

The immunological effects of continuous veno-venous haemodiafiltration in critically ill patients

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Background: Haemodynamic instability is common in septic patients with acute renal failure. Continuous veno-venous haemodiafiltration (CVVHD) is therefore used as an alternative to conventional haemodialysis. Haemodialysis is associated with an activation of the immune system. The aim of the present study was to test the hypothesis that initiation of CVVHD influences the immune system with release of proinflammatory cytokines followed by a decrease in granulocyte activation, as assessed by the expression of adhesion molecules.

Results: Fifteen patients were included. Mean Acute Physiology and Chronic Health Evaluation-2 score before CVVHD was 19 (range 8–27). Mean duration of CVVHD treatment was 9 days (1–21 days). Tumour necrosis factor- α and interleukin-8 were detectable in plasma in all patients, whereas interleukin-10 was detectable only in a few patients. Proinflammatory and anti-inflammatory cytokines were detected in the ultrafiltrate. Large intraindividual and interindividual variations were demonstrated for all of the immunological parameters studied.

Conclusion: The hypothesis that CVVHD induces the release of proinflammatory cytokines followed by a decrease in granulocyte activation was not confirmed in the present study. The heterogeneous group of patients studied, with different underlying diseases and various durations of illness before the start of CVVHD, might have contributed to the difficulty in demonstrating the proposed immunological effect of CVVHD.

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Introduction

Acute renal failure as part of the multiorgan dysfunction syndrome (MODS) is a severe complication in critically ill patients. Haemodynamic instability is common in these patients and is exacerbated by haemodialysis. Continuous veno-venous haemodiafiltration (CVVHD) is therefore used as an alternative to conventional haemodialysis.

It is well documented that different types of extracorporeal circulation, such as haemodialysis using cuprophane membranes and cardiopulmonary bypass (CPB), are associated with complement activation [1,2]; granulocytopenia followed by granulocytosis [3], granulocyte activation and degranulation [3,4]; increased production of proinflammatory and anti-inflammatory cytokines; and increased acute-phase protein synthesis [5,6]. In fact, CPB elicits a well defined, temporary, systemic inflammatory response, which may contribute to organ dysfunction postoperatively. CPB using a membrane oxygenator is, however, used for 1–2 h during cardiocirculatory arrest, and thus

differs from CVVHD and haemodialysis. The above mentioned effects of haemodialysis are largely due to the use of cuprophane membranes. With the use of more biocompatible membranes, this pronounced activation of the immune system is not observed. Haemodialysis is used in (acute and chronic) renal failure for approximately 4h three to four times a week.

CVVHD is a long-term procedure that is carried out continuously for several days to weeks. It has recently been demonstrated [7] that CVVHD does not activate the complement system and the intrinsic coagulation pathways. The immunological effects of CVVHD have, however, not yet been thoroughly described. Whether it is possible to perform continuous extracorporeal circulation for days or weeks, even with biocompatible membranes, without affecting the immune system has not been determined.

The aim of the present study was therefore to test the hypothesis that initiation of CVVHD in critically ill

patients induces an activation of the immune system with release of proinflammatory cytokines, followed by a decrease in granulocyte activation, as assessed by the expression of adhesion molecules. Finally, we investigated whether proinflammatory and anti-inflammatory cytokines were removed by the ultrafiltrate.

Material and methods

Patients

The present study was approved by the Regional Ethics Committee and all patients or their relatives gave their informed consent. Over a period of 10 months we studied 15 consecutive critically ill patients with acute renal failure treated with CVVHD. The patients were included if they developed acute oliguric (<400 ml urine/day) or anuric renal failure as part of MODS and sepsis. The clinical diagnosis of sepsis and MODS were according to the international consensus definition of sepsis in 1992 [8]. In all patients, an Acute Physiology and Chronic Health Evaluation-2 score was calculated on admission to the intensive care unit and an assessment of the number of failing organs performed at the time of initiation of CVVHD. The patients were followed until they died or were discharged from the intensive care unit.

