SERUM BILE-ACIDS AND ESTERIFIED BILIRUBIN IN EARLY DETECTION AND DIFFERENTIAL-DIAGNOSIS OF HEPATIC-DYSFUNCTION FOLLOWING ORTHOTOPIC LIVER-TRANSPLANTATION

Journal of Hepatology, 1993; 17: 141–145

HEPAT 01227

Serum bile acids and esterified bilirubin in early detection and differential diagnosis of hepatic dysfunction following orthotopic liver transplantation[1]

M. Muraca[a], K. Kohlhaw[b], M.T. Vilei[a], B. Ringe[b], H. Bunzendahl[b], G. Gubernatis[b], K. Wonigeit[b], G. Brunner[c] and R. Pichlmayr[b]

[a] *Istituto di Medicina Interna, Università di Padova, Padova, Italy,* [b] *Abteilung für Transplantations- und Abdominalchirurgie, Medizinische Hochschule, Hanover, Germany and* [c] *Zentrum für Innere Medizin, Medizinische Hochschule, Hanover, Germany*

(Received 6 June 1990)

Routine laboratory tests are of little help for early detection and differential diagnosis of hepatic dysfunction following orthotopic liver transplantation (OLT). In the present study, serum levels of esterified bilirubin, total bilirubin and bile acids were investigated in 20 patients after OLT. Twenty episodes of liver dysfunction were observed: 10 rejection episodes, 3 cases of thrombosis of the hepatic artery, 3 cases of septic shock, and 4 episodes of cyclosporin toxicity. During rejection, the median increase in esterified bilirubin was 3.2-fold (range 1.6–24.9), while total bilirubin increased 1.5-fold (range 0.7–3.4). Bile acids increased 3.6-fold (range 2.5–6.6; peak levels 25–87 μM). Both bile acids and esterified bilirubin increased 1–3 days earlier than serum transaminases and decreased only after successful anti-rejection treatment. The response of bile acids to successful treatment was usually more rapid than the response of esterified bilirubin. Hepatic artery thrombosis and septic shock were associated with a sharp increase in esterified bilirubin and very high bile acid levels (peak levels 80–185 μM). During cyclosporin toxicity, a characteristic pattern of progressively increasing bilirubin with no change in the bile acid levels was observed. Both esterified bilirubin and bile acids are very sensitive indicators of hepatic graft dysfunction. In particular, serum bile acids are useful for identifing cyclosporin toxicity and monitoring the response to anti-rejection treatment.

Key words: Serum analysis; Bilirubin; Bile acids; Liver transplantation

Hepatic dysfunction, possibly leading to progressive jaundice, is a frequent finding in the early postoperative course of orthotopic liver transplantation (OLT) (1). Etiology includes primary liver non-function, rejection, drug toxicity, infection and thrombosis of the hepatic blood vessels. Early recognition and classification of deteriorating liver function are important for successful treatment. Unfortunately, routine laboratory tests are of little value for such purposes (2–4), and invasive techniques increase the risk of serious complications. The use of the alkaline methanolysis-HPLC (AM-HPLC) procedure to analyze serum bilirubins (5) provides more information on the pathophysiology of hyperbilirubinemia. The esterified bilirubin fraction has proved to be a very sensitive and specific index of hepatobiliary disease (6,7). Serum bile acids have also been extensively used as sensitive markers of liver dysfunction (8,9), and preliminary reports suggest that they help to monitor graft function after OLT (10,11). In the present study, serum concentrations of individual bile pigments and of total bile acids were correlated with the different complications occurring in the early postoperative course after OLT.

Patients and Methods

Patients

Twenty consecutive patients undergoing OLT in the transplant unit of the 'Medizinische Hochschule' in Hanover were prospectively studied. Diagnosis and

Correspondence to: Maurizio Muraca, M.D., Ph.D., Istituto di Medicina Interna, Policlinico universitario, Via Giustianini 2, 35128 Padova, Italy.
[1]Part of this work was presented at the meeting of the European Association for the Study of the Liver, Munich, 1989.

