ELSEVIER

European Journal of Cardio-thoracic Surgery 17 (2000) 77–83

EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY

www.elsevier.com/locate/ejcts

Modified ultrafiltration lowers adhesion molecule and cytokine levels after cardiopulmonary bypass without clinical relevance in adults ☆

Jürg Grünenfelder[a], Gregor Zünd[a,*], Andreina Schoeberlein[a], Friedrich E. Maly[b], Ulrich Schurr[a], Silvia Guntli[c], Katja Fischer[c], Marko Turina[a]

[a]*Clinic for Cardiovascular Surgery, University Hospital, Rämistrasse 100, 8091 Zürich, Switzerland*
[b]*Division of Clinical Chemistry, University Hospital, Rämistrasse 100, 8091 Zürich, Switzerland*
[c]*Division of Surgical Research, University Hospital, Rämistrasse 100, 8091 Zürich, Switzerland*

Received 7 September 1999; received in revised form 19 November 1999; accepted 29 November 1999

Abstract

Objective: Cardiac surgery with cardiopulmonary bypass (CPB) results in expression of cytokines and adhesion molecules (AM) with subsequent inflammatory response. The purpose of the study was to evaluate the clinical impact of modified ultrafiltration (MUF) and its efficacy in reducing cytokines and AM following coronary artery bypass grafting (CABG) in adults. **Methods**: A prospective randomized study of 97 patients undergoing elective CABG was designed. Fifty patients were operated on using normothermic and 47 patients using hypothermic CPB. The normothermic group was subdivided into a group with modified ultrafiltration ($n = 30$) and a group without MUF ($n = 20$). In the hypothermic group 30 patients received MUF compared to 17 patients serving as controls. MUF was instituted after CPB for 15 min through the arterial and venous bypass circuit lines. Cytokines (IL-6, IL-8, TNF-α, IL-2R) and adhesion molecules (sE-selectin, sICAM-1) were measured preoperatively, pre-MUF, in the ultrafiltrate, 24 h, 48 h and 6 days after surgery by chemiluminescent enzyme immunometric assay or enzyme-linked immunosorbent assay (ELISA). Clinical parameters were collected prospectively until discharge. **Results**: In all patients AM and cytokines were significantly elevated after normothermic and hypothemic CPB. AM and cytokines were significantly higher in hypothermia compared to normothermia. In hypothermic CPB sE-selectin was decreased after 24 h by 37% ($P < 0.0063$) and by 40% ($P < 0.0027$) after 48 h postoperatively. ICAM-1 was reduced by 43% ($P < 0.0001$) after 24 h and by 60% ($P < 0.0001$) after 6 days. Similar results were seen in cytokines with reduction up to 60% after 24 h. Changes after 48 h were noticeable but not significant. Reduction of AM and cytokines after normothermic CPB was minimal. Neither in normothermia, nor in hypothermia has sIL-2R been effectively removed from the circulation. There were no significant differences in the clinical variables between the patients with or without MUF. **Conclusion**: AM and cytokines are significantly elevated after hypothermic CPB compared to normothermic CPB. MUF led to a significant reduction in cytokine and AM levels after hypothermic CPB, except for IL-2R. MUF showed minimal effect in normothermia. We conclude that MUF is an efficient way to remove cytokines and AM. However, we were unable to demonstrate any significant impact of MUF in outcome of adults after elective CABG.

Keywords: Cardiac surgery; Modified ultrafiltration; Adhesion molecules; Cytokines; Clinical outcome

1. Introduction

The systemic inflammatory response that occurs in humans after cardiopulmonary bypass (CPB) remains a major cause of morbidity and mortality. The most evident part in the whole-body inflammatory response is the systemic endothelial cell–neutrophil adhesion that results from the release of complement degradation products and cytokines after blood exposure to the artificial conduits of the cardiopulmonary circuits [1]. The systemic inflammatory response may have a particularly deleterious effect on the heart where an additional insult occurs from myocardial ischemia during cardiac arrest [2]. Myocardial ischemia, present in many patients preoperatively and as a result of cardioplegic arrest during cardiopulmonary bypass, activates the vascular endothelium to recruit neutrophils that accentuate injury upon reperfusion with oxygenated blood [3].

The cellular and molecular mechanisms of neutrophil–endothelial cell adhesion in ischemia/reperfusion injury are increasingly understood. Shreeniwas et al. [4] and Zünd et al. [5] demonstrated the induction of adhesion

☆ Presented at the 13th Annual Meeting of the European Association for Cardio-thoracic Surgery, Glasgow, Scotland, UK, September 5–8, 1999.

