Transpl Int (1995) 8: 340–345

ORIGINAL ARTICLE

Satoru Izumi
Robin D. Hughes
Peter G. Langley
J. Ricardo B. Pernambuco
Roger Williams

Acute phase response after liver transplantation for fulminant hepatic failure and cirrhosis

Received: 7 September 1994
Received after revision: 13 December 1994
Accepted: 16 February 1995

S. Izumi · R.D. Hughes · P.G. Langley
J.R.B. Pernambuco · R. Williams (✉)
Institute of Liver Studies,
King's College Hospital and
King's College School of Medicine
and Dentistry,
Bessemer Road, London SE5 9PJ, UK
Fax: + 44 1 71 346 3167

Abstract The hepatic acute phase response after orthotopic transplantation (OLT) was studied in patients with fulminant hepatic failure (FHF) and with cirrhosis, in relation to the pre-existing disease. Plasma levels of C-reactive protein (CRP) increased significantly on day 1 after OLT in both the FHF (Δ = 58 μg/ml) and cirrhosis (Δ = 94 μg/ml) groups and reached a peak 4–5 days post surgery. α_1-Antitrypsin reached normal levels on day 1 post-transplant and fibrinogen reached normal levels on the 3rd day. The main stimulator of acute phase protein synthesis IL-6 was significantly increased pre-OLT in plasma in both FHF (median 54 pg/ml) and cirrhosis (median 8.7 pg/ml) patients compared to controls (2.35 pg/ml, $P < 0.05$). After OLT, IL-6 decreased rapidly in patients with FHF, indicating either removal of the source of IL-6 or clearance by the transplanted liver. In patients with cirrhosis, plasma IL-6 remained low, except in three patients who developed infection/rejection and whose IL-6 levels rose above 100 pg/ml. In conclusion, there is a marked acute phase response in the liver graft after transplantation, irrespective of the aetiology of the liver disease for which the transplant was performed.

Key words C-reactive protein, liver transplantation · α_1-Antitrypsin, liver transplantation · Fibrinogen, liver transplantation · Interleukin-1, –6, liver transplantation · Liver transplantation, acute phase response

Introduction

The acute phase response is observed during sepsis and after surgery [7, 10] as a protective mechanism to limit the systemic effects of tissue injury to the organism. In patients with fulminant hepatic failure (FHF), there is a limited acute phase response as determined by small increases in the plasma levels of C-reactive protein (CRP) [9]. This is despite the presence of greatly increased levels of interleukin-6 (IL-6), a major stimulus of acute phase protein synthesis in the liver [4], and this presumably reflects a diminished synthetic capacity in FHF. Higher levels of α_1-antitrypsin and fibrinogen, both acute phase proteins with important functions, were found in patients with FHF who survived compared to those who died [9], suggesting that the residual synthetic capacity of the liver for acute phase proteins could represent an important mechanism of host defence. In patients with FHF who undergo orthotopic liver transplantation (OLT) [1, 14, 17], the stress of the major surgery involved would be expected to stimulate an acute phase response in the transplanted liver. The aim of this study was to investigate the extent of the acute phase response in patients with FHF and cirrhosis treated by OLT in relation both to the pre-existing condition of the patients and to circulating stimuli for acute phase protein synthesis.

Table 1 Routine laboratory parameters in patients prior to liver transplantation (*AST* serum aspartate aminotransferase, *WBC* white blood cell counts, *AHLT* auxiliary heterotopic liver transplantation)

	Prothrombin time (INR)	AST (IU/l)	Bilirubin (μmol/l)	Creatinine (μmol/l)	WBC ($\times 10^9$/l)	Platelets ($\times 10^9$/l)
FHF ($n = 6$)						
Median	12.2	1137	104	213	13.1	122
Range	7.3–15.0	255–7594	60–445	61–479	5.8–28.1	35–186
Liver cirrhosis ($n = 14$)						
Median	1.5	104	122	87	4.5	99
Range	1.3–5.0	83–155	21–494	64–251	2.3–12	16–286
Normal range	0.9–1.2	10–50	3–20	45–105	5–9	150–400
AHLT patient	5.3	–	64	206	14.7	197