serum total bilirubin before transplantation are reported in Table 1. All patients had T-tube biliary drainage throughout the study, and underwent the following immunosuppressive regimen: cyclosporin 1–2 mg/kg body wt. (aiming at whole blood trough levels of 120–200 ng/ml); azathioprin 1–2 mg/kg body wt.; anti-thymocyte globulin (ATG, rabbit, Fresenius AG, Germany) 5 mg/kg body wt.; prednisolone 1 mg/kg body wt. (days 1–2), 0.5 mg/kg (days 3–10), 0.4 mg/kg (days 11–20), further reduced by 0.05 mg/kg every 10 days down to 0.15 mg/kg (maintenance dose). In the case of rejection, 3–5 pulses of 0.5 g methylprednisolone were administered, followed by 5 mg of OKT3 for 7 days in cases of persistent rejection. One patient with persistent rejection even after OKT3 administration was successfully treated with ATG 20 mg/kg body wt. for 10 days. The mean follow-up time after OLT was 26 days. Twenty episodes of severe jaundice (defined as serum total bilirubin concentrations increasing above 100 μM for at least 3 days) were observed (Table 1).

Diagnosis of graft rejection

Liver biopsy was performed in the presence of clinical and laboratory data suggesting graft rejection (2,4). Histological diagnosis of rejection was made according to the criteria of Kemnitz et al. (12). Resolution of rejection following steroid treatment was diagnosed on the basis of clinical and laboratory data, and confirmed by follow-up. If steroid-resistant rejection was suspected due to persistent clinical and biochemical alterations, it was always confirmed by liver biopsy. Serum bilirubin levels (analysed by AM-HPLC) and bile acids were not included in the laboratory data evaluated at the time of diagnosis.

Diagnosis of vascular complications

Thrombosis of the hepatic artery was detected by Doppler ultrasound examination and confirmed by angiography (1 case) or by examination of the explanted liver (2 cases).

Diagnosis of hepatic cyclosporin toxicity

Hepatic cyclosporin toxicity was diagnosed on the following grounds: (a) low serum monoclonal specific cyclosporin levels (<100 ng/ml) and high cyclosporin monoclonal non-specific levels (>1250 ng/ml) in the presence of a low dosage of the drug, indicating altered cyclosporin metabolism (13); (b) resolution of jaundice and normalization of the metabolite versus parent drug level ratio (to about 5:1) by discontinuing the drug for 2 or 3 days (in 2 patients) or reduction of the dosage to 50% (in the other 2 patients); (c) a liver biopsy showing no evidence of rejection or cholangitis; (d) normal Doppler ultrasound of the graft; (e) no clinical evidence of sepsis.

Analytical methods

Total serum bilirubin was determined with an automated diazo-assay. Unconjugated and esterified serum

TABLE 1

Preoperative characteristics and postoperative complications in individual patients

Name	Diagnosis before transplantation	Pre-TB (μM)	Complications	Day	TB_{max}	EB_{max} (μM)	BA_{max}
M.A.	Cryptogenic cirrhosis	41	Steroid-resistant rejection	13	311	187	25
C.A.	Cirrhosis post-hepatitis B	771	Steroid-resistant rejection	18	269	108	69
M.P.	Acute hepatic failure	351	Steroid-resistant rejection	6	248	64	25
M.T.	Chronic graft rejection	178	Steroid-resistant rejection	5	188	66	87
M.G.	Haemangiomatosis	115	Steroid-resistant rejection	5	294	176	37
S.B.	Cirrhosis post-hepatitis B	320	Steroid-resistant rejection	5	514	140	64
E.K.	Cirrhosis post-hepatitis NANB	133	Steroid-sensitive rejection	5	195	89	79
A.G.	Glycogenosis I, adenomatosis	6	Steroid-sensitive rejection	10	154	53	38
J.H.	Cirrhosis post-hepatitis NANB	109	Steroid-sensitive rejection	6	121	42	46
M.W.	Cirrhosis post-hepatitis B	159	Steroid-sensitive rejection	10	221	66	37
C.O.	Cirrhosis post-hepatitis B	21	Septic shock	7	335	160	80
A.B.	Hepatocellular carcinoma	30	Septic shock	4	167	50	115
S.P.	Sclerosing cholangitis	171	Septic shock	4	280	145	115
S.S.	Primary biliary cirrhosis	233	Hepatic artery thrombosis	6	723	314	121
F.H.	Primary biliary cirrhosis	250	Hepatic artery thrombosis	6	600	332	185
S.M.	Budd-Chiary syndrome	55	Hepatic artery thrombosis	14	500	225	132
F.B.	Cirrhosis post-hepatitis B	24	Cyclosporin toxicity	7	131	80	19
J.B.	Cirrhosis post-hepatitis B	58	Cyclosporin toxicity	5	210	65	20
A.S.	Budd-Chiari syndrome	40	Cyclosporin toxicity	4	105	42	19
C.O.	Cirrhosis post-hepatitis B	17	Cyclosporin toxicity	17	208	94	17

