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ORIGINAL ARTICLE

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Bile acids analysis: a tool to assess graft function in human liver transplantation

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Abstract The expanding use of "sub-optimal" grafts due to donor organ shortage increases the importance of accurate graft assessment before liver transplantation. Bile secretion is an early sign of recovering hepatic function post-transplant. The role of bile acid analysis in assessing graft function before and immediately after liver transplantation has been investigated. Two hundred and sixteen samples of hepatic bile were collected from 35 donors and 13 recipients. Clinical data, bile flow, total bile acid concentration, apparent choleretic activity and bile acid composition were assessed. Sub-optimal donor livers showed a low apparent choleretic activity and a different bile acid composition when compared to normal grafts. In recipients, the pattern of recovery of bile secretion immediately after reperfusion was a useful predictor of graft function. This study characterises bile acid secretion of liver grafts and remarks the potential value of bile acid analysis to assess donor liver quality and early post-transplant graft function.

Keywords Liver transplantation · Bile acids · Bile secretion · Graft function

Introduction

The reliable assessment of donor livers prior to transplantation is of increasing importance as more "marginal" grafts are being utilised to try to overcome the current shortage of cadaveric donors [1, 2, 3]. Many clinical and laboratory parameters have been used to assess donor livers before transplantation [4], but the transplant surgeon currently relies on an overall assessment of donor data and the appearance of liver [5, 6, 7]

Bile secretion is an important physiological function of the liver and has been considered as a marker of early graft function following transplantation [8, 9]. However, the majority of studies have been confined to the analysis of T-tube bile post-transplant with prolonged interruption of the bile acid enterohepatic circulation [10]. To assess the bile acid secretion and composition of the hepatic bile from donors at organ retrieval and from recipients immediately after reperfusion, we developed a standardised bile collection technique [11] and determined bile flow, biliary bile acids concentrations, individual bile acid composition and apparent choleretic activity (ACA).

Materials and methods

Patients

Thirty-five randomly selected liver donors and corresponding transplant recipients were included in this

study after permission from the hospital ethics committee. Our surgical team retrieved all liver grafts and transplanted all recipients included in this study. Standard clinical and biochemical data were collected during organ retrieval and orthotopic liver transplantation (OLT). The development of primary graft dysfunction (PGD), a concept that includes early graft dysfunction (EGD) and primary non-function (PNF) [12], was monitored in all recipients using the parameters proposed by Clavien et al. [13]. These parameters included first day AST greater than or equal to 2000 IU/l or a transient increase in AST levels greater than or equal to 1000 IU/l or a persistent elevated prothrombin time greater than 20 s [or the equivalent international normalised ratio (INR) >1.4] for at least 3 days. Other relevant clinical, biochemical and operative data of donors and recipients are summarised in Table 1.

Hepatic bile collection

A total of 153 samples of hepatic bile were collected from 35 donors during organ retrieval using a standardised bile collection technique that gives access to hepatic bile under controlled conditions but without significant interruption of the enterohepatic circulation [11]. The technique included clamping of the cystic duct to avoid gallbladder bile contamination of the hepatic bile and the cannulation of the common bile duct with a standard feeding tube (size 8 FG, 75 cm length) allowing free drainage of hepatic bile from a well-perfused liver still in situ. The volumes of bile were collected at regular intervals (either 10 or 15 min) and the samples stored for biochemical analysis at −20°C. Hepatic bile was also collected from 13 recipients (63 samples) during OLT using the same technique, but collection was started

Table 1 Clinical data from donors and recipients. *D* donor number, *R* recipient number, *F* female, *M* male, *hypotension* period of hypotension (systolic <60 mmHg), *ITU* intensive therapy unit, Na^+ sodium, *ALT* alanyl aminotransferase, *AST* aspartate aminotransferase, *d* day, *m* month

