and two sepses on post-operative day 38 and 70). Fourty patients (34.2%) died in the SP group. ITBL-related complications were the most common reasons for death (n = 8, 20%).

Biliary complications

A total of 33 (19.4%) recipients of octogenarian grafts experienced at least one biliary complication. The DP group experienced biliary complications in five (9.4%) patients: anastomotic stricture (n = 3), ITBL and stones (n = 1), and stones alone (n = 1). Anastomotic strictures were treated with endoscopic retrograde cholangiopancreatography (ERCP): in two cases, patients required one and two procedures, respectively, while the third patient required four procedures and was eventually treated with hepatico-jejunostomy. The patient with ITBL and stones was treated with ERCP (1 procedure), and the patient with intracholedochal stones was treated with only one ERCP procedure.

The SP group experienced biliary complications in 28 (23.9%) patients: ITBL alone (n = 11); ITBL and stones (n = 5); ITBL and anastomotic stricture (n = 2); stones alone (n = 6); anastomotic stricture alone (n = 2); fistula (n = 1), and stenosis of the papilla (n = 1). Among patients with ITBL (n = 18), 10 (55.6%) were successfully treated with ERCP (median procedures = 2, IQR = 1–4); 6 (33.3%) still require endoscopic treatment (median procedures = 4, IQR = 2–10), while 2 (11.1%) were re-transplanted: one patient died intraoperatively and one died 6 months after surgery for pneumonia. All patients with stones required one or two ERCPs, while the patient with stenosis of the papilla was treated with ERCP and sphincterotomy. The two cases of anastomotic strictures were treated with ERCP in one case and hepatico-jejunostomy after post-ERCP stenosis recurrence in the other case.

The time-dependent risk of ITBL in recipients of octogenarian grafts is shown in Fig. 3. DP patients showed a 2.0% incidence risk at 1 and 3 years, while SP patients presented sevenfold higher incidence overall (14.3% and 15.2% at 1 and 3 years, respectively; P = 0.02).

At multivariable analysis, donor male gender (HR = 6.4; P = 0.001), donor haemodynamic instability (HR = 4.9; P = 0.008) and donor DM2 (HR = 3.0; P = 0.03) were all independent risk factors for ITBL in recipients of octogenarian donors, while double perfusion at procurement (HR = 0.1; P = 0.04) and longer donor ICU stay (HR = 0.7; P = 0.04) were protective factors (Table 4). As for graft loss, the recipient HCV positive status (HR = 2.2; P = 0.02) was an independent risk factor, while the recipient male gender (HR = 0.4; P = 0.03) and double perfusion at procurement (HR = 0.3; P = 0.48) were protective factors (Table 5).

Discussion

The use of very old donors is still limited due to the risk related to a potentially higher incidence of post-transplant complications. In a recent paper by Biancofiore *et al.*, [23] recipients of very old donors' grafts show an early postoperative course comparable to that of younger graft recipients. In the mid and long term, concerns focus on reported higher incidence of biliary complications, namely ITBL, and HCV recurrence. Even if this last scenario is changing due to the advent of the novel direct-acting antiviral agents, no specific prevention has been implemented to reduce the risk of ITBL. Treatment of ITBL may require multiple ERCP procedures, several re-admissions, and it is responsible for

Table 1. Donor demographics and clinical characteristics by age category.

