Studies on predictability of early graft function after liver transplantation
Maring, Jan Kornelis

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
chapter 6

Endotoxin and cytokines during liver transplantation: changes in plasma levels and their effects on clinical outcome

J.K. Maring¹, I.J. Klompmaker², J.H. Zwaveling¹, J. van der Meer², P.C. Limburg³, M.J.H. Slooff³

Liver transplant group. University Hospital Groningen
¹ Department of Surgery
² Department of Internal Medicine
Chapter 6

Abstract

**Background** Endotoxin, tumor necrosis factor α, interleukin-1 and interleukin-6 are thought to play a key role in liver transplantation. The origin and course of these factors is not completely known.

**Methods** In this prospective study in 40 patients by sampling at different sites and consecutively more understanding of the relations between these factors and the effects on clinical outcome was sought.

**Results** Endotoxemia was only present in 20% of the patients. In 75% of these patients it was present during the anhepatic phase and quickly resolved after reperfusion. Endotoxemia was not related to any clinical adverse event. Tumor necrosis factor α was released from the graft after reperfusion and initial levels after reperfusion were related to predonation levels in the donor. Only levels of tumor necrosis factor α in the recipient before transplantation were found to be predictive of postoperative complications. We conclude that monitoring endotoxin and these cytokines is of a very limited value in predicting outcome.

Introduction

Liver transplantation is standard treatment for patients with end stage liver disease. Though improvements over the years have increased survival, initial liver function is not optimal in all cases and infection and rejection are common. Research has focused on graft preservation and reperfusion injury as causative factors for these events. Reperfusion injury is characterized by a loss of endothelial cell viability associated with Kupffer cell activation. Pro-inflammatory cytokines, especially tumor necrosis factor α (TNFα), are presumed to play a key role in this process. Elevated levels of cytokines have been reported after liver transplantation, as well as after other types of surgery. The exact dynamics in cytokine concentrations are not clear.

Several factors may influence levels of cytokines, like the presence of endotoxin during the liver transplantation procedure, ischemia and manipulation of other
Endotoxin and cytokines during OLT

organs during transplantation, especially the splanchnic viscera. Postreperfusion systemic levels of cytokines in the recipient may be influenced by their serum levels in the donor. Finally, the preoperative systemic levels of cytokines in the recipient might be an important factor, since these are related to the nature and stage of the underlying disease.

The role of cytokines in human liver transplantation remains controversial. There is some evidence that cytokines are involved in rejection and infection in the early postoperative phase, but available data are not consistent. Before starting a trial with anti-TNFα more precise data from the course and effects in human transplantation should be available.

We performed this study in order to evaluate changes in levels of cytokines during and shortly after orthotopic liver transplantation and to assess their relation to clinical outcome.

Patients and Methods

The study was approved by the Institutional Review Board of our hospital and informed consent was obtained from each participating patient or a relative, in case of coma.

Liver transplantation
Donor livers were not accepted when the donor was older than 65 years; had a history of liver disease, alcoholism or drug abuse; had experienced hypotensive periods, unless the donor had recovered from a hypotensive period for more than 24 hours and revealed normal or near normal liver function tests afterwards; had received consistently more than 10 mg/kg/min. of dopamine; and liver function tests exceeded triple normal values. Standard harvesting techniques were used with in situ cooling of the abdominal organs with UW preservation solution. Anesthesia was induced with midazolam 0.1 mg/kg, vecuronium 0.1 mg/kg, sufentanil 1 μg/kg and maintained with continuous infusions of midazolam, vecuronium and sufentanyl. The trachea was intubated and the lungs ventilated throughout the procedure with 40% of oxygen in air. Tidal volume and ventilatory frequency were adjusted to maintain PaCO₂ at 4.5 kPa.

In all patients orthotopic liver transplantation was performed using a standard...
Chapter 6

surgical procedure. Venovenous bypass was performed using a centrifugal pump and heparinized tubing in 36 patients connecting the femoral vein and either the portal or inferior mesenteric vein with the axillary vein. Flow was maximized to levels where the inflow was obstructed because of collapse of the involved veins or tubing. In the remaining four patients the liver was transplanted using the so-called piggy-back technique during which the inferior caval vein is left in situ, without temporary porto caval shunt. No venovenous bypass was used in these patients.