During CVVHD a combination of haemodialysis and ultrafiltration is used. This means that molecules may be removed by diffusion (haemodialysis) as well as by convection (ultrafiltration). In the present study CVVHD was carried out in the postdilution mode, with a BSM 22 bloodpump and monitor (Hospal, Lyon, France) with a constant blood flow rate of 100–150 ml/min. An AN69 polyacrylonitrile 1.6 m² high-flux membrane (Hospal) was used in all cases. The AN69 polyacrylonitrile membrane has a mean pore size of 29 Å and a maximum of 55 Å, and is suited for diffusion of small molecules and for convection of larger molecules. Filters were used until the filtration rate was <3 ml/min or until a maximum of 2 days of use. Vascular access was provided by internal jugular silicon twin catheters (Quinton, Gambro, Lund, Sweden). Daily haemofiltration was tailored to the individual patient's needs to maintain urea below 20 mmol/l. The patients were continuously treated with heparin to obtain an activated clotting time of 150–220 s. The dialysate used was gambrosol 1.5% (calcium 1.75 mmol/l, chloride 96 mmol/l, magnesium 0.25 mmol/l, sodium 132 mmol/l, lactate 40 mmol/l; Gambro), the dialysate flow rate was 10–30 ml/min and the ultrafiltrate flow rate was 10.4–4.3 ml/min.

Laboratory measurements

Arterial blood was sampled in EDTA tubes 1 h before the start of CVVHD; 2, 24 and 48 h after the start; and then every second day until the CVVHD treatment was stopped, either because of death of the patient or return of renal function. Patients who were treated with CVVHD

for more than 2 weeks had blood samples taken every fourth day after the second week. Blood samples were immediately refrigerated on ice and plasma was isolated by centrifugation at 1500 rpm for 10 min. The samples were subsequently stored at –80°C until they were assayed. Ultrafiltrate was sampled simultaneously with the blood samples. The ultrafiltrate samples were also frozen at –80°C until they were assayed.

Immunological methods

The adhesion/activation molecules on granulocytes and lymphocyte subpopulations were measured using flow cytometry and monoclonal antibodies. Heparinized peripheral blood was incubated with monoclonal antibodies for 15 min in the dark at room temperature. The following antibodies were used: anti-CD3-fluorescein-isothiocyanate (FITC) [mouse against human (MaH) IgG₁], anti-CD4-FITC (MaH IgG₁), anti-CD8-FITC (MaH IgG₁), CD11a-FITC (MaH IgG₁), anti-CD11b-FITC (MaH IgG₁), anti-CD62-FITC (MaH IgG₂), anti-CD16-FITC (MaH IgG₁), anti-CD18-FITC (MaH IgG₁) and anti-CD44-FITC (MaH IgG₁; all from Dako, Glostrup, Denmark). Red cells were lysed using ammonium chloride. Cells were then washed twice with phosphate-buffered saline and fixed in 1% formalin in phosphate-buffered saline before being analyzed by flow cytometry (Coulter Elite, Luton, UK). Both the granulocytes and lymphocytes were identified on the basis of their forward and side scatter characteristics. Cells positive for the specific antigens were scored on the basis of Simultest control (MIgG₁-FITC; Becton-Dickinson, San Jose, California, USA). The total leucocyte count was determined using a Coulter Counter S (Coulter Electronics, Luton, UK) and the differential count was measured automatically (Hematrack Model 360; Geometric Data, Luton, UK).

The cytokine concentration of tumour necrosis factor (TNF)- α , interleukin (IL)-8 and IL-10 in plasma and ultrafiltrate were measured by double-sandwich enzyme-linked immunosorbent assay. The sensitivities of these assays for cytokines in plasma were 10–30 pg/ml.

Determination of cortisol

The serum cortisol concentration was determined using a radioimmunoassay technique.

