Transpl Int (2003) 16: 465–470
DOI 10.1007/s00147-002-0512-z

ORIGINAL ARTICLE

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Analysis of systemic and regional procalcitonin serum levels during liver transplantation

Received: 9 October 2001
Revised: 5 June 2002
Accepted: 8 July 2002
Published online: 1 May 2003

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Abstract Elevated procalcitonin (PCT) levels are observed early after orthotopic liver transplantation (OLTx). The aim of this study was to evaluate the changes in systemic and regional PCT serum levels from the time of organ harvesting until the early postoperative phase of OLTx ($n=28$) and to investigate the prognostic suitability of postoperative changes in PCT level for the outcome of OLTx ($n=61$). Only in seven of 28 donors were higher PCT levels found (0.84 ± 0.43 ng/ml). During organ preservation, hepatectomy, and in the anhepatic phase, the PCT levels were in the normal range; in 11 of 28 cases hepatic vein PCT levels were higher during graft flush with own blood than the systemic or portal vein samples at the same time (1.27 ± 0.43 ng/ml vs 0.16 ± 0.26 ng/ml and 0.23 ± 0.15 ng/ml, respectively, $P<0.02$). The elevation of PCT levels began immediately after graft reperfusion (1.04 ± 0.77 ng/ml vs 0.27 ± 0.22 ng/ml, $P<0.001$), and the levels at postoperative day 2 were significantly higher in the case of postoperative complications (30.6 ± 19.6 ng/ml vs 4.8 ± 3.6 ng/ml, $P<0.001$).

Keywords Liver transplantation · Procalcitonin · Organ donation · Multiple organ failure syndrome · Postoperative complications · Infection

Introduction

Infection can often be difficult to diagnose in transplant medicine, where the classic fever response and leukocytosis may be absent. Procalcitonin (PCT), as a marker of sepsis, is an infection-induced protein, a specific marker of severity in response to bacterial infection, which may be useful for differential diagnosis in the case of complications following organ transplantation [8]. The main stimulus of production of PCT is endotoxin, but other cytokines were also observed to induce PCT secretion [11]. The peak level of cytokines in the blood is too short for clinical use, as opposed to PCT, where the peak level is reached after 6 h and is maintained for 8–24 h [1]. The liver may be a significant source of PCT during systemic inflammation and sepsis [4, 10]. PCT is considered a potent inflammatory-response indicator; therefore, elevated PCT serum levels after orthotopic liver transplantation (OLTx) can usually help in the differentiation between acute rejection and infection [5, 7].

The aim of the study was to analyze the changes in systemic and regional PCT serum levels from the time of organ harvesting until the early postoperative phase of OLTx to determine the possible source of PCT after liver transplantation and to investigate the prognostic suitability of postoperative changes in PCT levels for the outcome of OLTx.

Patients and methods

Patients

Included in this study were 61 patients who underwent 62 liver transplantations at the Department of Transplantation and Surgery, Semmelweis University, Budapest, from December 1998 to May 2001. The study was reviewed by the appropriate ethics committee and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All patients were thoroughly examined for signs and symptoms of infection. Clinical data and general severity scores (APACHE II, SAPS II, organ failure number) were recorded daily during the first 5 postoperative days. The patients were divided into two groups: group A ($n=32$), patients without, and group B ($n=29$), patients with, major postoperative complications including hepatic artery thrombosis, renal or respiratory failure, coagulopathy, and sepsis. The demographic data, indications for OLTx, Child-Pugh scoring, cold ischemia time, and the preservation solutions used, are presented in Table 1. Sepsis was diagnosed if positive cultures were associated with the appropriate clinical picture.

Table 1 Demographic data of patients belonging to group A (without) and group B (with major postoperative complications) (*HCV* hepatitis C virus, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis, *AIH* autoimmune hepatitis, *ALD* alcoholic liver disease, *SBC* secondary biliary cirrhosis, *HCC* hepatocellular carcinoma, *HTK* Custodiol, *UW* ViaSpan)

Demographic data		Group	
		A	B
Number of patients		32	29
Male/female		17/15	15/14
Age (years)		41 ± 10	40 ± 11
Child-Pugh score	A	4	3
	B	20	14
	C	8	12
Etiology	HCV	14	13
	PBC	5	1
	PSC	3	0
	AIH	1	2
	Budd-Chiari syndrome	1	1
	Toxic	1	1
	ALD	4	5
	SBC	1	0
	HCC	0	3
	Unknown	2	3
Preservation solution	HTK	17	15
	UW	15	14
Cold ischemia time (min)		489 ± 95	507 ± 70
Surgical procedure	Piggyback	15	16
	Cross-clamping	17	13

Surgical procedure

The explanted liver was always part of a multi-organ donation. HTK solution was used for graft preservation in 30 patients, and University of Wisconsin solution was used in the remainder. Liver transplantation was performed by the classic technique of cross-clamping ($n=30$) or by piggyback technique ($n=31$). A veno-venous bypass was used in only six patients. The graft was flushed with the recipient's own blood.