Materials and methods

Two groups of patients were studied between September 1991 and June 1992. They comprised 7 cases of FHF, as defined by Trey and Davidson [19], who had been listed for emergency transplantation and were receiving intensive care and a group of 14 cases in a stable condition undergoing elective transplantation for end-stage liver cirrhosis. Selection for transplantation in the patients (three male, four female; mean age 34 years, range 20–57 years) with FHF was on the basis of poor prognostic criteria, as used at King's College Hospital [15]. Aetiology of the FHF was paracetamol overdose in five patients and non-A non-B viral hepatitis in two patients. All patients were in grade 4 coma prior to transplantation. Emergency liver transplantation was performed in six patients on day 2 and in one on day 3 after admission. The remaining patient, a 36-year-old female who had taken an overdose of paracetamol, underwent auxiliary heterotopic liver transplantation [13] on the basis that recovery of the liver was considered possible in the longer term.

The 14 patients with end-stage liver cirrhosis (6 male, 8 female; mean age 45 years, range 17–65 years) had primary biliary cirrhosis ($n = 5$), autoimmune chronic active hepatitis ($n = 2$), hepatitis C with cirrhosis ($n = 2$), primary sclerosing cholangitis ($n = 1$), Wilson's disease ($n = 1$), and cryptogenic cirrhosis ($n = 3$). The differences in condition pre-transplant of the two groups of patients is reflected in the INR values (median 12.2 in FHF and 1.5 in cirrhosis) and the AST values (1137 IU/l and 104 IU/l), respectively (Table 1).

Mean cold ischaemic time of the donor liver was 11.8 h (range 6–20 h). All patients received transfusion of blood products during the transplant procedure and the mean amounts (range) given were as follows; whole blood 6.0 (3–9) units, packed cells 5.2 (1–8) units, platelets 3.6 (0–6) units, fresh frozen plasma 6.2 (2–12) units, cryoprecipitate 6.0 (0–12) units. Immunosuppression post-transplant was with methylprednisolone (40 mg i.v.), azathioprine (75 mg oral) and cyclosporin (50 mg b.i.d.). Two cases, one in each patient group, received FK 506 (0.15 mg/kg i.v.) instead of cyclosporin. Therapy was switched to oral administration on day 2 or 3 when the patient was eating, with prednisolone (20 mg) substituted for methylpredisolone and the dose of cyclosporin adjusted to give a trough blood level in the range of 100–250 μg/ml.

Blood samples were taken from patients prior to transplantation and then daily until 7 days after transplantation or 12 days in the case of the patient who underwent auxiliary heterotopic liver transplantation. Citrated blood samples were collected on ice and were immediately centrifuged for 10 min at 2000 g at 4 °C. The resulting plasma was stored in aliquots at −70 °C until assay. Normal control samples were obtained from 20 laboratory staff (10 male, 10 female; mean age 32 years). Approval for the study was obtained from the local ethics committee.

Assays

The acute phase proteins, CRP, α_1-antitrypsin and fibrinogen, and IL-6 were determined in plasma prior to OLT and then serially for 1 week after surgery. CRP was assayed by an enzyme linked immunosorbent assay (ELISA) based on the method of Highton and Hessian [8]. Antisera, peroxidase conjugated antisera and CRP standard were obtained from Dako (High Wycombe, UK). Microelisa plates (Dynatech, Billingshurst, UK) were coated with antibody to CRP (diluted 1:5000 in barbitone buffer pH 8.8) by incubation overnight. Sera were diluted (1:1000 and 1:10000) in phosphate buffered saline pH 7.4 containing 0.05 % Tween and 0.1 % bovine serum albumin. The peroxidase substrate was 3,5',5,5' tetramethyl benzidine in citrate/acetate buffer pH 6.0. A standard curve was run on each plate and all samples were measured in duplicate. The final colour change was measured at 450 nm using a MR-5000 microplate reader (Dynatech, Billingshurst, UK).