Variables	Overall			Donor <60			Donors 60–69			Donors 70–79			Donors ≥80		
	DP group (*n* = 160)	SP group (*n* = 859)		DP group (*n* = 34)	SP group (*n* = 305)		DP group (*n* = 22)	SP group (*n* = 181)		DP group (*n* = 51)	SP group (*n* = 256)		DP group (*n* = 53)	SP group (*n* = 117)	
	Median (IQR) or *n* (%)		*P*	Median (IQR) or *n* (%)		*P*	Median (IQR) or *n* (%)		*P*	Median (IQR) or *n* (%)		*P*	Median (IQR) or *n* (%)		*P*
Donor characteristics															
Male gender	79 (49.4)	465 (54.1)	0.3	19 (55.9)	184 (60.3)	0.7	10 (45.5)	104 (57.5)	0.2	28 (54.9)	127 (49.6)	0.5	22 (41.5)	50 (42.7)	1.0
Age (years)	75 (58–84)	67 (55–79)	<0.001	49 (47–59)	46 (35–57)	<0.001	66 (63–69)	65 (62–68)	0.3	76 (74–78)	75 (73–77)	0.6	83 (81–86)	83 (81–85)	0.2
Height (cm)	170 (165–175)	169 (161–177)	0.8	170 (164–176)	170 (162–178)	0.8	165 (158–174)	170 (164–177)	0.04	170 (164–176)	170 (162–178)	0.06	165 (160–170)	165 (160–175)	0.4
Weight (kg)	70 (62–78)	72 (64–80)	0.6	75 (66–84)	75 (67–83)	0.2	70 (59–81)	75 (65–85)	0.06	72 (64–80)	70 (62–78)	0.4	70 (60–79)	70 (60–80)	0.8
BMI (kg/m^2)	25 (23–28)	25 (23–27)	0.7	26 (22–30)	25 (20–30)	0.07	25 (22–28)	25 (23–27)	0.4	25 (22–28)	25 (23–27)	0.8	25 (23–28)	25 (23–27)	0.4
ICU stay (days)	3 (1–5)	3 (1–5)	0.7	4 (1–7)	3 (1–5)	0.2	4 (1–7)	2 (1–4)	0.04	4 (2–6)	3 (1–5)	0.03	3 (2–5)	2 (1–4)	0.003
CVA as cause of death	126 (78.8)	613 (71.4)	0.07	18 (52.9)	162 (53.1)	1.0	19 (86.4)	142 (78.5)	0.3	47 (92.2)	213 (83.5)	0.1	42 (79.2)	96 (82.1)	0.7
Dyslipidaemia	38 (23.8)	111 (12.9)	0.001	4 (11.8)	24 (7.9)	0.5	5 (22.7)	36 (19.9)	0.5	17 (33.3)	32 (12.5)	0.001	12 (22.6)	19 (16.2)	0.4
Hypertension	94 (58.8)	409 (47.6)	0.02	11 (32.4)	67 (22)	0.2	13 (59.1)	104 (57.5)	0.5	32 (62.7)	162 (63.3)	1.0	38 (71.7)	76 (65.0)	0.5
Diabetes mellitus	24 (15.0)	79 (9.2)	0.06	3 (8.8)	7 (2.3)	0.07	5 (22.7)	24 (13.3)	0.2	7 (13.7)	40 (15.6)	0.8	9 (17.0)	8 (6.8)	0.05
Cardiopathy	55 (34.4)	217 (25.3)	0.04	6 (17.6)	35 (11.5)	0.3	5 (22.7)	55 (30.4)	0.3	23 (45.1)	88 (34.4)	0.8	21 (39.6)	39 (33.3)	0.5
Extraregional procurement	17 (9.1)	185 (21.5)	0.01	9 (12.3)	70 (23)	0.7	2 (9.1)	43 (76.2)	0.4	5 (9.8)	56 (21.9)	0.3	1 (1.9)	15 (12.8)	0.02
Shipped grafts	4 (2.5)	18 (2.1)	1	2 (5.9)	9 (3.0)	0.7	1 (4.5)	3 (1.7)	0.9	1 (2.0)	5 (2.0)	0.6	0 (0)	1 (0.9)	0.7
Liver biopsy at procurement	70 (43.7)	409 (47.6)	0.4	14 (41.2)	93 (30.5)	0.3	13 (59)	86 (47.6)	0.4	19 (37.2)	138 (54.0)	0.04	24 (45.2)	92 (78.6)	<0.01
Arteriolar thickening															
Mild	11 (15.7)	48 (11.7)	0.6	3 (21.4)	8 (8.6)	0.1	1 (7.7)	6 (7.0)	0.7	3 (15.8)	13 (9.4)	0.9	4 (16.7)	21 (22.8)	0.1
Moderate	10 (14.3)	19 (4.6)	0.01	0 (0)	0 (0)	–	3 (2.3)	4 (4.7)	0.02	1 (5.7)	7 (5.1)	0.9	6 (25.0)	8 (8.7)	0.5
Blood tests															
Peak															
SGOT (IU/l)	33 (14–53)	35 (18–53)	0.5	67 (27–107)	45 (18–72)	0.4	30 (8–58)	34 (16–53)	0.5	30 (17–43)	29 (17–41)	0.7	31 (24–41)	30 (23–45)	1.0
SGPT (IU/l)	24 (11–41)	24 (10–40)	0.7	65 (31–99)	33 (6–87)	0.3	30 (10–50)	25 (14–36)	0.9	22 (13–41)	22 (9–45)	0.3	18 (15–33)	20 (15–31)	0.7
Total bilir. (mg/dl)	0.8 (0.3–1.3)	0.8 (0.3–1.3)	0.6	0.8 (0.3–1.3)	0.8 (0.3–1.3)	0.7	0.5 (0.4–0.6)	0.7 (0.2–1.2)	0.3	0.7 (0.2–1.2)	0.8 (0.3–1.3)	0.8	0.8 (0.6–1.0)	0.8 (0.6–1.0)	0.9
Serum sodium (mEq/l)	152 (146–158)	152 (145–159)	0.7	152 (145–159)	154 (147–161)	0.5	154 (148–160)	152 (145–159)	0.8	149 (142–156)	150 (143–157)	0.6	150 (143–157)	148 (144–153)	0.3
Haemodynamics															
Haemodynamic instability	115 (71.9)	515 (60.0)	0.005	27 (79.4)	198 (64.9)	0.1	16 (72.7)	105 (58.0)	0.3	35 (68.6)	149 (58.2)	0.2	37 (69.8)	63 (53.8)	0.06

