

CLINICAL INVESTIGATIONS

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High-volume, Zero-balanced Hemofiltration to Reduce Delayed Inflammatory Response to Cardiopulmonary Bypass in Children

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Background: In previous studies, researchers suggested a beneficial role of hemofiltration performed during cardiopulmonary bypass in children. This study was performed to assess both clinical effects and inflammatory mediator removal by high-volume, zero-fluid balance ultrafiltration during rewarming (Z-BUF).

Methods: Twenty children undergoing cardiac surgery were assigned randomly to Z-BUF or a control group. Plasma C3a, interleukin (IL)-1, IL-6, IL-8, IL-10, tumor necrosis factor, myeloperoxidase, and leukocyte count were measured before (T1) and after (T2) hemofiltration and 24 h later (T3). The intensive care unit staff was blinded to the patient's group. Postoperative alveolar-arterial oxygen gradient, time to extubation, body temperature, and postoperative blood loss were monitored.

Results: Ultrafiltration rate was 4,972 (3,183–6,218) mL/m² (median [minimum-maximum]) in the Z-BUF group, where sig-

nificant reductions were observed in postoperative blood loss, time to extubation (10.8 [9–18] vs. 28.2 [15–58] h) and postoperative alveolar-arterial oxygen gradient (320 [180–418] vs. 551 [485–611] mmHg at T3). In the Z-BUF group, significant removal of tumor necrosis factor, IL-10, myeloperoxidase, and C3a were observed at T2. Interleukin 1, IL-6, IL-8, and myeloperoxidase were decreased at T3, suggesting earlier removal of factor(s) that may trigger their release.

Conclusions: These results suggest that hemofiltration exerts some beneficial clinical effects that are not due to water removal. The role of the early removal of factors triggering the inflammatory response, rather than a direct removal of cytokines, deserves further investigation. (Key words: Cardiopulmonary bypass; Pediatric cardiac; hemofiltration; ultrafiltration; Cytokines: interleukin-8; interleukin-6 interleukin-1; tumor necrosis factor; complement 3a. Leukocytes: macrophages; monocytes; Alfentanil [blood]; Midazolam [administration and dosage]).

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CARDIOPULMONARY bypass (CPB) induces fluid overload in pediatric patients associated with activation of the systemic inflammatory response that may lead to severe postoperative complications.^{1–4} Therefore, CPB has been proposed as a model for studying the inflammatory pathways involved in the Systemic Inflammatory Response Syndrome.^{5,6} Due to the ease with which accumulated extracellular water is removed by hemofiltration, this technique is now commonly used in pediatric cardiac surgery to concentrate the blood at the end of CPB.⁷ The use of hemofiltration during rewarming or after CPB has been associated with a marked improvement in hemodynamics,^{3,8} cardiac contractility,² and oxygenation,^{3,7,9} and a reduction in postoperative blood loss and duration of mechanical ventilation.^{3,7} These beneficial effects of hemofiltration were attributed initially to a reduction in tissue edema.⁷ However data from experimental models^{9–11} and evidence of removal by hemofiltration of several major inflammatory mediators^{3,4,12} suggested that water removal is not the only mechanism involved.

Nevertheless, no study has confirmed that the removal of these mediators is of clinical benefit once the inflammatory process is triggered. It would seem that hemofiltration would be most beneficial by eliminating the early precursors of the inflammatory response, such as complement fragments, rather than efficiently removing other mediators without regard to their biologic importance. Maximal complement and cytokine release coincides with the period of rewarming.^{1,13} Therefore, the preferred time for hemofiltration, to limit the effects of the inflammatory response, would be during the rewarming period.

This study was undertaken to assess the clinical and biologic effects of substance removal in an interventional group that received a pure high-volume, zero-fluid balance hemofiltration (Z-BUF) during the rewarming phase of CPB followed by a standard post-CPB hemofiltration technique, compared with a control group that received only post-CPB hemofiltration.

Methods

After Institutional Review Committee approval, 20 consecutive children undergoing an elective surgical correction of various congenital heart diseases under hypothermic CPB were enrolled in this study. Children having had previous cardiothoracic surgery or children whose lungs were mechanically ventilated preoperatively were excluded from the trial.

Anesthetic Techniques

All patients were premedicated with atropine and 30 $\mu\text{g/kg}$ oral flunitrazepam. Anesthesia was induced and maintained with a continuous infusion of 2 $\mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ midazolam, 2 $\mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ alfentanil, and 120 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ vecuronium. The lungs were ventilated with $\text{FiO}_2 = 1.0$, maintaining an end-tidal fraction of CO_2 (FECO_2) of 35 mmHg. Rectal and nasopharyngeal temperatures were monitored continuously, using 9F Mon-a-therm temperature probes (Mallinckrodt Medical, St. Louis, MO).