* Corresponding author. Tel.: + 41-1-255-3801; fax: + 41-1-255-4369.
E-mail address: gregor.zund@chi.usz.ch (G. Zünd)

1010-7940/00/$ - see front matter
PII: S1010-7940(99)00355-3

molecules (E-selectin, ICAM-1) on the surface of endothelial cells rendered hypoxic and being injured when these cells are reoxygenated, emphasizing the importance of reperfusion in this syndrome. In addition, postoperative episodes of myocardial ischemia were significantly correlated with peak elevations of cytokines (TNF-α, IL-1, IL-6, IL-8) which are responsible for direct cell damage as well as increased activation of neutrophils.

The technique of modified ultrafiltration has been claimed to reduce the serum level of inflammatory mediators generated during CPB and to improve myocardial postoperative function as well as postoperative convalescence in infants and children [6]. The purpose of this prospective randomized study is to evaluate the efficacy of modified ultrafiltration in adults to reduce the plasma levels of cytokines and adhesion molecules as well as clinical outcome in elective cardiac surgery with cardiopulmonary bypass.

2. Material and methods

2.1. Patient groups

After approval of the Ethics committee for Surgery and Anesthesia at the University Hospital Zürich 97 patients were recruited for this study undergoing elective coronary artery bypass grafting using normothermic (37°C) or hypothermic (26–28°C) cardiopulmonary bypass. Between July 1997 and May 1999 we enrolled 50 patients with normothermic CPB (mean age 62.1 years) and 47 patients with hypothermic CPB (mean age 58.7 years). Both groups were subdivided into a group with modified ultrafiltration ($n = 30$) and a group without modified ultrafiltration ($n = 20$ and $n = 17$). Patients with diabetes, preoperative ischemia or previous coronary artery bypass surgery were excluded from the study. Cardiopulmonary bypass time in the normothermia group was mean 77 ± 17 min (46 ± 15 min of cardiac arrest time). In the hypothermia group cardiopulmonary bypass time was 98 ± 23 and 60 ± 20 min of cardiac arrest time. Within the two groups there were no significant differences in cardiopulmonary bypass time as well as in cardiac arrest time. Informed consent was obtained from each patient according to the protocol of the Ethics committee of the University Hospital of Zürich.

2.2. Operative techniques

Cardiopulmonary bypass was performed with a Stöckert roller pump system (Stöckert Instrumente GmbH, Munich, Germany) and a Shiley-Dideco Maxima hollow fiber oxygenator (Dideco, Mirandola, Italy). Myocardial protection was accomplished using Buckberg cardioplegia with an initial dose of 15 ml/kg. Infusions of 300 ml were repeated every 20 min or earlier if electrical activity returned. Before aortic declamping 500 ml warm blood cardioplegia was administered at 37°C for 2 min at a pressure of 50 mmHg. The operations were performed under normothermic by one surgeon (M.T.) and under hypothermic conditions by three different surgeons according to the usual practice of our clinic. Rewarming was achieved using the heat-exchange oxygenator, warming blanket, and heated humidified gases to reach a rectal temperature of >34°C before terminating CPB.

2.3. Modified ultrafiltration

After completion of CPB an ultrafiltration modified in 1991 by Elliott and coworkers [7] was performed in heparinized patients between the arterial and the venous tubings of the CPB circuit, using a Gambro FH22 filter with an effective membrane are of 0.2 m^2 and a pore size of 5 nm (Gambro Dialysatoren GmbH and Co. KG, Hechingen, Germany). In general, if the molecular weight is between 0 and 8 kDa, all molecules are removed, no matter what their structure is. Between 8 and 40 kDa not all molecules are removed, only those with a certain configuration. The blood flow through the filter was about 200 ml/min, which was maintained by a roller pump on the inlet part of the filter. Suction was applied to the filtrate port to achieve an ultrafiltration rate of 100–150 ml/min. The process was carried out until an ultrafiltration volume of 50% of the net CPB-volume balance has been achieved.

2.4. Blood sampling

Blood samples (10 ml) required for determination of IL-6, IL-8, IL-2R, TNF-α, sE-selectin, and sICAM-1 were obtained form a venous line of the patient at the following times: preoperatively after induction of anesthesia, before instituting modified ultrafiltration, the ultrafiltrate itself, after modified ultrafiltration, 24 h, 48 h, and 6 days postoperatively. Blood samples were allowed to coagulate, centrifuged for 20 min at 4°C with 4000 U/min and the serum was aliquoted and stored at −70°C and analyzed within a tolerable time frame. Soluble adhesion molecules in the serum were measured using commercially available ELISA kits (R&D Systems, Abingdon, UK). Standards of known concentration were run in parallel together with a control serum. The optical density (OD) was read at 450 nm with a correction wavelength set to 630 nm. The absorbency was plotted against a standard curve of known concentrations and expressed as nanograms per milliliter. The values were corrected by hemodilution using the hematocrit.

