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Balance of pro- and anti-inflammatory cytokines in cirrhotic patients undergoing liver transplantation ☆

Gianni Biancofiore [a,*], Lucia Bindi [a], Mario Miccoli [b], Maria R. Metelli [c], Erica Panicucci [c], Angelo Baggiani [b], Franco Filipponi [d]

[a] Liver Transplant Anaesthesia and Critical Care Medicine, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy
[b] Epidemiology and Biostatistics Unit, Department of Experimental Pathology, University School of Medicine, Pisa, Italy
[c] Department of Laboratory Medicine, University School of Medicine, Pisa, Italy
[d] Liver Transplant Surgery, University School of Medicine, Pisa, Italy

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ABSTRACT

This study was prospectively aimed at having better information about the natural history of serum cytokines in cirrhotic patients undergoing liver transplant surgery and at assessing their ability to set up an appropriate dynamic relationship between pro-inflammation and anti-inflammation. The levels of six cytokines (TNF-α, IL-1, IL-2, IL-6, IL-8, IL-10) were measured in blood samples collected at different time points before, during and after (48 h) the transplant procedure from the radial artery of 62 consecutive cirrhotic patients who underwent orthotopic liver transplantation.
IL-1 always stayed within the normal range; IL-2 showed elevated baseline levels but decreased up to half at the end of the study ($p < 0.0001$). IL-6 peaked at the end of surgery and returned to baseline 48 h afterwards. The same happened to IL-8 concentrations. IL-10 levels shown above the normal threshold at baseline, peaked at the end of surgery ($p < 0.0001$) and were halved at the end of the study ($p < 0.0001$). TNF-α peaked at the end of surgery without, however, being different from baseline levels ($p = 0.6$). The physiologic pattern of cytokine release and their dynamic relationship was found to be preserved with a quick return to a balance between pro-inflammation and anti-inflammation as shown by the IL-6/IL-10 and TNF-α/IL10 ratios (used to assess the inflammatory balance). A correlation was found between perioperative pro-inflammatory cytokine levels and the severity of the liver disease necessitating OLT. In summary, cirrhotic patients can achieve a balanced inflammatory response to surgery which is considered a primary requirement for uneventful grafts and patients' postoperative recovery.

1. Introduction

Cytokines are a group of endogenous proteins which play a pivotal role in regulating the inflammatory response to surgery through a predictable set of adaptive events. This process, which is designed to maximize the organism's healing potential, is initiated locally, at the site of surgical trauma, by macrophages and monocytes that release pro-inflammatory cytokines. In particular, tumour necrosis factor alpha (TNF-a) and interleukin-1 (IL-1) initiate a cascade of mediators which are directly responsible for the various events associated with inflammation [1–6]. The increase in the circulating blood of pro-inflammatory cytokines triggers immune cells to release anti-inflammatory cytokines (e.g., IL-10) with the aim of downregulating, through complex feedback mechanisms, the pro-inflammatory process so to maintain homeostasis [1–6]. From the clinical point of view, the role of circulating inflammatory cytokines is not much clear yet. They might serve as biomarkers for certain clinical events, such as infections (i.e., IL6 in sepsis) or inflammation [7]. In addition, they might serve as marker for metabolic changes or stress [3–5]. It has been also reported that pre-existing chronic diseases can interact with the patients' capability to set up a balanced inflammatory response to surgery which, therefore, may result in being exaggerated (leading to hyper-inflammation) or inadequate (leading to immunosuppression) possibly causing compromised outcomes and organ dysfunction [2,3,7,8]. In particular, it has been shown that liver cirrhosis leads to an over-expression of various pro- and anti-inflammatory cytokines resulting in a significant derangement of the whole inflammatory response [9–12]. Therefore, we designed a study to obtain better information about the natural history of serum cytokines in cirrhotic patients undergoing liver transplantation with the aim of assessing if they are still capable to set up an appropriate physiologic relationship between pro-inflammation and anti-inflammation leading to a balanced response to surgery trauma.