DP, double perfusion; SP, single perfusion; IQR, interquartile ranges; BMI, body mass index; ICU, intensive care unit; CVA, cerebrovascular accident; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; bilir., bilirubin.

Table 2. Recipient and transplant characteristics by donor age.

	Overall			Donor <60			Donors 60–69			Donors 70–79			Donors ≥80		
	DP group (*n* = 160)	SP group (*n* = 859)		DP group (*n* = 34)	SP group (*n* = 305)		DP group (*n* = 22)	SP group (*n* = 181)		DP group (*n* = 51)	SP group (*n* = 256)		DP group (*n* = 53)	SP group (*n* = 117)	
Variables	Median (IQR) or *n* (%)		*P*	Median (IQR) or *n* (%)		*P*	Median (IQR) or *n* (%)		*P*	Median (IQR) or *n* (%)		*P*	Median (IQR) or *n* (%)		*P*
Recipient characteristics															
Male gender	117 (73.1)	653 (76.0)	0.5	28 (82.4)	216 (70.8)	0.2	15 (68.2)	151 (83.4)	0.1	36 (70.6)	194 (75.8)	0.5	38 (71.7)	92 (78.6)	0.3
Age (years)	55.5 (50.5–60.5)	53.0 (47–59)	<0.001	56 (51–61)	49 (42–56)	<0.001	54 (48–60)	54 (49–59)	0.3	54 (49–59)	55 (49–61)	0.4	57 (51–62)	56 (51–61)	0.6
HCV positive serologyy	86 (53.8)	444 (51.7)	0.5	21 (61.8)	152 (49.8)	0.2	9 (40.9)	96 (53)	0.4	28 (54.9)	144 (56.3)	0.9	28 (52.8)	52 (44.1)	0.3
HCC	63 (39.4)	378 (44.0)	0.3	9 (26.5)	98 (32.1)	0.6	12 (54.5)	91 (50.3)	0.8	16 (31.4)	125 (48.8)	0.03	26 (49.1)	60 (51.3)	0.6
MELD	11 (8–14)	12 (9–13)	0.4	12 (9–15)	12 (8–16)	0.8	11.5 (7–16)	11 (8–14)	0.3	12 (9–15)	11 (8–14)	0.6	11 (9–14)	11 (8–15)	0.6
D-MELD	702 (499–905)	823 (587–1059)	0.01	602 (436–768)	517 (351–683)	0.05	782 (500–1064)	744 (557–931)	0.4	888 (685–1091)	843 (450–1039)	0.6	920 (709–1127)	902 (688–1223)	0.7
Transplant															
CIT (min)	450 (410–490)	455 (403–507)	0.5	436 (394–478)	475 (416–534)	0.01	458 (425–491)	441 (393–489)	0.7	470 (430–510)	450 (396–504)	0.2	440 (419–501)	440 (391–490)	0.4
>8 h	49 (30.6)	314 (36.6)	0.4	9 (28.1)	144 (48.0)	0.04	5 (26.3)	56 (31.8)	0.8	18 (40.0)	84 (33.3)	0.4	17 (32.1)	30 (25.6)	0.5
Anhepatic phase (min)	78.2 (68–85)	79.2 (70–90)	0.5	74.5 (65–84)	78.1 (69–85)	0.1	80.7 (71–87)	82.8 (70–91)	0.5	77.3 (70–85)	79.6 (70–88)	0.3	76.3 (70–86)	78.1 (70–90)	0.5

