Systemic inflammatory response syndrome is reduced by preoperative plasma-thrombo-leukocyte aphaeresis in a pig model of cardiopulmonary bypass

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Objectives. The systemic inflammatory response syndrome (SIRS) after cardiac surgery with cardiopulmonary bypass (CPB) exacerbates organ dysfunction and increases postoperative mortality. The aim of this study was to reduce SIRS after CPB in a pig model by profoundly decreasing all blood defence factors (complement, coagulation and fibrinolytic and contact systems, leukocytes and thrombocytes) using pre-operative aphaeresis.

Methods. Thirty-three pigs underwent 3 h of hypothermic CPB with 2 h of cardioplegic arrest, followed by 4 days of observation. One half of the sample underwent prebypass plasma-thrombo-leukocyte aphaeresis with the adjuvant leukofiltration.

Results. In the control group, there were classical signs of SIRS (tachycardia, tachypnea and leukocytosis) postoperatively. There was also myocardial ischaemia and the need for inotropic support in 90% of the control animals. Neutrophils showed an increase in superoxide anion production ($P < 0.001$), and surface neutral protease activity ($P < 0.001$) and blood endotoxin levels increased ($P < 0.01$) compared with preoperative levels. In contrast, in the aphaeretic group, there were no classical signs of SIRS; no myocardial ischaemia; minimum neutrophil production of the superoxide anion and protease activity were recorded ($P < 0.001$); and endotoxin levels were also decreased ($P < 0.05$) compared with the controls. In the control group, the haemodynamic problems associated with disconnecting from CPB correlated with the histologic findings in the myocardium (leukocyte endothelial adhesion and leukodiapedesis).

Conclusions. Pre-operative plasma-thrombo-leukocyte aphaeresis significantly reduces the major symptoms of SIRS and organ dysfunction after 3 h of CPB without adverse effects, such as bleeding and infection, during the postoperative course.

Key words: systemic inflammatory response syndrome, plasma-thrombo-leukocyte aphaeresis, cardiopulmonary bypass

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INTRODUCTION

Systemic inflammatory response syndrome (SIRS) after cardiac surgery remains clinically evident despite improved surgical procedures¹. SIRS is linked to temporary depression in all organ functions, especially vital organs such as the heart, lungs, brain and splanchnic organs¹. Despite long lasting debates about its clinical importance, it is now clear that SIRS after cardiac surgery contributes to longer intensive care unit stays due to the exacerbation of organ dysfunction and increases early postoperative mortality².

The cause of SIRS is complex and includes prolonged contact of the blood with the non-endothelial surface of the bypass system and specific milieu during cardiopulmonary bypass (CPB): nonpulsatile blood flow, hypothermia and haemodilution¹. Other major factors include surgical trauma, ischaemia-reperfusion injury and contamination with bacterial endotoxins. The proposed mechanism of

SIRS has led to the formulation of an immunologic hypothesis. A defensive inflammatory reaction, initially localized to a site of injury or infection, spreads throughout the organism via the cardiopulmonary bypass system and becomes deleterious after exhausting the natural regulators of inflammation. A pathological interaction, strengthened by protein systems, occurs between the vascular endothelium and the activated leukocytes and thrombocytes, resulting in the loss of vascular integrity, the loss of intravascular volume, the transition of activated cells into the tissues and hypoxia due to microcirculation failure^{3,4}. The pathophysiology of SIRS allows the possibility of prevention and therapy: prevention includes refinement of surgical techniques and improved biocompatibility of extracorporeal systems or systemic preconditioning⁵. Such methods have achieved reasonable success but for complex and longer surgical procedures, they have not been effective. Therapy influences the inflammatory response

pharmacologically and decreases the levels and activity of activated host defence factors⁶. The latter method was used to decrease blood loss by preoperatively collecting thrombocyte-rich plasma⁷. Another study showed that leukocyte removal by filters is suitable and safe for use during cardiac surgery. Early clinical reports appeared to confirm these findings, but reviews in recent years have concluded that the clinical effects have not been evaluated vigorously enough and that no recommendations can be made regarding the use of leukocyte-depleting filters in routine cardiac surgery⁸. The aim of this experimental study was to prevent SIRS by reducing all blood components of the host protection system (plasma protein systems, leukocytes and thrombocytes) by using pre-operative aphaeretic-filtration in a pig model of SIRS elicited by CPB, with 4 days of postoperative observation.

