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# A validated technique for the analysis of biliary bile acid secretion in donor livers prior to transplantation

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**Abstract** Many parameters currently used for the pre-transplant assessment of liver allografts, are not reliable enough in predicting the likelihood of early graft dysfunction or non-function. It is generally accepted that bile secretion is a sign of hepatic function post-transplant and that bile flow shows a close linear relationship to the secretion of bile acids ("apparent choleretic activity"). We have studied bile flow, biliary bile acid concentrations and composition and measured apparent choleretic activity from hepatic bile collected with a new technique under controlled conditions at the time of retrieval from 18 donor livers. More than three samples were collected from each of 13 donors and a total of 65 samples of hepatic bile were analysed. Of these, ten showed typical apparent choleretic activity with a positive slope in the regression line analysis (correlation coefficient of 0.9), validating our collection technique.

**Key words** Bile acids, donors, liver transplantation · Bile flow, liver transplantation, donor · Liver transplantation, bile flow, donor Donor bile flow, liver transplantation

## Introduction

Liver transplantation is now firmly established as a treatment for patients with end-stage liver disease. The reliable assessment of the donor liver prior to transplantation has been a subject of much research and is increasingly important as donor numbers remain limited. Many clinical and laboratory parameters have been used to assess the graft pre-transplantation [10, 12, 19, 22, 24], but there is no single criterion that is reliable and so the transplant surgeon must rely on the overall assessment of donor data and liver appearance [16, 18].

Bile secretion is an important physiological function of the liver, and good bile flow has been considered as an early sign of hepatic function following transplantation [8, 25]. Bile acids are quantitatively the major component of human bile and their secretion influences bile flow and the biliary concentrations of phospholipids and cholesterol. Hepatic bile formation occurs at two levels, canalicular and ductular. Canalicular bile flow is mainly dependent on bile acid secretion [bile acid-dependent bile flow (BADBF)] and accounts for 70 %–85 % of the total canalicular flow. Bile acid-independent bile flow (BAIBF) is probably driven by inorganic ion transport [13, 21] and is both canalicular and ductular.

Measurement of bile secretion in transplant patients presents considerable difficulties. Previous attempts have predominantly been confined to the analysis of bile obtained by T-tube drainage in post-transplant patients whose bile acid enterohepatic circulation (EHC) was interrupted for varying time periods [4, 8, 9, 11, 17, 26]. Reports of the measurement of bile secretion without EHC interruption, using duodenal perfusion, have been confined to two studies in patients 6–8 weeks post-transplant [25, 29]. There are no reports of analysis of bile secretion from donor livers before transplantation. To assess the potential value of pre-transplant assessment of the hepatic bile from donor livers, we used a new technique to collect 65 hepatic bile samples from 18 donors at the time of organ retrieval. We then analy-

**Table 1** Clinical and biochemical data at organ retrieval (*No.* patient number, *Age* patient age (years), *AST/ALT* aspartate aminotransferase/alanine aminotransferase (IU/l), *Bil* bilirubin (μmol/l), *ALP* alkaline phosphatase (IU/l), *GGT* gamma glutamiltransferase (IU/l), *Alb* albumin (g/l), *INR/PT* international normalized ratio/prothrombin time (s), *Hb* haemoglobin (mg/dl), *WCC* white cell count ($x10^9$), *CMV* cytomegalovirus status, *ITU* days in intensive care unit, *Fatty liver* assessment of hepatic fatty infiltration by surgeon, *M* male, *F* female, *S.A.H.* subarachnoid haemorrhage, *I.C.B.* intracraneal bleeding, *Pos* positive, *Neg* negative, – no data available, *Mild-Mod.* mild to moderate fatty infiltration)