α_1-Antitrypsin activity was determined using an indirect chromogenic substrate assay (Quadratech, Epsom, UK). Plasma was initially diluted in buffer containing methylamine in order to degrade α_2-macroglobulin. The colour change was measured at 405 nm using an Ultrospec III spectrophotometer (Pharmacia LKB, Milton Keynes, UK). Fibrinogen was measured using the method of Clauss [5] with a Fibrintimer (Behring, Hounslow, UK). IL-6 was measured using a quantitative 'sandwich' enzyme immunoassay – Quantikine Human IL-6 Immunoassay – with a monoclonal antibody specific for IL-6 coated onto a microtitre plate. The kit was obtained from British Bio-technology (Abingdon, UK). IL-1 was assayed with an ACE enzyme immunoassay based on a double-antibody 'sandwich' technique kindly supplied by SPI BIO (Saclay, France).

Statistical analysis

Significance was assessed between groups using the Mann Whitney U-test. Correlation between parameters was assessed with the Spearman rank correlation coefficient. Results are reported as either median and range or mean ± SEM, as appropriate.

Table 2 Plasma levels of acute phase proteins and cytokines in patients prior to liver transplantation (*CRP* C-reactive protein, *AAT* α_1-antitrypsin, *AHLT* auxiliary heterotopic liver transplantation)

	CRP (μg/ml)	AAT (%)	Fibrinogen (g/l)	IL-1 (pg/ml)	IL-6 (pg/ml)
FHF ($n = 6$)					
Median	12.0*	56.6**,***	0.76**	1.18	54*
Range	0.9–19.7	26.3–116	0–1.30	0–5.86	0–303
Liver cirrhosis ($n = 14$)					
Median	13.4**	139	–	–	8.7**
Range	2.4–46.6	46.3–203			4.2–32
Controls ($n = 20$)					
Median	0.80	126	2.48	1.03	2.35
Range	0.30–2.90	75.4–148	1.82–3.39	0–4.48	1.50–8.2
AHLT patient	15.5	97.5	1.12	0	2.6

* $P < 0.001$ and ** $P < 0.01$ vs controls; *** $P < 0.05$ vs liver cirrhosis

Results

Despite the differences in liver function prior to transplantation between the two groups of patients, levels of plasma CRP, the most sensitive acute phase protein measured, were similar in both groups, with values significantly higher than in controls (Table 2). After OLT, there was an immediate increase in plasma CRP in both groups (CRP on day 1, FHF median 61.5 μg/ml, range 43.2–210 μg/ml; cirrhosis median 107 μg/ml, range 38.0–256 μg/ml). Levels then increased further to reach a peak on days 4–5 after OLT, which was followed by a slight decrease by day 7 (Fig. 1). Levels in the FHF and cirrhosis patients were not significantly different.

Plasma α_1-antitrypsin, a less sensitive acute phase protein, was significantly lower in the FHF group before OLT than in normal controls (Table 2). In the cirrhosis group, the levels were significantly higher than in the FHF group, and were marginally higher than those in the controls. Following OLT, plasma α_1-antitrypsin in patients with FHF returned to normal levels on day 1 after OLT (median 103 %, range 60.0 %–165 %) and continued to increase gradually thereafter (Fig. 2). In patients with cirrhosis, levels were unchanged for the first 2 days after OLT (on day 1 median 137 %, range 65.0 %–203 %) and then increased to values significantly ($P < 0.05$) greater than those in controls (day 3 median 168 %, range 73.1 %–223 %; Fig. 2). Comparison of the plasma α_1-antitrypsin in the FHF and cirrhosis groups showed no significant differences, although the levels in the cirrhotic cases tended to remain higher than in those with FHF.