PRE-TB = serum concentration of total bilirubin on the day before transplantation; Day = post-operative day in which the described complicatio occurred; TB_{max}, EB_{max}, BA_{max}: peak serum concentrations of total bilirubin, esterified bilirubin and bile acids during the described episode hepatic dysfunction. Normal range values: total bilirubin = 2–17 μM; esterified bilirubin = 0.08–0.4 μM; bile acids = 3–8 μM.

bilirubin were measured by AM-HPLC (5). Bilirubin–protein conjugate was analyzed according to the method of Blanckaert et al. (14). Serum bile acids were determined with an enzymatic fluorimetric assay (Sterognost 3-alpha-Flu, Nygaard, Oslo, Norway). Cyclosporin and its metabolites were measured with the monoclonal specific and non-specific radioimmunoassay as whole blood 12-h levels (Sandoz Ltd., Basel, Switzerland). Results are expressed as medians and ranges.

Results

Serum bile pigments

During the first 3 postoperative days the median concentration of total bilirubin was 98 μM (range 14–218 μM). The median concentration of esterified bilirubin was 22 μM (range 2–78 μM). The concentration of bilirubin–protein conjugate was high (22–41 μM) in 10 patients who had been jaundiced for several months before OLT, while it was low (2–5 μM) in the other patients. Total bilirubin and esterified bilirubin decreased during the first 3 days, but thereafter all patients had at least 1 episode of severe hyperbilirubinemia (Table 1). In every case, AM-HPLC analysis of serum demonstrated that this was mainly due to the increase in the esterified pigment, while the unconjugated fraction accounted for only 9–30% of the total increase. Diesterified bilirubin accounted for 20–50% of the esterified pigment. During episodes of jaundice, the bilirubin–protein conjugate increased slowly and in proportion to the severity and duration of hyperbilirubinemia. No characteristic profile of bile pigment fractions could be identified in relation to the different causes of hyperbilirubinemia. A sharp increase in esterified bilirubin (from 2.5–9.6-fold) was observed during the rejection episodes (Table 1). The median increase in esterified bilirubin on the day of biopsy was 3.2-fold (range 1.6–24.9) versus an increase in total bilirubin of 1.5-fold (range 0.7–3.4). Moreover, the increase in esterified bilirubin usually became evident 1–3 days earlier than the increase in serum transaminases (see Fig. 1 for a representative case). Transaminases increased in 8 out of 10 patients with acute graft rejection. In these patients, the median increase in AST and ALT were 2.1-fold (range 0.8–5.9) and 1.9-fold (range 0.8–3.1) compared to pre-rejection values. After successful anti-rejection treatment, esterified bilirubin returned to baseline levels within 2–10 days (Fig. 2). When treatment was not successful, as determined by persistent and specific alterations in control liver biopsies, esterified bilirubin remained high or increased even further (Fig. 2). A sharp increase in esterified bilirubin was also observed

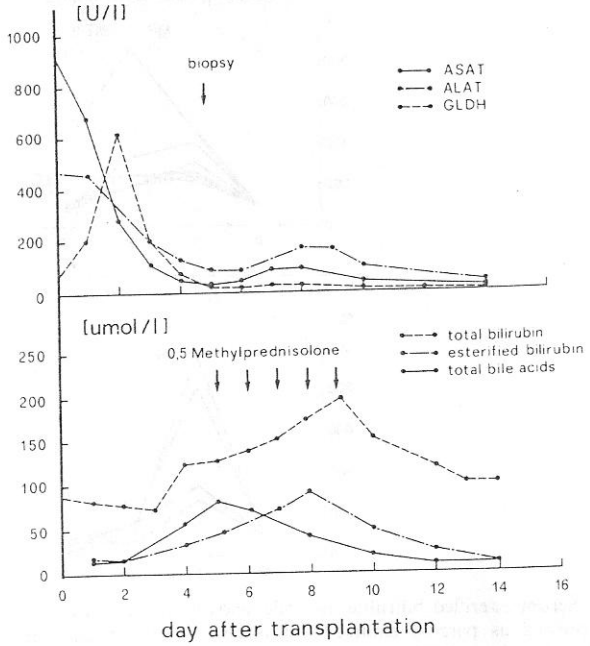

Fig. 1. Serum concentrations of total bilirubin, esterified bilirubin, total bile acids, lactic dehydrogenase (GLDH) and transaminases (ASAT, ALAT) in a patient with steroid-sensitive graft rejection.

in cases of hepatic artery thrombosis, septic shock (3.4–10.7-fold) and during drug toxicity (2.5–13.8-fold). In these conditions the extent of increases were similar to those observed during rejection.