D	Age	Gender	Sub-optimal graft (criteria)	Procedure	R	Age	Gender	PGD	Survival
D1	23	F	Yes (hypotension, moderate steatosis)	OLT	R1	42	M	No	Alive
D2	31	M	No	Split graft	R2a	12	M	Yes	Alive
				Split graft	R2b	37	F	Yes	Died (m 2)
D3	15	F	Yes (9 days ITU stay)	OLT	R3	40	F	No	Alive
D4	62	M	Yes (age, mild steatosis)	OLT	R4	60	F	Yes	Died (d 14)
D5	45	F	No	OLT	R5	16	M	No	Alive
D6	38	F	Yes (mild steatosis)	OLT	R6	50	F	No	Died (m 15)
D7	36	M	Yes (mild steatosis)	Split graft	R7a	2	F	Yes	Alive
				Split graft	R7b	47	M	Yes	Alive
D8	41	F	Yes (7 days ITU stay)	OLT	R8	49	M	No	Alive
D9	42	F	No	OLT	R9	1	M	No	Alive
D10	53	F	Yes (Na^+ 182 μmol/l)	OLT	R10	57	F	Yes	Died (m 2)
D11	47	M	Yes (mild steatosis)	OLT	R11	46	M	Yes	Alive
D12	55	F	No	OLT	R12	68	F	No	Alive
D13	54	F	Yes (mild steatosis)	OLT	R13	59	M	No	Alive
D14	48	F	Yes (mild steatosis)	OLT	R14	48	M	No	Alive
D15	48	F	No	OLT	R15	54	M	No	Alive
D16	33	F	No	OLT	R16	71	F	No	Alive
D17	49	F	Yes (ALT 218 IU/l)	OLT	R17	45	M	No	Alive
D18	21	M	No	OLT	R18	40	F	No	Alive
D19	44	M	Yes (127 kg, hypotension, mild steatosis)	OLT	R19	59	M	No	Died (m 11)
D20	43	F	No	OLT	R20*	56	M	No	Alive
D21	37	M	Yes (110 kg, mild steatosis)	OLT	R21*	40	M	Yes	Alive
D22	13	F	No	OLT	R22*	4	M	No	Alive
D23	56	F	No	OLT	R23*	70	F	No	Died (m 21)
D24	41	F	No	OLT	R24*	53	F	No	Died (m 2)
D25	45	F	Yes (8 days ITU stay)	OLT	R25*	55	F	No	Alive
D26	45	M	Yes (AST 217 IU/l, hypotension)	OLT	R26*	57	F	No	Alive
D27	57	F	Yes (hypotension, severe steatosis)	Graft not used	–				
D28	42	F	Yes (hypotension, adrenaline 6 μg/k/min)	OLT	R28*	60	M	No	Alive
D29	53	M	Yes (mild steatosis)	OLT	R29	55	M	Yes	Alive
D30	57	F	Yes (mild steatosis)	OLT	R30*	59	M	Yes	Alive
D31	12	F	No	OLT	R31*	25	F	No	Alive
D32	44	M	Yes (hypotension, severe steatosis)	Graft not used	–				
D33	34	F	Yes (moderate steatosis)	OLT	R33*	60	M	Yes	Alive
D34	47	M	Yes (mild steatosis)	OLT	R34*	65	M	No	Died (m 5)
D35	36	M	Yes (hypotension, severe steatosis)	OLT	R35*	55	M	Yes	Died (m 1)

*Recipients had their hepatic bile collected for bile acid analysis

immediately after portal reperfusion and ended at the time of biliary reconstruction.

Laboratory methods

A total of 216 samples of hepatic bile were analysed. Initially, total bile acid (TBA) concentrations were measured using the 3α-hydroxysteroid dehydrogenase enzymatic procedure [14] and biliary bile acid composition was analysed by reverse ion-pair high-performance liquid chromatography technique (HPLC) [15]. To improve the sensitivity for bile acid detection, total and individual bile acid concentrations were measured by column gas chromatography (GC) [16, 17] using nordeoxycholic acid and coprostanol as internal standards.

Statistical analysis

Regression line analysis and the correlation coefficients were calculated using the Microsoft Excel Statistical Pack, version 7.0 for Windows 95 and non-parametric statistical test using SPSS 7.0 for Windows.

Results

Analysis of clinical data

There were 23 female and 12 male liver donors in total with a median age of 44 years (range: 12–62). Of these 35 liver donors, 12 provided normal and 23 "sub-optimal" grafts. This second group included those grafts with any degree of fat infiltration (as qualified by the retrieving surgeon) and those considered "marginal" grafts, as defined elsewhere [2].

Of the 35 grafts retrieved, two were not transplanted due to severe (>60%) fatty infiltration ("sub-optimal" grafts). Of the remaining 33 grafts, 31 were used as whole grafts for OLT and two were divided on the back table to provide four grafts for split liver transplantation, giving a total of 35 patients transplanted with these grafts. Of these 35 recipients, 14 were female and 21 male with a median age of 53 years (range: 1–71). It was considered that the splitting of a liver may alter the post-transplant outcome and the four recipients that received a "split" graft were not included in this analysis.