Hemodynamic measurements included heart rate, mean arterial pressure, central venous pressure, pulmonary artery pressure, pulmonary artery occlusion pressure, and cardiac output. Cardiac output was calculated from triplicate measurements by thermodilution method. Surgical data, i.e. time (warm and cold ischemia as well as operation time) and blood loss, were collected.

All patients received the same immunosuppressive medication, since the type of medication might influence systemic cytokine concentrations.

Immunosuppressive therapy consisted of methylprednisolon 1000 mg on the day of operation, cyclophosphamid for 7 days (100 mg), prednisolon 200 mg for 3 days, then 100 mg for 4 days and then tapered guided by liver tests and clinical condition. Azathioprine was given in a dose of 125 mg/day. All patients received cyclosporine. However, cyclosporine was only started when creatinine clearance was over 50 ml/min, but not before day three after transplantation. This implicated that it was not given within the period endotoxin and cytokines were measured. All patients received cefotaxime 3 g/day, metronidazole 1.5 g/day and tobramycin 240 mg/day intravascularly for 3 days, or imipenem 4 g/day when creatinine clearance was below 30 ml/min.

Fifteen patients also received selective bowel decontamination within the framework of a randomized trial investigating the efficacy of selective bowel decontamination. These patients received a suspension 4 times a day containing colistine sulfate 200 mg, tobramycin 80 mg and amphotericin B 500 mg and oral paste containing 2% solution of the same drugs for at least one week before transplantation till thirty days after the operation. None of the patients received aprotinin in this study.
Measurements of cytokines and endotoxins

Serial measurements of TNF\(\alpha\), interleukin-1\(\beta\) (IL-1\(\beta\)) interleukin-6 (IL-6) and endotoxin concentrations in systemic blood were performed in adult liver transplant patients, who underwent their first orthotopic liver transplantation. In order to clarify changes in cytokine concentrations, measurements were done simultaneously in arterial, hepatic and portal vein blood.

Samples from portal vein, hepatic vein and arterial blood, were taken at the start of the operation (pre), 5 minutes before the start of venovenous bypass (bypass), 5 minutes before recirculation (t=-5'), 5(t=5'), 30 (t=30'), 60 (t=60') and 120 minutes (t=120') after recirculation. Additionally an arterial sample was taken 12 hours (t=12hrs) after recirculation. Donor arterial blood samples were taken before perfusion with preservation fluids. All venous samples were taken by repetitive punctures. The arterial samples were collected via a radial artery catheter. Blood was kept on ice until centrifugation at 1000xg at 4°C for 15 min. Plasma was frozen at -80°C until measurements were performed.

TNF\(\alpha\) and IL-1\(\beta\) plasma levels were measured using the high sensitivity Quantakine\textsuperscript{TM} ELISA according to the manufacturers instructions (R&D systems, Minneapolis MN)(normal values 1.3 ng/L range 0.22-4.12 ng/L and 0.51 ng/L range 0.14-1.63 ng/L respectively).

Levels of IL-6 were measured using a sandwich ELISA with a monoclonal and polyclonal antibody (both from CLB, Amsterdam, NL)(normal value 6 ng/L range 1.1-14 ng/L) as previously described\textsuperscript{17} with modifications. Shortly, after incubation with the biotinylated second polyclonal antibody the conjugated streptavidin Strepta-E+ (CLB, Amsterdam, NL) was used and visualized with the chromogen TMB (Roth, Karlsruhe, FRG)(normal value

Endotoxin levels were determined in platelet rich plasma following the manufacturer instructions(Kabi Diagnostics, Stockholm, Sweden)(normal value <0.05 EU/mL). Blood was collected in Endotubes\textsuperscript{®}, kept on ice in order to avoid degradation and centrifuged at 200xg for 15 min at 4°C. Plasma was stored at -80°C. In order to avoid possible underestimation of endotoxin levels, we also measured recovery of a known amount of endotoxin spiked to the platelet rich plasma of each patient.
Chapter 6