MATERIALS AND METHODS

Perioperative procedure

All manipulations with the animals respected the European Convention for experimentation with laboratory animals, and the concrete experimental protocols were approved by the local Constitutional Commission for Ethics. Thirty-three minipigs, 8-12 months old, with an average weight of 40 ± 7 kg, were used. Eight animals were used for the development and validation of the pig model of SIRS after CPB. Sixteen minipigs received the standard CPB, and seventeen minipigs underwent aphaeretic-filtration before bypass. Five animals were excluded from the experiment (three cases for surgical bleeding complications; one pig had malignant hyperthermia, and one pig experienced serious postinfective pericardial and pleural structural changes (Fig. 1)). After pre-medication with atropine, ketamine and dehydrobenzperidol, the animals were intubated under local anaesthesia, relaxed with pipercuronium, and maintained at a tidal volume of 10 mL/kg with an inspiratory oxygen fraction of 50%. Anaesthesia was maintained by 1-2% isoflurane. Then, brachial arteries and veins were secured to measure the systemic and central venous pressures and the connection of the aphaeretic apparatus. Via a right-sided thoracotomy, the upper and lower vena cava and ascending aorta were prepared for the connection of the bypass. A vent was also applied into the v. pulmonalis for the decompression of the left side. All of the animals received an initial dose of heparin at 3 mg/kg (Zentiva, CZ), and ACT (Hemochron, ITC, USA) was maintained for more than 400 s. A Capiiox SX 18 membrane oxygenator (Terumo, Japan) was used without heparin coated lines. The priming was a mixture of crystalloids and a starch derivate (Tetraspan 10%, Braun, Germany) calculated for HCT 20%. After initiating perfusion, the heart was fibrillated electrically; the aorta was clamped, and 500 mL of St. Thomas solution was administered and then repeated after 30 min. Systemic cooling was performed to reach a temperature of 27°C, and local cooling of the heart was performed to reach 20°C. The flow after systemic cooling was reduced from 80 mL/kg to 50 mL/kg per min. After aortic declamping (2 h) and heating to

38 °C, disconnection from the bypass was carried out, and after the cannulae were removed, protaminsulphate was added for heparin neutralization. After weaning off CPB, cardiopulmonary stabilization and extubation, the pigs were observed until the 4th post-operative day and then euthanized. Their tissue samples were subsequently taken for histological analysis.

Aphaeretic and filtration procedure

After ensuring arterial access, the separation was initiated using a PCS plus apparatus (Haemonetics, Mas. USA). During 2 h of aphaeresis, during the preparation to connect the extracorporeal bypass, 70% of the calculated plasma volume was gained, and the numbers of thrombocytes and leukocytes were reduced to 40% of their original quantities. The volume of blood flowing back from the centrifuge corresponded to the calculated blood volume that came through the Pal RC 400 (Pall Corp. Italy) leukocyte filter, which was replaced after the overflow of 1 L of blood to ensure a 95% leukocyte catch. The separated volume was replaced with crystalloids and a starch derivate during aphaeresis and, after the procedure had finished, by a 5% solution of pig albumin (Bioveta, Czech Rep.). Intravascular blood volume was determined by central venous pressure (CVP) measurement, and the systemic pressure was maintained at the initiating level using a dopamine infusion.

Myocardial ischaemia detection and pulmonary function

At minimum, two of three ECG changes (leg leads) were considered to be positive for myocardial ischaemia: ST segment fluctuation (more than 2 mm), QRS interval prolongation and the presence of any rhythm other than sinus rhythm. Inotropic support was defined as follows: the need for dopamine at a rate of more than 10 µg/kg/min and a duration of longer than 2 h. Lung mechanics (static compliance and inspiratory resistance) were subtracted from the analytical unit of the anaesthesia machine (Datex-Ohmeda).

Leukocyte determination

Leukocytes were isolated by sedimentation from citrate blood in dextrate 100 (Serva) in a ratio of 4 portions of blood to one portion of 6% dextran for 30 min at 37 °C. The cells from the dextran were washed, and the erythrocytes were removed by hypotonic lysis and again washed twice in phosphate buffered saline (PBS) with bivalent Ca and Mg ions.