Clinical assessment of the remaining 31 recipients showed that 64% of them received "sub-optimal" grafts and 36% received normal grafts. From the group with "sub-optimal" grafts, 40% developed PGD post-transplantation, whilst there were no instances of PGD in those who received normal grafts and this difference was statistically significant ($P=0.015$, chi square 5.93, d.f. = 1).

The cumulate proportional patient survival rate was calculated at a median follow up of 40 months. It was 63% for the recipients that developed PGD and 78% for the recipients who had good early graft function. However, this difference increased when the patient survival rate was calculated for the first 3 months post-transplant (63% for the recipients with PGD and 96% for those with normal function), indicating that the excess mortality in the group with PGD occurred in the early period post-transplant.

Donor bile assessment

Bile flow [volume of hepatic bile collected during regular intervals of time (ml/min)] and TBA output rate [total amount of bile acids excreted into bile per minute (μmol/min)] were calculated for each subject. The median bile flow rate for all the donors ($n=31$) was 0.20 ml/min (range 0.07–0.85) and the median TBA output rate was 1.25 μmol/min (range 0.17–11.08). There was no difference between the median bile flow of the donors with normal and "sub-optimal" grafts (0.20 ml/min). However, the median TBA output rate was greater in the group of sub-optimal grafts (1.56 μmol/min) than in the group of normal grafts (0.67 μmol/min). This difference was statistically significant ($P=0.037$, Mann–Whitney $Z=2.09$).

The relationship between bile flow (μl/min) and TBA output rate (μmol/min) is known as the "apparent choleretic activity" (ACA) and is calculated from the equation of a positive slope of a linear regression, indicating that greater bile acid secretion produces more bile flow [18, 19]. Due to the nature of the regression analysis, only donors with more than three bile samples collected were studied ($n=27$) and a total of 130 samples were plotted in the regression analysis (Fig. 1). The coefficient of linear correlation (r) was 0.84, indicating a strong relationship between these two variables. The

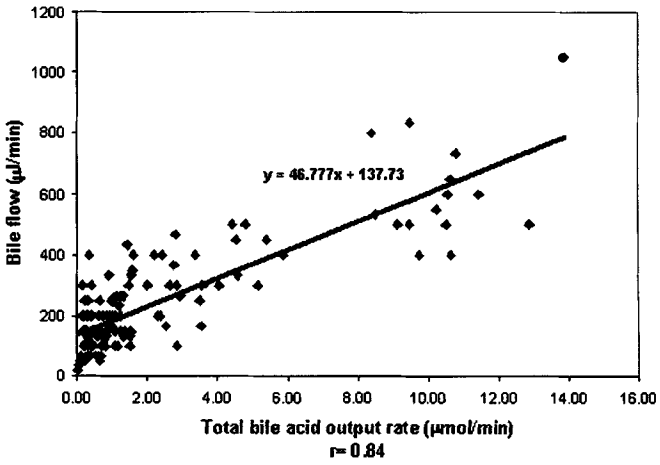

Fig. 1 Apparent choleretic activity (35 donors, 130 samples)

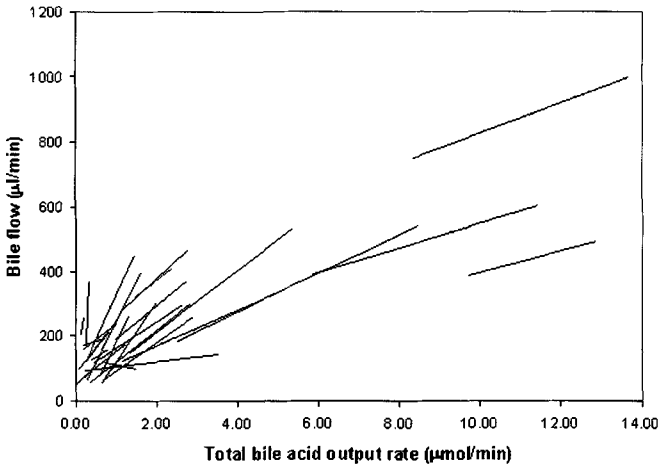

Fig. 2 Individual ACA (35 donors)

slope of the linear regression (ACA) was 46.78 µl/µmol and the intercept of the slope on the Y-axis was 137.73 µl/min. The individual ACA showed a wide inter-patient variation and is represented in Fig. 2. The median ACA of the donor grafts considered to be sub-optimal ($n=17$) was 81.30 µl/µmol and for the group considered as normal ($n=10$) 100.57 µl/µmol. There was a statistically significant difference between these two groups ($P=0.027$, Mann–Whitney $Z=2.21$).