| No. | Age | Sex | Diagnosis | ALT/AST | Bil | ALP | GGT | Alb | INR/PT | Hb | WCC | CMV | ITU | Fatty liver |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 23 | F | Meningitis | 0/45 | 5 | 46 | – | 20 | 1.6/20 | 8.2 | 14.3 | Pos | 1 | Mild-Mod. |
| 2 | 31 | M | S.A.H. | 0/91 | 8 | 53 | 9 | 26 | 1.3/0 | 14 | 16.5 | Neg | 2 | Normal |
| 3 | 15 | F | Encephalitis | 0/68 | 7 | 4.1 | – | – | 0/17 | 8.7 | 14.2 | Neg | 9 | Normal |
| 4 | 62 | M | S.A.H. | 0/14 | 13 | 141 | 12 | 30 | 0/15 | 11 | 11.9 | Pos | 1 | Mild |
| 5 | 45 | F | I.C.B. | 12/0 | 15 | – | – | 41 | – | 15 | 13.9 | Pos | 2 | Normal |
| 6 | 38 | F | S.A.H. | 0/16 | 13 | 69 | – | 28 | 0/15 | 11 | 15.4 | Neg | 3 | Mild |
| 7 | 36 | M | CO poisoning | 76/0 | 12 | 48 | – | 30 | 0/18 | 14 | 14.3 | Neg | 3 | Mild |
| 8 | 41 | F | S.A.H. | 0/39 | 8 | 40 | 173 | 26 | 1.1/0 | 11 | 14.6 | Neg | 7 | Normal |
| 9 | 42 | F | Brain tumour | 0/27 | 3 | 121 | – | 23 | – | 10 | 14 | Neg | 5 | Normal |
| 10 | 53 | F | I.C.B. | 0/22 | 3 | 48 | – | 16 | 1.2/0 | 11 | 6.8 | Neg | 3 | Mild |
| 11 | 47 | M | I.C.B. | 0/16 | 15 | 59 | – | 33 | 0/15 | 12 | 12.7 | Pos | 2 | Mild |
| 12 | 55 | F | S.A.H. | 24/0 | 5 | 88 | – | 21 | – | 8.4 | 12 | Pos | 3 | Normal |
| 13 | 54 | F | Head injury | 0/15 | 8 | 76 | – | 44 | – | 14 | 12.5 | Neg | 2 | Mild |
| 14 | 48 | F | Amytriptiline | 27/36 | 5 | 47 | – | 24 | 0/17 | 11 | 13.2 | Pos | 3 | Mild |
| 15 | 48 | F | S.A.H. | 80/39 | 7 | 80 | – | 24 | 1.6/16 | 10 | 6.3 | Pos | 2 | Normal |
| 16 | 33 | F | S.A.H. | 0/9 | 15 | 105 | – | 43 | 2.1/36 | 9.5 | 9.7 | – | 3 | Normal |
| 17 | 49 | F | Cardiac arrest | 218/0 | 6 | 6.6 | – | 27 | 1/0 | 13 | 18.3 | Pos | 2 | Mild |
| 18 | 21 | M | S.A.H. | 0/21 | 38 | – | – | 21 | 2.1/0 | 6.6 | 57 | Pos | 2 | Normal |

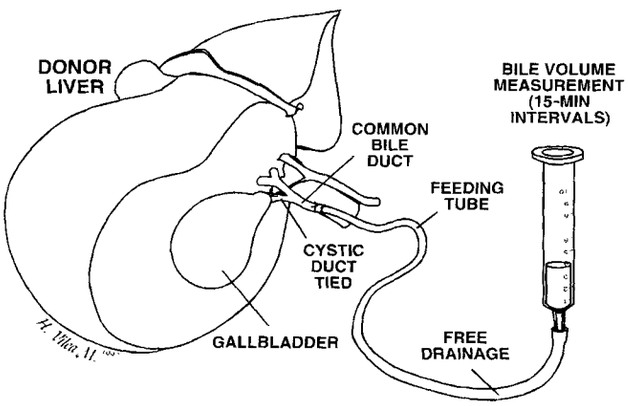


**Fig. 1** Hepatic bile collection during organ retrieval

sed bile flow, biliary bile acid concentration and composition and measured "apparent choleretic activity" (ACA).

## Materials and methods

Eighteen liver donors were assessed after Hospital Ethical Committee permission was granted. Relevant clinical and biochemical data are summarized in Table 1. Hepatic bile samples were collected with a new technique during the standard surgical retrieval of the liver. The cystic duct was ligated to exclude gallbladder bile and the common bile duct was cannulated with an 8 FG feeding tube, allowing free drainage of bile (Fig. 1). These additional simple steps did not significantly extend the time of the normal procedure. The volume of bile was measured at 15-min intervals while the dissection of the liver and other organs was being performed, and this continued until multi-organ perfusion with preservation solution.