Cardiopulmonary Bypass Techniques

Cardiopulmonary bypass was performed with a stretching roller pump (RP06 Rhone-Poulenc, Lyon, France) and an appropriately sized Dideco hollow fiber oxygenator (Dideco Laboratories, Mirandola, Italy). The tubing of the extracorporeal circuit was made of sili-

cone (Dideco Laboratories). The extracorporeal circuit was primed with 40 g/l albumin solution (CNTS, Paris, France), hydroxyethylstarch (Elohes, Biosedra Laboratories, Louviers, France) or fresh frozen plasma and fresh erythrocytes according to patient needs, 8% volume of molar sodium bicarbonate, and 5% aprotinin (10,000 IU/mL, Trasylol, Bayer Laboratories, Puteaux, France), to reach a total prime volume of 1,250 ml/m² with a minimum of 520 mL. Heparin was added to this priming solution (2 IU/ml).

Hypothermia was induced in all patients. The pump flow rate was modified to provide a blood flow linearly adjusted to body temperature between 2.4 l $\cdot \text{min}^{-1} \cdot \text{m}^{-2}$ at 37°C and 1.7 l $\cdot \text{min}^{-1} \cdot \text{m}^{-2}$ at 24°C. Blood gasses were regulated according to alpha-stat regimen, and sodium bicarbonate was administered when the base excess was less than -2.5 mmol/l during CPB. When considered necessary for surgery, deep hypothermic circulatory arrest was commenced when the nasopharyngeal temperature decreased to at least 20°C, after a period of cooling of at least 20 min.

Anticoagulation was achieved with an initial bolus of heparin (Beef lung sodium heparin, Léo Laboratories, Paris, France) of 250 IU/kg injected into the right atrium before cannulation, followed by a continuous infusion of 62.5 IU $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ until the end of CPB. Aprotinin was administered in all children at a dose of 30,000 IU/kg after the induction of anesthesia and then infused continuously at a rate of 1.35 KIU $\cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. After CPB, protamine (Choay Laboratories, Paris, France) was administered at a rate of 10 mg/min to a total dose of 3.5 mg/kg. A cell separator system (Autotrans, Dideco Laboratories, Mirandola, Italy) was used (in both groups) to wash and centrifuge the blood aspirated in the surgical field before and after CPB.

Myocardial preservation was achieved using cold blood cardioplegia with an initial dose of 30 ml/kg repeated every 20 min. Before aortic declamping, 15 ml/kg warm blood cardioplegia was administered. Cardioplegia solution was aspirated from the right atrium to the cell separator system (during administration) to prevent blood dilution. Rewarming was achieved using a heat-exchange oxygenator, warming blanket, and heated humidified gases to reach a rectal temperature of 36.5°C before terminating CPB. No vasodilator was used throughout the operation.

For all patients, a polyacrylonitrile hemofilter with a surface area of 0.6 m² (Multiflow, Hospal Laboratories, Meyzieu, France) was rinsed with 1,000 ml saline, then

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placed with a tubing inserted in an occlusive roller pump between the arterial tubing and the cardiomy reservoir of the CPB circuit. Blood from the patient was circulated through this circuit for exactly 5 min at the beginning of CPB, with the ultrafiltrate line clamped.

Zero-balanced Ultrafiltration

Patients were assigned randomly to a Z-BUF group or a control group just before rewarming. When patients were assigned to the Z-BUF group, two tubings with similar silicone compressible tubings were inserted in opposite directions in a single, double-way high precision occlusive roller pump (ESPA Laboratories, Savigny, France). The occlusiveness of this pump was checked systematically before each operation. One tubing conducted the ultrafiltrate from the hemofilter to a sterile, pyrogen-free reservoir. The other tubing delivered a replacement solution commonly used for hemofiltration in the intensive care unit (ICU) and contained 35 mmol/l bicarbonate, with 140 mmol/l sodium, 3.5 mmol/l potassium, 105 mmol/l chloride, and 2 mmol/l calcium (SLF 114, Biosedra Laboratories, Paris, France) into the cardiomy reservoir. A single pump for both tubings ensured that the ultrafiltrate and substitution volumes were equal. Blood flow was maintained at $200 \text{ ml} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ of body surface through the hemofilter throughout the rewarming period. The level of the cardiomy reservoir was monitored continuously to detect any discrepancy between ultrafiltration and substitution rates that could lead to changes in circulating blood volume. The CPB flow was adjusted continuously according to body temperature and mixed venous blood oxygen saturation until completion of rewarming. Administration of red separator concentrate cells or albumin during rewarming was allowed.

Modified Ultrafiltration

At CPB completion, patients' blood in both groups was ultrafiltered between the arterial and the venous tubings of the CPB circuit, using the hemofilter mentioned earlier, according to the technique described by Naik *et al.*,⁷ to achieve an ultrafiltration volume of 750 ml/m². The blood remaining in the CPB circuit, at completion, was washed and centrifuged using the cell-separator system and reinfused in the operating room or in the ICU.