Superoxide anions of neutrophils. These levels were determined on the basis of the ability to reduce cytochrome c (cyt c). In the reaction mixture, 3×10^5 leukocytes, 100 nmol of cyt c (type III, Sigma Aldrich) and 0.5 mg of zymosan (Serva) were opsonized by 7.5% autologous serum for a period of 30 min before the experiment. Parallel samples were incubated either with or without 120 µg of superoxide dismutase – SOD (Serva) for a total volume of 0.7 mL of PBS. After 30 min of incubation, the samples were immediately cooled in ice and centrifuged. The cell supernatant was measured for changes in extinction at 550 nm. The differences between the values/levels at E

550 with and without SOD yielded the specific differences in the reduction in cyt c by the superoxide anions formed by the neutrophils.

The surface neutral protease activity of the neutrophils was determined with the chromogen substrate azocoll (Calbiochem) by particles of collagen with the bound/attached azocolour. The reaction mixture contained 3.10^6 cells and 1.5 mg of azocoll per sample at a total volume of 0.7 mL in PBS, which was incubated for 30 min at a temperature of 30 °C. Then, the samples were immediately cooled in an ice bath and centrifuged, and the cell supernatant was measured for changes in extinction at 550 nm. Leukocyte incubation was carried out in 5 mL of polypropylene test tubes (NUNC).

The phagocytic activity of the leukocytes was assessed by microspheric hydrophilic particle engulfment (MSHP, Czech Rep). The blood samples were processed immediately after being collected in the following manner: 0.1 mL of heparinized blood (max. 10 I.U. of heparin per 1 mL of blood) was added to 0.005 mL of the diluted suspension of microspheric particles in polystyrene test tubes (KOH-I-NOR, Czech Rep.) and incubated with occasional shaking for 60 min at 37 °C. After the incubation, coats of paint were placed on microscopic glasses and coloured by a time-modified May-Grundwald and Giemsa solution. In every sample, 100 cells were counted: phagocytosing and non-phagocytosing neutrophil granulocytes and monocytes were counted. The phagocytic index (total number of phagocytosing particles per 100 cells) was determined.

Complement protein determination

The micromodification of the haemolytic method was used in a system containing 3.10^7 erythrocytes in a total volume of 0.5 mL. For the determination of the alternative path, we used guinea pig erythrocytes; for the diluting solution, we used veronal buffer containing ethyleneglycol-bis-(beta-amino ethylether)-N,N-tetraacetic acid (EGTA) at a final concentration of 1 mM. The grade/level of haemolysis was determined by using the photometric method at 412 nm, and the AH50 index (alternative pathway complement haemolysis units) was counted.

Endotoxin and microbiological estimations

Endotoxin levels: the blood samples were collected in pyrogen-free tubes, centrifuged at low speed and stored in a refrigerator. The samples were then analysed using a Limulus amebocyte lysate test (Sigma-Aldrich, USA). Endotoxin concentrations were not corrected for haemodilution because the haematocrits were maintained at the same levels in both groups. *Microbiological estimation*: the blood samples were collected on the second postoperative day, and lymphatic nodes near the large intestine were excised after stopping the experiment using the imprint method on the sensitive cultivation medium.

Histological examinations were performed with fixation by 8% forbol, paraffine cuts, and colouring by Giemsa. The kidneys were impregnated by Ag (Jones), PAS (Periodic Acid Schiff) and IF (Immersion Fixation). Tissue samples were withdrawn from the left ventricle (anterior wall), lung (right lower lobe), brain (frontal lobe) and kidney (upper pole).

Statistical analysis

Continuous data are presented as the average $\pm$ standard error of the mean. Categorical variables are expressed as percentages of categories (%). Comparisons between the groups were carried out by two-way analysis of the variance for repeated measures. Comparisons within groups were performed with a one-way analysis of variance, followed by a paired Student's t-test. Statistical significance was computed using the χ^2 -test. $P < 0.05$ was considered to be statistically significant.

RESULTS

The validation branch of the experiments showed the classical signs of SIRS after 3 h of CPB: tachycardia, tachypnea and leukocytosis in the early postoperative period compared with the prebypass period. The same trends were confirmed in the control group in the main experimental branch: elevation of heart + respiratory rates 6 h postoperatively and leukocytes 12 h postoperatively

Table 1. Physiological data of animals.