The total bile acid concentration was measured using an enzymatic procedure [28] and biliary bile acid composition by high-performance liquid chromatography [32]. Regression line analysis and the correlation coefficients were calculated using Microsoft Excel 7.0 for Windows 95 and the statistical significance of the results was calculated by an analysis of variance using SPSS 7.0 for Windows.

## Results

### Liver donors

There were 5 males and 13 females with a median age of 44 years (range 15–62 years) and a median weight of 64 kg (range 50–85 kg). The median period in the intensive care unit prior to organ donation and days of ventilatory support were the same: 2.5 days (range 1–9 days). During this period, these patients were not fed enterally or intravenously. The median urinary output for the 24 h prior to donation was 6.5 l (range 2.3–10 l). Six donors were treated for diabetes insipidus. Two patients were resuscitated after cardiac arrest (donors 12 and 17) and one after respiratory arrest (donor 4). Ten patients had at least one episode of transient systemic hypotension and were supported with inotropes (six requiring dopamine at > 5 μg/kg per hour and one also receiving adrenaline). Haemodynamic instability was not consid-

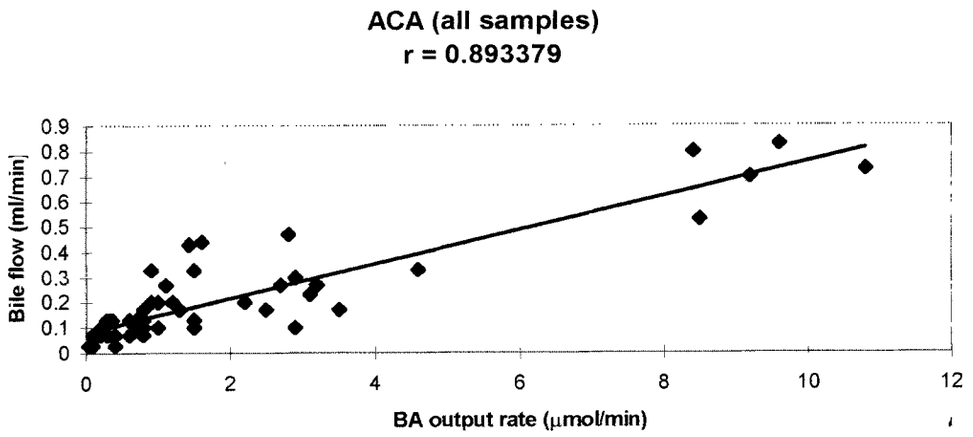


**Fig. 2** Apparent choleretic activity (13 donors, 56 samples)

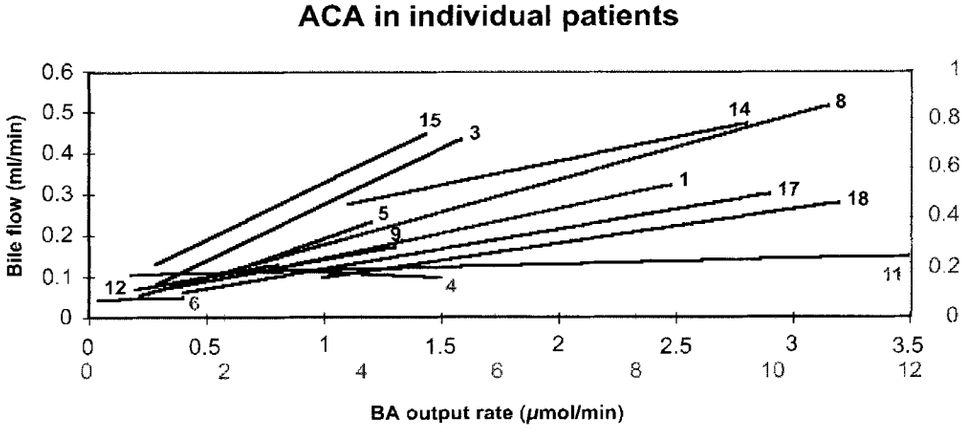


**Fig. 3** Individual ACA (13 donors). Donors 1 and 8 are on the bottom axis scale; the rest are on the upper axis scale. Donors 4, 6 and 11 have a correlation coefficient ($r$) < 0.85

ered sufficiently significant to preclude organ donation. One donor had liver congestion secondary to a raised central venous pressure (donor 6).