Cytokines (TNF-α, IL-6, IL-8, IL-2R) were analyzed by the technique of a solid-phase, two-site chemiluminescent enzyme immunometric assay (Immulite, Euro/DPC Ltd., Gwynedd, UK).

2.5. Clinical variables

Patient demographic data and medical history were collected prospectively, as well as the postoperative course including intubation time (h), blood loss in 24 h (ml), given transfusion (ml), alveolar-arterial oxygen gradient ($PAaO_2$),

inotropic requirement, pulmonary infection requiring antibiotic treatment, atrial fibrillation, body temperature (max, min), ICU stay (days), leukocytes count and CRP for 6 days (min, max), length of hospital stay (days).

2.6. Statistics

Data were processed with Statview software (Abacus Concepts Inc., Berkeley, CA). All data were expressed as mean with one standard deviation and were graphically presented as bar charts. Intergroup comparison was performed with the Mann–Whitney test for unpaired data. Intragroup comparison was done using the Wilcoxon test for paired data. Differences between groups at specific time intervals were inspected by repeated-measures analysis with multivariate analysis of variance, as well as correlation analysis (SPSS Software Inc., Chicago, IL). *P*-values <0.05 were considered statistically significant.

3. Results

In all patients sICAM-1, sE-selectin, IL-6, IL-8, IL-2R, as well as TNF-α were significantly elevated postoperatively after normothermic and hypothermic CPB compared to preoperative levels.

3.1. Modified ultrafiltration (MUF) in hypothermic CPB

Adhesion molecules after hypothermic CPB were significantly lowered by MUF. Soluble E-selectin was decreased by 37% 24 h postoperatively from mean 302.1 ± 123.9 to 191.7 ± 184.4 ng/ml ($P < 0.0063$), by 40% 48 h postoperatively from mean 241.8 ± 98.9 to 150.3 ± 137.9 ng/ml ($P < 0.0027$). After 6 days sE-selectin levels were almost reduced to baseline from mean 146.2 ± 62.5 to 98 ± 73.5 ng/ml ($P < 0.0084$).

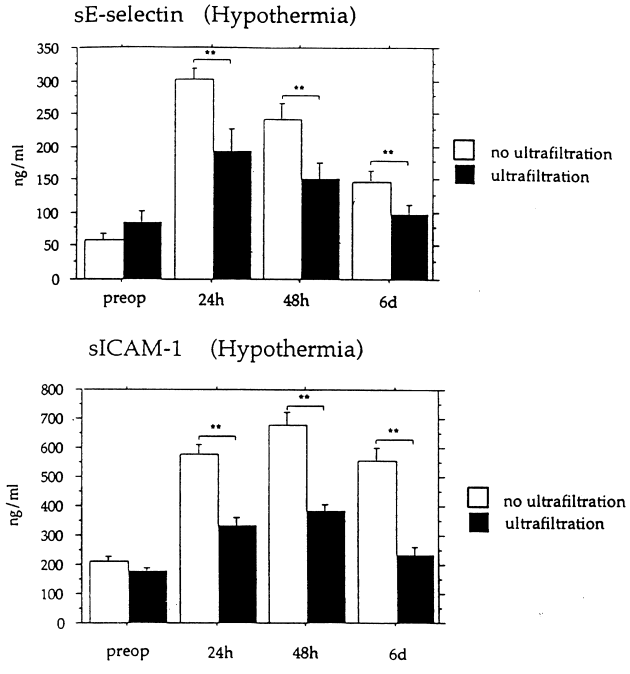

Fig. 1. Expression of sE-selectin and sICAM-1 after hypothermic CPB at different time intervals (**$P < 0.01$).

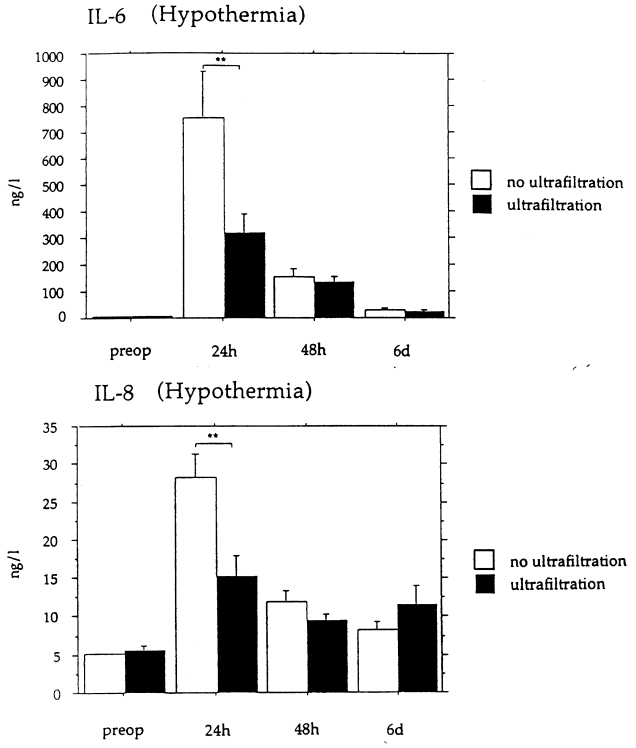

Fig. 2. Expression of IL-6 and IL-8 after hypothermic CPB at different time intervals (**$P < 0.01$).

