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Coagulation, fibrinolysis, and cell activation in patients and shed mediastinal blood during coronary artery bypass grafting with a new heparin-coated surface

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Objectives: Heparin coating of the cardiopulmonary bypass circuit is shown to improve the biocompatibility of the surface. We have studied a new heparin surface, the Corline Heparin Surface, applied to a complete set of an extracorporeal device used during coronary artery bypass grafting in terms of activation of inflammation, coagulation, and fibrinolysis in patients and in shed mediastinal blood.

Methods: Sixty patients scheduled for coronary artery bypass grafting were randomized to one of 3 groups with heparin-coated devices receiving either a standard, high, or low dose of systemic heparin or to an uncoated but otherwise identical circuit receiving a standard dose of systemic heparin. Samples were drawn before, during, and after the operation from the pericardial cavity and in shed mediastinal blood. No autotransfusion of shed mediastinal blood was performed.

Results: The Corline Heparin Surface significantly reduced the activation of coagulation, fibrinolysis, platelets, and inflammation compared with that seen with the uncoated surface in combination with a standard dose of systemic heparin during cardiac surgery with cardiopulmonary bypass. Both a decrease and an increase of systemic heparin in combination with the coated heparin surface resulted in higher activation of these processes. A significantly higher expression of all studied parameters was found in the shed mediastinal blood compared with in systemic blood at the same time.

Conclusions: The Corline Heparin Surface used in cardiopulmonary bypass proved to be more biocompatible than an uncoated surface when using a standard systemic heparin dose. The low dose of systemic heparin might not be sufficient to maintain the antithrombotic activity, and the high dose resulted in direct cell activation rather than a further anti-inflammatory and anticoagulatory effect.

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During cardiac surgery with cardiopulmonary bypass (CPB), the body is subject to extensive trauma, and the blood is exposed to a large artificial surface, leading to activation of inflammation and coagulation. Many factors during CPB can induce complex inflammatory response involving complement and cellular activation, along with the production of cytokines. It has been suggested that the systemic inflammatory response in patients undergoing coronary artery bypass grafting (CABG) is caused mainly by the surgical procedure.[1] Thrombin generation during cardiac surgery with CPB might be caused by the surface of the extracorporeal unit, with the oxygenator, pump, and connections between the patient and the extracorporeal device.[2] The surgical trauma with exposure of tissue factor

(TF), the principal initiator of blood coagulation, is also suggested to be of major importance for thrombin generation.[3-5] Previously, it was thought that the activation of coagulation during CPB occurred mainly through contact activation. However, current evidence suggests that activation of the TF-induced pathway might be the most important mechanism of thrombin generation during this type of operation.[6-8] Heparinization is required to prevent clotting in the extracorporeal circuit during cardiac surgery, and heparin coating has been reported to improve the biocompatibility of artificial surfaces in contact with circulating blood.[9] Moreover, the enhanced biocompatibility of CPB circuits by means of heparin coating has led to the idea of reducing the systemic heparinization during cardiac surgery. This was shown to be clinically safe, with a lower incidence of homologous transfusion and thromboembolic complications, although no differences in markers for inflammation, coagulation, and fibrinolysis.[10-12]

Retransfusion of shed mediastinal blood (SMB) was introduced by Schaff and colleagues[13] in 1978 and has become a widely used method during and after cardiac surgery.[14,15] However, a number of studies have shown that the composition of the shed blood is far from normal.[16,17] SMB after CABG is characterized by extraordinary activated coagulation and fibrinolysis.[18-20] Furthermore, high levels of proinflammatory cytokines are found in shed blood.[20] However, the interpretations of the outcome of retransfusion of this blood differs; some studies have concluded that retransfusion of shed blood deteriorates hemostasis, fibrinolysis, and inflammation, whereas others suggest that it does not.[16,17,20-22]

A new heparin surface, the Corline Heparin Surface (CHS), has been prepared by the use of a macromolecular conjugate of covalently bonded heparin. In this study we have, for the first time, investigated the effects in CPB of the CHS in combination with different concentrations of systemic heparin on leukocytes, platelets, coagulation, and inflammatory responses compared with effects achieved with an uncoated device. The aim was to further study the combination of cell and tissue activation, inflammation, and coagulation in both systemic and shed blood during and after cardiac surgery with CPB.