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* Corresponding author at: Liver Transplant Anaesthesia and Critical Care Medicine, Azienda Ospedaliera Universitaria Pisana; Ospedale Cisanello; 56100 Pisa, Italy. Tel.: +39 050995409; fax: +39 050996984.
E-mail address: g.biancofiore@med.unipi.it (G. Biancofiore).

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2. Materials and methods

This study involved all of the patients who underwent orthotopic liver transplantation (OLT) at our Centre during 9 consecutive months who gave their informed consent. Anaesthetic and intraoperative management were the same in all cases as previously described [13]. According to the standard protocol at our Centre, a centrifugal pump-driven veno-venous extracorporeal by-pass with heparinised tubing was placed to drain the infra-diaphragmatic venous circulation into the superior vena cava to allow better haemodynamic stability during the anhepatic phase. All of the transplant procedures were performed by the same surgeon. Routine postoperative medications included antibiotics (Cefuroxim 1 g 3 times/day for 2 days) and Morphine. The immunosuppressive protocol included oral cyclosporin A (Sandimmun Neoral®, Novartis Pharma S.A., Huningue, France) at a dose of 15 mg/day on the day of surgery, subsequently titrated to maintain blood trough levels of 200–250 ng dL; intravenous Basiliximab (Simulect®, Novartis Pharma S.A., Huningue, France) 20 mg in postoperative days 1 and 4; oral mycophenolate mophetil (Cellcept®, Roche Pharma S.A., Milan, Italy) at a dose of 1 g twice a day and methylprednisolone (Solu-Medrol®, Pharmacia & Upjohn, Puurs, Belgium) at an intra-operative dose of 10 mg/kg subsequently reduced by 50% per day to a prednisolone dose of 20 mg/day. Patients with HCV-related cirrhosis did not receive steroids. Liver transplant recipients were classified according to the Model for End Stage Lived Disease (MELD). MELD is a scoring system assessing the severity of chronic liver disease that was found to be useful in determining prognosis and for prioritizing allocation of liver transplants [14].

2.1. Study protocol

The levels of six cytokines (TNF-α, IL-1, IL-2, IL-6, IL-8, IL-10) were measured in blood samples collected from the radial artery of the studied patients at the following time points: T0 (2 h before surgery), T1 (before the start of the veno-venous bypass), T2 (immediately before the by-pass was discontinued), T3 (15 min after graft's reperfusion), T4 (30 min after ICU admission), T5 (12 h from ICU admission), T6 (24 h from ICU admission), and T7 (48 from ICU admission).

2.2. Laboratory techniques

Blood samples were collected in tubes kept in ice, centrifuged at 0 °C and the plasma was stored at −70 °C until assayed. Measurements of cytokines were performed quantitatively by an enzyme-linked immunosorbent assay kit (ELISA, Bender MedSystems GmbH, Vienna, Austria).

2.3. Statistical analysis

Values are presented as mean ± standard deviation. Pro- vs. anti-inflammatory balance was assessed by calculating IL-6/IL-10 and TNF-α/IL10 ratios at each time point. During the preliminary phase we performed the Shapiro–Wilk test and the Hartley test to verify normality and homoscedasticity of distributions.

Subsequently we used ANOVA with Bonferroni's correction to compare variable values at each time. We calculated the Pearson's coefficient (with specific p-values) to study the correlations between IL peak concentrations and AST/ALT levels and to analyse the correlations between IL peak concentrations and INR. We performed statistical power analysis (ex post) to estimate the sample sizes used for the statistical tests. The 1-β values of the significant variables were >0.8, assuring a low risk of type II error and appropriate sample sizes. The level of significance was 0.05 and the analysis was carried out using the SPSS software (version 12.0, SPSS Inc., Chicago, USA).