Soluble ICAM-1 showed similar changes after 24 h with a reduction of 43% from mean 575.9 ± 212.9 to 333.5 ± 143.8 ng/ml ($P < 0.0001$), by 44% after 48 h from 675.7 ± 269.7 to 383.6 ± 123.9 ($P < 0.0001$), and by 60% from 553.5 ± 261.9 to 232.2 ± 153.4 ng/ml ($P < 0.0001$) 6 days postoperatively (Fig. 1).

The measured cytokine levels in patients treated with MUF were also significantly different postoperatively. Interleukin-6 levels decreased by 60% from mean 792.9 ± 580.4 to 318.4 ± 393.4 ng/l ($P < 0.0018$). After 48 h and 6 days changes in IL-6 after MUF were noticeable, but not statistically significant. Similar results were found in IL-8 after 24 h ($P < 0.0004$) and in TNF-α after 24 h ($P < 000.3$) without significance after 48 h or 6 days (Fig. 2).

3.2. Modified ultrafiltration in normothermic CPB

After normothermic CPB the reduction of adhesion molecules with modified ultrafiltration was still present, but not as remarkable as in the hypothermic patient group. Soluble E-selectin peaked after 24 h (mean 193 ± 93 ng/ml) in the patient group not undergoing MUF. The difference after 24 h in the patients with MUF was 35% ($P < 0.007$). No significant changes were seen after 48 h and 6 days. Soluble

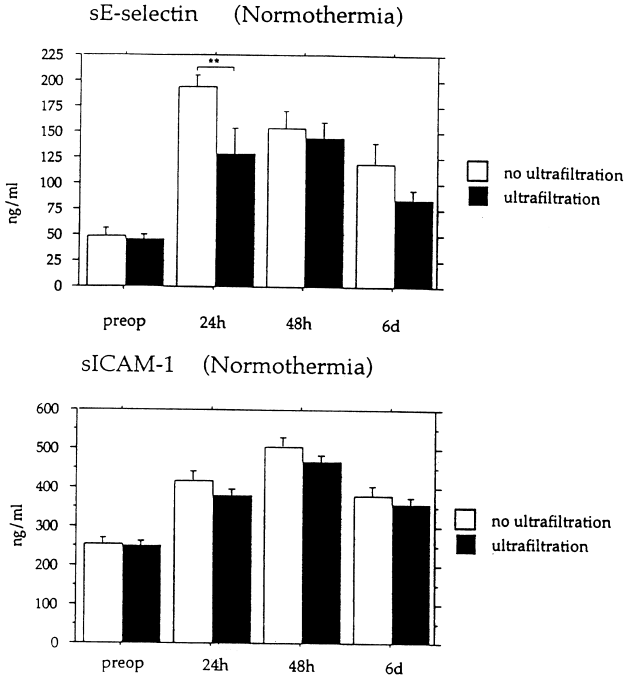

Fig. 3. Expression of sE-selectin and sICAM-1 after normothermic CPB at different time intervals (**$P < 0.01$).

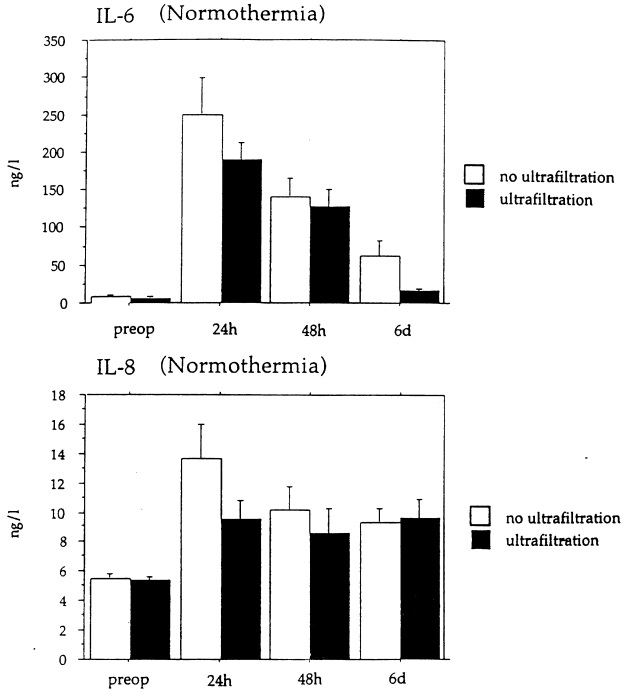

Fig. 4. Expression of IL-6 and IL-8 after normothermic CPB at different time intervals.