Gastric mucosal pH
After induction of anesthesia a tonometer (Tonometrics, Worcester MA) was positioned in the lumen of the stomach. The correct position was confirmed by intraoperative palpation.
Gastric mucosal pH was assessed by filling the balloon with 3 ml of saline and after 30 minutes measuring pCO₂ in the aspirate after 1 ml was discarded. With the other 2 ml. of saline, the pCO₂ was measured with a Radiometer type 330 blood gas analyzer (Radiometer, Copenhagen, Denmark) within 10 minutes of sampling. An arterial blood sample was taken simultaneously to evaluate blood gasses. Using the Henderson-Hasselbalch formula the gastric mucosal pH (pHi) was calculated. For correction of low arterial pH (pHa) the standard pH, was calculated as standard pHₐ=7.4-(pHa-pHi). Samples were taken after induction, 30 and 60 minutes after start of bypass, 30 minutes before end of bypass, and 30, 60 and 120 minutes after reperfusion of the graft. Sodium bicarbonate was not administered to avoid possible interference with pHi determination.

Clinical outcome
Clinical endpoints were graft function at seven consecutive days after transplantation, the occurrence of infection and rejection in the first 30 days after transplantation, duration of ventilation and length of intensive care (ICU) stay. Graft function was assessed by serial measurements of alanine amino transferase (ALAT), aspartate amino transferase (ASAT), prothrombin time (PTT), activated partial thromboplastin time (APTT), fibrinogen, antithrombin III (ATIII) and total bilirubin and by a monoethylglycinexylidide (MEGX) test that was performed at 12 and 36 hours after reperfusion. Apart from these individual parameters, patients were grouped according to graft function using the criteria defined by Ploeg. Patients were thought to suffer from primary dysfunction if ASAT > 2000 U/l and PTT > 16 s on days 2-7, or death or regrafting occurred on days 1-7. All others were diagnosed to have immediate function.
Primary non function of the graft was defined as the need for regrafting within one week after transplantation. The indication for regrafting was based on a combination of parameters, including levels of transaminases and bilirubin, coagulation tests, hypoglycemia, hypothermia, production and quality of bile, circulatory and mental status, and, if applicable, histological findings.
Infection was observed for 30 days after transplantation or until discharge, if before day 30. Types of infection assessed were cholangitis, abdominal infection, pneumonia or bacteremia. Cholangitis was diagnosed in case of fever > 38°C, chills, contaminated bile and obstruction of the biliary system. Criteria for abdominal infection were fever > 38°C and a positive ascites culture or surgical drainage of an abdominal abscess. Pneumonia was diagnosed when the clinical pulmonary infection score (CPIS) was $\geq 7$. In ventilated patients a bronchoalveolar lavage was performed when the CPIS score was $\geq 7$. Bacteremia was defined by positive blood cultures, taken on clinical grounds. Rejection was diagnosed by the pathologist in a biopsy, routinely performed at one week and when clinically indicated, using the classification described by Snover 21.

Statistical methods
Statistical analysis was performed using SPSS 6.0 for Windows® (SPSS, Chicago, IL). Changes in and differences between cytokine concentrations were assessed by the paired student-t-test for normally distributed variables or Wilcoxon signed rank sum test for nonparametrically distributed variables. Possible associations between continuous variables and cytokine concentrations were evaluated by linear regression analysis. Patients were grouped according to clinical outcome and differences in cytokine and endotoxin concentrations during transplantation were assessed. Comparisons between groups were performed using the Student-t-test and the Wilcoxon signed rank-sum test where appropriate. Normal distributions were tested using the Kolmogorov-Smirnov test. The P value was calculated using Dallal and Wilkinson’s approximation to Lilliefors’ method. Pearson chi-square test was used to compare dichotomous variables between groups. Analysis of variance for repeated measures and Scheffe’s method for significance of comparisons between groups were applied for multiple comparisons between the groups. Two tailed p-values below 0.05 were considered significant.
Chapter 6

Results

We studied 22 male and 18 female adult patients. The diagnosis of the underlying disease is indicated in Table 1. One year patient survival was 95%, one year graft survival was 90%.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Number of patients</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>sclerosing cholangitis</td>
<td>8</td>
<td>42 (23-60)</td>
</tr>
<tr>
<td>cryptogenic cirrhosis</td>
<td>8</td>
<td>38 (21-54)</td>
</tr>
<tr>
<td>autoimmune chronic active hepatitis</td>
<td>4</td>
<td>31 (21-52)</td>
</tr>
<tr>
<td>acute liver failure</td>
<td>4</td>
<td>44 (32-56)</td>
</tr>
<tr>
<td>hepatitis C</td>
<td>3</td>
<td>50 (36-59)</td>
</tr>
<tr>
<td>alcoholic cirrhosis</td>
<td>3</td>
<td>47 (41-55)</td>
</tr>
<tr>
<td>sec. biliary cirrhosis</td>
<td>3</td>
<td>46 (36-56)</td>
</tr>
<tr>
<td>familial amyloid polyneuropathy</td>
<td>2</td>
<td>48 (46-49)</td>
</tr>
<tr>
<td>miscellaneous</td>
<td>5</td>
<td>46 (27-60)</td>
</tr>
</tbody>
</table>

Age is given as mean and range in brackets.