Immunosuppression

Intra-operative immunosuppression was started with i.v. administration of 500 mg methylprednisolone; thereafter, the patients were treated with different immunosuppression protocols. The majority of patients received cyclosporin A ($n=53$); the others were treated with FK 506 ($n=8$). Patients who received OKT-3 were excluded from the study [6]. In the case of infection, immunosuppression therapy was adjusted according to severity.

Collection of blood samples

Donor blood samples ($n=28$) were taken before the donation procedure from the central venous line (D). Samples from the outflowing perfusion solution were taken at the end of donation (P_1) and at the beginning of back-table work (P_2). Systemic blood samples of the recipients were taken from the arterial line (COLD system, Pulsion). In the case of regional measurements ($n=28$), portal vein (PV) blood samples were taken at the end of the anhepatic phase. Hepatic vein blood samples (HV_1–HV_4) were taken before venous declamping from the outflow of flushing blood (before venous graft perfusion the graft was flushed with 400 ml of the recipient's own blood, and blood samples were taken from every 100 ml outflowing blood). Intra-operative systemic PCT measurements ($n=40$) were performed before surgery (AS), hepatectomy (H_1–H_2), the anhepatic phase (AH), and venous reperfusion (BRP) as well as after venous reperfusion (PRP), end of surgery (ES), and the first postoperative hour (ICU). Different laboratory data and inflammatory response indicators ($n=62$) were noted before surgery and during the first 5 postoperative days (POD_1–POD_5).

PCT measurement

Measurement was carried out with the LUMItest PCT kit for quantitative determination of PCT in human serum or plasma (B.R.A.H.M.S., Hennigsdorf, Germany) with immunoluminometric assay (ILMA); the equipment necessary for running the procedure consisted of a luminometer (AutoCliniLumat LB 952). Analytical assay sensitivity was approximately 0.1 ng/ml. Functional assay sensitivity, the lowest value measured with a precision of 20% maximum inter-assay variation, was approximately 0.3 ng/ml.

Statistical analysis

Data are presented as mean ± SD. Wilcoxon signed rank test or Student's paired t-test were used to compare the different serum samples of the two groups. The χ^2-test was used for non-parametric data; multiple regression analysis was used to compare PCT with other prognostic markers. Significant differences were stated at $P<0.05$.

Table 2 Postoperative complications with regard to the development of sepsis in group B (with major postoperative complications)

Postoperative complication	Group B	
	Patients with sepsis ($n=13$)	Patients without sepsis ($n=14$)
Hepatic artery thrombosis	2	0
Renal failure	13	4
Respiratory failure	13	8
Coagulopathy	13	14
Mortality by day 30	5*	0

*$P<0.05$

Results

Groups A and B were homogeneous in terms of age, gender, Child-Pugh scoring, and etiology of liver disease, and there were no significant differences between them regarding type of preservation solution used, cold ischemia time, or surgical technique (Table 1). There were significant differences between the two groups in general severity scores (pre-operative APACHE II in group B: 11.6 ± 4 vs group A: 8.5 ± 3.1, and SAPS II in group B: 29.3 ± 8 vs group A: 20.5 ± 4.2, $P<0.05$; and postoperative day 1 APACHE II in group B: 15.1 ± 3.1 vs group A: 11.6 ± 4, and SAPS II in group B: 29.3 ± 8 vs group A: 20.5 ± 4.2, $P<0.05$).