ICAM-1 levels in patients with MUF and without MUF differed between 5 and 10% (Fig. 3).

In line with adhesion molecules were the values for cytokines which revealed a maximal reduction after MUF of 25% for IL-6 after 24 h (NS), 8% after 48 h (NS), and of 20% after 6 days (NS). IL-8 was lowered by 30% after 24 h, however without being statistically significant. The range of reduction otherwise varied between 5 and 10% (Fig. 4).

Soluble IL-2 receptor has neither in the normothermic, nor in the hypothermic group effectively been removed from the circulation (data not shown).

All clinical variables showed no significant differences between patients with or without MUF (Table 1).

4. Discussion

This study has demonstrated that surgery with cardiopulmonary bypass is correlated with a significant increase of soluble adhesion molecules and cytokines in the serum compared to preoperative levels. As we have shown in a previous study [8] we found that hypothermia in CPB represents a much stronger stimulus to endothelial cells which subsequently leads to significantly higher levels of adhesion molecules as well as cytokines levels compared to normothermia. Modified ultrafiltration after CPB in adult patients undergoing coronary artery bypass grafting is an

Table 1
Postoperative clinical variables in normothermic and hypothermic bypass with or without MUF[a]

	Normothermic CPB			Hypothermic CPB		
	MUF ($n = 30$)	No MUF ($n = 20$)	$P<$	MUF ($n = 30$)	No MUF ($n = 17$)	$P<$
$PAaO_2$	301.3 ± 116.6	286.6 ± 138.2	NS	308.4 ± 115.7	364.0 ± 137.1	NS
Blood loss (ml)	1100 ± 400	1130 ± 250	NS	1140 ± 370	1450 ± 633	NS
Transfusion (unit)	1.2 ± 1.1	1.4 ± 1.3	NS	1.4 ± 1.5	1.5 ± 1.1	NS
Temp max (C)	38.0 ± 0.6	37.9 ± 0.7	NS	38.0 ± 0.5	38.1 ± 0.6	NS
Lc max (×1000)	11.2 ± 3.1	10.2 ± 4.0	NS	11.4 ± 2.5	11.8 ± 3.0	NS
CRP max	164 ± 58	133 ± 36	NS	189 ± 81	166 ± 55	NS
Inotropic support (n)	13	10	NS	15	12	NS
Atrial fibrillation (n)	15	9	NS	15	8	NS
Pulmonary infection (n)	8	3	NS	4	7	NS
Intubation (days)	13.3 ± 5.8	14.0 ± 3.4	NS	17.5 ± 11.5	15.9 ± 5.8	NS
ICU stay (days)	2.0 ± 1.9	2.2 ± 3.3	NS	2.4 ± 1.9	2.4 ± 2.3	NS
Hospital stay (days)	11.0 ± 2.1	10.4 ± 4.3	NS	9.5 ± 2.9	10.8 ± 4.0	NS

[a] $PAaO_2$, pulmonary-alveolar O_2-gradient; Lc, leukocyte; CRP, C reactive protein; NS, not significant.

effective tool to significantly lower adhesion molecules and cytokines from the blood immediately after termination of cardiopulmonary bypass.

The release of proinflammatory cytokines (TNF-α, IL-6, IL-8, IL-2R), as well as upregulation of adhesion molecules may play a vital role in the whole body inflammatory response encountered after cardiopulmonary bypass. Ultrafiltration has been proposed to be a way to reduce at least some of the inflammatory mediators as well as the amount of accumulated tissue water generated during pediatric cardiac surgery [9–11]. Hemofiltration also showed to be effective in controlling water balance, concentration of clotting factors and improved early postoperative PaO_2 and time of extubation as well as postoperative blood loss [12]. However, there has been no report to date of a prospective randomized study in an adult patient population demonstrating a significant impact in lowering adhesion molecules and cytokines after CPB as well as its correlation to clinical outcome.

Casey et al. studied the adverse effects of experimental cytokine administration and the prognosis related to high plasma concentrations of cytokines in sepsis [13]. However, the clinical benefit of cytokine neutralization or removal has not been proven. In addition, other studies suggest that plasma cytokine concentrations are only circulating markers of an inflammatory process occurring at tissue level [14].