Control							
n=10	Normal range	Preop	Postop.	Day 1.	Day 2.	Day 3.	Day 4.
Heart rate	70-90/min	88 $\pm$ 7	138 $\pm$ 12 *	115 $\pm$ 12 *	110 $\pm$ 15	105 $\pm$ 18	99 $\pm$ 19
Resp. rate	10-30/min	23 $\pm$ 6	38 $\pm$ 5*	30 $\pm$ 4	28 $\pm$ 6	25 $\pm$ 5	26 $\pm$ 7
Body Temp.	38-40 °C	37.7 $\pm$ 2.2	35.7 $\pm$ 2.4	36.8 $\pm$ 4.1	37.9 $\pm$ 3.3	39 $\pm$ 5.4	39.5 $\pm$ 5.2
Leukocytes	7.5-13.5x10 ⁹ /L	12.8 $\pm$ 2.1	15.6 $\pm$ 3.2	17.2 $\pm$ 2.4 *	12.6 $\pm$ 3.4	13.1 $\pm$ 3.6	14.2 $\pm$ 3.6
Aphaeretic							
n=11							
Heart rate	70-90/min	90 $\pm$ 10	107 $\pm$ 14	94 $\pm$ 12	95 $\pm$ 11	98 $\pm$ 11	87 $\pm$ 12
Resp. rate	10-30/min	28 $\pm$ 4	22 $\pm$ 5	26 $\pm$ 4	20 $\pm$ 7	22 $\pm$ 6	25 $\pm$ 4
Body Temp.	38-40 °C	38.6 $\pm$ 0.2	36.2 $\pm$ 2.4	37.5 $\pm$ 0.6	39.5 $\pm$ 0.8	39.2 $\pm$ 0.5	38.2 $\pm$ 0.7
Leukocytes	7.5-13.5x10 ⁹ /L	8.3 $\pm$ 2.2	11.1 $\pm$ 2.1	9 $\pm$ 1.9	10.3 $\pm$ 2.4	9.8 $\pm$ 2.2	8.1 $\pm$ 2.4

The control group shows a postoperative increase in three of the four physiological parameters elevated in SIRS: heart + respiratory rates 6 h postop. and leukocytes 12 h postoperatively ($P < 0.05$ for preoperative measurements versus 6 h and the 1st postop. day by one-way analysis of variance).

compared with preoperative levels ($P < 0.05$). (Table 1). The average time of the aphaeretic procedure was 110 ± 18 min, and the procedure was completed before initiating CPB. The efficiency of plasmapheresis was validated by measuring the fibrinogen level, which decreased to 25% of the amount measured prior to plasmapheresis (Table 2.). The thrombocyte concentration reached its predicted level ($\times 10^{11}/L$). The blood returning from the centrifuge through the leukocyte filter contained 800 leu/L, 500 thr/L and 9.9×10^{12} ery/L. Despite this high level of separation, the numbers of thrombocytes and leukocytes decreased to 40% of their initial levels on average. The decreases in platelets and leukocytes were the same in both groups, which corresponded to haemodilution (Table 2). A smaller decrease in the blood elements than in the plasma proteins (fibrinogen) was caused by bone marrow compensation. The differences in the peripheral blood lymphocytes were larger than in the neutrophils, likely due to more efficient aphaeresis and lower bone marrow compensation. Table 2 also shows a significant difference in the decrease in complement proteins (alter-

native path), which was noticeable after aphaeresis was performed ($P < 0.001$). A further decrease was shown by their consumption during CPB by the differences between the groups ($P < 0.05$). The control measurements after surgery showed normalisation in both groups.

Fig. 1. records the activation of the neutrophil granulocytes by the measurement of superoxide anion production and surface neutral protease activity. The superoxide anions were increased in the control group as early as when CPB was initiated, and they increased to as much as 100% of their initial level, whereas in the aphaeretic group minimal production was recorded during the operative phase. During the early postoperative phase, however, this trend reversed, and the increase in activity was significantly higher in the aphaeretic group during the first two postoperative days. A similar trend was observed for the second marker of neutrophil activation, i.e., the surface neutral proteases. The phagocytic activity of the neutrophils was not affected in both groups; there was only a small increase at the end of the operation, which was not statistically significant (Table 2).

Table 2. Perioperative data of animals.