According to the selection criteria, all patients from group B had some kind of severe postoperative complication (Table 2). Of the patients from group B, 13 of 29 developed sepsis due to urinary tract (four cases), pulmonary (three), abdominal (three), or blood stream (three) infections. Organ failure was more frequent and mortality on day 30 was significantly higher in the patients that developed sepsis. All patients with renal failure needed renal replacement therapy, and 21 of 29 patients needed prolonged mechanical ventilation. All patients from group B suffered major coagulopathy during the first postoperative days. No significant differences in PCT levels were found between patients with and without sepses in group B (maximum values on postoperative day 2: patients with sepsis, 30.89 ± 18.32 ng/ml, those without, 30.5 ± 15.4 ng/ml).

Pre-operatively, the patients in group B had worse liver function values than those in group A, but significant differences between the groups were found only in albumin levels and renal function (Table 3). In the postoperative phase the patients in group B showed significantly worse kidney function parameters and higher bilirubin levels. Multiple regression analysis was performed on all patients in groups A and B, involving the following parameters: PCT, serum albumin, creatinine, and APACHE-II score (Table 4). The other parameters of the inflammatory system, such as CRP, leukocytes, and body temperature changes, were without any significance between the two groups.

Serum PCT levels of the donors were mostly in the normal range; only in seven of 28 cases were elevated PCT levels observed (range: 2.1–30.4 ng/ml). No correlation was found between these values and the postoperative course. The PCT levels of the perfusion solution were not measurable, or were in the normal range for all the donors (D: 0.28 ± 0.18 ng/ml), and these levels remained unchanged during cold ischemia time (P_2: 0.2 ± 0.1 ng/ml).

There were no changes in PCT serum levels during hepatectomy and the anhepatic phase (H_2: 0.34 ± 0.25, AH: 0.35 ± 0.22 ng/ml). Significant elevation of systemic PCT levels was found in all patients after graft reperfusion. PCT serum levels increased to approximately four times the baseline value (PRP: 1 ± 0.43 vs BRP: 0.27 ± 0.22 ng/ml, $P<0.01$) and rose continuously until the first postoperative day (Fig. 1). Analyzing the postoperative phase, we found significant differences between groups A and B for the first four postoperative days (Fig. 2).

There were no differences between systemic and PV PCT levels (BRP: 0.16 ± 0.26, PV: 0.23 ± 0.15 ng/ml). Of 28 patients, 11 had higher hepatic vein (HV_2 and HV_3) than systemic or PV PCT levels at the same time (HV_3: 1.27 ± 0.43 vs BRP: 0.16 ± 0.26 and PV: 0.23 ± 0.15 ng/ml, respectively, $P<0.02$; Fig. 3). Intra-operative and regional PCT levels did not show significant differences in relation to postoperative complications.

Discussion

The magnitude of PCT levels in patients after solid-organ transplantation is a better indicator of systemic infection than changes in acute-phase protein levels, the presence of fever, and the increase in white blood cell count [2]. Fever of unknown origin after OLTx may suggest the possibility of infection or rejection. Usually, serum PCT levels rise proportionally after surgery with type or extent of surgical procedure [9]. This also happens in the case of OLTx, and, in the case of fever without rise in PCT, rejection may be suspected [7, 8].

The exact biological role of PCT is still not clear. This protein is part of the neuro-endocrine-immune axis and obviously has important biological functions within the infection defense mechanism and systemic inflammation. PCT, especially, is induced during sepsis and systemic inflammation caused by bacterial infection. However, numerous other stimuli may contribute to PCT induction. Since the mediators during reperfusion injury and in systemic inflammatory response syndrome as well as

Table 3 Pre- and postoperative laboratory data of the patients in groups A and B (*Pre-op* pre-operative, *POD 1–5* post-operative days 1–5)

Substance	Group	Pre-op	POD 1	POD 3	POD 5
Albumin (g/l)	A	32.7 ± 3.7*	32.6 ± 3.1	35.1 ± 2.4	32.8 ± 3.2
	B	29.6 ± 5.4	31 ± 4.8	35.1 ± 3.7	33 ± 3.6
ALT (U/l)	A	106 ± 83	550 ± 368	467 ± 311	247 ± 132
	B	79 ± 60	602 ± 418	465 ± 327	211 ± 127
ALP (U/l)	A	710 ± 478	245 ± 99	252 ± 102	243 ± 82
	B	571 ± 342	201 ± 72	272 ± 113	262 ± 83
Serum bilirubin (mmol/l)	A	98 ± 76	85 ± 37	90 ± 46	97 ± 50
	B	130 ± 128	97 ± 52	126 ± 65	175 ± 108*
Creatinine (μmol/l)	A	69 ± 20	110 ± 32	103 ± 33	96 ± 30
	B	109 ± 50*	162 ± 58**	198 ± 95**	168 ± 81**
BUN (mmol/l)	A	4.5 ± 1.6	7.8 ± 2.3	15.2 ± 5	16.2 ± 5.7
	B	8.8 ± 5.4*	11 ± 4.6*	22.1 ± 9.5*	26 ± 11.3*