Table 1  Patient characteristics.

Plasma concentrations of cytokines and endotoxin

In recipients at the start of the operation and in donors at heptatectomy a wide range of TNFα plasma concentrations was found. In recipients the mean TNFα concentration was lower just before recirculation (5.1 ng/L) than at the start of the operation (7.0 ng/L) (p<0.03). After recirculation TNFα levels in all recipients increased significantly up to a maximum at two hours after recirculation (17 ng/L) (Fig 1).

During the first hour (ie. at 5′,30′ and 60′) after reperfusion, the TNFα levels in hepatic vein blood were significantly higher than in portal and arterial blood at the same time point (p<0.02 in all cases) (Fig 2: 5 min. after reperfusion). At other time points (before heptatectomy and 2 hours after reperfusion) there were no differences in plasma levels.
Endotoxin and cytokines during OLT

**Figure 1** Arterial TNFα concentrations during liver transplantation depicted in box and whiskers plot. The vertical dotted line represents time of reperfusion. * paired t-test revealed p< 0.01 when compared with pre, ** paired t-test revealed p< 0.01 when compared with -5'.

**Figure 2** TNFα concentrations in arterial, hepatic vein and portal vein blood respectively 5 minutes after reperfusion. Wilcoxon signed rank sum test revealed significantly higher TNFα concentrations in hepatic vein blood compared to arterial and portal vein blood (p<0.01)
Chapter 6

Changes in IL-1β concentrations showed an almost identical pattern as compared to TNFα (Fig. 3) except that IL-1β concentrations before recirculation (t= -5’) were similar to baseline values (t=pre).

![Box and whiskers plot of IL-1β concentrations in plasma collected from the radial artery at sequential points in time and from the organ donor. The vertical dotted line represents time of reperfusion. * Wilcoxon matched-pairs signed-ranks test revealed significant rise when compared to pre or -5’ (p< 0.02).]

IL-1 concentrations at the start of hepatectomy in were normal or close to normal in almost all of the donors and recipients. After recirculation the mean IL-1β concentration rose from 3.2 ng/L 5 min. before recirculation to 6.5 ng/L at one hour (p<0.04). No differences were observed in hepatic vein, portal vein or arterial IL-1β plasma levels at any time point during transplantation.

IL-6 levels also varied widely between recipients at the start of operation and in donors at hepatectomy. IL-6 concentrations increased immediately after start of the operation and further increased during the anhepatic phase (from a mean value of 160 ng/L to 400 ng/L and 840 ng/L respectively, p<0.05). Immediately after reperfusion IL-6 levels decreased to 720 ng/L (p<0.04), but they increased
again to 1350 ng/L at 30 minutes. Peak levels of 2250 ng/L were reached 2 hours after recirculation of the implanted liver (Fig. 4).

Endotoxemia was demonstrated in 9 patients (23%) at any point during transplantation. In six patients endotoxemia became apparent during the anhepatic phase and it resolved within an hour after reperfusion. In one patient it was only present during the anhepatic phase and disappeared within 5 min after reperfusion. Another patient experienced endotoxemia for at least half an hour after reperfusion. Differences between portal, hepatic vein and systemic arterial blood endotoxin levels were not observed at any time point during transplantation. Despite the fact that the fifteen patients with selective bowel decontamination all had cultures of throat and faeces without gram negative bacteria at the time of transplantation, there was no difference in incidence of

*Figure 4* Box and whiskers plot of IL-6 arterial plasma concentration. The vertical dotted line represents time of reperfusion. * paired t-test revealed significant increase when compared with pre (p<0.03). # paired t-test revealed significant decrease when compared with 5' (p<0.04). ** paired t-test showed significant increase when compared with -5'(p<0.02).
endotoxemia in the group receiving selective bowel decontamination (3/15 = 20%) and the patient not receiving selective bowel decontamination (6/25 = 24%) (p>0.75).

