

# Cytokine Removal during Continuous Hemofiltration in Septic Patients

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**Abstract.** A potential application of the continuous renal replacement therapies is the extracorporeal removal of inflammatory mediators in septic patients. Cytokine elimination with continuous renal replacement therapies has been demonstrated in several clinical studies, but so far without important effects on their serum concentrations. Improved knowledge of the cytokine removal mechanisms could lead to the development of more efficient treatment strategies. In the present study, 15 patients with septic shock and acute renal failure were observed during the first 24 h of treatment with continuous venovenous hemofiltration (CVVH) with an AN69 membrane. After 12 h, the hemofilter was replaced and the blood flow rate ( $Q_B$ ) was switched from 100 ml/min to 200 ml/min or *vice versa*. Pre- and postfilter plasma and ultrafiltrate concentrations of selected inflammatory and anti-inflammatory cytokines were measured at several time points allowing the cal-

ulation of a mass balance. Cytokine removal was highest 1 h after the start of CVVH and after the change of the membrane (ranging from 25 to 43% of the prefilter amount), corresponding with a significant fall in the serum concentration of all cytokines. The inhibitors of inflammation were removed to the same extent as the inflammatory cytokines. Adsorption to the AN69 membrane appeared to be the main clearance mechanism, being most pronounced immediately after installation of a new membrane and decreasing steadily thereafter, indicating rapid saturation of the membrane. A  $Q_B$  of 200 ml/min was associated with a 75% increase of the ultrafiltration rate and a significantly higher convective elimination and membrane adsorption than at a  $Q_B$  of 100 ml/min. The results indicate that optimal cytokine removal with CVVH with an AN69 membrane could be achieved with a combination of a high  $Q_B$ /ultrafiltration rate and frequent membrane changes.

Sepsis is induced by local or systemic release of microorganism components, leading to the production of proinflammatory cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and IL-6. They initiate an inflammatory cascade mediated by several cell lines and involving the complement, coagulation, and fibrinolytic systems (1,2). These phenomena are counterbalanced by a complex system of naturally occurring inhibitors of inflammation: Anti-inflammatory cytokines such as IL-10, soluble cytokine receptors such as soluble TNF receptors (sTNFR-I and sTNFR-II), and cytokine receptor antagonists such as IL-1 receptor antagonist (IL-1ra). Sepsis is characterized by release of excessive amounts of proinflammatory cytokines in the circulation, leading to a generalized and uncontrolled host response, overwhelming the natural inhibitors of inflammation (3).

It has been hypothesized that this excessive inflammatory response could be downregulated with the continuous renal replacement therapies (CRRT), by nonspecific extracorporeal

removal of cytokines and other mediators (4). *In vitro* studies indicate that cytokine removal with current synthetic membranes is more convective than diffusive (5–8). Moreover, these membranes have a high adsorptive capacity for cytokines (5–10). The extracorporeal removal of inflammatory mediators has been confirmed in clinical studies (11–20), but few reported important effects on their plasma concentration (19,20). Knowledge of the relative contribution of membrane adsorption and convective elimination could lead to more efficient removal strategies, but so far a detailed quantitative assessment of adsorption and convection has not been performed *in vivo*. Furthermore, little is known about the concomitant removal of inhibitors of inflammation. If these are removed to the same extent as the proinflammatory cytokines, the pro/anti-inflammatory balance might remain unaffected. Finally, cytokines exert their effects at the tissue level and the significance of their presence in the circulation is undefined. Whether removal of cytokines from the systemic circulation has a beneficial effect on the short-term hemodynamic status and respiratory function of the septic patient is at present unclear.

To address these questions, a prospective trial was conducted in critically ill patients with sepsis and acute renal failure, treated with continuous venovenous hemofiltration (CVVH). The removal of selected inflammatory cytokines and inhibitors of inflammation was studied. To determine the relative importance of convective removal and membrane adsorp-

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tion, mass balance studies were performed, with comparison of different flow rates and analysis of the effect of replacement of the AN69 hemofilter. In addition, the influence of CVVH on hemodynamic status and respiratory function was examined.

## Materials and Methods

### Study Population

The study was approved by the Ethical Committee on Human Research of the University Hospital of Gent. A total of 15 consecutive patients with septic shock, as defined by the ACCP/SCCM Consensus Conference (21), and acute renal failure, as defined by a rise of the serum creatinine  $>2$  mg/100 ml and a urine output  $<20$  ml/h despite volume correction and intensive diuretic therapy, was studied during the first 24 h of treatment with CVVH. Informed consent was obtained from a close family member. Clinical, hematologic, and biochemical data were collected for calculation of the APACHE II score and creatinine clearance.

### Studies of Hemodynamic and Respiratory Function

All patients were mechanically ventilated and had an arterial line and a Swan-Ganz thermodilution catheter connected to a continuous cardiac output measurement device (Vigilance, Baxter, McGaw Park, IL). Heart rate, mean arterial BP (MABP), cardiac output (CO), systemic vascular resistance (SVR), pulmonary artery occlusion pressure (PAOP), central venous pressure, pulmonary artery pressure, and central body temperature were measured 4 h, 2 h, and immediately before the start of CVVH, and every 2 h after the start of treatment. Arterial blood gasses were analyzed every 4 h. Inspired oxygen fraction ( $\text{FiO}_2$ ), positive end expiratory pressure, dose of vasopressor and inotropic agents, and administered colloid volume were recorded.