DP, double perfusion; SP, single perfusion; IQR, interquartile ranges; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; D-MELD, donor – model for end-stage liver disease; CIT, cold ischaemia time.

Table 3. Incidence of biliary complications by donor age.

	Overall			Donor <60			Donors 60–69			Donors 70–79			Donors ≥80		
	DP group (*n* = 160)	SP group (*n* = 859)		DP group (*n* = 34)	SP group (*n* = 305)		DP group (*n* = 22)	SP group (*n* = 181)		DP group (*n* = 51)	SP group (*n* = 256)		DP group (*n* = 53)	SP group (*n* = 117)	
	n (%)		*P*	*n* (%)		*P*	*n* (%)		*P*	*n* (%)		*P*	*n* (%)		*P*
Re-LT	2 (1.3)	24 (2.8)	0.41	0 (–)	7 (2.3)	1.0	0 (–)	2 (1.1)	1.0	1 (2.0)	10 (3.9)	0.46	1 (1.9)	5 (4.3)	0.76
Patients with biliary complications	13 (8.1)	138 (16.1)	0.01	1 (2.9)	42 (13.7)	0.10	3 (13.6)	26 (14.4)	1.0	4 (7.8)	42 (16.5)	0.14	5 (9.4)	28 (23.9)	0.04
Any biliary complication*															
Anastomotic stricture	4 (2.5)	50 (5.8)	0.12	0 (–)	19 (6.2)	0.24	1 (4.5)	14 (7.7)	1.0	0 (–)	13 (5.1)	0.51	3 (5.7)	4 (3.4)	0.68
Stones	6 (3.8)	62 (7.2)	0.12	0 (–)	18 (5.9)	0.24	2 (9.1)	14 (7.7)	0.69	2 (3.9)	19 (7.5)	0.55	2 (3.8)	11 (9.4)	0.35
Fistula	1 (0.6)	17 (2.0)	0.34	0 (–)	5 (1.6)	1.0	0 (–)	3 (1.6)	1.0	1 (2.0)	8 (3.1)	1.0	0 (–)	1 (0.9)	1.0
Papilla dysfunction	1 (0.6)	1 (0.1)	0.29	0 (–)	0 (–)	1.0	0 (–)	0 (–)	1.0	0 (–)	1 (0.4)	1.0	0 (–)	1 (0.9)	1.0
ITBL	6 (3.8)	63 (7.3)	0.12	1 (2.9)	16 (5.2)	1.0	0 (–)	12 (6.6)	0.37	4 (7.8)	17 (6.7)	0.76	1 (1.9)	18 (15.4)	0.008
One-year competing risk adjusted cumulative incidence of ITBL (%)	3.8	6.0	0.38	2.9	4.9	0.97	0	6.5	0.45	8.0	7.0	0.96	2.0	16.0	0.02

DP, double perfusion; SP, single perfusion; HCV, hepatitis C virus; LT, liver transplantation; ITBL, ischaemic-type biliary lesions.

*More than one complication could have been reported for a single patient.