	Normal range	preop	Aph.-end	CPB-10' Control	CPB-end	Postop.	Day 1
n=10							
Fibrinogen	1.5 - 4 g/L	3.3 ± 0.9	$3.1. \pm 0.8$	$1.3. \pm 0.2$	1.7 ± 0.3	1.8 ± 0.4	2.9 ± 0.9
Complement	>1.5AH50 u/mL	3.9 ± 0.9	$3.8. \pm 0.8$	1.8 ± 0.6	1.5 ± 0.4	1.6 ± 0.4	2.8 ± 0.6
Thrombocytes	$190-500 \times 10^9/L$	235 ± 70	230 ± 65	130 ± 20	100 ± 15	85 ± 15	92 ± 10
Neutrophils	100%	95 ± 5	90 ± 10	55 ± 7	37 ± 6	60 ± 7	46 ± 6
Lymphocytes	100%	90 ± 10	40 ± 10	30 ± 7	40 ± 10	40 ± 9	47 ± 9
Phagoc. Index	Particle/cell	10 ± 2	11 ± 1	8 ± 1	10 ± 1	14 ± 3	13 ± 2
n=11				Aphaeretic			
Fibrinogen	1.5 - 4 g/L	2.6 ± 0.8	$0.8 \pm 0.2^{*}\#$	$0.4 \pm 0.1^{*}\#$	$0.3 \pm 0.1^{*}\#$	$0.3 \pm 0.1^{*}\#$	2.4 ± 0.4
Complement	>1.5AH50 u/mL	3.4 ± 0.7	$1.4 \pm 0.4^{*}\#$	0.8 ± 0.3	$0.5 \pm 0.2^{*}\#$	$0.5 \pm 0.2^{*}$	2.4 ± 0.5
Thrombocytes	$190-500 \times 10^9/L$	240 ± 65	$85 \pm 15^{*}\#$	$55 \pm 10^{*}\#$	$50 \pm 12^{*}$	$50 \pm 10^{*}$	$110 \pm 15^{*}$
Neutrophils	100%	95 ± 5	$60 \pm 7^{*}\#$	$35 \pm 5^{*}$	$25 \pm 4^{*}\#$	$30 \pm 5^{*}\#$	$55 \pm 5^{*}$
Lymphocytes	100%	95 ± 5	$20 \pm 5^{*}\#$	$20 \pm 5^{*}\#$	$15 \pm 5^{*}\#$	$15 \pm 5^{*}\#$	$35 \pm 5^{*}$
Phagoc. Index	Particle/cell	11 ± 2	12 ± 2	11 ± 2	11 ± 1	13 ± 3	14 ± 4

AH50: (alternative pathway complement haemolysis units); Neutrophils, Lymphocytes: data are expressed as percentage of initial levels. Phag. index: Phagocytic index of leukocytes- the total number of phagocytizing particles per 100 leukocytes. * $P < 0.05$ for time point Preop versus the next points by one-way analysis of variance in the aphaeretic group. # $P < 0.05$ for time points Aph-end, CPB-10, CPB-end, Postop. between both the groups by a two-way analysis of the variability for repeated measurements.

Table 3. Early postop. data of animals.

	Parameter	Control n=10	Aphaeretic n=11	significance
PaO ₂	% of change	13 ± 5	14 ± 6	NS
Static. compliance	% of change	33 ± 5	24 ± 3	$P < 0.05$
Inspir. resistance	% of change	22 ± 5	3 ± 2	$P < 0.003$
Myocard. ischaemia	percentage	90	10	$P < 0.003$
Inotropic support	percentage	90	25	$P < 0.007$
Extubation	hours	6 ± 1.5	5 ± 1.2	NS
Blood loss	mL/18 h	375 ± 95	490 ± 150	$P < 0.05$

Pulmonary parameters are expressed as a percentage of the change of the initial value. Myocardial ischaemia was defined as an ST segment fluctuation (more than 2 mm), QRS interval prolongation (leg leads) or a rhythm other than sinus rhythm. Inotropic support: the need for dopamine at more than $10 \mu\text{gr}/\text{kg}/\text{min}$. Myocardial ischaemia and inotropic support were significantly more common in the controls ($P < 0.007$ control versus aphaeretic group by Fisher's exact test). Blood loss was higher in the aphaeretic group ($P < 0.05$ for aphaeretic versus control group by one-way analysis of variance). NS = non-significant.