### CVVH Procedure

Vascular access was obtained via a double-lumen catheter (Medcomp, Harleysville, PA) in the subclavian or femoral vein. CVVH was performed with an AN69 hollow fiber hemofilter (effective surface area  $0.9$  m<sup>2</sup>) (Multiflow 100, Hospal, France), connected to a flow-controlled blood roller pump (BSM22-VPM, Hospal, France). The AN69 membrane has an *in vivo* cutoff value of 35 to 40 kD (22). The blood flow rate ( $Q_B$ ) was randomly set at 100 or 200 ml/min. The ultrafiltration rate was not pump-controlled. An electrolyte solution (Clearflex D6, Bieffe Medital, Grosotto, Italy) was warmed to 38°C and infused in a postdilution mode to maintain a zero fluid balance. The circuit was anticoagulated with a continuous unfractionated heparin infusion in the afferent limb. The activated clotting time (ACT) was measured every hour, and the heparin infusion was adjusted to maintain the ACT between 160 and 180 s. After 12 h, the hemofilter was replaced and  $Q_B$  was switched from 100 to 200 ml/min or *vice versa*.

### Cytokine Analyses

Simultaneous samples of filter inlet and outlet blood and ultrafiltrate (from the drainage tubing within 10 cm of the hemofilter) were drawn at  $t$  (hours) = 0, 1, 6, 12, 13, 18, 24 and immediately transported on ice to the laboratory, where they were centrifuged at 2200 rpm for 10 min, frozen, and stored at  $-70^\circ\text{C}$  until assay.

TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were analyzed with the Immulite solid-phase, two-site chemiluminescent enzyme immunometric assay from EURO/DPC Ltd. (Glyn Rhonwy, United Kingdom) on an Immulite automated analyzer (DPC, Los Angeles, CA), according to the manufacturer's specifications. Detection limits and expected values are 4 and  $<25$  pg/ml for TNF- $\alpha$ , 5 and  $<12$  pg/ml for IL-1 $\beta$ , and 4 and

$<28$  pg/ml for IL-6, respectively. IL-10 was analyzed with the Milenia end point enzyme immunometric assay in a microplate format (DPC Biermann, Bad Neuheim, Germany). Detection limit and expected values are 10 and  $<24$  pg/ml. IL-1ra was measured with the quantitative sandwich enzyme immunoassay obtained from R&D Systems Europe (Abingdon, Oxon, United Kingdom). Detection limit and expected values are 20 and 100 to 1500 pg/ml. sTNFR-I and sTNFR-II were measured with the Twin ELISA test kit, which is a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle (HyCult Biotechnology, Uden, The Netherlands). Detection limits and expected values are for both receptors 25 and 1000 to 4000 pg/ml. For the measurement of the plasma samples, plasma-containing standards were used.

The mass balance of the cytokines at each time point was calculated as follows:

$$Q_I = Q_B(1 - \text{hematocrit}), Q_O = Q_I - Q_{UF},$$

$$M_I = Q_I \times C_I, M_O = Q_O \times C_O$$

$$M_{TR} = M_I - M_O, M_{UF} = Q_{UF} \times C_{UF}, M_{AD} = M_{TR} - M_{UF}$$

$$SC = 2 \times C_{UF}/C_I + C_O,$$

where  $Q_I$  is inlet plasma flow rate (ml/min);  $Q_O$  is outlet plasma flow rate (ml/min);  $Q_{UF}$  is ultrafiltrate flow rate (ml/min);  $M_I$  is inlet mass rate (pg/min);  $C_I$  is concentration in inlet plasma (pg/ml);  $M_O$  is outlet mass rate (pg/min);  $C_O$  is concentration in outlet plasma (pg/ml);  $M_{TR}$  is total mass removal rate (pg/min);  $M_{UF}$  is mass removal rate by ultrafiltration (pg/min);  $C_{UF}$  is concentration in ultrafiltrate (pg/ml);  $M_{AD}$  is mass removal rate by membrane adsorption (pg/min); and SC is the sieving coefficient.

### Statistical Analyses

Data for each time point are expressed as mean  $\pm$  SEM. For the evaluation of the hemodynamic parameters, one-way repeated measures ANOVA and the Student–Newman–Keuls test for pairwise multiple comparison were performed. For the evaluation of the cytokine plasma concentrations, ANOVA and a Wilcoxon signed rank test were used. The total cytokine removal was analyzed with a Wilcoxon signed rank test. To study the impact of the  $Q_B$ ,  $M_{UF}$  and  $M_{AD}$  were compared for  $Q_B = 100$  ml/min and  $Q_B = 200$  ml/min, and the results for the corresponding time points after use of a new hemofilter ( $t = 1$  and 13,  $t = 6$  and 18, and  $t = 12$  and 24) were pooled. Analysis was done with a Wilcoxon signed rank test. Differences were considered statistically significant at  $P < 0.05$ .

## Results

### Patient Characteristics

The clinical characteristics are summarized in Table 1. The overall mean ACT was  $170.5 \pm 0.36$  s. The ACT did not change significantly throughout the study period.