Tumor necrosis factor alpha (molecular weight 17–50 kDa) was one of the first cytokines to be implicated in the activation of endothelial cells, causing hypotension and leukopenia. IL-6 (molecular weight 20–30 kDa) is produced via inflammatory response including a protease-mediated pathway from myocardium during postischemic reperfusion in patients undergoing coronary artery bypass grafting [15]. In myocardial infarction serum level of IL-6 are significantly elevated [16] and IL-6 mRNA are expressed in the area of infarcted myocardium or in hypoxic neonatal myocytes [17]. The production of IL-8 (molecular weight 8–10 kDa) during CPB has been described in several studies [18]. This cytokine has been shown to be a most powerful neutrophil and T-lymphocyte chemotactic factor. It has also been found to stimulate neutrophil adherence to endothelial cells and extracellular matrix proteins [19]. It has been demonstrated that IL-8 plays a role of in the pathogenesis of adult respiratory distress syndrome, which has also been reported as a complication of postbypass surgery [18].

Although our results clearly demonstrate that modified ultrafiltration significantly lowers adhesion molecules and cytokines after CPB, we were unable to detect any significant impact of MUF in all clinical variables studied or in the patients outcome. Ultrafiltrated patients showed less blood loss within 24 h and subsequently less transfusion had to be given compared to the patients not treated with MUF. A reduction in postoperative bleeding by MUF was reported by Naik and colleagues. They speculated that the effect of hemoconcentration itself and increased concentration of clotting factors, including platelets, after MUF lead to improved coagulation [7]. Probably the most changes postoperatively was seen in the improvement in postoperative PaO_2 and the reduction in duration of mechanical ventilation. However, no difference between the groups was observed regarding the duration of ICU stay or hospital stay. The slight increase in the neutrophil count seen in the hypothermic patients postoperatively compared to the normothermic patients may be due to circulating C3a and C5a promoting release of leukocytes and precursors from bone marrow [20]. Our findings of increased adhesion molecules and cytokines in the hypothermia group may in part be explained by a higher leukocyte count and therefore stronger inflammatory response. Royston and coworkers observed that up to 50% of circulating polymorphonuclear leukocytes are selectively trapped in the pulmonary capillaries during the rewarming period, with subsequent degranulation of additional inflammatory mediators contributing to endothelial damage [21]. However, not only pro-inflammatory but also anti-inflammatory cytokines are released during cardiopulmonary bypass, which may play a protective role by actually suppressing the production of pro-inflammatory mediators. IL-10 is a potent inhibitor of the production of TNF-α, as well as IL-1b, IL-6, and IL-8 [22]. This cytokine is produced during septicemia and exerts a protective role while down-regulating TNF-α production in tissue macrophages [23]. Its neutralization or removal from the circulation through MUF may therefore theoretically be deleterious. More apparent disadvantages in performing modified ultrafiltration after terminating cardiopulmonary bypass are that decannulation is delayed for about 15 min, and there is also a potential danger of hypotension caused by excessive rate of ultrafiltration jeopardizing adequate coronary blood flow and myocardial perfusion. Also, exposure of blood to filter and its connecting circuit might theoretically increase the inflammatory stimuli and potentiate complement activation and cytokine release during the use of hemofiltration. However, no studies showed any additional increase in complement activation during the procedure of ultrafiltration itself [12] as long as no filter which contain nylon are used.

As we already pointed out [8] adhesion molecule and cytokine levels were significantly elevated in patients after hypothermic cardiopulmonary bypass compared to patients operated on under normothermic conditions. Interestingly, MUF turned out to be significantly more effective in the hypothermia group lowering cytokine levels up to 60% and adhesion molecules up to 37% after 24 h. Under normothermic conditions the reduction through MUF was minimal showing only a significant decrease in sE-selectin levels after 24 h while all other parameters were almost unchanged. However, no significant differences have been seen after MUF between normothermia and hypothermia. A possible explanation for this finding is the fact, that since the amount of circulating adhesion molecules and cytokines released after hypothermic CPB exceeds significantly the amount after normothermic CPB, obviously more mole-

cules get removed in a given time. The ultrafilter used in our study, apparently, was able to remove adhesion molecules and cytokines from the circulation to a certain degree regardless of how high the levels were to begin with. To prove the efficacy of the filter we also analyzed the ultrafiltrate, in which showed almost identical levels pre-ultrafiltration and in the ultrafiltrate. The fact, that sIL-2R with a molecular weight of 40–50 kDa was not removed from the circulation by MUF, couldn't be explained by different molecular weights. We believe that different conformations of molecules might contribute to the efficiency of the ultrafilter to remove the substances in the plasma. This finding, however, is in line with other reports of patients in which the usually high sIL-2R levels in renal failure were unaffected by dialysis [24]. Therefore, any possible adverse effects of IL-2 secretion by activated T-cells cannot be altered by MUF.