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Material and Methods

Patients

This randomized study comprised 60 patients younger than 75 years of age scheduled for elective CABG. Patients with inflammatory or renal disease or severely low ejection fraction were not considered for the study. Informed consent was obtained from all patients, and the study was approved by the Swedish Medical Product Agency and the ethical committee of the Medical Faculty at Uppsala University.

The study was planned and performed in 2 parts, with a short delay between the 2 parts giving time for analyses of some important parameters to survey the in vivo effects of the new surface. In the first part patients were randomly assigned to group 1 (n = 15) for the use of an uncoated circuit (Jostra Medizintechnic AG, Hirrlingen, Germany) and to group 2 (n = 15) for a completely heparin-coated (Corline Heparin Surface; Corline Systems AB, Uppsala, Sweden) but otherwise identical device. Both groups received a standard dose of systemic heparin, corresponding to an activated clotting time (ACT) of 480 seconds. In the second part of the study, the CHS was used in all patients (groups 3 and 4). In group 3 (n = 15) patients were randomized to a low dose of systemic heparin (ACT of 300 seconds) and group 4 (n = 15) to a high dose of systemic heparin (ACT of 600 seconds).

No autotransfusion of SMB was performed during this study.

Heparin Surface

The CHS was applied to a complete set of an extracorporeal circuit (tubing, cannula, oxygenator, and reservoir). The CHS uses a unique macromolecular conjugate in which approximately 70 heparin molecules are covalently linked to a polymer carrier having a molecular weight of 50 kd. The specificity of the covalent linkages ensures that the antithrombin III-binding pentasaccharide sequence of heparin are left intact. The CHS coating comprises a conditioning layer of a polymeric cationic amine onto which the macromolecular conjugate is irreversibly attached by means of multiple ionic interactions. The surface concentration of heparin is 0.5 to 1 $\mu g/cm^2$, and the capacity to bind antithrombin is 2 to 4 $pmol/cm^2$.[23] An extracorporeal closed-loop system modified with CHS used during extracorporeal circulation for 24 hours in pigs without any systemic dose of heparin had no effect on the clotting time (data submitted to the Swedish Medical Products Agency).

Anesthesia and Analgesia

Patients were premedicated with morphine or morphine-scopolamine on the morning of the operation. Anesthesia followed conventional procedures with thiopental sodium used for induction and a combination of pancuronium bromide and inhaled isoflurane used during the operation. After weaning from CPB, propofol in continuous infusion was used as an anesthetic agent. Alfentanil hydrochloride and fentanyl citrate were used for intraoperative analgesia.

Cardiopulmonary Bypass

Techniques and equipment for CPB were similar in all patients. The standard set consisted of a Stöckert roller pump (Stöckert Instrumente GMBH, Munich, Germany) with a Quadrox hollow-fiber oxygenator (Jostra) and a hard-shell venous reservoir (Jostra). The tubing, including cannula, was CHS coated from tip to tip (9 patients had uncoated cannulas because of surgeons' preferences). The circuit was primed with 1500 to 2000 mL of asanguineous fluid containing 5000 IU of heparin. The operation was performed with moderate hypothermia (32°C-35°C), and the patients were rewarmed to a rectal temperature of 36°C before weaning from CPB. The flow was nonpulsatile, initially 2.4 $L \cdot m^{-2} \cdot min^{-1}$, and gradually reduced to 2.0 $L \cdot m^{-2} \cdot min^{-1}$ during hypothermia.

Anticoagulation and Operative Procedures

Patients in groups 1 and 2 were anticoagulated by means of intravenous administration of heparin administered as a bolus of

300 IU/kg body weight after completed dissection of the internal thoracic artery and before cannulation for CPB. In groups 3 and 4 the bolus doses of heparin were 200 and 400 IU/kg body weight, respectively. If necessary, additional doses of heparin were given to maintain the desired ACT. After decannulation, heparin was reversed with protamine chloride in a 1:1 proportion to the administrated heparin dose. ACT was measured repeatedly throughout the operation with an ACT II (Medtronic Inc, Minneapolis, Minn) to assess adequate anticoagulation. Cardioplegic arrest was achieved with antegrade infusion of modified St Thomas' cardioplegic solution at 4°C through the aortic root. During the CPB procedure, cardiotomy suction was used. Blood remaining in the CPB circuit after decannulation was collected in an infusion bag and immediately retransfused. Postoperatively, shed blood was collected in the reservoir, but no retransfusion was performed.