*$P < 0.05$, **$P < 0.001$, mean significant differences between group A (without) and group B (with major postoperative complications)

Table 4 Multiple regression analysis correlation coefficients between PCT and serum albumin, creatinine levels, and APACHE-II scores for all patients, group A (without), and group B (with major postoperative complications)

Parameter	All patients	Group A	Group B
PCT—albumin	0.20	0.22	0.17
PCT—serum creatinine	0.42	0.52	0.44
PCT—APACHE-II score	0.58	0.79	0.50

decrease. The source of PCT is not unambiguously clear; there is some relationship between leukocytes or monocytes and PCT production [3, 11]. The hepatosplanchnic region itself may be a producer of PCT. In patients undergoing coronary artery bypass grafting, PCT concentrations in liver venous samples were significantly higher than in time-matched arterial samples [12]. This observation was reinforced by other studies: in the septic anhepatic baboon model, no PCT elevation

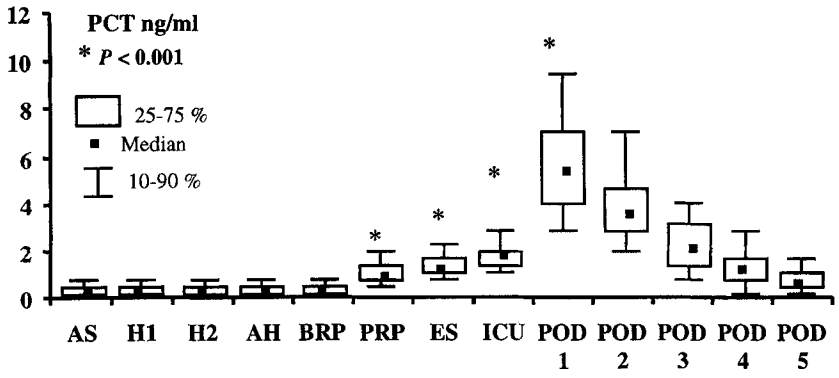

Fig. 1 Changes in intra- and postoperative PCT levels ($n = 40$). *$P < 0.001$, significant differences compared with the previous value. *BRP* 5 min before reperfusion, *PRP* 20 min after reperfusion, *ICU* arrival at the intensive care unit

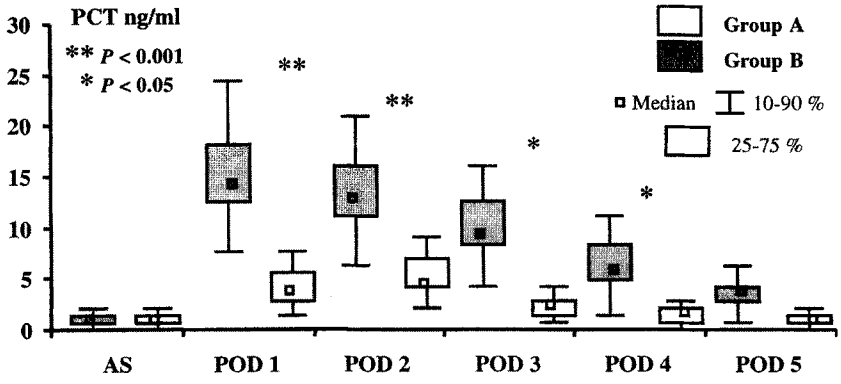

Fig. 2 Changes in postoperative PCT levels in groups A and B. *$P < 0.05$, **$P < 0.001$, significant differences between group A (without) and group B (with major postoperative complications)

multiple organ failure syndrome are similar, this may cause an overlap in these processes. As a consequence, the specificity of PCT in the diagnosis of sepsis could was found after endotoxin injection [10], and in patients after liver surgery the hepatic vein PCT levels were found to be elevated [4].