Serum bile acids

In the first 3 days after OLT, serum bile acids ranged from 3 to 37 μM (normal values: 3–10 μM). These levels increased sharply during rejection episodes (Table 1; see Fig. 1 for a representative case). Peak levels varied from 22 to 87 μM, corresponding to a 2.5- to 6.6-fold increase over pre-rejection values (median 3.6-fold). These increases were observed for 1–3 days before a liver biopsy was performed. Successful anti-rejection treatment was associated with a rapid drop in serum bile acids towards pre-rejection levels within 2–6 days (Fig. 2). In contrast, serum bile acids remained elevated or even increased when control biopsies demonstrated persisting rejection (Fig. 2). In the 6 patients with impaired hepatic blood flow (septic shock or hepatic artery thrombosis), serum bile acids rapidly reached the highest levels recorded in the present series (Table 1). No increase in serum bile acids was observed during the 4 episodes of hyperbilirubinemia associated with deranged cyclosporin metabolism. In fact, in 3 out of 4 patients serum bile acids decreased slightly despite rapidly rising esterified bilirubin levels (bile acid levels before jaundice 19, 20, 22, 17 μM; peak values during jaundice 17, 15, 19, 17 μM, respectively).

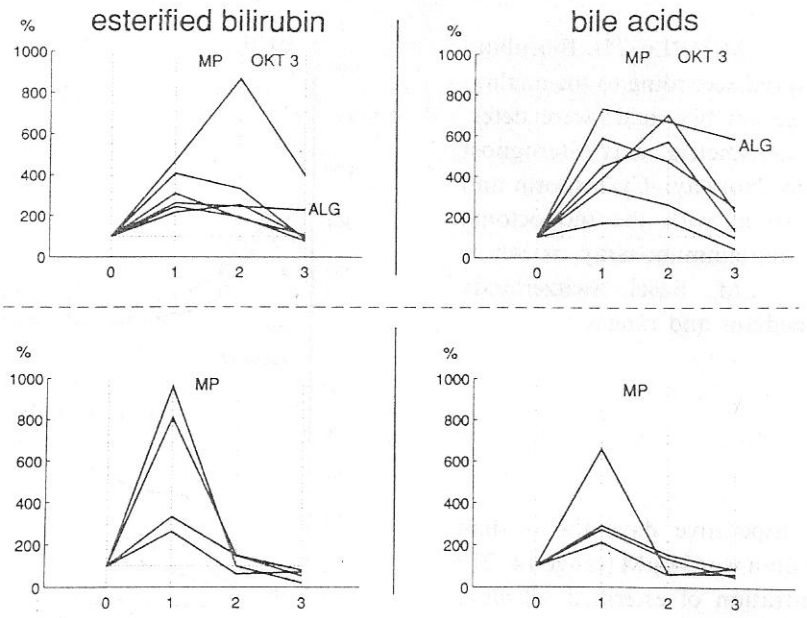

Fig. 2. Serum esterified bilirubin and bile acids in patients with steroid-sensitive (lower panel) and steroid resistant (upper panel) rejection. Data are expressed as percent change with respect to pre-rejection levels. 0 = pre-rejection values; 1 = peak during rejection; 2 = after 3 days of methylprednisolone (MP) treatment; 3 = after 1 week of OKT3 treatment (steroid-resistant rejection) *or* 1 week after end of MP treatment (steroid-sensitive rejection). ALG = Patient with steroid *and* OKT3-resistant rejection treated with anti-lymphocyte globulins.