As with α_1-antitrypsin, plasma fibrinogen in the FHF group prior to transplantation was significantly lower than in controls (Table 2). After OLT, plasma fibrinogen gradually increased (day 1 median 1.70 g/l, range 1.37–4.0 g/l) to reach normal levels on day 3 (day 3 median 1.82 g/l, range 0.8–3.59 g/l).

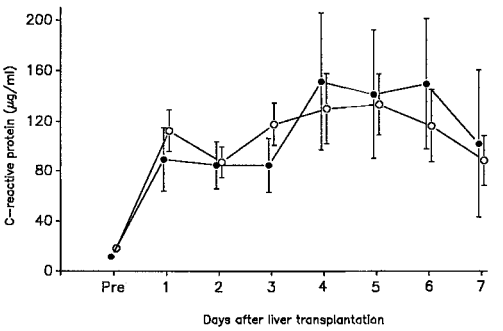

Fig. 1 Changes in plasma C-reactive protein levels after orthotopic liver transplantation (mean ± SEM). (● fulminant hepatic failure, ○ liver cirrhosis)

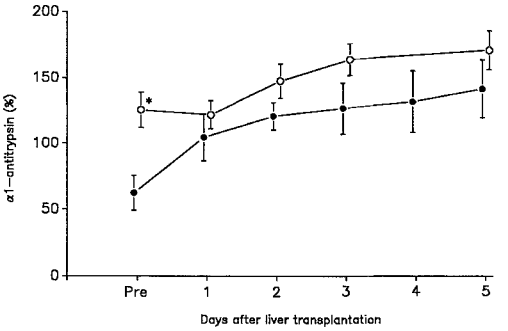

Fig. 2 Changes in plasma α_1-antitrypsin levels after orthotopic liver transplantation (mean ± SEM). (● fulminant hepatic failure, ○ liver cirrhosis) * $P < 0.05$ vs liver cirrhosis

Cytokines levels pre- and post-OLT

Plasma IL-6 in patients with FHF was significantly higher pre-transplant than in control subjects (Table 2). After OLT, IL-6 levels decreased significantly (on day 2 median 5.85 pg/ml, range 3.1–19.4 pg/ml, $P < 0.05$), but continued to be at significantly higher levels than in

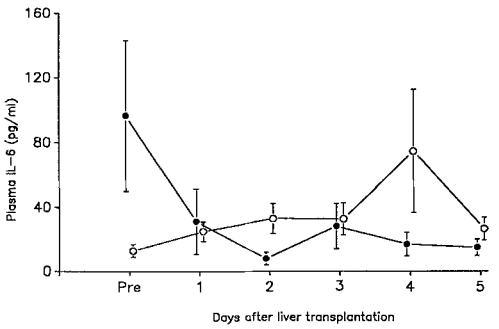

Fig. 3 Changes in plasma interleukin-6 levels after orthotopic liver transplantation (mean ± SEM). (● fulminant hepatic failure, ○ liver cirrhosis)

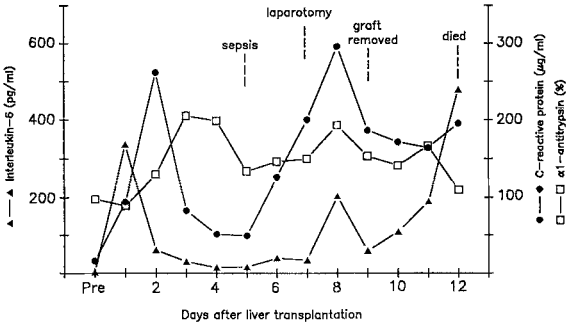

Fig. 4 The clinical course of patient who underwent auxiliary heterotopic liver transplantation

controls for 5 days after OLT (on day 5 median 11.95 pg/ml, range 3.9–38.2 pg/ml, $P < 0.01$; Fig. 3). There was no correlation between the increased plasma IL-6 and the plasma creatinine. In the cirrhosis patients, the plasma IL-6 pre-OLT level was significantly elevated to approximately three times normal, and after transplantation IL-6 increased slightly to values similar to those found in the FHF patients. On day 4, plasma IL-6 values were greater than 100 pg/ml in 3 of the 14 cases transplanted for cirrhosis (Fig. 3).