Total blood volume (TBV) was defined as prime volume + estimated patient blood volume.⁷ The ratio of total ultrafiltration volume (Z-BUF + post-CPB ultrafil-

tration) to TBV (ultrafiltrate/TBV) was calculated, to compare the relative ultrafiltration volume in each patient and correlate it with other measured variables.

Biologic Variables

Arterial blood samples were withdrawn before rewarming (T_1), at completion of rewarming (T_2), and 24 h later in the ICU (T_3). Plasma was recovered immediately from these samples, aliquoted, and frozen at -70°C until use. Assays were performed within 2 months. Interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tumor necrosis factor (TNF α), myeloperoxidase, and C3a were determined in duplicate by enzyme-linked immunosorbent assay (Medgenix, Rungis, France). Neutrophil, lymphocyte, and monocyte counts were measured at these three time points. To be comparable, the concentration values were corrected for hemodilution.¹⁴ Usual hemostasis parameters, including platelet count and celite-activated clotting time, were recorded at ICU admission and 24 h later.

Anesthetic Assays

To evaluate the removal of the administered anesthetic agents by hemofiltration, midazolam and alfentanil plasma concentrations were assayed by high performance liquid chromatography before (T_1) and after rewarming $\pm$ hemofiltration (T_2) in the two groups.

Postoperative Clinical Variables

The team of ICU physicians that provided the postoperative care of the study patients was totally different from the team involved in the operating room. They were blinded to group assignment until the end of the study. They received only a summary of the perfusionist record, on which only the net fluid balance obtained at the end of the procedure was noted. The original CPB files were hidden from them for the duration of the study. The Pediatric Risk of Mortality score was recorded 6 h postoperatively.¹⁵ Maximum body temperature was monitored during the first 24 h, and the mean body temperature was calculated by integrating body temperature curves (Biopac Systems, Goleta, CA). Postoperative arterial oxygen tension was measured on admission to the ICU and 3 h later under an FiO_2 of 1.0, to calculate the alveolar-arterial oxygen gradient (PA-aO₂). A systematic echocardiographic examination was performed at this time to exclude patients with right-to-left shunt from oxygen gradient analysis. Postoperative

blood loss from chest tubes was recorded every 6 h. Each patient's trachea was extubated as soon as our usual criteria for extubation were met. The time to meet these criteria was recorded.

Statistical Methods

All continuous data were tested to conform to a normal distribution using the Kolmogorov-Smirnov test (SPSS 4.0.5 software, Chicago, IL, run on a Macintosh Quadra 950, Apple Computer, Cupertino, CA). All normally distributed data are expressed as mean $\pm$ SD. The remaining variables are expressed as median $\pm$ [25-75 percentiles] or [minimum-maximum] when specified. The data were analyzed using the Mann-Whitney nonparametric method for unpaired data. All tests were two-sided. Correlations were studied with the nonparametric Spearman rank correlation test. For all statistical analyses, statistical significance was set at $P < 0.05$.

Results

Ten patients were included in each group, and their individual perioperative and demographic characteristics are reported in table 1. There was no difference between groups in preoperative and intraoperative data. No differences were observed between groups regarding flow rates (at either hypothermia or normothermia) or amounts of administered bicarbonate (taking into consideration bicarbonate contained in the replacement solution). No differences were observed between control and Z-BUF group regarding Pediatric Risk of Mortality scores, body surface area (respectively, 0.27 [0.20-1.07], CPB priming volume (625 [500-1210] and 630 [520-800]), CPB duration (147 [92-250] and 117 [145-210], or rewarming duration (44 [37-51] and 46 [36-49]).

Hemofiltration Results

The volume of ultrafiltrate obtained during rewarming in the Z-BUF group was 1,960 ml (1,235-2,137; 4,972 [3,183-6,218] ml/m² body surface area). The ultrafiltrate/TBV ratio was 1.30 (1.10-1.82). One death on the operating table, due to the underlying cardiac disease, occurred in the control group (table 1). No differences were observed between groups regarding net bicarbonate balance or post-CPB acidosis.

Clinical Variables

The alveolar-arterial oxygen gradient was significantly less in the Z-BUF group on ICU admission and 3 h later

(table 1). Two patients from the control group were excluded from this analysis (one died before admission in the intensive care unit and the other had a right-to-left shunt). A correlation was observed between postoperative PA-aO₂ values and the ultrafiltrate/TBV ratio (Spearman's coefficient = -0.62, $P = 0.011$). Time to meet extubation criteria after operation was less in the Z-BUF group than in the control group (table 1). Maximum and mean body temperatures during the first postoperative day were less in the Z-BUF group than in the control group (table 1). There were no reoperations for bleeding. Cumulative blood loss was significantly less in the Z-BUF group compared with the control group at 6, 12, and 24 h (table 1).