Relation between plasma levels of endotoxin and cytokines in recipients and donors

Peak TNFα, IL-1β and IL-6 levels in the recipient, as mentioned before shortly after reperfusion, were correlated with arterial (Table 2) and portal plasma levels in the donor. The higher the donor value, the higher the levels were in the recipient after reperfusion. TNFα and IL-1β levels at 5 minutes and 30 min. after reperfusion were correlated (R²=0.89, p<0.02, R²=0.9, p<0.02 respectively) as were IL-6 levels at 60 and 120 minutes (R²=0.8, p<0.025 for both). In all recipients levels of TNFα were correlated to those of IL-6 at corresponding time points (R² ranging from 0.4-0.9, p<0.05 at all points in time). TNFα was also correlated with IL-6 at the preceding sample time point. For example, TNFα levels at 5 minutes before recirculation were correlated to IL-6 levels at that time, but also at 5 minutes before start of venovenous bypass (-5').

IL-1β concentrations 5 and 30 min after reperfusion were correlated with the duration of the cold ischemia time (mean 11 hours, min: 4 and max 17 hours) (Table 2). IL-1β levels were not correlated with the levels of the two other cytokines, except at the start of the operation, when IL-1β and TNFα levels were weakly correlated (R² 0.54, p<0.005).

Endotoxin or cytokine concentrations in the recipient were not correlated to endotoxin concentrations in the donor. Perioperative variables like cold ischemia time, warm ischemia time (mean 1 hour, min: 30' max: 105') (table 2), presence of corrected pH, lower than 7.32 (table 3), duration of surgery (mean 10 hours, min: 7 max: 15 hours) or blood loss (mean 10 L, min: 1 max: 57 L) (table 2) were no determinants of endotoxin or cytokine levels at any time point.

Relation between plasma cytokine, endotoxin concentrations and clinical outcome

None of the assessed cytokines nor endotoxin levels were associated with any of the parameters of graft function in the first postoperative week. Primary non function did not occur in this group of patients. No differences were found in cytokine nor in endotoxin levels in donors nor in recipients between the group with immediate good function and the group with initial poor function. Abdominal infection occurred in 13 of 40 patients. The group of patients with
Endotoxin and cytokines during OLT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>donor TNFα</th>
<th>donor IL-1β</th>
<th>donor IL-6</th>
<th>blood loss</th>
<th>operation duration</th>
<th>CIT</th>
<th>WIT</th>
<th>ICU stay</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα pre</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.74</td>
</tr>
<tr>
<td>TNFα 5'</td>
<td>0.89</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>TNFα 30'</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>TNFα 60'</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.41</td>
</tr>
<tr>
<td>TNFα 120'</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.03</td>
</tr>
<tr>
<td>TNFα 24 hrs</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.74</td>
</tr>
<tr>
<td>IL-1β pre</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.39</td>
</tr>
<tr>
<td>IL-1β 5'</td>
<td>0.85</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.4</td>
<td>ns</td>
<td>ns</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β 30'</td>
<td>ns</td>
<td>0.92</td>
<td>ns</td>
<td>ns</td>
<td>0.37</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β 60'</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IL-1β 120'</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.52</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β 24 hrs</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IL-6 pre</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IL-6 5'</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IL-6 30'</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>IL-6 60'</td>
<td>ns</td>
<td>ns</td>
<td>0.83</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 120'</td>
<td>ns</td>
<td>ns</td>
<td>0.88</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 24 hrs</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Table 2  \( R^2 \) (above) and correlation (below) from several perioperative parameters.

abdominal infection had higher IL-1β concentrations after reperfusion for at least two hours. This difference was no longer present after 12 hours (Table 3).

Peak IL-1β concentrations (for two hours after reperfusion) were higher in the group of patients who developed postoperative abdominal infection (10.8 vs. 3.8 ng/L) than in the group who did not develop this complication.
Chapter 6

In the group with abdominal infection also TNFα levels at 5 min. after reperfusion were higher (8.8 vs. 5.5 ng/L) than in the group of patients who did not suffer from an abdominal infection. Despite the earlier noted relation between TNFα levels in the donor and the TNFα levels at 5 and 30 min. after reperfusion, there was no correlation between TNFα levels in the donor and clinical outcome.