The balance of the physicochemical properties of individual bile acids accounts for the different ways in which bile acids interact with each other and with biliary lipids [20, 21] and the determination of the bile acid profile is fundamental to understanding their physiology. In this study, the proportions of individual bile acids in the donor samples ($n=28$) were expressed as percentages of the main bile acids for each donor and the median values for these were: ursodeoxycholic acid (UDCA) 2%, cholic acid (CA) 38%, chenodeoxycholic acid (CDCA) 37% and deoxycholic acid (DCA) 18%. Lithocholic acid was not detected in these bile samples. The bile acid composition of the samples from "sub-optimal" grafts ($n=19$) was UDCA 2%, CA 39%, CDCA 34% and DCA 19% and for the group considered to be normal ($n=9$) was UDCA 2%, CA 35%, CDCA 39% and DCA 13%. There was a reversal of the proportions of CA and CDCA between these two groups (CA/CDCA ratio of 1.21 for "sub-optimal" and 0.88 for normal grafts) and this difference was statistically significant ($P=0.039$, Kruskal–Wallis test, chi square $=4.27$, d.f. $=1$) (Fig. 3).

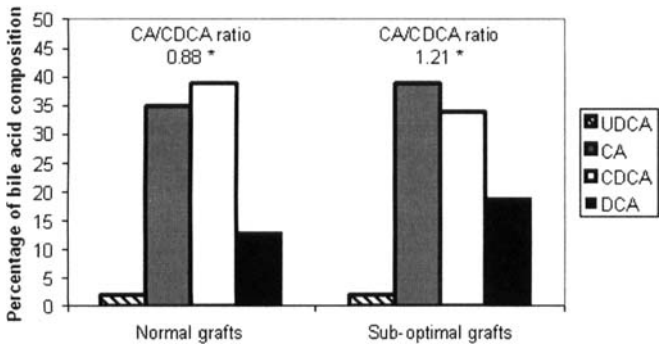

Fig. 3 Bile acid composition in donors. *Difference statistically significant ($P=0.039$, Kruskal–Wallis test, chi square $=4.27$, d.f. $=1$)

Recipient bile assessment

The secretion of bile in donors is characterised by a nearly constant flow during the period of bile collection. In recipients, however, as soon the liver is perfused with portal blood, hepatic cells resume their metabolic activity and start secreting bile from the stand-by status

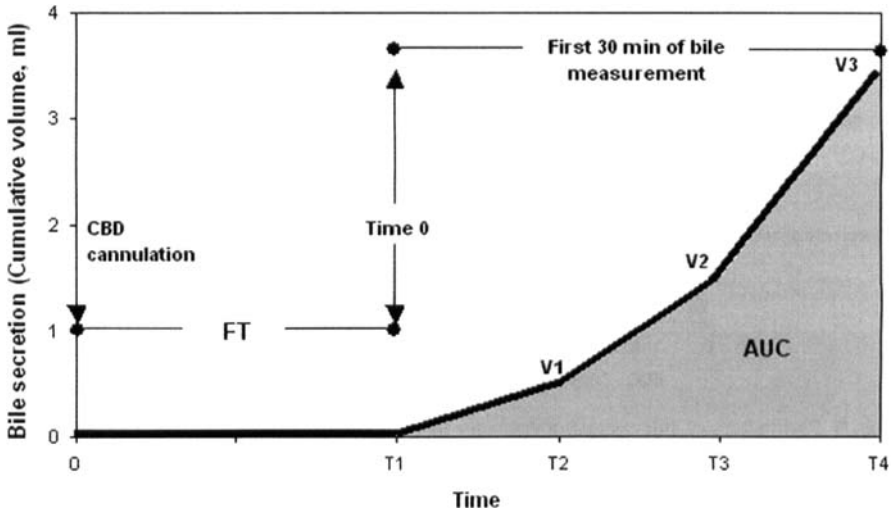

Fig. 4 Pattern of bile secretion recovery in recipients. FT = T1. AUC = 1/2×V1×(T2−T1) + 1/2×(T3−T2[V1 + V2]) + 1/2×(T4−T3[V2 + V3] where *Vn* is the volume measured at 10 min intervals (*Tn*). *CBD* common bile duct

caused by the cold preservation, and bile production slowly recovers [22]. The pattern of recovery of bile secretion in transplant recipients was assessed by measuring, immediately after reperfusion, the time taken by the bile secreted to fill the total length of the standard feeding tube inserted in the common bile duct [this time was called the "filling" time (FT)]. The cumulative bile volume (ml) and TBA output (μmol) were also plotted against time of bile collection (for the first 30 min after reperfusion) and the area under the curve (AUC) calculated for individual recipients (Fig. 4).