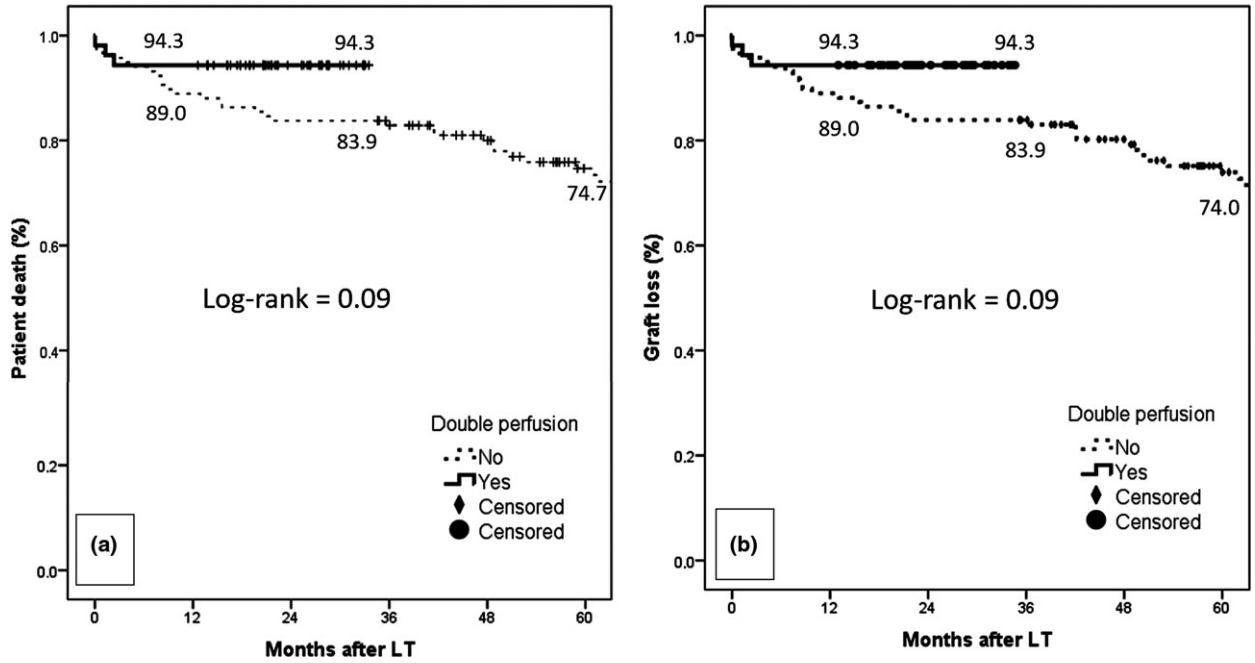

Figure 2 Patients (a) and grafts (b) survival of recipients of octogenarian livers.

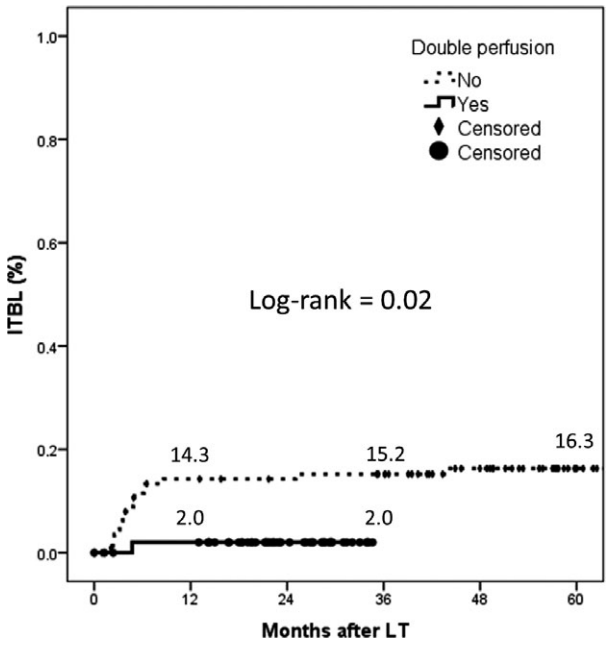

Figure 3 Incidence of ischaemic-type biliary lesions among recipients of octogenarian grafts.

poorer quality of life [16]. Patients often need aggressive antibiotic treatment for recurrent cholangitis, which may lead to selection of multidrug-resistant bacterial strains and to development of unresponsive sepsis, thus precluding the option of liver re-transplantation [16].