TNFα concentrations at baseline were higher in the 16 of 40 patients that developed bacteremia postoperatively (9.4 vs. 5.1 ng/L) when compared to the other 24 patients. TNFα concentrations at baseline were a major predictor of length of ICU stay (table 2).

Since infection was defined as the presence of abdominal infection, cholangitis, pneumonia or bacteremia, it is not surprising that infections were correlated with the same peak or baseline levels.

Only 5 of the 40 patients developed pneumonia. This complication was not associated with a difference in any of the parameters as compared with patients without a pneumonia.

Rejection, grade ≥ 1 was demonstrated in 9 of 26 patients. In the remaining 14 patients (of the total of 40 patients included in the study) a biopsy was not performed because of either the bleeding risk due to impaired hemostasis (mostly a result of thrombocytopenia) or it was considered to be not beneficial because the clinical course was so excellent that a biopsy was not thought to be

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>TNFα pre</th>
<th>TNFα 30'</th>
<th>TNFα 24 hrs</th>
<th>IL-1β 30'</th>
<th>IL-1β 60'</th>
<th>IL-1β 120'</th>
<th>any IL-6</th>
<th>any endotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH i &lt;7.32</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>endotoxemia</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>major infection</td>
<td>8.5</td>
<td>4.3</td>
<td>7.1</td>
<td>5.1</td>
<td>10.5</td>
<td>4.5</td>
<td>6.6</td>
<td>2.9</td>
</tr>
<tr>
<td>bacteremia</td>
<td>9.4</td>
<td>5.1</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>abdominal infection</td>
<td>ns</td>
<td>8.8</td>
<td>5.5</td>
<td>ns</td>
<td>7.2</td>
<td>3.8</td>
<td>9</td>
<td>3.9</td>
</tr>
<tr>
<td>rejection</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>transplant function</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Group I is the group that experienced the complication, while group II did not. The number of patients in each group is shown in the text. If data are displayed concentrations differed significantly (p<0.05) in the student t-test.

Table 3  Different concentrations between groups are displayed.

In the group with abdominal infection also TNFα levels at 5 min. after reperfusion were higher (8.8 vs. 5.5 ng/L) than in the group of patients who did not suffer from an abdominal infection. Despite the earlier noted relation between TNFα levels in the donor and the TNFα levels at 5 and 30 min. after reperfusion, there was no correlation between TNFα levels in the donor and clinical outcome.
indicated. None of the tested parameters was associated with the occurrence of grade ≥ 1 rejection after one week. IL-6 concentrations nor the change in IL-6 plasma concentration were related to postoperative infection, organ function or rejection. Endotoxin concentrations nor the presence of endotoxemia ((i.e. > 0.05 IU) at any point were related to postoperative infection, organ function or rejection.

Discussion

Cytokine levels are known to increase during surgical procedures including liver transplantation. Many factors may influence peroperative cytokines levels in recipients of liver grafts. Examples are: presence of endotoxin, donor cytokines levels, manipulation or ischemia of the bowel. In animal experiments cytokines have been shown to play a key role in post operative graft function and rejection. In human liver transplantation their importance remains controversial.

Our data show that TNFα levels increase immediately after reperfusion. This increase in TNFα apparently originated from the implanted graft, since hepatic vein plasma levels of TNFα showed to be higher than arterial and portal vein plasma levels at simultaneous measurements. Whether TNFα was released or instantly produced by the donor liver after reperfusion remains to be clarified. Since donor plasma levels of TNFα in this study were related to the postreperfusion TNFα levels in the recipients, it is likely that at least part of the observed peak is due to mechanisms initiated before reperfusion.