The FT was variable between recipients ($n=14$) with a median time of 31 min (range 9–123). The median FT of the recipients considered to have received sub-optimal grafts was 36.5 min (range 16–123) and for those with normal grafts 22.5 min (range 9–34). Furthermore, the median FT of recipients who developed PGD post-transplant was longer than the median FT of recipients with good early graft function (43 and 27 min, respectively) and this difference was statistically significant ($P=0.039$, Mann–Whitney $Z=2.07$). Perioperative factors such as prolonged cold ischaemia time or increased blood loss during the transplant did not alter the FT, as these factors were similar in both groups (Fig. 5).

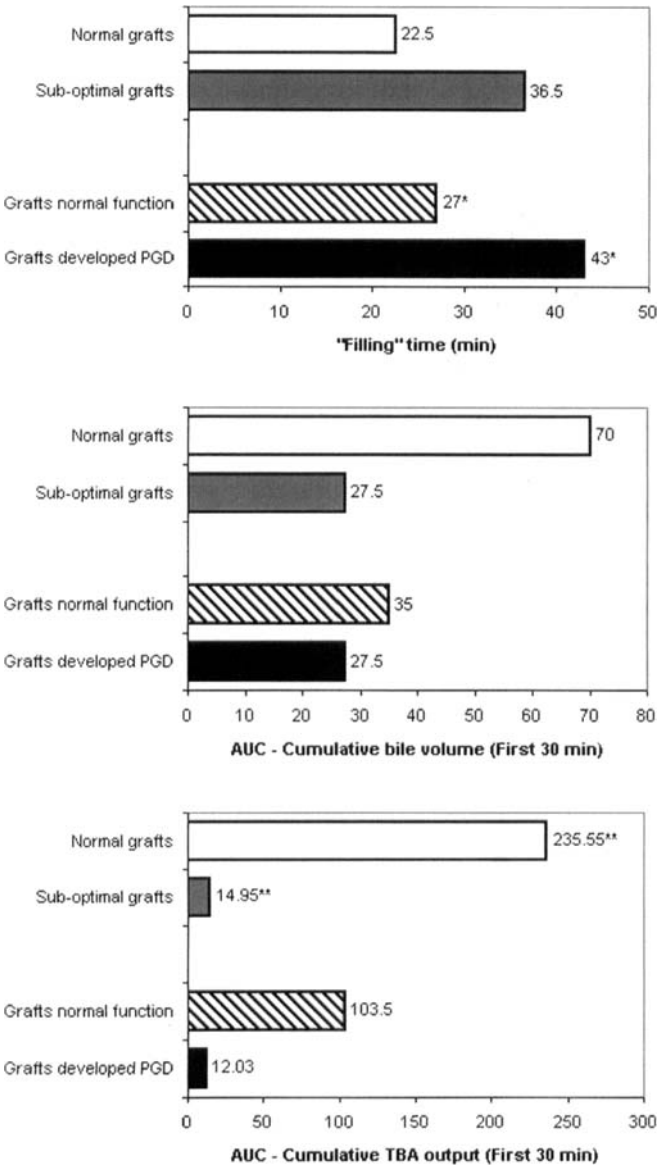

Fig. 5 Parameters of bile secretion recovery in recipients. *Difference statistically significant ($P=0.039$, Mann–Whitney $Z=2.07$), **difference statistically significant ($P=0.030$, Mann–Whitney $Z=2.17$)

The median AUC of the accumulated bile volume of recipients with "sub-optimal" grafts was 27.5 (range 17.5–45) and for recipients with normal grafts 70 (range 32.5–75); but this difference did not reach statistical significance. However, the median AUC of the accumulated TBA output was 14.95 (range 3.70–126) for "sub-optimal" grafts and 235.55 (range 121.75–269.6) for normal grafts, this difference was statistically significant ($P=0.030$, Mann–Whitney $Z=2.17$). The same parameters also showed differences between grafts that developed PGD and those with good early graft function post-transplant; however, these finding were not statistically significant (Fig. 5).