In literature, the incidence of ITBL has been reported ranging from 3.9% to 25% according to donor, transplant, graft and recipient characteristics [16]. Several risk factors for ITBL are well known, but the weight of each of them and their mode of interaction in clinical practice is still unclear. Donor, procurement, transplant and recipient-related factors all play a role in the development of biliary complications [14]. To date, there is no definitive consensus on the best procurement technique for LT, and the percentage of transplant centres using dual versus single perfusion is unknown. A recently published systematic review and meta-analysis of cold *in situ* perfusion [24], showed that over the 19 studies considered for the analysis, dual perfusion was the only procurement technique used in 12, single perfusion was the only technique performed in two and both were used in five. It might be consequently estimated that up to 37% of transplant centres procure liver grafts using a single perfusion technique. Nevertheless, the paper showed that aortic-only perfusion of DBD livers do not compromise transplantation outcomes, and it otherwise may be favoured because of its simplicity [24]. In our procurement technique, portal perfusion is performed simultaneously with aortic perfusion, while reperfusion through the portal vein once the liver is already in the ice or at the back table was not a matter of investigation.

In promoting a graft procurement protocol targeted to reduce the risk of ITBL, at our institution we focused on: (i) optimising graft perfusion; (ii) minimising bile

Table 4. Risk factors for ischaemic-type biliary lesions in recipients of octogenarian grafts. Univariable and multivariable Cox regression analyses: backward conditional method.

	Univariable				Multivariable			
		95% CI				95% CI		
Variables	HR	Lower	Upper	*P* value	HR	Lower	Upper	*P* value
Donor male gender	4.0	1.5	11.2	0.008	6.4	2.1	19.7	0.001
Donor haemodynamic instability	3.9	1.3	9.8	0.007	4.9	1.5	15.8	0.008
Donor DM2	2.8	1.1	6.3	0.03	3.0	1.1	7.9	0.03
Double perfusion at procurement	0.1	0.02	0.9	0.04	0.1	0.01	0.9	0.04
Donor ICU stay (per day)	0.7	0.5	1.0	0.04	0.7	0.5	0.9	0.04
Recipient age (per decade)	1.2	0.7	2.3	0.5	–	–	–	–
HCV-positive status	0.9	0.4	2.2	0.8	–	–	–	–
Recipient presence of HCC	1.3	0.5	3.1	0.6	–	–	–	–
Male recipient gender	0.9	0.3	2.6	0.8	–	–	–	–
Recipient MELD (per point)	1.0	0.9	1.2	0.4	–	–	–	–
Donor age (per year)	0.9	0.8	1.1	0.4	–	–	–	–
Donor BMI (per point)	1.1	0.7	1.6	0.6	–	–	–	–
CVA as donor cause of death	1.2	0.5	1.7	0.7	–	–	–	–
Donor sGOT peak (per 10 IU/l)	0.9	0.7	1.1	0.3	–	–	–	–
Donor sGPT peak (per 10 IU/l)	0.7	0.5	1.1	0.1	–	–	–	–
Donor total bilirubin peak (per 1.0 mg/dl)	1.5	0.8	2.9	0.2	–	–	–	–
Donor sodium peak (per 10 mEq/l)	1.3	0.8	2.0	0.2	–	–	–	–
Donor story of arterial hypertension	1.2	0.6	1.7	0.4	–	–	–	–
Donor story of cardiopathy	0.9	0.3	2.1	0.7	–	–	–	–
Cold ischaemia time (per hour)	1.0	0.7	1.5	0.8	–	–	–	–

−2log likelihood: 151.2.

HR, hazard ratio; CI, confidence intervals; DM2, diabetes mellitus type 2; ICU, intensive care unit; HCV, hepatitis C virus; HCC, hepatocellular cancer; MELD, model for end-stage liver disease; BMI, body mass index; CVA, cerebrovascular accident; sGOT, serum Glutamyl oxaloacetic transaminase; sGPT, serum Glutamyl pyruvic transaminase.

salt-related damage to cholangiocytes and (iii) avoiding excessive ischaemia/reperfusion damages.