Determination of C3d and C4d

The concentrations of C3d and C4d were determined using immunoelectrophoresis. First an anti-C3d antibody and anti-C4d antibody gel were produced. Human IgG was added to activate the serum pool and controls were made. The specific concentration values of C3d and C4d were measured by electrophoresis. The samples were water cooled to 16–18°C, and coloured with Coomassie Brilliant Blue.

Determination of mannose-binding lectin

The complement can be activated via three pathways: the classical, the alternative and the lectin pathways. The lectin pathway is initiated by the binding of lectin to mannose or to carbohydrates from bacteria or viruses. The concentration of mannose-binding lectin (MBL) was determined using time-resolved immunofluorometric assay. A plate was coated with monoclonal antibodies to MBL and incubated with the samples overnight at 4°C. Then the samples were washed and Eu-anti-MBL was added. After washing and overnight incubation the fluorescence were measured using a Wallac 1232 Delfia Fluorometer (Wallac, Turku, Finland).

Statistical analysis

Kolmogorov Smirnov's test was used to determine whether the data had a normal distribution. Because only the adhesion molecules were normally distributed, Friedman's analysis of variance was used to test for changes within the group. If Friedman's test demonstrated a difference, data were compared with initial values obtained before CVVHD was started, using Wilcoxon's rank test. $P < 0.05$ was considered statistically significant. The results are expressed as median values (25 and 75% quartiles, or range).

Results

Ten men and five women were included. Eleven patients developed MODS after surgery, while four patients suffered from medical diseases. The medical diseases included two cases of pneumonia; one case of endocarditis; and one case of haemolysis, elevated liver enzymes, low platelets (HELLP) syndrome. The postoperative patients included two patients with a perforated ulcer, one with colon cancer, one with pancreas cancer, one with cholecystolithiasis, three patients with aneurisma of the abdominal aorta and one trauma patient. Two patients developed MODS after open heart surgery. One of these patients was admitted with a congenital heart malformation, and the other with unstable angina. Mean age was 59 years (range 25–75 years). Mean duration of CVVHD was 9 days (range 1–21 days).

The mean Acute Physiology and Chronic Health Evaluation-2 score before the start of CVVHD was 19.4 (range 8–27). The leucocyte count varied between 4.2 and $26.0 \times 10^9/l$ before the start of CVVHD. At the end of the CVVHD treatment the leucocyte count varied between 2.9 and $31.0 \times 10^9/l$. Initiation of CVVHD influenced neither the total leucocyte count nor the granulocyte or lymphocyte count in peripheral blood (Fig. 1).

Before the start of CVVHD the median plasma cortisol value was 782 pg/ml (range 419–1537 pg/ml). CVVHD did not influence the cortisol value, and at the end of the study a median plasma cortisol of 857 pg/ml (range 373–961 pg/ml) was measured.

TNF- α was detectable in the plasma of all the patients. Before the start of CVVHD the median plasma TNF- α concentration was 70 pg/ml (25 and 75% quartiles 38.5 and 93.0 pg/ml). CVVHD did not influence the TNF- α concentration in plasma, and at the end of the study a median TNF- α level of 66 pg/ml (25 and 75% quartiles 41 and 89 pg/ml) was measured (Fig. 2). TNF- α was excreted in the ultrafiltrate by all patients. After 2 h CVVHD the median TNF- α concentrations in the ultrafiltrate was 12 pg/ml (range 0.2–48 pg/ml) compared with 12 pg/ml (range 0–22 pg/ml) at the end of the study (Fig. 2).