Biologic Variables

No difference was found in hemostasis variables between groups either at ICU admission or 24 h postoperatively. Platelet counts were 112 10⁹/mm³ (range, 79-171) in the Z-BUF group and 121 10⁹/mm³ (range, 94-156) in the control group at ICU admission and 125 10⁹/mm³ (107-176) in the Z-BUF group and 153 10⁹/mm³ (127-170) in the control group at 24 h. Celite-activated clotting times were 72 s (64-88) in the Z-BUF group and 75 s (60-84) in the control group at ICU admission and 64 s (57-80) in the Z-BUF group and 66 s (58-82) in the control group at 24 h.

The complement fragment C3a concentration was significantly less in the Z-BUF group at T₂ (table 2). In this group, the change in C3a concentration did not correlate with the ultrafiltrate/TBV ratio ($P = 0.32$).

The studied cytokines were detected in the plasma of all patients from both groups. Although IL-1 β , IL-6, and IL-8 concentrations did not change significantly during rewarming in either group, there was a marked difference between groups at 24 h (T₃) (table 2). Tumor necrosis factor alpha and IL-10 plasma concentrations were lower in the Z-BUF group at T₂, in contrast with the increase observed in the control group (table 2, figure 1). The change in cytokine concentrations during rewarming in the Z-BUF group did not correlate with the ultrafiltrate/TBV ratio, except for IL-1 β (Spearman's coefficient = -0.86, $P = 0.029$).

Table 3 shows the evolution of leukocyte counts and myeloperoxidase concentration over time. Both the neutrophil and monocyte counts increased throughout the study in both groups. The neutrophil count was significantly less in the Z-BUF group at 24 h after surgery. The lymphocyte count decreased in the control

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Table 1. Individual Clinical Data by Group

Patient	Diagnosis	Age (yr)	Cross-clamp Time (min)	DHCA Duration (min)	Minimal CPB Temperature (°C)	PRISM Score (%) 6th hr	Cumulative Blood Loss			P(A-a)O2 at ICU Admission (mmHg)	P(A-a)O2 after 3 hr (mmHg)	Max. 24 h Temperature (°C)	Mean 24 h Temperature	Time to Extubation (h)
							6th hr (mL/m ²)	12th hr (mL/m ²)	24th hr (mL/m ²)					
Control Group														
	median	0.49	55	—	21	7	234	351	531	551	527	38.3	37.9	28
	25%/75%	0/1	49/69	—	19/23	5/8	195/303	328/422	421/764	519/575	497/554	38.0/38.4	37.5/38.0	15/58
1	Tetralogy of Fallot	0.74	69	—	23	5	240	422	764	570	543	38.4	38.0	54
4	Truncus Arteriosus	0.06	98	34	18	11	234	364	897	590	581	38.4	37.9	59
6	Fontan for SV	4.72	54	—	23	8	377	765	925	†	†	38.2	37.8	12
8	VSD	1.42	50	—	27	4	195	310	648	485	456	38.4	38.0	15
9	Norwood for HLH	1.02	14	14	17	*	*	*	*	*	*	*	*	*
10	Tetralogy of Fallot	10.54	69	—	20	7	303	345	462	523	510	38.4	38.0	10
13	VSD	0.24	56	—	22	6	179	244	379	550	545	37.8	37.3	59
16	VSD	0.24	49	—	22.5	5	184	351	390	552	502	37.4	36.9	16
18	VSD	0.13	49	—	20	8	362	430	531	611	603	38.0	37.5	28
19	TGA	0.03	74	—	17.5	9	220	328	421	505	581	38.3	37.9	58
Z-BUF group														
	median	1.10	49	—	22.7	6	133	218	294	320	319	37.1	36.8	11
	25%/75%	0.28/2.10	46/57	—	20/26	4/8	78/176	158/260	229/369	195/390	198/360	37.1/37.6	36.6/37.2	9/18
2	VSD	5.51	69	—	23.4	4	58	153	184	183	179	37.1	36.6	11
3	TGA	0.08	50	39	20.7	8	108	236	297	370	360	37.5	37.1	10
5	VSD	2.10	17	—	27.5	2	68	132	151	313	302	37.1	36.8	6
7	VSD	0.90	37	—	26.7	7	28	162	243	180	184	37.6	37.2	15
11	Tetralogy of Fallot	2.10	84	17	20	6	222	261	292	210	205	36.6	36.1	11
12	IAA	0.03	47	45	15	10	176	254	299	418	360	37.7	37.3	19
14	VSD	1.30	59	—	26	5	205	311	392	396	380	37.6	37.2	9
15	Tetralogy of Fallot	0.88	50	—	25	6	141	200	409	190	195	37.1	36.7	22
17	IAA	0.06	45	45	20	9	174	332	421	405	390	36.9	36.5	19
20	Tetralogy of Fallot	3.74	48	—	22	4	125	157	225	327	335	37.0	36.7	9
Mann-Whitney test		NS (0.47)	NS (0.23)	—	NS (0.27)	NS (0.46)	P = 0.019	P = 0.019	P = 0.019	P = 0.002	P = 0.002	P = 0.0008	P = 0.0009	P = 0.02