The analysis of the apparent choleretic activity in transplant recipients was performed by regression analysis using the data from 43 bile samples acquired during the first 60 min of bile collection. The coefficient of linear correlation (r) was 0.69, the slope of the linear regression was 87.01 μl/μmol and the intercept of the slope on the Y-axis was 61.88 μl/min. The individual ACA of the recipients studied ($n=10$) showed a wide inter-patient variation and there was no statistically significant difference between the median ACA of recipients who received sub-optimal (118.87 μl/μmol) or normal grafts (45.89 μl/μmol).

The proportions of individual bile acids in the bile samples of each recipient ($n=12$) were expressed as percentages of the main bile acids and the median values for these were: CA 41%, CDCA 40% and DCA 7%. Interestingly, it was found that UDCA was the predominant bile acid in the bile samples collected after reperfusion of two recipients receiving oral UDCA before transplantation. LCA and other bile acid metabolites were not detected in these bile samples. There was no statistically significant difference in the percentage of individual bile acids between recipients who received sub-optimal from those who received normal grafts.

Discussion

The analysis of donor and recipient clinical data revealed that patients receiving "sub-optimal" grafts had an increased incidence of PGD compared to those receiving normal grafts. Furthermore, the survival

analysis showed that recipients with PGD are at higher risk of dying in the early post transplant period (first 3 months) indicating the importance of being able to predict PGD prior to transplantation.

The analysis of hepatic bile of all donors gave median values of bile flow, bile acid output and ACA similar to those reported in previous studies [11]. Under normal conditions the secretion of a determined amount of bile acids is accompanied by an equivalent amount of water into the canaliculus, which provides the driving force for generating bile flow [23]. In this study, the isolated measurement of bile flow in donors was not found to be a reliable parameter for differentiating "sub-optimal" from normal grafts. However, when bile flow was correlated to the secretion of TBA in individual subjects, the group with "sub-optimal" grafts showed lower ACA values indicating that their bile flow did not increase appropriately with the increased TBA output rate. The explanation for this is unknown, but it is likely to be multi-factorial. Higher TBA output rate in "sub-optimal" grafts may be an expression of impaired water secretion at a canalicular level, or due to a reduction in bile acid-independent promoters of bile flow such as glutathione, bicarbonate, calcium, sodium, potassium, glucose, amino acids and organic acids. Canalicular bile flow depends not only on the amount of bile acids secreted, but also on bile acid composition [24]. If "more hydrophobic" bile acids (CDCA and DCA) are found in a higher concentration in bile than in "less hydrophobic" bile acids (CA), then greater bile flow is expected [25]. Therefore, it is possible that despite an increased TBA output rate, the flow was proportionally reduced in "sub-optimal" grafts because of differences in bile acid composition, particularly increased CA levels. Furthermore, bile acids have been shown to be direct regulators of gene transcription in hepatocytes with effects on bile acid biosynthesis and cholesterol homeostasis and variations in gene expression are in response to the interaction of different bile acids with receptors that modify gene transcription [26, 27, 28, 29]. Thus, the difference in the bile acid profile observed in "sub-optimal" and normal livers may be an expression of changes at a molecular level.

The analysis of biliary bile acids in liver transplant recipients has generally been limited to the study of T-tube bile in the early post-transplant period [10]. In clinical practice, it is well recognised that grafts that have delayed production of bile after reperfusion may have a degree of graft dysfunction. In this study, we found a delayed recovery of hepatic bile secretion in those grafts that later developed PGD (as indicated by prolonged FT and lower cumulative bile volume and TBA output), these observations can be used as an early indicator of PGD. Other parameters of bile secretion in liver transplant recipients such as individual ACA and bile acid composition varied significantly between patients and were not reliable markers.

In summary, bile acids analysis of normal and sub-optimal donor grafts has shown significant differences between these two groups. Low ACA is characteristic of sub-optimal grafts and may be tightly related to differences in bile acid composition. Analysis of bile acids in transplant recipients provides information on the pattern of recovery of bile secretion immediately after graft reperfusion and may be an early indicator of the development of PGD. The importance of analysing parameters of graft function has been highlighted in this study and may have a clinical application in the future as HPLC and GC technologies became automated and less complex. The expanding knowledge of gene expression regulation (genomics) and the determination of bile acids' behaviour in different body compartments (integrative metabonomics) may help to further potentiate the role of bile acids analysis as a tool to assess graft function during liver transplantation.

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