IL-8 was also detectable in the plasma of all of the patients. Before the start of CVVHD the median IL-8 concentration in plasma was 310 pg/ml (25 and 75% quartiles 193 and 640 pg/ml). At the end of the study period a median IL-8 concentration of 260 pg/ml (25 and 75% quartiles 112 and 465 pg/ml) was measured. CVVHD did not induce changes in the IL-8 concentration in plasma. IL-8 was detectable in the ultrafiltrate in all the patients. After 2 h of CVVHD the median IL-8 concentration in the ultrafiltrate was 13 pg/ml (range 0–52 pg/ml) compared with 49 pg/ml (range 0–302 pg/ml) at the end of the study. There was no correlation between the concentration of IL-8 in plasma and ultrafiltrate. This is in accordance with other investigations [9].

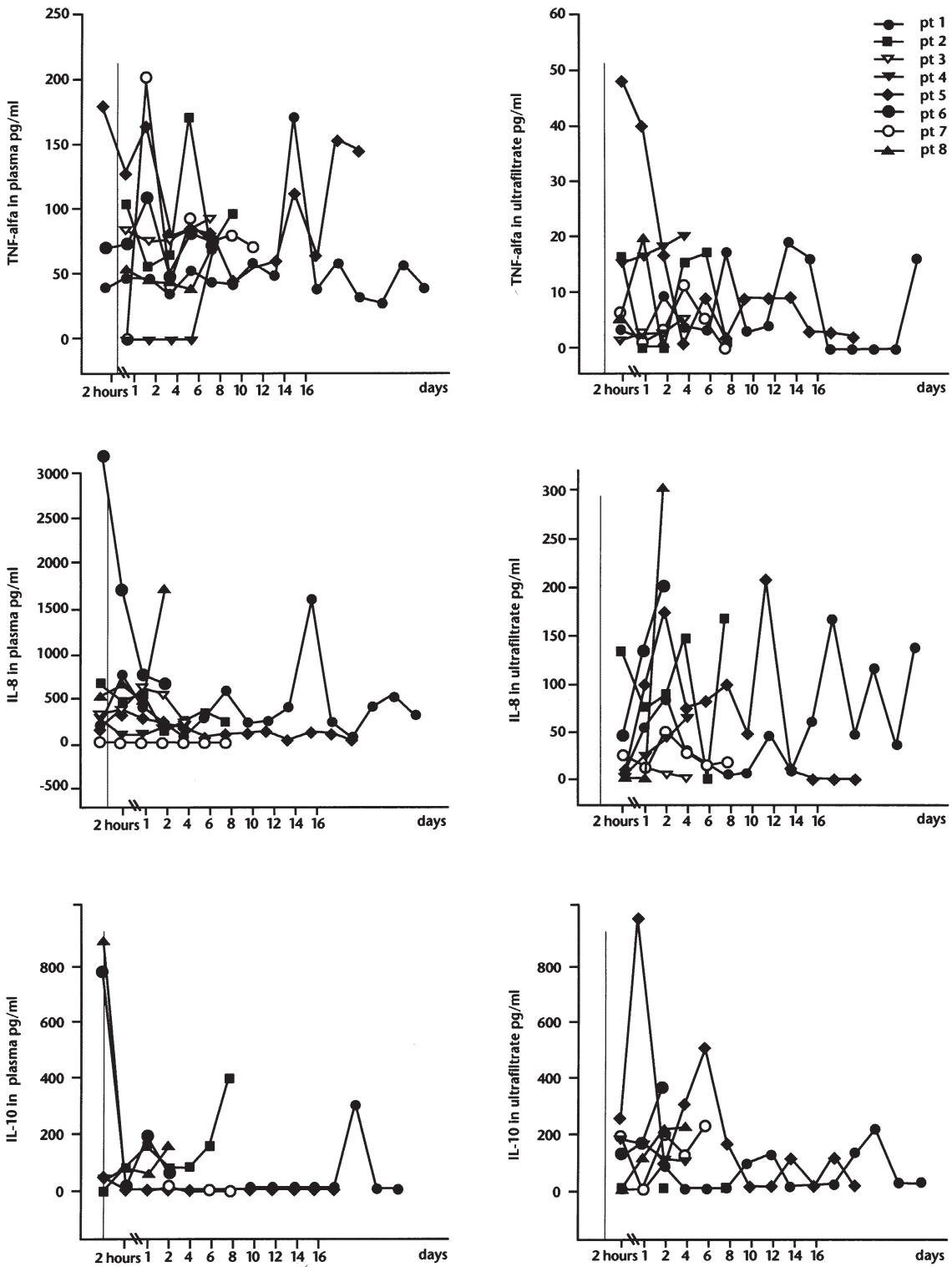
Only in 11 out of 15 patients was IL-10 detectable in the plasma. The median concentration of IL-10 in the plasma before CVVHD was 0 pg/ml (25 and 75% quartiles 0 and 104 pg/ml, range 0–1000 pg/ml). IL-10 was excreted in the ultrafiltrate. Two hours after the start of CVVHD the median IL-10 concentration in the ultrafiltrate was 180 pg/ml (range 0–800 pg/ml) compared with 95 pg/ml (range 0–380 pg/ml) at the end of the study.