Our findings are in contrast to those from a previous report that showed a relation of TNFα levels with postoperative graft function. In concordance to a previous report we found that patients who developed postoperative infections (abdominal and bacteremias) had higher peak TNFα levels, compared with patients who did not experience infections. Remarkably, preoperative TNFα levels were already higher in this group. In this case also duration of ICU stay is prolonged. The higher baseline levels of TNFα suggests a triggered immune system, maybe due to a subclinical infection or deterioration of the patient’s preexistent condition. In patients with septic shock it has been shown that the persistently elevated TNFα and IL-6 levels rather than peak levels predict a poor
outcome. This might explain that in our study the TNFα concentrations at baseline and at 12 hours after transplantation were predictors of postoperative complications rather than its peak levels after reperfusion. Interleukin-1 and IL-6 in our patients showed changes that were comparable to TNFα. However, in individual patients IL-1β was not related to the other cytokines. This is not surprising since it has been reported previously that IL-1β is not released after TNFα infusion. Another explanation for this finding might be that peak IL-1β levels were related to duration of cold ischemia time and IL-1β levels in donor plasma, in contrast to other cytokines. A previous study revealed also higher IL-1β levels after long cold ischemia times, but in contrast to that report we found a significant increase in IL-1β concentrations in all our patients. Patients with postoperative abdominal infection and/or major infections had higher peak IL-1β concentrations after reperfusion than patients who had no infection. This might be explained by an impaired cellular immune response.

From our data it is clear that IL-6 concentrations increase already during the anhepatic phase and continue to rise after recirculation of the graft. This pattern is independent of any of the data monitored in this study. Only TNFα concentrations at the preceding sampling time point and at the same time were related to IL-6 levels, which is easily understood by the mechanism of release and induction of IL-6 and has been shown in healthy volunteers as well. The peak values nor the percentage of change in IL-6 concentrations were related to the outcome parameters assessed.

The fact that our data do not confirm previous studies can not be explained by different levels of the measured cytokines, since in general our mean levels and, as far as has been published, ranges do not differ much except for the study published by McNicol et al, who found levels about 6-10 times as high when compared to ours and many other studies.

One might wonder whether our immunosuppressive protocol could have influenced our negative findings. It is known from the literature that corticosteroids can inhibit TNFα production as well as IL-1 production. Not only corticosteroids influence cytokine levels, also FK 506 is associated with reduced levels of TNFα following reperfusion. Most studies that show a negative effect of the studied cytokines on outcome either do not mention their immunosuppressive protocol or use antithymocyte globulin. A study that also did not show a role for TNFα, like ours used both anti-thymocyte globulin as well as methylprednisolone.
On the basis of our data it is doubtful whether blocking TNFα or IL-1β is worthwhile in clinical practice. Maybe subgroups of patients could benefit from such treatment, the way current studies in sepsis are performed. For example patients with high TNFα before transplantation could benefit from either investigating the reason for their high levels or maybe benefit from interventions. We observed endotoxemia in only 20% of patients at any point during transplantation and it did not affect clinical outcome. This finding is in agreement with other reports, but in contrast to the original report by Miyata et al., who showed an increased incidence of postoperative pneumonia in patients with peroperative endotoxemia. In our study, the five patients that developed pneumonia had no endotoxemia during the transplantation procedure. This discrepancy might be explained by our rigid protocol to identify pneumonia or by differences in treatment of the patient, with immunosuppressive or antibiotic drugs. Selective bowel decontamination did not influence endotoxemia as has been shown previously. The fact that we found no relation between endotoxemia and postoperative complications, might be explained by the low frequency of endotoxemia, though some groups have used almost the same methods to determine endotoxemia and found more patients with increased endotoxin concentrations perioperatively. In contrast to a study in cardiac surgery patients, in our patients no relation was noted between gastric mucosal pH and systemic cytokine concentrations. Endotoxemia was not associated with a corrected pH, below 7.32. This finding is identical to a previous report by Welte et al. who also could not show an association between gastric mucosal pH and endotoxemia, also probably because of a lack of endotoxemia.

In conclusion in our study endotoxin is hardly present and therefore does not play a key role in inducing cytokines nor in predicting or causing postoperative complications. TNFα is produced by the graft. TNFα concentrations after recirculation correlated with donor concentrations of this cytokine. High TNFα concentrations at the start of the operation are associated with postoperative infections and duration of ICU stay. IL-6 at least for a large part is produced independent of the liver. IL-6 concentrations appear to reflect surgical injury and are not associated with postoperative complications. In contrast to animal experiments endotoxemia and high cytokine levels in humans are not uniformly present and a relation with early graft function and short term clinical outcome is not found. 

Endotoxin and cytokines during OLT
References


17. Helle M, Boeye I, de Groot E, de Vos A, Aarden LA. Sensitive ELISA for interleukin-6. Detection of...


Chapter 6

Endotoxin and cytokines during OLT
Niets is genoeg of het is goed genoeg