The results of the current analysis showed that the advantages of DP in reducing the incidence of ITBL are concentrated for recipients of very old grafts. Based on our experience, it can be speculated that blood supply has a primary role in the aetiology of ITBL. Blood supply to the biliary tree is almost exclusively arterial, with no significant contribution from the portal vein in physiological conditions [14,25–27]. However, some evidence supports the hypothesis that the peribiliary vascular plexus derives blood not only from the hepatic artery but also from the portal vein [28]. Since ITBL may occur in the absence of hepatic artery thrombosis, it has been suggested that portal venous blood flow may contribute to ITBL [28]. Farid *et al.* [28] showed that patients with partial portal vein thrombosis and intact hepatic arterial blood supply developed ITBL in the hepatic segments affected by portal vein thrombosis. Thus, the contribution of portal blood flow to biliary microcirculation is not negligible, and a compromised portal venous blood supply can favour the development of ITBL [28]. It can be speculated that in some clinical scenarios – that is, very old donors; donors with diabetes mellitus, or in very steatotic livers – the peribiliary vascular plexus might be impaired along with major arteries, and arterial perfusion alone might be largely insufficient. Unfortunately, routine histological evaluation of arteriolar thickening was not sufficient to draw definitive conclusions.

Our data support the protective role of portal perfusion, especially when acute and chronic co-morbidities exist. Two acute variables seem to be crucial, as well: haemodynamic instability and the length of ICU stay. A large use of vasopressors during procurement surgery may determine an insufficient or sub-optimal perfusion, and a recent cardiac arrest may be responsible for acute ischaemic damages. On the contrary, a longer ICU stay might be a surrogate of a general better biological condition of the donor and of cardiovascular stability. Recently, we showed that, when using octogenarian donors with grafts procured with aortic perfusion only [16], donor haemodynamic instability, diabetes mellitus and D-MELD were statistically significant risk factors

Table 5. Risk factors for graft loss in recipients of octogenarian grafts. Cox regression analysis: backward conditional method.

	Univariable				Multivariable			
		95% CI				95% CI		
Variables	HR	Lower	Upper	*P* value	HR	Lower	Upper	*P* value
HCV-positive status	2.3	1.2	4.2	0.008	2.2	1.1	4.1	0.02
Male recipient gender	0.4	0.2	0.9	0.03	0.4	0.2	0.9	0.03
Double perfusion at procurement	0.4	0.1	1.1	0.08	0.3	0.08	0.9	0.048
Donor male gender	1.1	0.8	1.6	0.5	–	–	–	–
Recipient age (per decade)	1.4	0.9	2.1	0.1	–	–	–	–
Recipient presence of HCC	0.9	0.5	1.6	0.7	–	–	–	–
Recipient MELD (per point)	1.0	0.9	1.1	1.0	–	–	–	–
Donor age (per year)	1.0	0.9	1.1	0.8	–	–	–	–
Donor ICU stay (per day)	0.9	0.8	1.1	0.3	–	–	–	–
Donor BMI (per point)	1.5	1.0	2.2	0.053	–	–	–	–
CVA as donor cause of death	1.2	0.8	1.4	0.4	–	–	–	–
Donor sGOT peak (per 10 IU/l)	1.0	0.9	1.1	0.3	–	–	–	–
Donor sGPT peak (per 10 IU/l)	1.0	0.8	1.1	0.6	–	–	–	–
Donor total bilirubin peak (per 1.0 mg/dl)	1.2	0.7	1.9	0.5	–	–	–	–
Donor sodium peak (per 10 mEq/l)	1.2	0.9	1.5	0.3	–	–	–	–
Donor haemodynamic instability	1.3	0.9	1.5	0.3	–	–	–	–
Donor DM2	1.1	0.9	1.6	0.3	–	–	–	–
Donor story of arterial hypertension	1.2	0.8	1.6	0.4	–	–	–	–
Donor story of cardiopathy	0.9	0.3	2.1	0.7	–	–	–	–
Cold ischaemia time (per hour)	1.1	0.9	1.3	0.5	–	–	–	–

−2log likelihood: 379.5.