The complement split products C3d, C4d and MBL were within reference values and were not influenced by the CVVHD at any time (Fig. 2).

The integrin adhesion molecules CD11a, CD18 and CD 16 on granulocytes were below reference values before the start of CVVHD, whereas CD11b was above and CD44 was within the normal range. We observed no significant change in any of these molecules during CVVHD. The median channel value of the selectin adhesion molecule CD62L decreased from 416 (25 and 75% quartiles 336 and 491) before the start of CVVHD to 360 (25 and 75% quartiles 340 and 463) and 389 (25 and 75% quartiles 330 and 491) 2 and 24 h later (not significant).

The lymphocyte subpopulations CD4 and CD8 were below reference values before the start of CVVHD, and did not change during CVVHD. The percentage of CD3⁺ T lymphocytes was 38.2% (25 and 75% quartiles 25 and 44%)

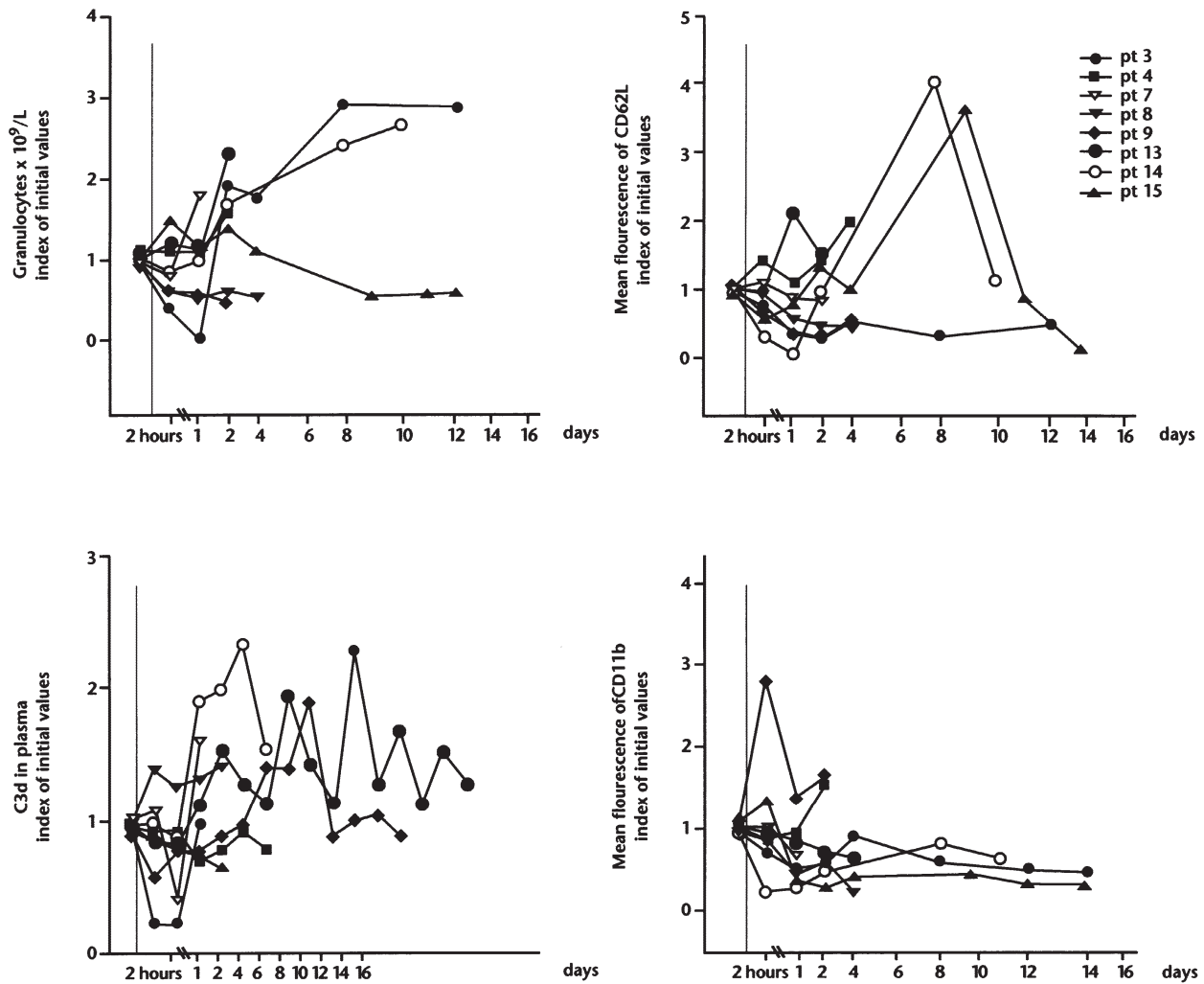
Figure 1



The concentration of the proinflammatory cytokines tumour necrosis factor (TNF)- α and interleukin (IL)-8 and the anti-inflammatory cytokine

IL-10 in plasma and ultrafiltrate for eight patients. \uparrow Start of continuous veno-venous haemodiafiltration.

Figure 2



The number of granulocytes, C3d in plasma, and the adhesion molecules CD62L and CD11b on granulocytes in relation to continuous veno-venous haemodiafiltration (CVVHD) for eight patients.

The values are an index of the initial value before CVVHD. ↑Start of CVVHD.

before CVVHD, but increased significantly to 44.8% (25 and 75% quartiles 35 and 50%) after 2 h of CVVHD ($P < 0.05$).

Discussion