During organ retrieval, the macroscopic features of the liver were assessed by the retrieving surgeon and included nine normal livers, eight mildly fatty livers and one with mild to moderate fatty infiltration (Table 1). All donor livers perfused well with University of Wisconsin solution except one (donor 1) with suboptimal liver perfusion at laparotomy and initial patchy perfusion with the preservation solution. All livers had normal biliary anatomy and one donor had gallstones (donor 6). Two donors also had heart and lung retrieval with an in situ cooling technique (donors 3 and 8).

## Bile assessment

Sixty-five samples of hepatic bile were collected. The median bile volume at 15-min intervals was 2 ml (range 0.5–12.5 ml). The median bile flow rate was 0.13 ml/min (range 0.03–17 ml/min). The median total bile acid concentration was 6 μmol/ml (range 0.9–34 μmol/ml). The median total bile acid output was 14.7 μmol (range 0.7–188 μmol). The median total bile acid output rate was 1 μmol/min (range 0.04–12.5 μmol/min).

It was possible to collect at least three consecutive hepatic bile samples in 13 of the 18 donors. A particularly high total bile acid concentration was detected in the first sample of one patient (donor 6), probably caused by passage of gallbladder bile into the common bile duct prior to the collection; this sample was discarded. The median ACA of 56 samples from these 13 patients was 175 μl/μmol (range 68–366 μl/μmol) and showed a positive slope with the correlation coefficient ($r$) of 0.9 (Fig. 2). The BADBF was 0.0953 ml/min and the BAI-BF was 0.037 ml/min. When the ACA was calculated for individual patients, there was a wide inter-patient variation. All but three patients (donors 4, 6 and 11) had a positive slope; the latter had slopes with a correlation coefficient of less than 0.85 (Fig. 3). The ten patients with positive slopes (group 1) showed statistically significant differences (Student's $t$ test) regarding bile flow and bile acid output when compared with the other

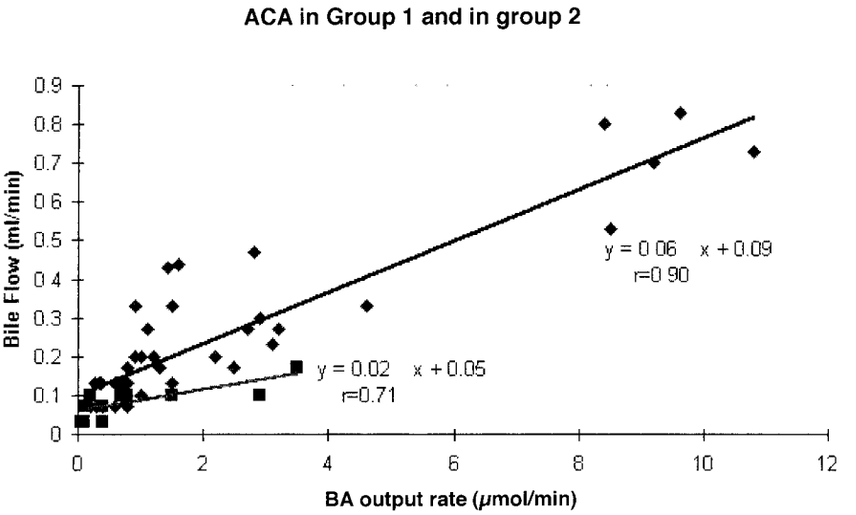


**Fig. 4** ACA for group 1 (◆), $n = 10$) and group 2 (■, $n = 3$)

three patients (group 2), but the difference between the respective ACA was not statistically significant (analysis of variance; Fig. 4). Primary bile acids predominated (up to 70%) and all of the samples contained secondary bile acids. There was a wide inter-patient variation in the composition of the individual bile acids. Glycine conjugates predominated over taurine conjugates (median glycine-taurine ratio was 2.3).