HR, hazard ratio; CI, confidence intervals; DM2, diabetes mellitus type 2; ICU, intensive care unit; HCV, hepatitis C virus; HCC, hepatocellular cancer; MELD, model for end-stage liver disease; BMI, body mass index; CVA, cerebrovascular accident; sGOT, serum Glutamyl oxaloacetic transaminase; sGPT, serum Glutamyl pyruvic transaminase.

for ITBL. The current analysis showed that two chronic factors such as donor diabetes mellitus and male gender have a role in promoting ITBL as well. It might be speculated that both factors are associated to a more severe atherosclerotic disease, thus, supporting the hypothesis of the relevance of portal perfusion during procurement. Moreover, even if the advantage of DP in terms of ITBL is demonstrated in very old donor grafts only, ITBL incidence in octogenarian DP group is lower than any of the SP groups, regardless of age, supporting the hypothesis of a potential advantage in younger decades as well. In accordance to the meta-analysis by Hameed *et al.*, [24] larger samples are needed to evaluate if DP advantage in terms of biliary complication are confirmed when procuring younger grafts or if its use should be considered only in specific donors categories (e.g. octogenarian, DCD, steatotic livers, etc..), where costs are justified.

The direct toxic effect of bile salts on cholangiocytes has already been recognised [29]. Although such injury is hard to assess, we suggest flushing the bile duct with room temperature normal saline before cross-clamping and with cold normal saline after reperfusion. Prolonged cold ischaemia time (CIT) is another independent risk factor for the development of biliary complications [30]. Cold graft preservation longer than 14 h has been associated with a twofold increase in preservation injury, resulting in biliary strictures and decreased graft survival [30,31]. Recently, Detry *et al.* [12] reported a 3.9% incidence of post-transplant ITBL, and identified a CIT threshold of 10 h to avoid ITBL. Despite our efforts were always aimed at minimising CIT, about 30% of our octogenarian grafts experienced a CIT longer than 8 h. Recent evidence highlights that elderly grafts are more susceptible to the negative effects of prolonged CIT due to impairment of cellular ATP content [32], thus promoting apoptosis [33], and ultimately ending more severe injuries after reperfusion. To this regard, machine perfusion might help to minimise biliary complications by protecting bile ducts or increasing ATP-content during preservation [34,35]. Prolonged WIT is another risk factor for ITBL. Unfortunately,

WIT data were not available for the study, but the anhepatic time (which is a valuable surrogate of WIT) was found similar among groups.

This study has some other limitations, mainly related to the small sample size and the shorter follow-up period of the DP group. Even if the 1-year incidence of ITBL is statistically significant, longer follow-up may play a role in influencing graft survival, and it is therefore required to validate our preliminary results. However, in this single-centre experience our graft allocation policy and even donor selection remained basically the same throughout the entire observation period, except for a more aggressive acceptance of multiple donor comorbidities in DP group. Moreover, the rigorous variable selection procedure applied in this study may have missed to identify some important risk factors and may have overestimated the real effect sizes and underestimated the *P*-values of the selected risk factors [36]. Several other variables may have a relevant weight in promoting or reducing the risk of ITBL, such as surgical technique, mode of graft revascularisation, and intraoperative use of vasopressors. Further studies will be required to explore all these issues, but DP should be considered standard of care when procuring very old donors.

Authorship

Davide Ghinolfi: designed study, wrote the paper, analysed data and performed the study. Giovanni Tincani: designed study, performed the study. Erion Rreka: collected data. Niccolo' Roffi: collected data, analysed data. Laura Coletti: performed the study. Emanuele Balzano: performed the study. Gabriele Catalano: performed the study. Sonia Meli: collected data. Paola Carrai: collected data. Stefania Petruccelli: collected data. Gianni Biancofiore: designed study, analysed data. Franco Filipponi: designed study, performed the study. Paolo De Simone: performed the study, wrote the paper.

Funding

The authors have declared no funding.

Conflicts of interest

The authors have declared no conflicts of interest.

Acknowledgement

The authors owe a debt of gratitude to the nursing staff of Pisa liver transplant centre.

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