We did not observe any increased production of proinflammatory or anti-inflammatory cytokines during CVVHD, which is in contrast to the increased production of cytokines that occurs after CPB and haemodialysis [5,6]. The presence of the proinflammatory cytokines TNF- α and IL-8 in the peripheral blood of all septic patients and the presence of the anti-inflammatory cytokine IL-10 in some of the patients are in accordance with findings from other investigations [9,10]. The present study shows that not only the

proinflammatory cytokines, but also the anti-inflammatory cytokines are excreted in the ultrafiltrate. The amount of TNF- α excreted in the ultrafiltrate varies widely. Bellomo *et al* [11] observed a daily excretion of TNF- α of 15.9 mg with CVVHD, whereas Sanders *et al* [12] measured an excretion of only 1.7 mg/48 h with continuous arteriovenous haemofiltration. Although the cytokines in the present study were excreted at low concentrations in the ultrafiltrate, the plasma concentrations did not decrease. This is in accordance with the findings of Bellomo *et al* [11] and Sanders *et al* [12], and those of a study by Tønnesen *et al* [9] with continuous arteriovenous haemofiltration. In addition to ultrafiltration, the polyacrylonitrile filters might adsorb

not only complement mediators [2], but also cytokines, especially TNF and IL-6 [13]. The lack of a decrease in the plasma levels of cytokines during CVVHD might be due to a minor activation of the extracorporeal circulation that is neutralized by adsorption and excretion into the ultrafiltrate. So far, however, the clinical significance of the limited removal of proinflammatory and anti-inflammatory cytokines in the ultrafiltrate remains unknown.