## Liver recipients

The 13 donor livers in which ACA was calculated were implanted in eight male and five female recipients (median age 46 years, range 1–68 years). The diagnosis, graft appearance at retrieval, early graft function and patient outcome are summarised in Table 2. The early graft function was classified as "good", when the initial international normalized ratio (INR) was less than 2.5 and the serum aspartate aminotransferase (AST) levels less than 1500 IU/l on day 1 post-transplant, "moderate" when the INR was less than 3 or AST less than 2000 IU/l and "poor" when the INR was more than 3 or AST more than 2000 IU/l. Graft appearance at retrieval and early graft function did not correlate. Of seven donor livers considered "normal", three had good and four had moderate graft function. Of five "mildly" fatty livers, two had moderate, two poor and one good graft function. One graft was considered as mild to moderately fatty and had good early graft function. Donor livers 4, 6 and 11 (group 2) had an ACA with a correlation coefficient of less than 0.85; all three were considered

**Table 2** Liver transplant recipient data (*Don* donor number, *Rec* recipient reference, *M* male, *F* female, *HCV* hepatitis C virus, *FHF* fulminant hepatic failure, *HCC* hepatocellular carcinoma, *PBC* primary biliary cirrhosis, *ALD* alcoholic liver disease, *HAT* hepatic artery thrombosis, *IAB* intra-abdominal bleeding post-transplantation)

| Don | Rec | Age | Sex | Diagnosis | Fatty liver | Early graft function | Outcome |
|---|---|---|---|---|---|---|---|
| 1 | A | 42 | M | HCV cirrhosis | Mild-Mod. | Good | No complications |
| 3 | B | 40 | F | Sec. biliary cirrhosis | Normal | Good | Early HAT, retransplantation |
| 4 | C | 60 | F | FHF No A-No B hepatitis | Mild | Poor | IAB, sepsis, died |
| 5 | D | 16 | M | FHF No A-No B hepatitis | Normal | Moderate | No major complications |
| 6 | E | 50 | F | Cryptogenic cirrhosis | Mild | Moderate | Cholestasis, died |
| 8 | F | 49 | M | HCV cirrhosis + HCC | Normal | Good | Recurrent HCV |
| 9 | G | 1 | M | Neonatal hepatitis | Normal | Moderate | No major complications |
| 11 | H | 46 | M | Recurrent HCV | Mild | Poor | Renal failure, IAB |
| 12 | I | 68 | F | PBC | Normal | Good | No major complications |
| 14 | J | 48 | M | HCV cirrhosis + ALD | Mild | Good | No major complications |
| 15 | K | 54 | M | ALD | Normal | Moderate | Bile duct dilatation |
| 17 | L | 45 | M | HCV cirrhosis | Mild | Moderate | Cholestasis, steatosis |
| 18 | M | 40 | F | FHF No A-No B hepatitis | Normal | Moderate | Renal failure, sepsis |

Donor livers 4, 6 and 11 have "apparent choleretic activity" with a correlation coefficient of less than 0.85

**Table 3** Normal values for human bile secretion (*BAIBF* bile acid-independent bile flow, *ACA* apparent choleretic activity)

| | Year | Patient | Method | Bile flow (ml/min) | BAIBF (ml/min) | BA output rate (μmol/min) | ACA (μl/μmol) |
|---|---|---|---|---|---|---|---|
| Prandi [21] | 1975 | Gallstone | T-tube | 0.46 | 0.16 | 22.2 | 10 |
| Ericzon [8] | 1990 | Post-OLT | T-tube | 0.06–0.16 | 0.04 | 0.5–2.4 | 34 |
| Shiffman [26] | 1991 | Post-OLT | T-tube | 0.06–0.33 | 0.09 | 0.4–4.5 | 51 |
| Theilman [29] | 1991 | Post-OLT | Duodenal perfusion[a] | | | 43.3 | |
| McCashland [17] | 1994 | Post-OLT | T-tube[b] | 0.09–0.16 | 0.07 | 0.4–1.1 | 129 |
| | | | T-tube[c] | 0.08–0.2 | 0.02 | 0.3–0.9 | 220 |
| Baumgartner [4] | 1995 | Post-OLT | T-tube[d] | 0.53 | | 7.7 | |
| | | | T-tube[e] | 0.21 | | 1.6 | |
| This study | 1996 | Donor | Short-term drainage | 0.13 | 0.04 | 1 | 175 |

[a] Patients between 6–12 weeks post-transplant
[b] Cyclosporin group
[c] FK506 group
[d] Group 1, uneventful recovery post-transplant
[e] Group 2, raised AST between days 5 and 8 post-transplant

mildly fatty at retrieval, but two had poor and one had moderate graft function. Two of these three recipients had post-operative intra-abdominal bleeding (recipients C and H) and one had persistent cholestasis (recipient E). Two patients subsequently died; recipient C of multi-organ failure with systemic sepsis and recipient E of heart failure.