PRISM = pediatric risk of mortality; CPB = cardiopulmonary bypass; DHCA = deep hypothermic circulatory arrest; TGA = transposition of the great arteries (switch procedures); HLH = hypoplasia of the left ventricle; SV = single ventricle; IAA = interrupted aortic arch.

* Patient died before this measurement.

† Patient excluded from this analysis.

Table 2. Complement Fragment C3a and Cytokines Concentrations

	Limit of Detection	T1 (Before Hemofiltration + Rewarming)			T2 (After Hemofiltration + Rewarming)			T3 (24 h after the Operation)		
		Z-BUF (n = 10)	Control (n = 10)	P	Z-BUF (n = 10)	Control (n = 10)	P	Z-BUF (n = 10)	Control (n = 9)	P
C3a (mg/ml)	0.03	3.1 1.2/2.7/4.1/6.4	3.0 1/2.1/3.2/12.8	N.S. (0.34)	2.8 1/2.2/3.5/4.5	4.2 1.8/3.3/5.4/8.8	0.021	0.5 0.2/0.3/0.8/1.5	0.4 0.3/0.3/0.5/5.6	N.S. (0.76)
TNF (pg/ml)	1	0/1/3/6 2	0/0/3/29 1	*	0/1/2/26 1.2	0/2/18/49 4	0.045	0.5/1/10/72 2	4/7/31/421 8	0.028
IL-1 β (pg/ml)	1	0/0/1/4 0	0/0/0/3 0	*	0/0/1/8 0	0/0/0/8 0	*	0/0/1/4 0	1/11/33/45 31	0.0003
IL-6 (pg/ml)	2	0/1/15/185 10	0/3/40/202 4	N.S. (0.70)	3.5/13/72/300 30	10/30/67/2100 46	N.S. (0.54)	4.5/14/50/210 30	0/13/476/2100 302	0.019
IL-8 (pg/ml)	0.5	0/0/1/16 0.8	0/0/4/44 2	*	0/0/5/22 1	0/2/33/238 12	*	0/0/2/5 0	0/6/40/236 15	*

Mann-Whitney test. Actual measured values are reported. Values under the limit of detection were set to 0 for statistical testing.

* Statistics results are reported when the 25th percentile of at least one group is above the detection limit.

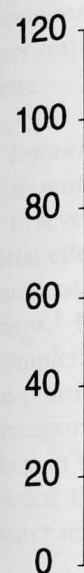


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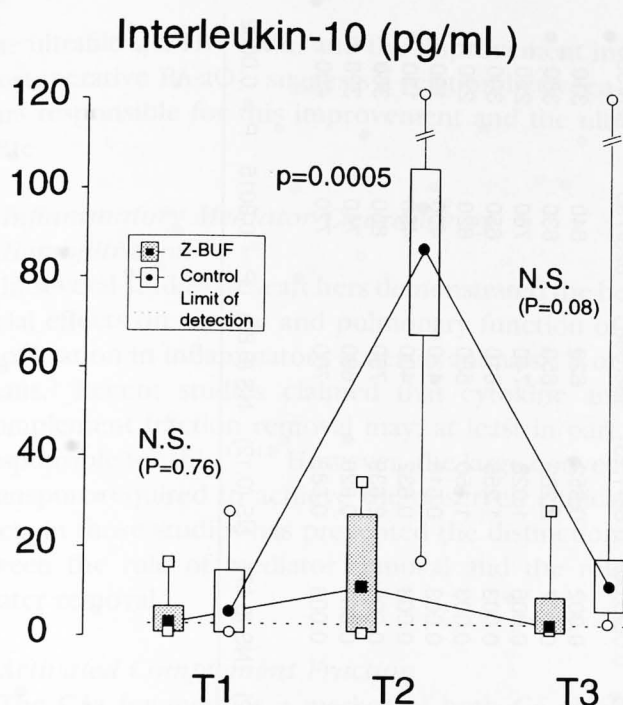


Fig. 1. Comparative changes in plasma interleukin-10 concentration over time (Mann-Whitney test). Data are expressed as median, with 25th, 75th percentiles, minimum, and maximum.

group but not in the Z-BUF group. The plasma myeloperoxidase concentration was significantly less in the Z-BUF group at the end of rewarming and at 24 h.