### Studies of Hemodynamic and Respiratory Function

At baseline, all patients had a hemodynamic profile, characteristic for hyperdynamic shock, with a low MABP, a high CO and a low SVR. Most patients required vasopressor (13 of 15) and/or inotropic (9 of 15) drugs. The start of CVVH was associated with a significant and sustained fall in CO and a rise in SVR (Figure 1). A trend toward a lower PAOP was observed (Figure 1). The other hemodynamic parameters did not change significantly. Central body temperature, the dopamine, dobut-

Table 1. Clinical characteristics of the study population<sup>a</sup>

Case	Gender	Age	Origin of Sepsis	Microbiology	APII	C <sub>Cr</sub> (ml/min)	Outcome	
							ICU	Hospital
1	M	70	Pulmonary	<i>Serratia marcescens</i>	24	8	Died	
2	M	66	Pulmonary	Negative <sup>b</sup>	34	5.4	Died	
3	F	79	Abdominal	Negative <sup>b</sup>	23	1.6	Survived	Survived
4	M	69	Abdominal	<i>Candida albicans</i>	30	0	Survived	Died
5	M	60	Unknown	Negative <sup>b</sup>	41	0	Died	
6	F	73	Pulmonary	<i>Pseudomonas aeruginosa</i>	26	0	Died	
7	M	75	Urinary	<i>Escherichia coli</i>	35	4.4	Survived	Survived
8	M	37	Abdominal	Negative <sup>b</sup>	26	2.3	Survived	Survived
9	F	66	Ruptured oesophagus	Polymicrobial	23	0	Died	
10	F	56	Necrotizing fasciitis	<i>Streptococcus pyogenes</i>	18	1.3	Survived	Survived
11	F	68	Abdominal	Negative <sup>b</sup>	24	5.3	Died	
12	M	62	Pulmonary	MRSA	29	4.8	Died	
13	F	46	Pulmonary	Negative <sup>b</sup>	25	3.2	Survived	Survived
14	M	36	Abdominal	Polymicrobial	35	1.4	Died	
15	M	35	Sinusitis	<i>Acinetobacter</i>	29	0	Survived	Survived

<sup>a</sup> APII, APACHE II score; C<sub>Cr</sub>, creatinine clearance; ICU, Intensive care unit; MRSA, methicillin resistant *Staphylococcus aureus*.

<sup>b</sup> Cultures of blood or body fluids obtained from sites of suspected infection did not grow any organisms.

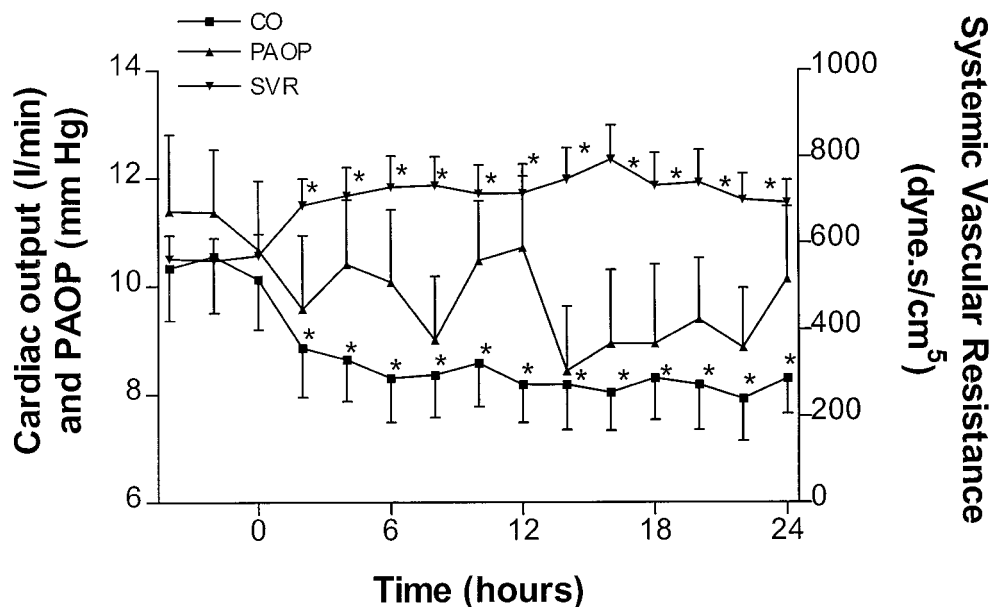


Figure 1. Response of cardiac output (CO), systemic vascular resistance (SVR), and pulmonary artery occlusion pressure (PAOP) to the treatment with continuous venovenous hemofiltration. CO was significantly higher and SVR was significantly lower during hemofiltration than at baseline, as defined by both  $t = 0$  and the mean of  $t = -4$ ,  $t = -2$  and  $t = 0$ . \* $P < 0.05$ . A trend toward a lower PAOP was observed.  $P = NS$ .

amine, epinephrine, and norepinephrine requirements, and the administered colloid volume remained constant throughout the study period.  $PaO_2/FiO_2$  and positive end expiratory pressure did not change during the observation period.

