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TNF-α depuration is a predictor of mortality in critically ill patients under continuous veno-venous hemodiafiltration treatment

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ABSTRACT

Introduction: Critically ill patients with acute kidney injury (AKI) present high mortality rates. The magnitude of inflammatory response could determine the prognosis of such patients. Continuous renal replacement therapy (CRRT) may play an important role in removing inflammatory mediators in patients with AKI.

Aim: To investigate whether the magnitude of inflammatory mediator's removal is associated with mortality among critically ill patients on CVVHDF, a CRRT modality.

Methods: This study consisted of 64 critically ill patients requiring CVVHDF. Plasma levels of C3a, TNF-α, IL-10, IL-6, IL-1β, sTNFRI and sTNFRII were determined by enzyme-linked immunosorbent assay (ELISA) at the beginning of CVVHDF and after 24 h (outlet). Clearance of cytokines during the first 24 h of CVVHDF was calculated. Clinical and laboratory data were acquired from patient's records data.

Results: Mean age of patients requiring CVVHDF was 63 years, 67.2% were men and 87.3% were Caucasian. Thirty-five (35) patients (54.7%) died. Comparing non-survivors with the group of survivors we observed higher incidence of sepsis (68.6 versus 37.9%, $p < 0.05$), higher APACHE II score (34.8 ± 7.6 versus 29.2 ± 7.1, $p < 0.05$) and higher lactate levels (23.2 ± 17.6 versus 16.4 ± 6.6, $p < 0.05$). According to the inter-tertile range of TNF-α clearance (ITR1 (<0.54); ITR2 (0.54–2.93); ITR3 (>2.93)) we found that those patients with higher TNF-α removal by RRT (ITR3) had a better survival. Multivariable analysis showed that lower clearance of TNF-α remained independently associated with high mortality after adjustment for sex, age, use of vasoactive drugs, APACHE II score sepsis, creatinine and lactate before CVVHDF (HR: 0.179, 95% IC: 0.049–0.661, $p < 0.01$).

Conclusion: The attenuation of inflammatory response may be related to the lower mortality observed on those patients with higher TNF-α removal by CVVHDF.

1. Introduction

It is estimated that more than 30% of intensive care unit (ICU) patients develop acute kidney injury (AKI), often accompanied by clinical, surgical or traumatic complications, usually with multiple organ and system dysfunction [1,2]. The most frequent cause of AKI is sepsis, a primary cause of increased morbidity and mortality, especially in ICU patients [3,4]. Even with the latest advances and innovations in renal replacement therapy (CRRT), the mortality of ICU patients remains high [5,6]. It is suggested that this treatment modality, in comparison with conventional hemodialysis, provides better cardiovascular tolerance associated with minimal variability of plasma osmolality, better metabolic control, more efficient correction of acid-base and electrolyte disturbances, and slow and unlimited fluid removal that facilitates the administration of parenteral nutrition and fluids in critically ill patients with AKI [7].

Serum levels of inflammatory mediators are increased in patients with AKI, regardless of its cause. Pro- and anti-inflammatory mediators play important roles in regulating the immunologic response which mediates the severity of the disease and its complications [8]. The increased plasma levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), IL-6 and lipid mediators, including

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platelet activating factor and anti-inflammatory IL-10, are produced early in the course of sepsis [9]. The close relationship between high levels of inflammatory cytokines in plasma and mortality in septic patients is indicative that the intense activation of inflammatory mediators plays an important role in the development of organ dysfunction [10].

In CRRT, the removal of circulating inflammatory mediators is an attractive strategy, and it seems to be a logical extension of the therapeutic arsenal already available. While transmembrane transport by diffusion is dependent on the size of the molecules, convective transport is independent of particle size, and primarily depends on the hydraulic permeability and transmembrane water flow [11].

Several studies have attempted to determine whether the beneficial effect of CRRT in critically ill patients may be partially attributed to the convective and diffusive removal of pro-inflammatory cytokines, particularly TNF-α and IL-1 [12–15]. However, few and conflicting data exist about the removal of cytokines by CRRT in septic patients with AKI. The aim of this study was to determine whether and to what extent the removal of inflammatory cytokines by CRRT, specifically CVVHDF, impacts ICU mortality.

2. Materials and methods

2.1. Patients

The study population consisted of 64 patients admitted to the intensive care unit (ICU) of Hospital Israelita Albert Einstein (São Paulo, Brazil), who had AKI and required CRRT, specifically CVVHDF.

Acute Physiology and Chronic Healthy Evaluation (APACHE II) was routinely used as the severity of disease score [16] and was evaluated on admission at ICU and on the day of CVVHDF initiation. All patients were enrolled into this study under informed consent guidelines approved by the Investigation Review Boards of the Hospital. For comparison, we used a control group of fifteen critically ill patients without AKI.