Anesthetic Assays

Total plasma midazolam concentrations did not change significantly during rewarming in the control group (183 ± 25 ng/mL to 195 ± 26 ng/mL, $P = 0.56$), but decreased in the Z-BUF group (188 ± 17 ng/mL to 162 ± 25 ng/mL, $P = 0.02$). The same pattern was observed with the plasma alfentanil concentration (616 ± 50 ng/mL to 626 ± 64 ng/mL, $P = 0.87$ in the control group; 611 ± 64 ng/mL to 562 ± 26 ng/mL, $P = 0.01$ in the Z-BUF group).

Discussion

Study Limitations

Although group assignment was blinded to intensivists, the technique of hemofiltration made a total blinding impossible in the operating room. However, no clinical data were obtained during the operation, and this

partial lack of blinding is unlikely to have influenced the biologic end-points measured in the operating room. The impact of this study may be limited by the relatively low number of included patients and by their lack of homogeneity. Nevertheless, the effects of the interventional treatment on simple clinical variables, as well as on markers of the inflammatory reaction, were dramatic, and this allowed easy reach of the statistical significance level when comparing the two groups. This may be due to the use of a very high volume of hemofiltration, which was experimental and which would not be recommended until further studies have confirmed both its efficiency and safety. Further studies also are required to determine whether a reduced hemofiltration rate, which should be easier to achieve, is similarly efficient.

Clinical Variables

The principal findings of this study are an improvement in postoperative clinical state associated with a reduction in plasma concentrations of several inflammatory mediators after Z-BUF in children during CPB. Several studies already reported improvements in biologic end-points that are believed to reflect beneficial clinical effects. These improvements have been achieved by different techniques, such as hemofiltration,^{1,4} administration of antiinflammatory agents,¹⁶ or use of heparin-coated CPB circuits.¹⁷ However, none of these studies demonstrated a related clinical benefit.

Hemofiltration can reduce postoperative blood loss in the absence of major changes in coagulation factor concentrations.^{3,7} This study confirms that this effect is not due to hemoconcentration. Cytokines have been implicated in altered coagulation^{18,19} and fibrinolysis,²⁰ and a reduction in their production may be the mechanism involved.

Postoperative fever is often present after CPB even in the absence of infection.⁶ This phenomenon is attributed to the effects of various endogenous pyrogens, including IL-1 β , IL-6, and IL-8.^{21,22} The lower peaks and mean postoperative temperatures observed in the Z-BUF group may reflect a globally reduced inflammatory response.

The reduction in the period of ventilation seen in the Z-BUF group confirms the finding reported by Stein *et al.*⁹ using a pig model, whereby zero-balanced ultrafiltrate reduced impaired oxygenation seen with a major systemic inflammatory response. The correlation observed between the volume of ultrafiltrate, assessed by

Table 3. Individual Leukocytes Counts and Myeloperoxidase Concentrations by Group

Patient No.	Neutrophils (Count $\times 1,000/\text{mm}^3$)			Lymphocytes (Count $\times 1,000/\text{mm}^3$)			Monocytes (Count $\times 1,000/\text{mm}^3$)			Myeloperoxidase (ng/mL)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Control group	1.00	6.46	14.10	1.33	2.33	1.60	0.097	0.003	1.520	640	895	600
1	0.85/1.42	3.42/12.33	12.52/15.22	1.19/1.58	2.07/2.66	1.29/1.85	0.06/0.14	0.00/0.00	1.23/1.67	570/703	805/988	600/700
4	0.77	3.66	12.52	1.29	2.35	1.85	0.184	0.005	1.231	600	1,000	600
6	0.88	3.34	8.69	1.18	2.21	1.14	0.110	0.004	1.436	680	800	650
8	0.77	15.31	29.39	0.85	1.52	1.29	0.068	0.002	2.001	710	820	700
9	1.71	12.54	15.22	3.12	2.75	2.50	0.363	0.004	2.970	900	950	850
10	1.12	2.97	*	1.59	2.02	*	0.156	0.002	*	480	650	*
13	0.85	22.02	14.10	0.76	2.92	1.60	0.046	0.003	1.520	560	850	550
16	1.64	4.07	13.32	1.38	2.30	1.79	0.052	0.007	1.674	620	1,200	600
18	1.16	2.99	20.48	1.25	1.33	1.28	0.086	0.003	0.806	750	940	800
19	0.85	11.68	14.97	2.69	3.34	1.45	0.109	0.001	1.624	660	800	600
	1.50	8.85	10.04	1.57	2.36	2.33	0.054	0.001	0.679	540	1,350	450
ZBUF group	1.21	3.52	7.44	1.71	2.66	3.35	0.170	0.003	0.940	635	645	305
2	0.74/1.35	2.53/4.57	6.20/9.56	1.23/2.37	2.02/4.01	2.44/6.21	0.10/0.25	0.00/0.00	0.65/1.23	588/698	623/735	228/350
3	0.59	7.09	9.99	1.17	2.67	3.11	0.101	0.002	0.852	620	640	310
5	1.42	1.20	5.04	3.30	2.66	6.86	0.924	0.007	2.262	650	630	380
7	1.32	2.97	6.02	1.99	6.73	3.07	0.256	0.006	1.028	740	760	250
11	1.17	4.88	8.26	2.49	4.03	4.25	0.155	0.003	1.255	610	650	300
12	0.18	4.50	7.50	0.31	0.90	1.18	0.029	0.003	1.150	580	620	220
14	1.36	1.44	4.47	1.43	2.02	7.21	0.222	0.003	0.616	420	400	190
15	0.91	3.63	15.21	2.59	4.88	3.60	0.185	0.003	0.632	400	450	180
17	0.68	2.38	6.74	1.40	2.02	7.16	0.100	0.005	2.639	750	850	360
20	1.67	3.41	7.38	1.14	1.47	2.23	0.296	0.001	0.620	690	740	320
Comparison (Mann-Whitney)	NS (0.94)	NS (0.07)	P = 0.0033	NS (0.54)	NS (0.49)	P = 0.007	NS (0.32)	NS (0.67)	NS (0.12)	NS (0.82)	P = 0.0015	P = 0.0002