#### Cytokine Plasma Concentrations

TNF- $\alpha$ , IL-6, IL-10, IL-1ra, sTNFR-I, and sTNFR-II levels were above the detection limit in all patients. IL-1 $\beta$  was not

detectable in 10 patients throughout the study period. The plasma concentrations (pg/ml) before the start of CVVH ( $t = 0$ ) were:  $68.4 \pm 17.2$  for TNF- $\alpha$ ;  $16.4 \pm 2.8$  for IL-1 $\beta$ ;  $3997 \pm 2594$  for IL-6;  $495.5 \pm 121.3$  for IL-10;  $13581 \pm 4102$  for IL-1ra;  $19009 \pm 2442$  for sTNFR-I; and  $31165 \pm 2270$  for sTNFR-II. The inlet plasma concentrations of the inflammatory cytokines as well as of the inhibitors of inflammation decreased significantly in the first hour after installation of a

new hemofilter ( $t = 1$  versus  $t = 0$  and  $t = 13$  versus  $t = 12$ ) (Figure 2, A and B). No decreases of a similar consistency were observed at other time points. No changes were observed in the ratios between the serum concentrations of the soluble TNF receptors and of TNF- $\alpha$  and between the serum concentrations of IL-1ra and IL-1 $\beta$  (Figure 3).

**Cytokine Removal**

The mean ultrafiltration volume was  $18.3 \pm 1.6$  L/12 h ( $25.4 \pm 0.7$  ml/min) with  $Q_B = 100$  ml/min, and  $31.9 \pm 3.0$  L/12 h ( $44.3 \pm 1.5$  ml/min) with  $Q_B = 200$  ml/min. TNF- $\alpha$ , IL-6, IL-1ra, sTNFR-I, and sTNFR-II were detectable in the ultrafiltrate of all patients. IL-1 $\beta$  was found only in the ultrafiltrate of the patients with detectable plasma levels. IL-10 could not be detected in any of the ultrafiltrates despite high plasma levels. The overall mean SC were:  $0.16 \pm 0.02$  for TNF- $\alpha$ ;  $0.22 \pm 0.02$  for IL-1 $\beta$ ;  $0.18 \pm 0.01$  for IL-6;  $0 \pm 0$  for

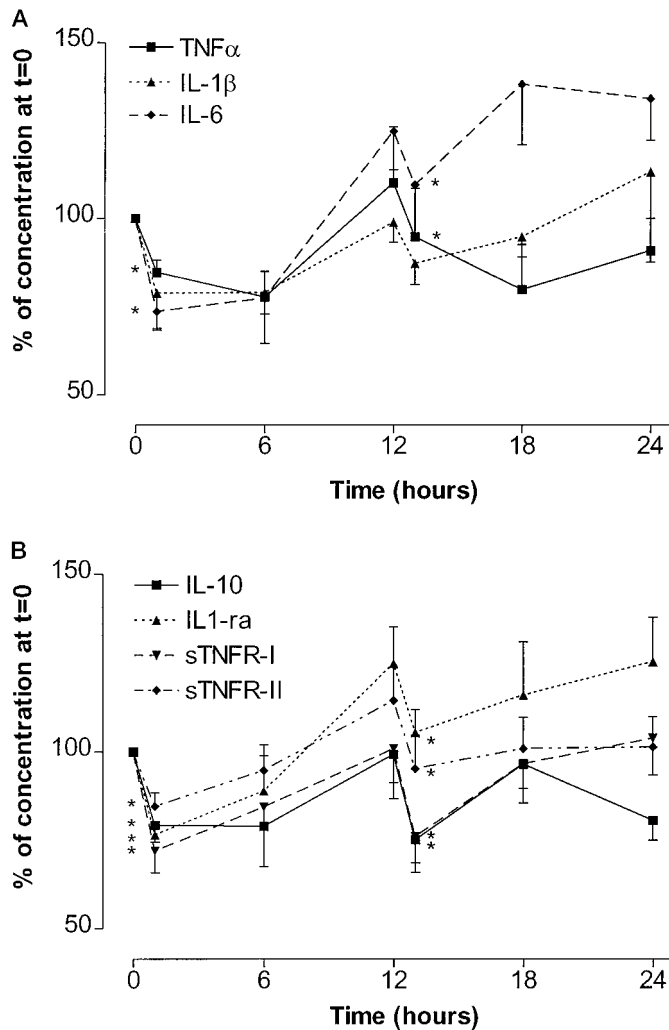


Figure 2. Cytokine plasma concentrations expressed as a percentage of the concentration at  $t = 0$ . A significant fall in the plasma concentrations of all cytokines was observed at  $t = 1$  and at  $t = 13$ . \* $P < 0.05$ . (A) Inflammatory cytokines. No statistical analysis was performed for interleukin-1 $\beta$  (IL-1 $\beta$ ), since it was detected in only five patients. (B) Anti-inflammatory cytokines.