Discussion

Routine laboratory tests are difficult to interpret after liver tranaplantation, since they are often non-specifically altered in the early postoperative course (2–4). The composition of bile pigments in patients with hepatobiliary diseases has been recently elucidated (for a review, see Ref. 7). In particular, the so-called direct-reacting fraction of bilirubin was found to consist of both esterified bilirubins and bilirubin–protein conjugate. The latter pigment appears and increases slowly during the course of icteric liver disease; due to its slow rate of breakdown and elimination it often persists for a long time after the resolution of the disease. In contrast, esterified bilirubin represents a pigment which is glucuronidated in the liver and normally excreted into bile. Thus, the concentration of this pigment fraction in serum directly reflects the actual function of the biliary excretory system. It has recently been suggested that direct measurement of the individual pigment fractions could yield useful information in liver-grafted patients (15–17). In most patients in the present series, total bilirubin was already more elevated than preoperative values in the first days following OLT. However, when total bilirubin was high, it consisted mainly of bilirubin–protein conjugate, thus indicating long-lasting preoperative jaundice. The concentration of esterified bilirubin seemed to represent a more specific index of actual liver function, and indeed it decreased in the absence of complications, and increased rapidly in any case of liver dysfunction. Determination of esterified bilirubin might be useful in cases of slowly-resolving hyperbilirubinemia after OLT. In this case, the existence of low or rapidly falling esterified bilirubin levels indicates that persisting jaundice is not due to graft dysfunction but to high concentrations of bilirubin–protein conjugate.

Serum bile acids are considered a very sensitive and specific indicator of both liver disease and hepatic graft dysfunction (8–11). This was confirmed in the present series, since bile acids increased along with esterified bilirubin during graft rejection and remained elevated until resolution of the episode. However, an interesting finding in the present study was that successful treatment was always associated with a rapid drop in serum bile acid concentrations (Fig. 2). This suggests that bile acids can be useful in monitoring the response to anti-rejection treatment. In fact, neither high-dose steroids nor OKT3 treatment decreased serum bile acids when the rejection episode was not resolved (Fig. 2). Esterified serum bilirubin and bile acids were also elevated in patients with impaired arterial hepatic blood supply. Bile acid concentrations reached levels higher than 100 μM in all cases, and were well above values observed during rejection episodes. High bile acid levels were also observed shortly after clinical manifestations of septic shock. The observed increase in esterified serum bilirubin and bile acids after hepatic artery thrombosis or septic shock could reflect hepatic hypoxia resulting in cholestasis (18). Graft rejec-

tion is mainly expressed by an immunological attack at the level of the small intrahepatic bile ducts and of the vascular epithelium with decreased perfusion (19,20). Both effects may decrease biliary excretion and lead to elevation of esterified serum bilirubin and bile acids.

The observation that serum bile acids did not vary in patients with cyclosporin toxicity, despite sharp elevations in esterified bilirubin, is of particular interest. Although cyclosporin hepatotoxicity in liver-grafted patients is difficult to define, there is abundant evidence for its existence (21,22). Based on the above-mentioned criteria (see 'Diagnosis of hepatic cyclosporin toxicity'), it is reasonable to suspect drug toxicity in the 4 patients of the present series. The different reactions of esterified bilirubin and bile acids during episodes of cyclosporin toxicity are difficult to explain, since both substances increase simultaneously during most hepatobiliary diseases. Moreover, both esterified bilirubin and bile acids increase in the serum of rats treated with cyclosporin (23,24). However, different reactions of serum bilirubin and bile acids have already been observed in recurrent intrahepatic cholestasis, with increasing bile acids in the presence of falling bilirubin levels (25,26). The present results suggest selective interference in biliary excretion for esterified bilirubin and cyclosporin and/or its metabolites, in contrast to the global deterioration of the hepatic excretory system which occurs during rejection or impaired blood supply. Clearly, further studies are necessary to confirm this hypothesis.

In conclusion, both esterified bilirubin and bile acids are sensitive indicators of graft dysfunction after OLT. Serum bile acids seem to be particularly useful in the early post-operative follow-up, since they help diagnose the occurrence of cyclosporin toxicity and monitor the response to anti-rejection treatment.

Acknowledgements

Part of this work was supported by the Consiglio Nazionale delle Ricerche, Rome.