* Patient died before this measurement.

the ultrafiltration postoperative factors responsible for the ultrafiltration.

Inflammatory Hemofiltration

In several studies, ultrafiltration has been shown to have beneficial effects on patients with acute renal failure. In a study by Mansueti et al.,³ ultrafiltration was shown to be responsible for the improvement in fluid balance and water removal.

Activated C3a

The C3a is a potent anaphylatoxin and generator of the complement cascade. It stimulates the release of histamine and other mediators from mast cells and basophils, leading to increased vascular permeability and edema formation. In severe cases, this can lead to a systemic inflammatory response syndrome (SIRS).

Its removal by ultrafiltration has been shown to be beneficial in patients with SIRS. In a study by Bellomo et al.,²⁴ ultrafiltration was shown to reduce the levels of C3a and other inflammatory mediators, leading to improved outcomes in patients with SIRS. This suggests that ultrafiltration may be a useful adjunct to medical therapy in the management of SIRS.

Cytokine Release

Both the ultrafiltration and plasma exchange techniques have been shown to reduce the levels of various cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor- α . This suggests that these techniques may be useful in the management of SIRS by reducing the levels of these pro-inflammatory mediators.

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the ultrafiltrate/TBV ratio, and the improvement in the postoperative PA-aO₂, suggests a relation between factors responsible for this improvement and the ultrafiltrate.

Inflammatory Mediator Clearance by Hemofiltration

In several studies, researchers demonstrated the beneficial effects on cardiac and pulmonary function of hemofiltration in inflammatory states in animals⁹⁻¹¹ or humans.³ Recent studies claimed that cytokine and/or complement fraction removal may, at least in part, be responsible for this.^{1,3,4,8} However, the large convective transport required to achieve the observed clinical effects in those studies has prevented the distinction between the role of mediator removal and the role of water removal.

Activated Complement Fraction

The C3a fragment is a marker of both C3 cleavage and generation of C3b. This fragment triggers the formation of the terminal complement attack complex, which stimulates neutrophil degranulation. The C3a fragment deposits on pulmonary vascular endothelium during CPB mediates increased expression of neutrophil CD18 adhesion protein and neutrophil sequestration.²³ This study shows that C3a removal is associated with a reduction in several adverse clinical effects linked to an activated complement system.

Its removal is not surprising, considering that it is hydrophilic and has a low molecular weight. The normal C3a concentration observed at 24 h in both groups confirms that C3a generation is limited to the period of CPB and supports the premise that any attempt to remove it from blood should be made at the time of its release. Unlike the traditional cuprophane hemofilter and the CPB circuit, which activate the complement cascade, polyacrylonitrile filters, which were used in this study, induce less complement activation and adsorb complement fragments onto the membrane surface.^{24,25}

Cytokines

Both the adverse effects of experimental cytokine administration and the poorer prognosis related to high plasma concentrations of cytokines in sepsis have been investigated.⁶ However, the clinical benefit of their neutralization or removal has not been proven. In addition, researchers in several studies suggest that plasma cyto-

kine concentrations are only circulating markers of an inflammatory process occurring at tissue level.²⁶ Therefore, cytokine assays were used in this study as markers of the effects of Z-BUF of the inflammatory response to CPB rather than to address their removal. One major finding of this study regarding cytokines was that concentrations of IL-1 β , IL-6, and IL-8 at T₃ were closely related to the use of high volume hemofiltration 24 h before, but not because hemofiltration removed them directly.