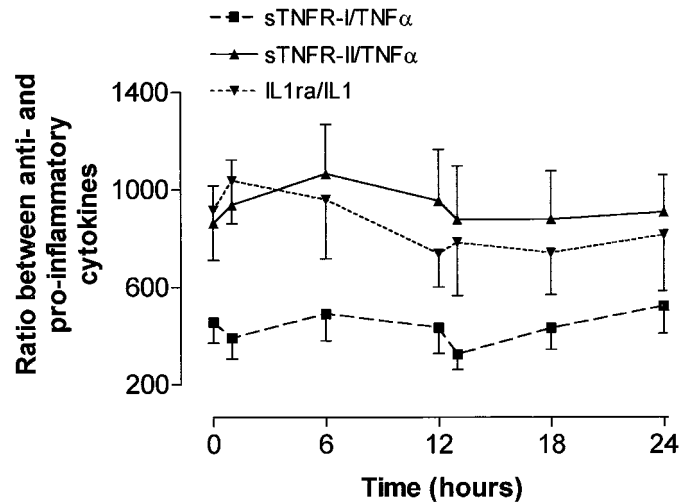
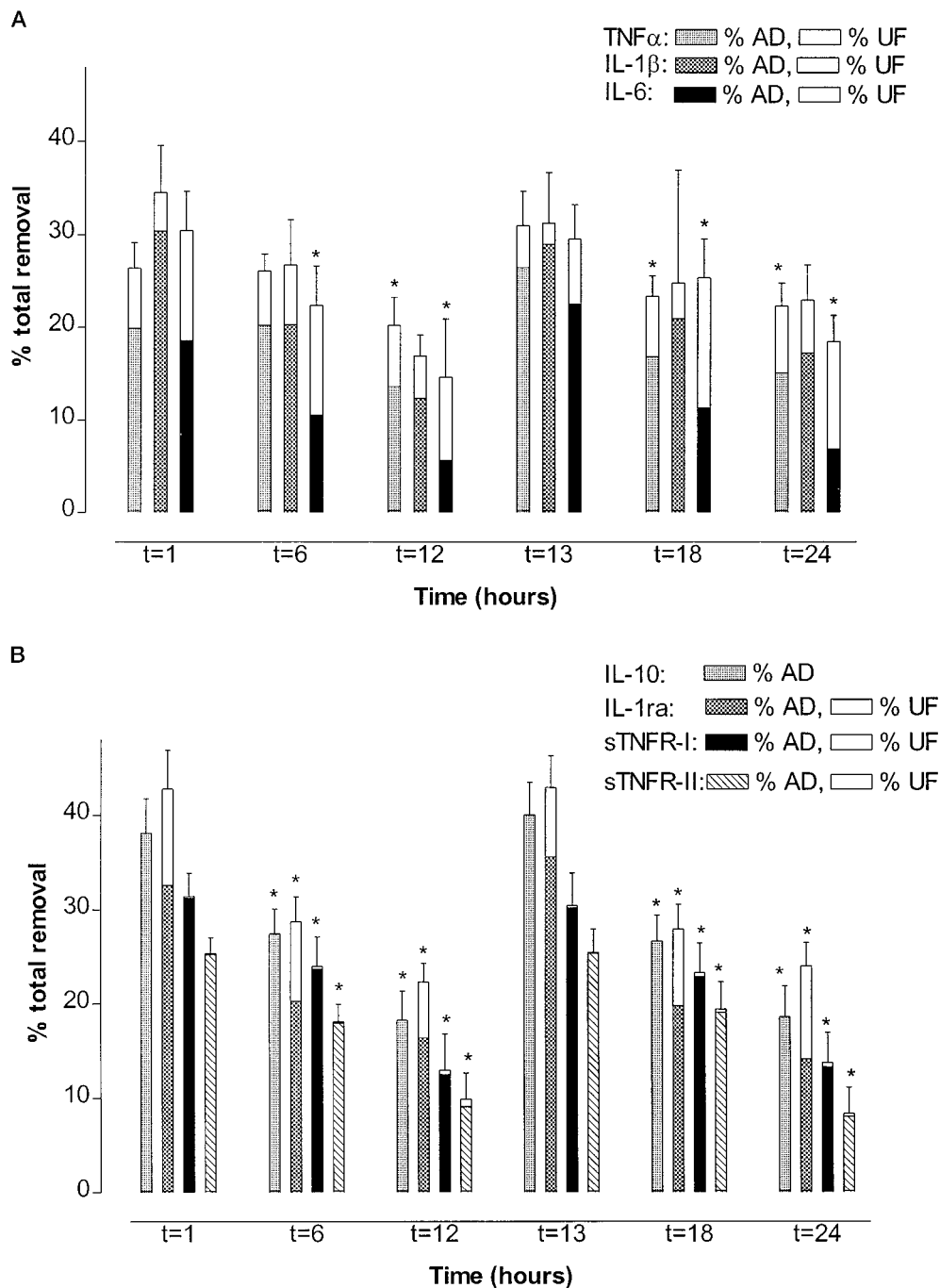


Figure 3. Ratios between the serum concentrations of the soluble tumor necrosis factor (TNF) receptors and of TNF- $\alpha$  and between the serum concentrations of IL-1 receptor antagonist (IL-1ra) and IL-1 $\beta$ . No significant changes in soluble TNF receptor I (sTNFR-I)/TNF- $\alpha$  and sTNFR-II/TNF- $\alpha$  were observed. No statistical analysis was performed for IL-1ra/IL-1 $\beta$ , since IL-1 $\beta$  was detected in only five patients.

IL-10;  $0.28 \pm 0.03$  for IL-1ra;  $0.006 \pm 0.0007$  for sTNFR-I; and  $0.003 \pm 0.0001$  for sTNFR-II.