Plasma IL-1, determined only in the FHF cases, remained within normal limits prior to and after OLT (on day 1 median 0.36 pg/ml, range 0–15.0 pg/ml). There were no significant correlations between IL-6 and IL-1 and any of the changes in acute phase proteins after OLT.

Relationship to post-transplant complications

Of the six FHF patients who underwent OLT, three had proven sepsis prior to transplantation and the other three had pyrexia after transplantation. Only one patient had a complication of clinical significance with acute graft rejection evident on day 7 post-transplant. The plasma CRP in this patient was considerably elevated on day 6 (313 µg/ml), although the plasma IL-6 had only reached 38 pg/ml before this. Of the 14 patients transplanted for cirrhosis, 3 developed complications and all had rises in IL-6 (> 100 pg/ml) on day 4, though IL-6 returned to the previous levels on day 5. One patient became pyrexial on day 2 and septicaemic on day 4 with a plasma IL-6 level of 530 pg/ml, the highest value observed in this study, and subsequently the plasma CRP on day 6 (382 µg/ml) was also the highest value observed after OLT. The second patient with increased IL-6 had an episode of infection together with graft rejection on day 4, and the other patient had acute rejection on day 4; however, in these two patients, the plasma CRP levels on day 5 were not greater than in those without complications (179 and 175 µg/ml, respectively).

In the one patient with FHF treated by auxiliary heterotopic liver transplantation, plasma CRP increased immediately after transplantation and reached a peak on day 2 (262 µg/ml), indicating an acute phase response. Levels then declined until day 5, when the patient developed fungal sepsis (Fig. 4), with a second peak of CRP on day 8, after a laparotomy to close the abdominal wound. This was different from the pattern seen after OLT, where the plasma CRP continues to rise gradually post-transplant to a peak at day 5. In the patient with the heterotopic graft, the CRP level decreased when the donor liver was removed on day 9 due to intractable sepsis. Plasma IL-6 in this patient was in the normal range pre-transplant (Table 2), but increased immediately after the transplant, a response not seen after OLT (Fig. 3), and it reached a peak on day 1 (336 pg/ml) before that of CRP (Fig. 4). The level of IL-6 subsequently decreased, followed by another peak on day 8 after the laparotomy (202 pg/ml). No change in plasma IL-1 was observed.

Discussion

The results of the present study show that there is a marked acute phase response in the recipient liver after transplantation, as shown by changes in CRP level, and that the response is similar irrespective of whether the patient was transplanted for FHF or for end-stage chronic liver disease. It is possible that stimulation induced in the donor liver during the surgery to retrieve the organ or in the subsequent preservation and storage process could have contributed to the effects observed. Increases in plasma CRP of a similar magnitude are seen after abdominal surgery of patients with liver cirrhosis [16]. CRP decreases towards normal levels within 5–7 days after major surgery [21], whereas after liver transplantation the CRP level only started to fall on day 7, in both groups of patients, suggesting a prolonged acute phase response. In the FHF patient who had

an episode of acute rejection, plasma CRP was greatly elevated; this was not seen in the cirrhotic patient who had a less severe rejection episode. This finding is in keeping with that of a previous study of patients with chronic liver disease undergoing transplantation, where plasma CRP was not found to be a good marker of acute graft rejection [12]. The highest CRP value was observed in a cirrhotic patient with infection, indicating that CRP levels can be further stimulated by the different complications of transplantation.