Tumor necrosis factor was one of the first cytokines to be implicated in the activation of endothelial cells, causing hypotension and leukopenia along with IL-1 β . The difference between the two groups at the end of rewarming, as with IL-1 β , and 24 h later, as with IL-1 β , IL-6, and IL-8, is likely to be due to blocking of the inflammatory response to CPB, thereby inhibiting the release of TNF.

Complement activation is known to induce IL-1 β gene expression,²⁷ and exposure to extracorporeal circuits leads to IL-1 β release approximately 20 h later.²⁸ The suppression of the 24th-hour IL-1 β response to CPB observed in this study may, therefore, be due to early clearance of the circulating complement fragments by Z-BUF.

Among other effects, IL-8 binds to specific receptors on neutrophils and augments their migration and degranulation.^{13,29} Therefore, IL-8 is regarded as one of the triggers of neutrophil-induced endothelial injury contributing to the "post CPB" syndrome.¹³ Interleukin-8 has been shown not to be cleared from blood by hemofiltration.^{3,4} One reason may be that IL-8 is significantly bound by red cells, so the free fraction that can be filtered is low.³⁰

Interleukin-10 is a potent inhibitor of the production of TNF α , IL-1 β , IL-6, and IL-8.^{31,32} This cytokine is produced during septicemia and exerts a protective role³³; its neutralization or removal may, therefore, be deleterious.

The significant increase in IL-10 only observed in the control group at the end of rewarming would suggest that Z-BUF has either removed it from plasma or suppressed some factors that contribute to its release. Given the inhibitory effect that IL-10 has on the release of other cytokines,^{31,32} the absence of a rebound increase in the other cytokines at T₃ would imply that such network interactions occur at a local tissue level and are not influenced by the insignificant changes induced by hemofiltration.

Changes in Leukocytes

Cardiopulmonary bypass also interferes with cell-mediated defenses. Lymphopenia is associated with a suppression in lymphocyte function.^{16,34,35} Rewarming induces neutrophil adhesion and pulmonary sequestration, followed by the release of oxygen intermediates, proteolytic enzymes, and cationic proteins.^{36,37} These changes can be prevented by removal of neutrophils by plasmapheresis before bypass.³⁸⁻⁴⁰

The increase in the neutrophil count seen in the control group at the end of rewarming may be due to circulating C3a and C5a promoting release of leukocytes and precursors from bone marrow.⁴¹ The less dramatic increase in the Z-BUF group may be related to the changes in complement. Royston *et al.*⁴² observed that as many as 50% of circulating polymorphonuclear leukocytes are selectively trapped in the pulmonary capillaries during the rewarming period, with subsequent degranulation contributing to endothelial damage.⁴³ The lower myeloperoxidase plasma concentration observed in the Z-BUF group is consistent with less neutrophil degranulation and may be partly responsible for the less severe PA-aO₂ gradient observed in this group.¹⁷

Anesthetic Agents Removal by Hemofiltration

Hemofiltration had no adverse effect on the anesthetic used during the study. Although plasma midazolam and alfentanil concentrations were reduced by Z-BUF, the decrease was not clinically significant and the drug concentrations remained in the high therapeutic range.⁴⁴ This may be due to the fact that the agents used are subject to high protein binding, thereby limiting their convective removal.⁴⁵ Nevertheless, with the introduction of high volume hemofiltration to perioperative management, its effect on other agents should be considered.

Management of Hemofiltration

Both studied groups received a standardized short duration "modified ultrafiltration" at the end of CPB to remove excess fluid. The beneficial effects of this method on pulmonary function, hemodynamics, oxygen transport, and reduction of myocardial edema have already been well demonstrated.^{2,7} We estimated that the importance of fluid removal, which is our routine technique, is such that withholding modified hemofiltration in an attempt to address Z-BUF alone would have been unethical. However, any benefits of this tech-

nique, as well as the use of aprotinin, was equally applicable to both groups.

If the preferred time for hemofiltration, to limit the effects of the inflammatory response, appears to be throughout the rewarming period, the "modified ultrafiltration" performed after CPB weaning is likely to be more efficient at water removal.⁷ It would, therefore, be best to combine both techniques in children undergoing CPB.

Conclusion

This preliminary study suggests that high-volume, zero-balanced hemofiltration can reduce the inflammatory response to CPB at an early stage. The reduction observed in plasma concentration of several mediators is, therefore, probably more a response to a reduced inflammatory process rather than a result of their removal by hemofiltration. Further clinical evaluations should address a larger population of patients to determine whether the biologic findings of this study are accompanied by improvements in outcome. Pediatric patients might, therefore, benefit in the future from new ways of dampening the inflammatory response of blood exposed to foreign surfaces. Until then, hemofiltration provides the opportunity to limit the clinical consequences of CPB while adjusting the body fluid balance.

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