The  $M_{TR}$ , expressed as a percentage of the  $M_I$ , was highest 1 h after the use of a new hemofilter (at  $t = 1$  and  $t = 13$ ), ranging from  $43.0 \pm 3.3\%$  for IL-1ra at  $t = 13$  to  $25.3 \pm 1.7\%$  for sTNFR-II at  $t = 1$  (Figure 4, A and B). Five hours later (at  $t = 6$  and  $t = 18$ ), total cytokine removal was already significantly lower, varying from  $28.7 \pm 2.7\%$  for IL-1ra at  $t = 6$  to  $18.1 \pm 1.8\%$  for sTNFR-II at  $t = 6$ . After 12 h of membrane use (at  $t = 12$  and  $t = 24$ ), total removal ranged from  $24.0 \pm 2.5\%$  for IL-1ra at  $t = 24$  to  $8.4 \pm 2.8\%$  for sTNFR II at  $t = 24$ . The contributions of convective elimination ( $M_{UF}$ ) and membrane adsorption ( $M_{AD}$ ) were calculated in absolute values (data not shown) and as a percentage of  $M_{TR}$  (Figure 4, A and B). Removal was due mainly to membrane adsorption for all inflammatory cytokines and especially for their inhibitors. Absolute  $M_{UF}$  remained stable over the observation period, whereas absolute  $M_{AD}$  was highest after installation of a new hemofilter and decreased steadily thereafter.  $M_{AD}$ , expressed as a percentage of  $M_{TR}$ , was 100% for IL-10 and nearly 100% for both soluble TNF receptors, at all time points. For the other cytokines, relative  $M_{AD}$  ranged from  $92.6 \pm 7.3\%$  (IL-1 $\beta$ ) to  $61.0 \pm 11.5\%$  (IL-6) at  $t = 1$  and 13, from  $84.4 \pm 9.5\%$  (IL-1 $\beta$ ) to  $44.3 \pm 4.1\%$  (IL-6) at  $t = 6$  and 18, and from  $75.1 \pm 9.5\%$  (IL-1 $\beta$ ) to  $37.0 \pm 9.5\%$  (IL-6) at  $t = 12$  and 24. At the latter two time points, each after 12 h of blood-membrane contact, a negative adsorptive removal rate was found for TNF- $\alpha$ , IL-1 $\beta$ , IL-6, sTNFR-I, and sTNFR-II in a few patients, indicating release from the membrane of previously bound cytokine.

The  $M_{UF}$  at  $Q_B = 200$  ml/min as compared to  $Q_B = 100$  ml/min was significantly higher for all cytokines, except for



**Figure 4.** The total amount of cytokine removed ( $M_{TR}$ ), expressed as a percentage of the amount present in the prefilter plasma ( $M_i$ ). The removed amount at  $t = 6$  and  $18$  and at  $t = 12$  and  $24$  is significantly lower than at  $t = 1$  and  $t = 13$ . \* $P < 0.05$ . For each value, the relative contribution of adsorption (% AD, hatched bars) and ultrafiltration (% UF, open bars) is indicated. (A) Inflammatory cytokines. No statistical analysis was performed for IL-1 $\beta$ , since it was detected in only five patients. (B) Anti-inflammatory cytokines.

IL-10, for sTNFR-I at  $t = 12/24$ , and for IL-1ra at  $t = 12/24$  (Table 2). The  $M_{AD}$  was also significantly higher with the higher  $Q_B$ , with TNF- $\alpha$  at  $t = 12/24$  and IL-10 at  $t = 12/24$  as exceptions (Table 2).

## Discussion

The inflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, are essential in the local host response to a microbial

challenge. However, their unbalanced production and systemic release may lead to diffuse tissue injury and the development of multiple organ failure (1–3). Several studies have found correlations between circulating levels of inflammatory cytokines and outcome of patients with sepsis (23). These mediators have therefore received attention as potential targets in the treatment of sepsis. Clinical trials with monoclonal antibodies and other anticytokine strategies have so far failed to show a

Table 2. Comparison of the mass removal rate by ultrafiltration ( $M_{UF}$ ) and by adsorption ( $M_{AD}$ ) for the two blood flow rates ( $Q_B$ )<sup>a</sup>