Baseline demographic and clinical data were obtained from the patients' hospital records.

The following criteria were used for eligibility: age over 18 years; admission to the ICU; fulfillment of the AKIN criteria (Acute Kidney Injury Network) for AKI, as defined: (a) serum creatinine increased 0.3 mg/dL or increased 1.5–2.0-fold from baseline or serum creatinine increased >2.0–3.0-fold from baseline or serum creatinine increased >3.0-fold from baseline or serum creatinine ($\geqslant$4.0 mg/dL) with an acute increase of at least 0.5 mg/dL or needed for RRT [17], or the calculated baseline creatinine for the individual according to the MDRD formula (Modification of Diet in Renal Disease) [18] and (b) elevation of at least 0.5 mg/dL compared to baseline creatinine at levels of up to 4 mg/dL; and indication of CVVHDF. Exclusion criteria were: past history of RRT, renal transplantation, previous participation in this study, or a DNR (do not resuscitate) order.

2.2. CVVHDF procedure

CVVHDF was conducted using a Prisma M100 machine (Gambro Renal Products, France). As previously described by our group, it was equipped with an AN 69 hemo filter, which was primed with 1 L of normal saline containing 5000 IU heparin followed by a second prime with only normal saline. Vascular access was provided by the insertion of a triple lumen catheter (Arrow International, PA) into either the internal jugular or femoral vein. Blood flow was constant at 100 mL/min. Four percent tri-sodium citrate was infused in the arterial line (through a three-way stopcock) and calcium replacement (0.75% $CaCl_2$) was done by infusion into the third lumen of the dialysis catheter, beginning with a flow rate of 140 and 70 mL/h, respectively. The dialysis solution was composed of 110 mEq/L sodium, 111.5 mEq/L chloride, 1.5 mEq/L magnesium, and 0.1% dextrose. Sodium bicarbonate (about 20–30 mEq/L) and potassium phosphate were added to the solution according to need, with a final sodium concentration between 130 and 140 mEq/L. The replacement solution (post-filter replacement) was composed of 0.45% NaCl, while 20% NaCl (about 10–15 mL/L) and 10% magnesium sulfate were added according to need, with a final sodium concentration between 111 and 128 mEq/L. The bags were carefully mixed in the hospitaĺs pharmacy [19].

The efficacy of the filter was measured by the effluent urea concentration/pre-filter urea concentration ratio.

2.3. Blood sampling

2.3.1. Patients

Blood samples collection (40 mL) was performed of filter inlet and outlet blood at times 0, 6 and 24 h with the machine in operation and the ultrafiltrate collection were drawn at 6 and 24 h at the time with the change in volume of 2000 ml ± 1000 ml (this volume of ultrafiltrate was collected in 40 ± 20 min). All samples were immediately transported on ice to the laboratory, where they were centrifuged at 3000 rpm for 10 min, frozen and stored at −70 °C until assay. Samples were collected in the first 24 h of CVVHDF.

2.3.2. Healthy volunteers (control group)

We collected 10 mL of whole blood from 35 healthy volunteers. After collection, the specimens were immediately centrifuged at 4 °C and 3000 rpm for 10 min, and samples of separated plasma were stored as 0.5-mL aliquots at −80 °C.

2.3.3. Critically ill patients not undergoing CVVHDF (critically ill control group)

We collected 10 mL of whole blood from 20 critically ill patients not undergoing CVVHDF which were held consecutively at times 0, 6 and 24 h after admission and presented an APACHE score of 23.5 ± 4.9. This collection was performed after collection of the 64 patients chosen for this study. After collection, the specimens were immediately centrifuged at 4 °C and 3000 rpm for 10 min, and samples of separated plasma were stored as 0.5-mL aliquots at −80 °C.

2.4. Biochemical methods

Renal function was evaluated by serum urea, creatinine, sodium and potassium using standard auto-analyzer techniques [20–23].

The C-reactive protein (CRP) was measured by the immune turbidimetry technique [24], and leukocytes were counted automatically in a CELL DYN 3200 (ABBOTT).

2.5. Cytokine analyses

Plasma levels of C3a were measured with the enzyme immunoassay kit C3aHuman September ELISA (BD Pharmingen) following the manufacturer's recommendations.

Plasma levels of the inflammatory mediators TNFRI and sTNFRII were measured with the enzyme immunoassay kit (ELISA) following the manufacturer's recommendations (Quantikine R&D Systems). The levels of TNF-α, IL-10, IL-6 and IL-1b were measured using high sensitivity kits (Quantikine high sensitivity – R&D Systems). The levels of inflammatory mediators were also measured in the ultrafiltrate as described above. The absorbance readings were measured using a spectrophotometer at 450 nm, and the results expressed in pg/mL.