Low concentrations of endotoxin were detectable during the prebypass period in both groups (Fig. 2). In the controls, endotoxin levels gradually increased after stopping CPB up to the 1st postoperative day compared with preoperative levels ($P < 0.001$) and then returned to prebypass levels. In the aphaeretic group, endotoxin levels were significantly lower at 6 h postoperatively and on the morning of the next day compared with the controls ($P < 0.05$).

The myocardial incisions from the controls exhibited significant signs of inflammation: leukostasis in the arterioles and capillaries and leukocyte perivascular, as well as perifibrillar infiltration (Fig. 3c, 3d). Moreover, after the 4th postoperative day, there were numerous areas characterized by parenchymatous dystrophy of the myocardial fibres (cloudy swelling). Such findings were not discovered in any incisions in the hearts of the aphaeretic group. In the kidneys, protein decrease in the controls were found both in the glomeruli and the tubuli, and leukodiapedesis was also present. In the aphaeretic group, the presence of protein drops was sporadic and without leukodiapedesis (Fig. 3e).

Similar histologic differences were found in the lungs; in the control group resorbing intra-alveolar edema was found (Fig. 3a, 3b). In the brain, there was extensive leukocyte glial infiltration which was found only in the control group (Fig. 3f). The behaviour of the animals in the postoperative period was also different: the control animals were apathetic up to the 2nd postoperative day and were in need of a parenteral fluid supply, whereas the aphaeretic group stood and drank as early as the first postoperative day.

During the process of weaning from the CPB, 90% of the animals in the control group showed signs of significant myocardial ischaemia requiring inotropic support, whereas in the aphaeretic group, these ischaemic changes were not present, and inotropic support was necessary in only 25% of the animals (Table 3). Blood loss up to the morning of the 1st postoperative day was higher in the aphaeretic group ($P < 0.05$).

DISCUSSION

We chose pigs for our model of SIRS after CPB because aside from the similarities in their immune systems, porcine physiology is also close to that of humans, making them a widely used model in medicine⁹. In fact, pigs are more sensitive to CPB and cardioplegic arrest than humans, whereas they are more tolerant of endotoxins. Our validation branch of experiments showed the presence of 3 of the 4 classical signs of SIRS (ref.¹⁰), and we confirmed organ inflammation by histology.

The ongoing research regarding the organism response to surgical trauma and the contact of blood with non-endothelial cells surfaces has added new information to the mosaic of present-day concepts regarding the nonspecific defence systems of an organism. CPB with surgical trauma activates at least 5 protein systems in the plasma and 5 cell types in the blood. The contact system ranks

Fig. 1. Neutrophil activation expressed by superoxide anion production and protease activity. 0: before aphaeresis-filtration, I: after aphaeresis-filtration, II: 10 min. after starting CPB, III: after stopping CPB, IV: after skin suture, V: 6 h postop. 1-4: postop. days. Data are expressed as a percentage of initial anion production and protease activity. $P < 0.001$ for time point 0 versus point III. and point 2 by one-way analysis of variance. $P < 0.001$ for points III. and 2 by two-way analysis of variance for repeated measurements.

Fig. 2. Endotoxin levels. 0: before aphaeresis-filtration, I: after aphaeresis-filtration, II: 10 min. after starting CPB, III: after stopping CPB, IV: after skin suture, V: 6 h postop. 1-4: postop. days. Stripped bars: Control group; Open bars: Aphaeretic group. In the controls, endotoxin levels increased at two time points: 6 h postoperatively and on the morning of the next day compared with preoperative levels ($P < 0.001$ for time point 0 versus points V. and 1 by one-way analysis of variance). In the aphaeretic group, endotoxin also increased significantly compared with preoperative levels but was lower than in the controls ($P < 0.05$ control versus aphaeretic group at time points V. and 1 by two-way analysis of variance for repeated measurements).

among the first¹¹, followed by the proteins of the intrinsic and extrinsic pathways, which are activated by the surgical incision by the expression of tissue factor (TF) on monocytes, as well as by CPB (ref.^{12,13}). The fibrinolytic system also plays an important role. Fibrin formation is impaired to a greater extent, and hyperfibrinolysis may also occur¹⁴. The complement system, especially its alternative amplification path and reactive intermediate products, also plays an important role¹⁵. Thrombocytes are activated during