3. Results

Data from 62 of the 67 enrolled patients were analysed (47 males, 15 females). Five patients were excluded because they were transplanted for liver cancer without cirrhosis. Our patients' age was 51.7 ± 8.6 years (range 26–64) and BMI was 24.7 ± 2.2 kg m^2. The underlying diseases necessitating liver transplantation were 59 cases of liver cirrhosis (45 viral, 10 alcoholic and 4 cryptogenic) and three of primary biliary cirrhosis. The MELD score of the study population was 19 ± 6.

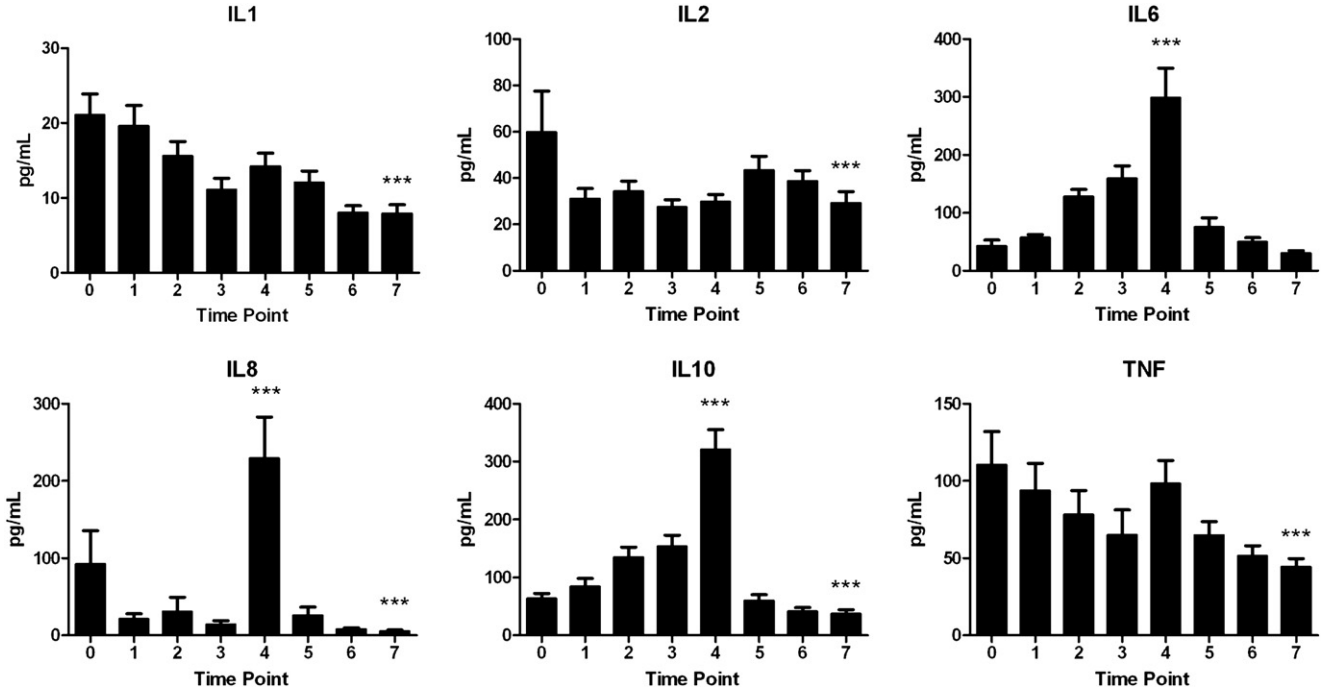

Fig. 1. Cytokine changes in the study population. 0: T0 (2 h before surgery). 1: T1 (before the start of the veno-venous bypass). 2: T2 (immediately before the bypass was discontinued). 3: T3 (15 min after graft's reperfusion). 4: T4 (30 min after ICU admission). 5: T5 (12 h from ICU admission). 6: T6 (24 h from ICU admission). 7: T7 (48 from ICU admission). *** = $p < 0.0001$ vs baseline (ANOVA).