Cytokine	Time <sup>b</sup>	$M_{UF}$ (pg/min) $Q_B = 100$ ml/min	$M_{UF}$ (pg/min) <sup>c</sup> $Q_B = 200$ ml/min	$M_{AD}$ (pg/min) $Q_B = 100$ ml/min	$M_{AD}$ (pg/min) $Q_B = 200$ ml/min
TNF- $\alpha$	$t = 1/13$	145.7 $\pm$ 16.0	221.9 $\pm$ 44.6*	1343.3 $\pm$ 388.2	2076.5 $\pm$ 497.0*
	$t = 9/18$	159.8 $\pm$ 23.7	284.5 $\pm$ 62.4*	749.6 $\pm$ 171.0	1524.9 $\pm$ 310.6*
	$t = 12/24$	138.3 $\pm$ 21.8	315.0 $\pm$ 54.2*	983.3 $\pm$ 242.9	1000.5 $\pm$ 303.4
IL-1 $\beta$	$t = 1/13$	91.3 $\pm$ 58.2	131.1 $\pm$ 86.3	331.6 $\pm$ 67.6	417.7 $\pm$ 123.7
	$t = 9/18$	89.0 $\pm$ 65.8	151.0 $\pm$ 100.3	226.5 $\pm$ 78.4	390.6 $\pm$ 143.1
	$t = 12/24$	99.3 $\pm$ 69.7	106.4 $\pm$ 106.4	192.5 $\pm$ 40.0	329.8 $\pm$ 157.0
IL-6	$t = 1/13$	13579 $\pm$ 6029	31887 $\pm$ 20941*	83421 $\pm$ 55676	132355 $\pm$ 73592*
	$t = 9/18$	21388 $\pm$ 10294	38409 $\pm$ 20735*	30800 $\pm$ 13429	68688 $\pm$ 31518*
	$t = 12/24$	19833 $\pm$ 11598	26668 $\pm$ 18383*	48636 $\pm$ 44722	91712 $\pm$ 47601*
sTNFR-I	$t = 1/13$	1928 $\pm$ 565	3242 $\pm$ 1067*	319835 $\pm$ 43965	514455 $\pm$ 74705*
	$t = 9/18$	3615 $\pm$ 1242	5981 $\pm$ 2271*	323228 $\pm$ 50420	452200 $\pm$ 64352*
	$t = 12/24$	4185 $\pm$ 1205	5205 $\pm$ 2232	170715 $\pm$ 44947	379496 $\pm$ 93805*
sTNFR-II	$t = 1/13$	1865 $\pm$ 48	3163 $\pm$ 135*	602245 $\pm$ 79116	900674 $\pm$ 109945*
	$t = 9/18$	2042 $\pm$ 82	3454 $\pm$ 161*	468669 $\pm$ 77832	671553 $\pm$ 45149*
	$t = 12/24$	1800 $\pm$ 65	3295 $\pm$ 121*	190996 $\pm$ 74328	485838 $\pm$ 89494*
IL-1ra	$t = 1/13$	51782 $\pm$ 20873	100356 $\pm$ 41529*	266462 $\pm$ 69479	402523 $\pm$ 112750*
	$t = 9/18$	65531 $\pm$ 16943	139372 $\pm$ 45081*	193912 $\pm$ 68635	278982 $\pm$ 79789*
	$t = 12/24$	64605 $\pm$ 17181	87370 $\pm$ 23687	159072 $\pm$ 45199	296765 $\pm$ 123296*
IL-10	$t = 1/13$	0 $\pm$ 0	0 $\pm$ 0	10523 $\pm$ 2510	17164 $\pm$ 4371*
	$t = 9/18$	0 $\pm$ 0	0 $\pm$ 0	8660 $\pm$ 2464	14028 $\pm$ 3625*
	$t = 12/24$	0 $\pm$ 0	0 $\pm$ 0	6632 $\pm$ 1729	9612 $\pm$ 3218

<sup>a</sup> TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-1 $\beta$ , interleukin-1 $\beta$ ; sTNFR-I and sTNFR-II, soluble tumor necrosis factor receptor I and II, respectively; IL-1ra, interleukin-1 receptor antagonist.

<sup>b</sup> The results for the corresponding time points after use of a new hemofilter ( $t = 1/13$ ,  $t = 9/18$ , and  $t = 12/24$ ) are pooled.

<sup>c</sup> Both  $M_{UF}$  and  $M_{AD}$  are significantly higher at a  $Q_B$  of 200 ml/min versus a  $Q_B$  of 100 ml/min (\* $P < 0.05$ ). No statistical analysis was performed for IL-1 $\beta$ , since it was detected in only five patients.

clear survival benefit (24). Blockade of only one mediator, however, may be insufficient to downregulate the inflammatory response.

The application of the CRRT to remove nonspecifically inflammatory mediators from the circulation therefore offers an attractive alternative. It has been suggested that the high endogenous production and clearance rate of the cytokines and their short half-life in the circulation preclude important extracorporeal removal (25). Several studies, examining removal of inflammatory mediators with CRRT in septic patients, reported the presence of mediators in the ultrafiltrate or a positive plasma clearance (11–20), but to our knowledge only two studies showed a significant lowering of their plasma concentrations (19,20). In the present study, a strikingly consistent fall in the plasma concentrations of all cytokines was found, 1 h after the start of CVVH as well as 1 h after the replacement of the AN69 hemofilter. At these time points, about one-third of the amount of cytokine present in the prefilter plasma was removed by passage through the hemofilter. For most cytokines, the fall in the plasma concentration was not sustained, corresponding with a progressive decrease in the removal rate with longer blood-membrane contact.

One of the objectives of the present study was to quantify convective transport and membrane adsorption and to deter-

mine their relative contribution to total cytokine removal, to delineate more efficient removal strategies. If removal is predominantly convective, the efficiency could be increased by inducing a higher ultrafiltration rate. If elimination is mainly due to adsorption, more benefit could be expected from frequent membrane changes or the use of alternating parallel membrane systems.

Convective removal of a solute depends on transmembrane pressure, on molecular weight (MW) and structure of the solute, as well as on the cutoff point of the membrane, which averages 35 to 40 kD for the AN69 membrane. TNF- $\alpha$  is present in the circulation as a biologically active trimer with a MW of 54 kD, together with the nonactive monomer of 17 kD (23). The TNF- $\alpha$  detected in the ultrafiltrate by the immunoassay is probably to a large extent the monomer, since only this form can pass the average pore size of the AN69 membrane. The MW values of IL-1 $\beta$  (17 kD), IL-6 (26 kD), and IL-1ra (17 to 22 kD) allow passage across the AN69 hemofilter, and these cytokines were indeed detected in the ultrafiltrate. None of the ultrafiltrate samples contained IL-10, which has a MW of 35 to 40 kD. sTNFR-I and sTNFR-II are the extracellular domains of the respective TNF receptors and have a MW of 30 and 33 kD. Their SC values were very low. The rate of convective cytokine removal remained stable during the 12-h hemofiltration at

a particular  $Q_B$ . Convective transport, however, represented only a relatively small fraction of total cytokine removal.