References

1 Wiliams JW, Vera S, Peters TG, et al. Cholestatic jaundice after hepatic transplantation. Am J Surg 1986; 151: 65–9.
2 Esquivel CO, Jaffe R, Gordon RD, Iwatsuki S, Shaw BW, Starzl TE. Liver rejection and its differentiation from other causes of graft dysfunction. Semin Liver Dis 1985; 5: 369–74.
3 Sankary HN, Williams JW, Foster PF. Can serum liver function tests differentiate rejection from other causes of liver dysfunction after hepatic transplantation? Transplant Proc 1988; 20, Suppl 1: 669–70.
4 Shaw BW, Stratta RJ, Donovan JP, et al. Postoperative care after liver transplantation. Semin Liver Dis 1989; 9: 202–30.
5 Muraca M, Blanckaert N. Liquid chromatographic assay and identification of mono- and diester conjugates of bilirubin in normal serum. Clin Chem 1983; 29: 1767–71.
6 Van Hootegem P, Fevery J, Blanckaert N. Serum bilirubins in hepatobiliary disease: comparison with other liver function tests and changes in the postobstructive period. Hepatology 1985; 5: 112–7.
7 Muraca M, Fevery J, Blanckaert N. Analytic aspects and clinical interpretation of serum bilirubins. Semin Liver Dis 1988; 8: 137–47.
8 Berry W, Reichen J. Bile acid metabolism: its relation to clinical disease. Semin Liver Dis 1983; 3: 330–40.
9 Mannes GA, Thieme C, Stellard F, et al. Prognostic significance of serum bile acids in cirrhosis. Hepatology 1986; 6: 50–3.
10 Visser JJ, Bom-van Noorloos AA, Meijer S, Hoitsma HF. Serum total bile acids monitoring after experimental liver transplantation. J Surg Res 1984; 36: 147–53.
11 Mora NP, Cienfuegos JA, Codoceo R, et al. Monitoring of serum total bile acids as an early indicator of graft function in clinical and experimental liver transplantation. Transplant Proc 1987; 19: 3840–1.
12 Kemnitz J, Gubernatis G, Bunzendahl H, et al. Criteria for the histopathological classification of liver allograft rejection and their clinical relevance. Transplant Proc 1989; 21: 2208–9.
13 Kohlhaw K, Wonigeit K, Schafer O, Ringe B, Bunzendahl H, Pichlmayr R. Association of very high blood levels of cyclosporin metabolites with clinical complications after liver transplantation. Transplant Proc 1989; 21: 2232–3.
14 Blanckaert N, Servaes R, Leroy P. Measurement of bilirubin protein conjugates in serum and application to human and to rat sera. J Lab Clin Med 1987; 108: 77–87.
15 Cox CJ, Valdiserri RO, Zerbe TR, Genter JL. Ektachem bilirubin fraction Bc as a predictor of liver transplant rejection. Transplant 1987; 44: 536–9.
16 Wu TW, Au JX, Greig PD, et al. Conjugated and delta bilirubin determinations in early liver allograft rejection. Transplant Proc 1988; 20: 383–90.
17 Wu TW, Levy GA, Ylu S, et al. Delta and conjugated bilirubin as complementary markers of early rejection in liver-transplant patients. Clin Chem 1990; 36: 9–14.
18 Becker SD, Lamont JD. Postoperative jaundice. Semin Liver Dis 1988; 8: 183–90.
19 Snover DC. The pathology of rejection. Transplant Proc 1988; 18: 123–7.
20 Houssin D, Fratacci M, Dupuy P, et al. One week of monitoring of portal and hepatic arterial blood flow after liver transplantation using implantable pulsed Doppler microprobes. Transplant Proc 1989; 21: 2277–8.
21 Kahan BD, Flechner SM, Lorber MI, et al. Complications of cyclosporin therapy. World J Surg 1986; 10: 348–60.
22 Kahan BD. Cyclosporine. N Engl J Med 1989; 321: 1725–38.
23 Stone BG, Udani M, Sanghvi A, et al. Cyclosporin A-induced cholestasis. The mechanism in a rat model. Gastroenterology 1987; 93: 344–51.
24 Nassuato G, Strazzabosco M, Muraca M, et al. Effect of cyclosporin A on hepatic bilirubin transport (Abstract). J Hepatol 1988; 7: S63.
25 Summerfield JA, Kirk AP, Chitranukroh A, Billing BH. A distinctive pattern of serum bile acid and bilirubin concentrations in benign recurrent intrahepatic cholestasis. Hepatogastroenterology 1981; 28: 139–143.
26 Finni K, Simila S, Heikura S, et al. Serum bile acid concentrations in the course of benign recurrent intra-hepatic cholestasis. Ann Clin Res 1980; 12: 168.