After auxiliary liver transplantation there was an early peak of CRP with a second peak on development of sepsis, as would be expected and CRP decreased when the implanted donor liver was removed, suggesting that the graft was the main site of acute phase protein synthesis. In terms of the other acute phase proteins studied, which have important physiological functions, the plasma levels of both α_1-antitrypsin and fibrinogen in FHF patients reached normal levels soon after transplantation. Plasma α_1-antitrypsin continued to increase in those with FHF, confirming an acute phase response post-transplantation. This was also observed in patients transplanted for liver cirrhosis who had normal levels of α_1-antitrypsin initially. Such levels are sufficient to protect against any release of elastase from activated neutrophils, which is expected after abdominal surgery [6] and has been found in the circulation of patients with FHF [11]. It is unlikely that the rise in plasma levels of acute phase proteins was due to the infusion of blood products during the operative procedure, as CRP is only present in trace amounts in normal plasma, although this may have contributed to the levels of α_1-antitrypsin and fibrinogen. The immunosuppressive drug regimen used, which was similar in both groups of patients, is also unlikely to have influenced the acute phase response observed, as the synthesis of CRP and α_1-antitrypsin have been shown not to be directly affected by corticosteroids [20], although there may have been effects due to the other immunosuppressive drugs administered.

The high plasma levels of IL-6 pre-transplantation in both the FHF and cirrhotic patients could provide an early stimulus for acute phase protein synthesis in the transplanted liver. As already mentioned, in FHF patients who die, plasma levels of IL-6 remain elevated during the course of the illness [9], despite the expected short half-life of this cytokine. In the present study there was no relationship between plasma IL-6 and renal failure in the small group of patients with FHF. This situation is similar to that found in our study of 50 FHF patients without liver transplantation [9], indicating that impaired renal elimination is not a major factor in the increased plasma IL-6. After transplantation in FHF there is a rapid reduction in the IL-6 level, probably due to clearance of circulating IL-6 by the graft, as the liver rather than the kidney has been shown to be the main site of IL-6 removal from the circulation [3]. In addition, removal of the necrotic liver at the transplant will have reduced the source or stimuli for IL-6 production. In the patient treated by an auxiliary liver graft, a different pattern of plasma IL-6 was seen, with a major increase on the 1st day post-transplantation. This may have been the stimulus for the peak in CRP 1 day later, and this further suggests that the necrotic liver is a source of IL-6 production. In patients without liver disease, plasma IL-6 reaches a peak at 24 h after surgery [16], and in sepsis it rises at onset and rapidly decreases to undetectable levels within approximately 24 h [2], whereas in our study IL-6 remained slightly increased after OLT during the study period in both patient groups. The reason for this is unknown; it may be part of the immune response to the graft and may reflect a low level of graft damage. This would provide a continuing stimulus to acute phase protein synthesis, which is prolonged in both groups, compared to conventional surgery. In three of the chronic patients there was a rise in IL-6, coincident with the development of infection and with an episode of rejection. However, in the FHF patient who developed severe graft rejection, no large rise in IL-6 was seen. A similar lack of diagnostic power for IL-6 after transplantation was also found in the recent study of Tilg et al. [18]. In conclusion, a similar marked acute phase response was observed after liver transplantation in patients with FHF and in those with liver cirrhosis.

Acknowledgement S.I. was a Visiting Clinical Research Fellow from the Department of Internal Medicine of Hyogo Prefectural Nishinomiya Hospital, Hyogo, Japan.