Modified ultrafiltration has shown to be efficient not only in removing soluble inflammatory mediators from the circulation, but also may indeed have an impact on clinical variables (i.e. hemostasis, $PAaO_2$) in children. We agree with Ramamoorthy and colleagues on their comment that we now do need to focus on clinical outcome measures, rather than the assay of cytokines [25]. Current evidence suggests, that MUF is an efficient way of removing inflammatory mediators and helping blood conservation, probably best suited to the pediatric patient population in whom blood loss and transfusion requirements are significant. However, our study failed to demonstrate any significant clinical benefit in adults after elective coronary artery bypass surgery except for better ΔAaO_2 values and slightly earlier extubation. Taking into account all advantages and disadvantages, we believe, that modified ultrafiltration might be more beneficial in patients with cardiogenic shock undergoing emergent cardiac operation and with a presumably longer postoperative course.

In conclusion, modified ultrafiltration is able to lower adhesion molecules and cytokine levels significantly after elective coronary artery bypass surgery in adults, although its role in reducing postoperative morbidity and increasing outcome is yet to be shown.

References

[1] Sacks T, Moldow CF, Craddock PR, Bowers TK, Jacob HS. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. J Clin Invest 1978;61:1161–1167.

[2] Verrier ED. The microvascular cell and ischemia-reperfusion injury. J Cardiovasc Pharmacol 1996;27:26–30.

[3] Johnson MJ, Pohlman TH, Verrier ED. Neutrophil antiadhesion therapy for myocardial ischemia: clinical potential. Clin Immunother 1995;3:8–14.

[4] Shreeniwas R, Koga S, Karakurum M, Pinsky D, Kaiser E, Brett J, Wolitzky BA, Norton C, Plocinski J, Benjamin W. Hypoxia-mediated induction of endothelial cell interleukin-1 alpha. An autocrine mechanism promoting expression of leukocyte adhesion molecules on the vessel surface. J Clin Invest 1992;90:2333–2339.

[5] Zünd G, Dzus AL, Prêtre R, Niederhäuser U, Vogt P, Turina M. Endothelial cell injury in cardiac surgery: salicylate may be protective by reducing expression of endothelial adhesion molecules. Eur J Cardio-thorac Surg 1998;13:293–297.

[6] Hennein HA, Kiziltepe U, Barst S, Bocchieri KA, Hossain A, Call DR, Remick DG, Gold JP. Venovenous modified ultrafiltration after cardiopulmonary bypass in children: a prospective randomized study. J Thorac Cardiovasc Surg 1999;117:496–505.

[7] Naik SK, Knight A, Elliott M. A prospective randomized study of a modified technique of ultrafiltration during pediatric open-heart surgery. Circulation 1991;84(Suppl III):422–431.

[8] Grünenfelder J, Zünd G, Schoeberlein A, Schmid AC, Frisullo R, Schmid ER, Turina M. Expression of adhesion molecules and cytokines after normothermic and hypothermic coronary artery bypass grafting. Eur J Cardio-thorac Surg 2000; (submitted).

[9] Andreasson S, Gothberg S, Berggren H, Bengtsson A, Eriksson E, Risberg B. Hemofiltration modifies complement activation after extracorporeal circulation in infants. Ann Thorac Surg 1993;56:1515–1517.

[10] Millar AB, Armstrong L, van der Linden J, Moat N, Ekroth R, Westwick J, Scallan M, Lincoln C. Cytokine production and hemofiltration in children undergoing cardiopulmonary bypass. Ann Thorac Surg 1993;56:1499–1502.

[11] Saatvedt K, Lindberg H, Geiran OR, Michelsen S, Pedersen T, Seem E, Mollnes TE. Ultrafiltration after cardiopulmonary bypass in children: effects on hemodynamics, cytokines and complement. Cardiovasc Res 1996;31:596–602.

[12] Journois D, Pouard P, Greeley WJ, Mauriat P, Vouhe P, Safran D. Hemofiltration during cardiopulmonary bypass in pediatric cardiac surgery: Effects on hemostasis, cytokines, and complement components. Anesthesiology 1994;81:1181–1189.

[13] Casey LC. Role of cytokines in the pathogenesis of cardiopulmonary-induced multisystem organ failure. Ann Thorac Surg 1993;56:92–96.

[14] Schetz M. Removal of cytokines in septic patients using continuous veno-venous hemofiltration. Crit Care Med 1994;22:715–716.

[15] Sawa Y, Ichikawa H, Kagisaki K, Ohata T, Matsuda H. Interleukin-6 derived from hypoxic myocytes promotes neutrophil-mediated reperfusion injury in myocardium. J Thorac Cardiovasc Surg 1998;116:511–517.

[16] Ikeda U, Ohkawa F, Seino Y, Yamamoto K, Hidaka Y, Kasahara T, Kawai T, Shimada K. Serum interleukin-6 levels become elevated in acute myocardial infarction. J Mol Cell Cardiol 1992;24:579–584.

[17] Herskowitz A, Choi S, Ansari AA, Wesselingh S. Cytokine mRNA expression in postischemic/reperfused myocardium. Am J Pathol 1995;146:419–428.