## Discussion

Successful liver transplantation starts with proper organ retrieval and adequate donor assessment [12]. Currently, donor organ selection is based on overall donor data, as there are no reliable tests for pre-transplant graft assessment. Many clinical parameters have been considered to predict graft function. These include donor age [3, 5, 31], past medical history (e.g. viral infection, alcohol intake, smoking, drug abuse and chronic liver disease) and medical status of the potential donor (e.g. cause of brain stem death, trauma complications, haemodynamic status, infections and length of hospital stay). Laboratory parameters considered to predict graft function include serum urea and creatinine and liver function tests and coagulation expressed by the prothrombin time or INR. Morphological changes found on liver biopsy at the time of organ retrieval do not usually predict graft viability [14], but they are useful in assessing the different degrees of fatty infiltration [2, 6, 30]. Endogenous metabolites that reflect liver function, such as serum bile acids [4], bile acid clearance [23], ketone body ratio [20, 27], the ratio between branched chain amino-acids and aromatic amino acids [23], cytochrome P450 activity measured by the lidocaine-MEGX (monoethylglycinexylidide) test [1, 7] and the adenosine triphosphate (ATP) content of the liver by magnetic resonance spectroscopy [33], have all been evaluated but have not found a place in clinical practice.

Bile secretion is an important physiological function of the liver and has been the subject of much research. Many studies suggest that bile secretion is a potentially useful marker of early liver function following liver transplantation [8, 26]. The placement of a T-tube after biliary reconstruction provides a direct source of hepatic bile; however, it interrupts the EHC, causing chronic bile pool depletion [11]. The predominance of primary bile acid conjugates and the near absence of secondary bile acids in samples collected using T-tubes [26] suggest that the diversion is significant and that the bile acid output is equivalent to bile acid biosynthesis from cholesterol [17] and does not reflect normal physiological recovery. The recognition that T-tube bile samples have limitations has led to the use of other methods of measuring bile acid secretion after human transplantation. Theilman et al. [29] used duodenal perfusion with a non-absorbable marker, without EHC interruption, 6 weeks post-transplant. Although their method was reliable, it is not suitable for early post-transplant studies.

The composition of bile produced by the liver graft is determined by many donor and recipient factors [11]. There are no studies of bile acid secretion by the donor liver prior to transplantation, and all post-transplant studies are based on the consideration that the livers start with a similar functional quality. However, this is not so, as it involves the removal of the liver from an assumed healthy environment (donor), the occurrence of preservation injury (denervation and cold ischaemic preservation) and implantation in a pathological environment (recipient) under the effects of previous cholestasis, surgical trauma, reduction of the bile pool and the effect of a variety of drugs.

Current knowledge of bile flow has been derived from studies of patients with T-tubes placed after cholecystectomy for gallstone disease [15, 21] and, more recently, from studies of transplant recipients. The

"choleretic index" [26], or "apparent choleretic activity" (ACA) [13, 17], is the expression of the relationship between the bile acid secretion and the bile flow and is calculated from the equation of a positive slope of a linear regression, indicating that more bile acid secretion produces more bile flow (bile acid-dependent bile flow). In humans there is a fraction of the total bile flow that is not bile acid-dependent (bile acid-independent bile flow) and this is calculated from the estimated intercept of the slope.

The results of hepatic bile analysis in the present study are compatible with the "normal" values for human bile flow, bile acid output rate and ACA reported in the literature and they validate the collection technique and methodology (Table 3). There was, however, a wide inter-patient variation between our 13 cases with more than three samples analysed. When the individual data is plotted on the regression line analysis, a statistically significant positive correlation is shown in ten patients (group 1) and not in three patients (group 2). When these groups are compared, the bile flow and bile acid output rate are significantly different, with median values in group 1 two times higher than those in group 2. The analysis of variance did not show a statistically significant difference when compared with the slopes (ACA) of these groups, probably due to the small number of cases; however, it was interesting to note abnormal early graft function and poor outcome in group 2 recipients (Table 2). The significance of variables such as donor age, degree of fatty infiltration or hospital stay will require the study of a larger number of donors and recipients to confirm the use of donor liver bile acid secretion as a discriminative tool for pretransplant liver function assessment.