If the white blood cells, especially the granulocytes, are activated they will increase the expression of adhesion molecules on their surface. The adhesion molecules are necessary for the adhesion and migration through endothelium into the tissues of activated cells. Two families of adhesion molecules are necessary for the adhesion and migration of granulocytes through endothelium into tissues, namely the integrin and the selectin family. The CD11a-c/CD18 adhesion molecules are the most important integrins, whereas CD62L is the most important member of the selectin family. CD44 is the most important member of a third adhesion molecule family termed the homing-associated cell adhesion molecules. Because the adhesion molecules enable activated cells to migrate into tissues, they play a key role in the development of adult respiratory distress syndrome and MODS. In addition, any major activation of the granulocytes in particular will be reflected in a change in the expression of adhesion molecules on their surface.

Several studies have demonstrated an increased expression of CD18 and CD11b adhesion molecules on granulocytes after 15–30 min of haemodialysis, whereas the expression of CD62L on granulocytes was decreased. After 3 h of haemodialysis the granulocyte adhesion molecules were nearly normalized [14,15]. To our knowledge the effect of CVVHD on the important adhesion molecules CD11a, CD11b, CD18 and CD44 has not been investigated before. We did not observe any significant changes in the adhesion molecules on granulocytes, but only a tendency to a decrease in CD62L levels 2 h after the start of CVVHD. This is in accordance with a recent study by Kellum *et al* [10] who failed to demonstrate any significant changes in soluble L-selectin during CVVHD.

The percentage of CD3⁺ T lymphocytes increased significantly 2 h after the start of CVVHD. In haemodialyses patients an increased proportion of CD3⁺ T lymphocytes has also been detected [16]. The absence of significant changes in the other lymphocyte subpopulations and in the number of natural killer cells is in contrast to findings with haemodialysis. During haemodialysis a decline in the number of natural killer cells and an increase in the proportion of CD4⁺ lymphocytes have been observed [17].

The initiation of CPB and haemodialysis with use of cuprophane membranes is associated with granulocyto-

penia followed within hours by granulocytosis [3,18]. In contrast, we did not observe any significant changes in the number of granulocytes or lymphocytes in peripheral blood during CVVHD. This may indicate that the granulocytes were activated to lesser extent with CVVHD than they are with CPB and haemodialysis.

The elevated serum cortisol level in these critically ill patients is in accordance with that found in other investigations [19]. The serum cortisol level did not increase during CVVHD, however. Thus no endocrine stress response was elicited by CVVHD.

Extracorporeal circulation in the form of CPB activates the complement system [1]. Several studies have also demonstrated an activation of the complement system during haemodialysis, although to a lesser degree. Andreassen *et al* [20] measured complement factors in the ultrafiltrate and a reduction in C3a and C3b in the blood during haemofiltration. In addition, it has been shown [2] that complement factors are adsorbed into the polyacrylonitrile filter used for CVVHD. In the present study we observed neither an activation of the complement system, nor a decrease in the levels of complement factors in the blood. With the polyacrylonitrile filters a minor activation of the complement system might be neutralized by adsorption into the filter and excretion in the ultrafiltrate.

The large intraindividual and interindividual variation in the measured immunological parameters has also been observed in other studies [10,11]. The hypothesis that CVVHD induces an activation of the immune system with release of proinflammatory cytokines followed by a decrease in granulocyte activation as assessed by the expression of adhesion molecules was not confirmed in the present study. The heterogenic patient population, with different underlying diseases and various durations of illness before the start of CVVHD, might have contributed to the large variation in the measured immunological parameters.

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