Fig. 3. Light microscope, magnification: x 440, colouring: haematoxylin. Nuclei of leukocytes are spheric with dense colouring. The 4th postoperative day. Left pulmonary lobe: a) Aphaeretic group: normal parenchyma, b) Control group: resorbing edema, interalveolar leukocyte infiltration. Left ventricle: c) Aphaeretic group: normal parenchyma with the arteriolar cross section, d) Control group: leukocyte endothelial arteriolar adhesion and leukocyte infiltration of the interfibrillar space. Kidney: e) Control group: glomerular cross section with protein drops and leukocytes. Brain, frontal lobe: f) Control group: leukocyte endothelial arteriolar adhesion and extensive leukocyte glial infiltration.

CPB, likely by thrombin, and they adhere to and aggregate on the non-endothelial surface. Then they may embolize and contribute to the systemic inflammatory reaction¹⁶. Neutrophils play a key effector role in systemic inflammation because their activation leads to the production of cytotoxic hydroxyl radicals and proteolytic enzymes¹⁷. The level of cytokines produced by monocytes increases during and after extracorporeal circulation and orchestrated pro-anti-inflammatory responses¹⁸. Endothelial cells, with their surface of 100 square metres, are effective excretion organs that are closely involved in inflammatory processes¹⁹. Lymphocytes are prone to depression, both functional and quantitative, due to the influence of the extracorporeal bypass²⁰. Several ways of controlling these processes must be considered. The development of biomaterials that does not activate blood components and

the protein systems of the plasma have not been entirely successful to date. Another way involves the temporary inhibition of host defences, particularly those involved in the initial activation, such as nuclear factor kappa B (NFkB) (ref.²¹). The influences of corticosteroids and proteolytic enzymes which have a certain effect but also have undesirable side-effects, have been examined thoroughly²². The use of thrombocyte inhibitors to mitigate SIRS is in the experimental stage²³. It is difficult to accomplish such a goal by the inhibition of one or more components of the inflammatory network. The innate defence of the organism has been refined over millions of years into a highly redundant activating network with many feedback points and co-stimulating branches. Our attempted method was the reduction of the blood defence systems by means of a separation technique. However, the effective influence

of the inflammatory process which is activated at many checkpoints, is possible only by changing the environment in which these actions proceed. Therefore, we changed 70% of the plasma and reduced the number of leukocytes and thrombocytes to 40% of their initial levels using separation and filtration. The reduction of the blood elements to 40% was established, together with the time limit of the separation, mainly by effective compensation from the bone marrow in the aphaeretic group. As little as 70% of plasmapheresis influenced haemocoagulation in a negative way, as we discovered in the validation branch. The level of depression of the inflammatory activity which was measured by the activation of neutrophils, confirmed our assumptions. The presence of blood endotoxin in connection with CPB was studied widely in the 1990s. Two sources have been found: the environment and the gut (endotoxin translocation due to increased intestinal permeability after CPB) (ref.²⁴). Endotoxaemia augments SIRS, but its true pathological role after CPB has been called into question²⁵. In accordance with other studies, we found blood endotoxin elevations after stopping CPB but we detected lower levels in the aphaeretic group. We hypothesized that the dampening of the systemic inflammation in the aphaeretic group restricted intestinal permeability and that such a method decreased endotoxin translocation.

Blood loss was of only moderate clinical significance, although the difference was statistically significant. Pulmonary function was influenced according to differences in lung static compliance and inspiratory resistance, although the decreases in PaO₂ were not significant between the groups. It is possible that any changes could be overlaid by high inspiratory oxygen concentration. However, we found no difference in the length of the ventilation support.

The limitations of this study are twofold: first, we did not measure cytokine levels, adhesive molecules or chemokines as we instead measured the differences in neutrophil activation. Second, the experimental results from young, healthy animals must be applied to clinical practice with caution.

CONCLUSION

In conclusion, reduction of blood defence systems by preoperative plasma-thrombo-leukapheresis profoundly reduces symptoms of SIRS and organ dysfunction after 3 h of CPB with 2 h of cardioplegic arrest. This method does not interfere with the standard process of a cardiac operation with respect to either time or technique and is worth verifying in a clinical trial.

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