In conclusion, this is the first study of bile acid secretion in liver donors using a new technique for hepatic bile collection. It allows access to hepatic bile under controlled conditions without interruption of the EHC, making this technique favourable for the study of bile composition and bile flow.

## References


1. Adam R, Azoulay D, Astarciuglu I, Bao YM, Bonhomme L, Fredj G, Bismuth H (1991) Reliability of the MEGX test in the selection of liver grafts. Tranplant Proc 23: 2470–2471
2. Adam R, Reynes M, Johann M, Morino M, Astarcioglu I, Kafetzis I, Castaing D, Bismuth H (1991) The outcome of steatotic grafts in liver transplantation. Transplant Proc 23: 1538–1540
3. Alexander JW, Vaughn WK (1991) The use of 'marginal donors' for organ transplantation. Transplantation 51: 135–141
4. Baumgartner U, Scholmerich J, Kremer B, Streckfuß G, Henne-Bruns D, Mergard BL, Kraemer-Hansen H, Farthmann EH (1995) Early detection of graft dysfunction after orthotopic liver transplantation in man by serum and biliary bile acid analysis. Hepatogastroenterology 42: 950–960
5. Buckel E, Sanchez-Urdazpal L, Steers J, Sterioff S, Wiesner R, Krom RAF (1993) Impaired initial function in liver grafts from donors > 50 years of age. Transplant Proc 25: 1558–1559
6. D'Alessandro AM, Kalayoglu M, Sollinger HW, Hoffmann RM, Reed A, Knechtle SJ, Pirsch JD, Hafez GR, Lorentzen D, Belzer FO (1991) The predictive value of donor liver biopsies for the development of primary non-function after orthotopic liver transplantation. Transplantation 51: 157–163
7. Diaz D, Pageaux GP, Fabre JM, Pichard L, Maurel P, Baumel H, Michel H (1990) The risk of short-term liver graft dysfunction may be correlated whit a low pre-transplant hepatic cytochrome P450IIIA level. J Hepatol 11: S19
8. Ericzon BG, Eusufzai S, Kubota K, Einarsson K, Angelin B (1990) Characteristics of biliary lipid metabolism after liver transplantation. Hepatology 12: 1222–1228
9. Farouk M, Branum GD, Watters CR, Cucchiaro G, Helms M, McCann R, Bollinger R, Meyers WC (1991) Bile compositional changes and cholesterol stone formation following orthotopic liver transplantation. Transplantation 52: 727–730
10. Greig PD, Forster J, Superina RA, Strasberg SM, Mohamed M, Blendis LM, Taylor BR, Levy GA, Langer B (1990) Donor-specific factors predict graft function following liver transplantation. Transplant Proc 22: 2072–2073
11. Haagsma EB, Huizenga JR, Vonks RS, Albers CJEM, Ground J, Krom RAF, Gips CH (1987) Composition of bile after orthotopic liver transplantation. Scand J Gastroenterol 22: 1049–1055
12. Heaton ND, Tan KC (1994) Retrieval team and national organization. In: Williams R, Portmann B, Tan KC (eds) A practical guide to liver transplantation. Churchill-Livingstone, London, pp 11–15
13. Hofmann AF (1990) Bile acid secretion, bile flow and biliary lipid secretion in humans. Hepatology 12[Suppl]: 17S-25S
14. Kakizoe S, Yanaga K, Starzl TE, Demetris AJ (1990) Evaluation of protocol before transplantation and after reperfusion biopsies from human orthotopic liver allografts: considerations of preservation and early immunological injury. Hepatology. 11: 932–941
15. Linbland L, Schersten T (1976) Influence of cholic and chenodeoxycholic acid on canalicular bile flow in man. Gastroenterology 70: 1121–1124
16. Makowka L, Gordon RD, Todo GS, Ohkohchi N, Marsh JW, Tzakis AG, Yokoi H, Ligush J, Esquivel CO, Satake M, Iwatsuki S, Starzl TE (1987) Analysis of donor criteria for the prediction of outcome in clinical liver transplantation. Transplant Proc 19: 2378–2382
17. McCashland TM, Donovan JP, Amelberg A, Rossi SS, Hofmann AF, Shaw BW Jr, Quigley EMM (1994) Bile acid metabolism and biliary secretion in patients receiving orthotopic liver transplants: differing effects of cyclosporine and FK506. Hepatology 19: 1381–1389
18. Mor E, Klintmalm GB, Gonwa TA, Solomon H, Holman MJ, Gibbs JF, Watemberg I, Goldstein RM, Husberg BS (1992) The use of marginal donors for liver transplantation. Transplantation 53: 383–386