References

1. Bismuth H, Samuel D, Gugenheim J, Castaing D, Bernuau J, Rueff B, Benhamou JP (1987) Emergency liver transplantation for fulminant hepatitis. Ann Intern Med 107: 337–341

2. Calandra T, Gerain J, Heumann D, Baumgartner JP, Glauser MP (1991) High circulating levels of interleukin-6 in patients with septic shock: evolution during sepsis, prognostic value, and interplay with other cytokines. Am J Med 91: 23–29

3. Castell JV, Geiger T, Gross V, Andus T, Walter E, Hirano T, Kishimoto T, Heinrich PC (1988) Plasma clearance, organ distribution and target cells of interleukin-6/hepatocyte stimulating factor in the rat. Eur J Biochem 177: 357–361

4. Castell JV, Gomez-Lechon MJ, David M, Andus T, Geiger T, Trullenque R, Fabra R, Heinrich PC (1989) Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett 242: 237–239
5. Clauss A (1957) Gerinnungsphysiologisches schnellmethode zur bestinnung des fibrinogens. Acta Haematol 17: 237–246
6. Duswald K, Jochum M, Schramm W, Fritz H (1985) Released granulocytic elastase: an indicator of pathobiochemical alteration in septicemia after abdominal surgery. Surgery 98: 892–899
7. Gordon AH, Koj A (eds) (1985) The acute-phase response to injury and infection. Elsevier, Amsterdam
8. Highton I, Hessian P (1984) A solid phase enzyme immunoassay for C-reactive protein: clinical value and the effect of rheumatoid factor. J Immunol Methods 68: 186–192
9. Izumi S, Hughes RD, Langley PG, Pernambuco JRB, Williams R (1994) Extent of the acute phase response in fulminant hepatic failure. Gut 35: 982–986
10. Kushner I (1982) The phenomenon of the acute phase response. Ann N Y Acad Sci 389: 39–48
11. Langley PG, Hughes RD, Rolando N, Williams R (1991) Increased elastase-α_1-antitrypsin complex in fulminant hepatic failure: relationship to bacterial infection and activation of coagulation. Clin Chim Acta 200: 211–220
12. Maury CPI, Teppo A-M, Hockerstedt K (1988) Acute phase proteins and liver allograft rejection. Liver 8: 75–79
13. Metselaar HJ, Hesselink EJ, Rave S de, Cate FJW ten, Lamens JS, Groenland THN, Reuvers CB, Weimar W, Terpstra OT, Schalm SW (1990) Recovery of failing liver after auxiliary heterotopic transplantation. Lancet I: 1157–1157
14. O'Grady JG, Alexander GJM, Thick M, Potter D, Calne RY, Williams R (1988) Outcome of orthotopic liver transplantation in the aetiological and clinical variants of acute liver failure. Q J Med 69: 817–824
15. O'Grady JG, Alexander GJM, Hayllar KM, Williams R (1989) Early indicators of prognosis in fulminant hepatic failure. Gastroenterology 97: 430–445
16. Ohzato H, Yoshizaki K, Nishimoto N, Ogata A, Tagoh H, Monden M, Gotoh M, Kishimoto T, Mori T (1992) Interleukin-6 as a new indicator of inflammatory status: detection of serum levels of interleukin-6 and C-reactive protein after surgery. Surgery 111: 201–209
17. Peleman RR, Gavaler JS, Thiel DH van, Esquivel C, Gordon R, Iwatsuki S, Starzl TE (1987) Orthotopic liver transplantation for acute and subacute hepatic failure in adults. Hepatology 7: 484–489
18. Tilg H, Nordberg I, Vogel W, Luger TA, Herold M, Autlitzky WE, Margreiter R, Huber C (1992) Circulating serum levels of interleukin 6 and C-reactive protein after liver transplantation. Transplantation 54: 142–146
19. Trey C, Davidson CS (1970) The management of fulminant hepatic failure. In: Popper H, Schaffner F (eds) Progress in liver disease, vol 3. Grune and Stratton, New York, pp 282–298
20. Whicher IT, Dieppe PA (1985) Acute phase proteins. Clin Immunol Allergy 5: 425–426
21. William RH (1989) C-reactive protein: the best laboratory indicator available for monitoring disease activity. Cleve Clin J Med 58: 126–130