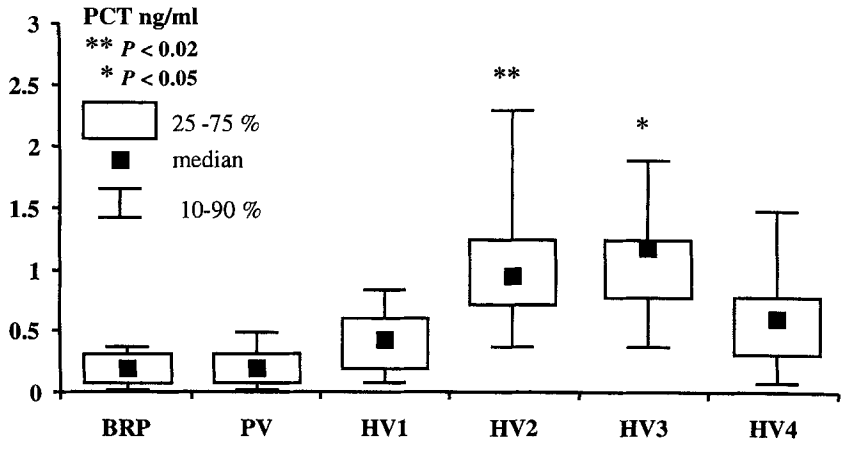

Fig. 3 Changes in regional PCT levels ($n = 28$). Hepatic vein blood samples (HV_1–HV_4) were taken at the end of the anhepatic phase, but before venous graft perfusion, the graft was flushed with 400 ml of the recipient's own blood, and blood samples were taken from every 100 ml outflowing blood. $^*P < 0.05$, $^{**}P < 0.02$, significant differences between BRP and HV_2–HV_3 (*BRP* systemic sample and PV samples at the end of the anhepatic phase)

Supranormal PCT levels of donors may suggest the presence of systemic inflammatory response syndrome or sepsis, which may have an effect on the donation process. In some of our donors elevated PCT levels were observed, but we could not prove any severe infection in these cases, and OLTx was performed without any infection problem, as opposed to an observation in cardiac transplant patients, where elevated PCT concentrations in donors were correlated with poor graft–patient survival [13]. An unchanged PCT level of the perfusion solution during back-table procedures is expected, due to minimal metabolism. Our results also support this hypothesis.

All types of major surgery, including OLTx, may induce non-specific inflammatory reactions causing a slight and proportional elevation in PCT level within the first postoperative days [8, 9]. However, in the majority of patients we found the first significant PCT level increase immediately after PV reperfusion. This observation suggests that in these cases PCT may come from the graft itself, since concentrations of PCT in hepatic vein blood were significantly higher than systemic or PV concentrations at the same time; however, the graft as a possible source of PCT after OLTx may be discussed. The elevation of PCT at the end of OLTx could be a sign of graft ischemia-reperfusion injury, surgical insult, or morbidity of the recipient, which contribute to PCT production, but we could not find any correlation between these parameters and increased levels of PCT.

Elevated PCT levels in OLTx patients are found in the early postoperative phase [5, 7]. A slight increase in PCT level is seen as a non-specific reaction to surgical insult, but levels above 5 ng/ml are considered an infection problem. We found similar results in our patients; however, increase of PCT during the surgical procedure might have been due to ischemia-reperfusion injury of the graft and might have derived from the liver in significant amounts. In the patients without major postoperative complications, only a slight increase in PCT level was observed, compared with the complication group where PCT levels were significantly higher. The occurrence of sepsis among these patients was less than half, despite their PCT levels having been equally high. The reason for this could be that acute severe organ failure alone could maintain highly elevated PCT levels without infection. The higher PCT levels with worse outcome were influenced by the magnitude of graft failure, as opposed to good graft function when the slight increase in PCT levels correlated better with serum creatinine levels and APACHE-II scores. This observation decreases the specificity of PCT as an infection marker, but gives it the other possibility of being a prognostic marker. This is supported by the fact that the values of the APACHE-II and SAPS-II prognostic factors were significantly higher in group B, where every patient had some kind of severe postoperative complication, than in group A, where only mild organ dysfunction occurred.

In summary, we found that hepatic vein PCT levels are significantly higher than systemic or PV concentrations before reperfusion (suggesting the possibility that the liver is able to produce PCT), that serum PCT levels start to increase significantly during OLTx immediately after PV reperfusion, and that postoperative PCT elevation may have a prognostic value beside its role as sepsis marker. Further investigation is necessary to clarify the exact role of PCT during different transplantations.

Acknowledgements The authors wish to thank Elek Dinya for the statistical analysis performed.

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