[18] Messent M, Sullivan K, Keogh BF, Morgan CJ, Evans TW. Adult respiratory distress syndrome following cardiopulmonary bypass: incidence and prediction. Anaesthesia 1992;47:267–268.

[19] Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin-8, a novel cytokine that activates neutrophils. J Clin Invest 1989;84:1045–1049.

[20] Quiroga MM, Miyagishima R, Haenschen LC, Glovsky M, Martin BA, Hogg JC. The effect of body temperature on leukocyte kinetics during cardiopulmonary bypass. J Thorac Cardiovasc Surg 1985;90:91–96.

[21] Royston D, Fleming JS, Desai JB, Westaby S, Taylor KM. Increased production of peroxidation products associated with cardiac operations. Evidence for free radical generation. J Thorac Cardiovasc Surg 1986;91:759–766.

[22] Eppinger MJ, Ward PA, Bollinger SF, Deeb GM. Regulatory effects of interleukin-10 on lung ischemia-reperfusion injury. J Thorac Cardiovasc Surg 1996;112:1301–1305.

[23] Marchant A, Deviere J, Byl B, De Groote D, Vincent JL, Goldman M. Interleukin-10 production during septicaemia. Lancet 1994;343:707–708.

[24] Shu KH, Lu YS, Cheng CH, Lian JD. Soluble interleukin 2-receptor in dialyzed patients. Artif Organs 1998;22:142–144.

[25] Ramamoorthy C, Lynn AM. Con: the use of modified ultrafiltration during pediatric cardiovascular surgery is not a benefit. J Cardiothorac Vasc Anesth 1998;12:483–485.

Appendix A. Conference discussion

Dr J. Fragata (*Lisbon*, *Portugal*): We have used modified ultrafiltration in around 100 patients after coronary artery bypass grafting, and what we noticed is that there is a marked clinical benefit, as expressed by less blood use, less bleeding, and less time on the ventilator. I wonder, and you haven't mentioned in your study, whether your results were due only to the increase in hematocrit because of the filtration. We think there is more than that, because you also remove inflammatory mediators. Could you please compare the hematocrits you achieved with both methods that you used, and, second, did you measure the concentration of inflammatory mediators in the ultrafiltrate?

Dr Grünenfelder: To the first question, the hematocrits changed. We did not actually look at that specifically, because, as you know, if you transfuse blood into a patient, usually, and that has been reported in several studies, interleukin-8 and -6 will be transfused as well. We actually did not have any specific data about when we have to transfuse, since the patients in the ICU will be treated in our institution by anesthesiologists. So we have no access exactly how to and when to transfuse.

Dr Fragata: The point I was trying to raise is that we can, of course, get rid of extra fluid just by giving a diuretic such as furosemide, but the clinical effect we get with modified ultrafiltration is different and more intense, when compared to the same amount of fluid you lose just by using a diuretic. So I think this method is very advantageous, and actually in our series that we are about to publish, we could clearly demonstrate the clinical benefit, expressed by less bleeding and transfusion and less time on the ventilator. The other point is, could you analyze what you were actually draining out in the ultrafiltrate?

Dr Grünenfelder: Yes. We specifically looked at the ultrafiltrate, too, and it showed that the amount of cytokines in the ultrafiltrate actually represents the same amount preultrafiltration. So we actually filtered the same amount out of the circulation as the amount of circulating proinflammatory mediators in the circulation before filtration.

Dr S. Wan (*Hong Kong*, *China*): How did you determine your sampling points? The current sampling points just exactly missed the peak release of those cytokines, especially TNF and interleukin-8.

Dr Grünenfelder: That may be correct for the cytokines, but from previous studies done at our institution, we know that specifically the adhesion molecules peak not before 12 h, and we have performed studies to confirm that, and we showed that the peak for E-selectin and ICAM-1 is at 18–24 h. It's true that the cytokines peak before that. But we think for that reason that we have a correlation to the adhesion molecules that wouldn't matter too much.

Dr Wan: In that case, how can you determine that reduced adhesion molecule is due to ultrafiltration and not relates to the other post-operative treatments?

Dr Grünenfelder: We think that since we are filtrating cytokines also, the stimulus after transporting the patient to the ICU is reduced. Since we found adhesion molecules in the ultrafiltrate, we know that we also filter adhesion molecules, although the peak of adhesion molecules is at 24 h.

Dr A.M. El Gamel (*London*, *UK*): You cannot extrapolate a lot out of your result because the number of your patients is so small and you cannot say the ultrafilter doesn't help. You need a large number of patients to prove the clinical value of it. Although the cytokine results are quite clear, you take the proinflammatory cytokines out, and I am just wondering before we make a clinical statement on the value of ultrafiltration, we have to continue to evaluate it.

Dr Grünenfelder: That's correct.