In the present study, membrane adsorption emerged as the main clearance mechanism for cytokines. *In vitro* studies have demonstrated that the synthetic membranes, and especially the AN69 membrane, avidly adsorb cytokines and complement components (5–8, 26,27). The AN69 membrane consists of a copolymer of acrylonitrile and sodium methallylsulfonate with a homogeneous, dense, and symmetric structure and a strong hydrophilicity. Due to these physicochemical properties, it has the consistency of a hydrogel. In contrast to the microporous asymmetric membranes, all polymeric chains over the entire breadth of the AN69 membrane are in contact with the blood compartment and thus accessible for adsorption. Adsorption is further favored by the strong negative charges provided by the methallylsulfonate groups. Clinical studies have confirmed that important adsorption also occurs *in vivo* (17,18,28). We found adsorption to be most pronounced immediately after the installation of a new hemofilter (at  $t = 1$  and  $t = 13$ ) with a steady decrease thereafter. After 12 h of blood-membrane contact (at  $t = 12$  and  $t = 24$ ), negative adsorptive removal was found for some cytokines in a few patients, indicating saturation of the membrane and release of previously bound cytokine. For the whole group, net adsorption remained positive.

$Q_B$  was randomly set at 100 or 200 ml/min at the start of CVVH and switched to 200 or 100 ml/min, respectively, after 12 h of hemofiltration. The ultrafiltration rate was 75% higher with the  $Q_B$  of 200 ml/min as compared to 100 ml/min. The  $M_{UF}$  of the cytokines increased more or less in parallel with the ultrafiltration rate. However, the removal rate by adsorption was also significantly higher when the  $Q_B$  was set at 200 ml/min. The higher convective driving force may increase the surface area accessible for adsorption, by pushing the molecules deeper into the hydrogel. In this respect, it is known that only minimal adsorption occurs when the ultrafiltrate line is clamped (29). Additionally, a higher load of cytokines is presented at the membrane per unit of time at higher blood flows and may thus also favor the adsorption process. Since we did not control ultrafiltration rate independently of  $Q_B$ , we cannot distinguish between their respective importance.

The present results indicate that optimal cytokine removal can be achieved with a combination of a high  $Q_B$ /ultrafiltration rate and frequent membrane changes. Frequent membrane changes, however, are impractical, labor-intensive, and expensive. Alternative solutions might be examined such as parallel alternating membrane systems or cartridges containing adsorptive microparticles. Whether a stable lowering of the cytokine plasma concentrations could be achieved with these measures remains to be determined.

CVVH indiscriminately removes all solutes that can pass across the membrane or adsorb to the membrane. In a few studies, the removal of inhibitors of inflammation has been investigated. Van Bommel *et al.* found an increase in the ratio between the soluble TNF receptors and TNF- $\alpha$  upon the start of continuous hemofiltration (13). In another study, no reduction in the plasma levels of various pro- and anti-inflammatory cytokines was achieved with CVVH (11). In the present study,

the removal rates of the inhibitors of inflammation paralleled those of the inflammatory cytokines. Furthermore, no significant changes in the ratio between the serum concentrations of TNF- $\alpha$  and its soluble receptors and between the serum concentrations of IL-1 $\beta$  and IL-1ra were found. The complex interplay between pro- and anti-inflammatory mediators, however, might not be entirely represented by a simple ratio between selected cytokines and their inhibitors. The present results therefore do not allow solid conclusions about the effect of CVVH on the pro/anti-inflammatory balance in sepsis.

Another objective of the study was the assessment of the impact of CVVH on respiratory and hemodynamic parameters during the first 24 h of treatment. No changes in oxygenation were observed. The hemodynamic response to CVVH was characterized by a stable MABP and heart rate. CO fell and SVR increased immediately after the start and remained stable thereafter. PAOP decreased, but this trend was not statistically significant. The fall in CO and the rise in SVR coincided with a decrease of the plasma concentration of all cytokines. The hemodynamic parameters did not change after the replacement of the hemofilter at  $t = 12$ , which was followed by a comparable fall of the cytokine plasma concentrations. Therefore, it appears improbable that the observed hemodynamic changes are caused by or related to an alteration in the inflammatory status. Although in our patients a zero fluid balance was aimed for, mild hypovolemia may have been induced by the extracorporeal circulation of blood and the time delay between ultrafiltration and infusion of the substitution fluid, and thus may have been responsible for the observed rise in SVR and fall in CO. Only a trend toward a lower PAOP was noted, but this parameter is a rough measure of left ventricular end-diastolic pressure and may not reflect left ventricular preload in sepsis and adult respiratory distress syndrome.

In conclusion, the present study demonstrates that CVVH with an AN69 membrane in septic patients removes cytokines from the circulation, mainly by membrane adsorption. The current data should not be generalized to other membranes, since their physicochemical characteristics and adsorptive capacities might be very different. Inhibitors of inflammation were removed to the same extent as the inflammatory cytokines, which may explain the lack of influence on short-term hemodynamics and respiratory function. Alternatively, the fall in the cytokine plasma concentrations obtained in our study might have been too small or too short-lived to produce a measurable effect on hemodynamic and respiratory parameters.

CVVH is a valuable tool in the treatment of acute renal failure in the intensive care unit patient. More work is required to determine whether optimization of the cytokine removal strategy leads to a sustained decrease of their plasma concentrations and improves the outcome of the patients.

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