19. Odom NJ (1990) Organ donation. I. Management of the multiorgan donor. BMJ 300: 1571–1573
20. Osaki N, Ringe B, Bunzendahl H, Taki Y, Gubernantis G, Oellerich M, Kuse E-R, Burdelski M, Uemoto S, Kimoto M, Yamaoka Y, Ozawa K, Pilchmayr R (1990) Postoperative recovery of mitochondrial function of the human liver graft procured and preserved with University of Wisconsin (UW) solution. Transpl Int 3: 128–132
21. Prandi D, Erlinger S, Glasinovic J-C, Dumont M (1975) Canalicular bile production in man. Eur J Clin Invest 5: 1–6
22. Pruim J, Woerden WF van, Knol E, Klompmaker IJ, Bruijn KM de, Persijn CG, Slooff MJH (1989) Donor data of liver grafts with primary non-function. A preliminary analysis by the European Liver Registry. Transplant Proc 21: 2383–2384
23. Pruim J, Vergert EM ten, Klompmaker IJ, Verwer R, Slooff MJH. (1991) Cellular damage and early metabolic function of transplanted livers stored in Eurocollins or University of Wisconsin solution. Eur Surg Res 23: 285–291
24. Pruim J, Klompmaker IJ, Haagsma EB, Bijleveld CMA, Slooff MJH (1993) Selection criteria for liver donation: a review. Transpl Int 6: 226–235
25. Sauer P, Stiehl A, Otto G, Theilmann L (1995) In patients with orthotopic liver transplantation, serum markers of cholestasis are unreliable indicators of biliary secretion. J Hepatol 22: 561–564
26. Shiffman ML, Carithers RL Jr, Posner MP, Moore EW (1991) Recovery of bile secretion following orthotopic liver transplantation. J Hepatol 12: 351–361
27. Takada Y, Ozaki N, Ringe B, Mori K, Gubernantis G, Oellerich M, Yamaguchi T, Kiuchi T, Shimahara Y, Yamaoka Y, Sakurai K, Ozawa K, Pichlmayr R (1992) Receiver operating characteristic (ROC) analysis of the ability of arterial ketone body ratio to predict graft outcome after liver transplantation – its sensitivity and specificity. Transpl Int 5: 23–26
28. Talalay P (1960) Enzymatic analysis of steroids hormones. Methods Biochem Anal 8: 119–143
29. Theilman L, Otto G, Arnold J, Gmelin K, Stiehl A (1991) Biliary secretion of bile acids, lipids and bilirubin by the transplanted liver. Transplantation 52: 1020–1023
30. Todo S, Demetris AJ, Makowka L, Teperman L, Podesta L, Shaver T, Tzakis A, Starzl TE (1989) Primary nonfunction of hepatic allografts with preexisting fatty infiltration. Transplantation 47: 903–905
31. Wall WJ, Mimeault R, Grant DR, Bloch M (1990) The use of older donor livers for hepatic transplantation. Transplantation 49: 377–381
32. Wildegube HJ, Fussel U, Lacier H, Stockhausen H (1983) Measurement of conjugated bile acids by ion-pair high performance liquid chromatography. J Chromatog 282: 603–608
33. Wolf RFE, Kamman RL, Mooyaart EL Haagsma EB, Bleichrodt RP, Slooff MJH (1993) 31-P Magnetic resonance spectroscopy of the isolated human donor liver: feasibility in routine clinical practice and preliminary findings. Transplantation 55: 949–951