Blood Sampling and Laboratory Procedures

Blood samples were drawn from a catheter in the radial artery after induction of anesthesia, at sternotomy, at the start and end of CPB, 30 minutes after protamine administration, and 3, 8, and 20 hours after CPB. Sampling was also performed from the pericardial cavity at the end of CPB and from the drainage reservoir (after gentle mixing of the reservoir) 3 hours after CPB. Vacutainer tubes containing Na-citrate or ethylenediamine tetraacetic acid K_3 (Becton Dickinson, Meylan Cedex, France) were used. One citrated sample was immediately brought to the laboratory for cell count and analysis of cellular surface antigens by means of flow cytometry. The other samples were immediately centrifuged at 2000*g* for 20 minutes, and plasma was frozen and stored in aliquots at −70°C until analysis.

Platelet and leukocyte differential counts were determined with an automatic cell counter (Coulter STKS; Coulter Electronics, Hialeah, Fla). Plasma levels of prothrombin fragment 1+2 (F_{1+2}), thrombin-antithrombin (TAT), plasmin/α_2-antiplasmin complex (PAP), human myeloperoxidase (MPO), and the cytokines interleukin (IL) 6, IL-8, IL-10, and soluble IL-2 receptor (sIL-2R) were quantified by means of sandwich enzyme-linked immunosorbent assay techniques (Enzygnost for F_{1+2}, TAT, and PAP from Behringswerke, Marburg, Germany; Bioxytech for MPO enzyme immunoassay from Oxis International, Inc Portland, Ore; and Quantikine for IL-6, IL-8, IL-10 and sIL-2R from R&D Systems, Abingdon, United Kingdom). In a reference group of 71 healthy individuals, reference intervals (2.5th-97.5th percentile) for F_{1+2} and TAT were 0.4 to 1.5 nmol/L and 1.2 to 5.0 μg/L, respectively, with respective medians of 0.8 nmol/L and 2.3 μg/L. For both assays, the intra-assay and interassay variations were 5% and 9%. In a reference group of 466 healthy individuals, reference intervals (2.5th-97.5th percentile) for PAP was 120 to 700 μg/L, with a median of 290 μg/L. The intra-assay and interassay variations were 4% and 7%, respectively. Plasma soluble fibrin (sF) was analyzed with an assay using a chromogenic, plasmin-specific substrate (Bericrom FM, Behring Diagnostics GmbH). In 73 healthy individuals aged 21 to 66 years (median, 28 years), the reference interval (2.5th-97.5th percentile) for sF was 17 mg/L or less (median, 9.0 mg/L; range, 3.2-16.6 mg/L), and the intra-assay and interassay variations had a total coefficient of variation of 7.9%. Reference intervals for the cytokines were according to the manufacturer:

- For IL-6, in a reference group of 40 healthy individuals, 33 had measurements of less than 3.13 pg/mL, and 7 had measurements of between 3.13 and 12.5 pg/mL.
- For IL-8, a reference group of 34 healthy individuals had measurements of less than 31.2 pg/mL.
- For IL-10, a reference group of 40 healthy individuals had measurements of less than 7.8 pg/mL.
- For sIL-2R, a reference group of 37 healthy individuals had measurements of 1057 pg/mL (404-2604 pg/mL).

The intra- and interassay variations were 1.7% and 3.3% for IL-6, 5.4% and 9.7% for IL-8, 4.3% and 7.5% for IL-10, and 6.1% and 6.0% for sIL-2R, respectively.

Determination of activated coagulation factor XI-antithrombin complex (FXIa-AT), activated coagulation factor XII-antithrombin complex (FXIIa-AT), and activated coagulation factor XII-C1-esterase inhibitor complex (FXIIa-C1 INH) were performed by using a solid-phase enzyme-linked immunosorbent assay according to the method of Sanchez and colleagues.[24] The intra-assay coefficient of variation was less than 4% in all assays. Cross-reactions between antibodies to FXIa and FXIIa or antibodies against AT and C1 INH were less than 5%.

No correction for hemodilution was made.

Flow Cytometry

Leukocytes. Surface antigen expression was analyzed by using a Coulter Epics XL-MCL flow cytometer (Beckman Coulter, Fullerton, Calif). Whole blood was labeled with a murine monoclonal flourescein isothiocyanate (FITC)-conjugated anti-TF antibody (TF4508-CJ; American Diagnostica, Greenwich, Conn) or an anti-CD11b antibody (Dakopatts A/S, Glostrup, Denmark). A murine FITC-conjugated irrelevant antibody of the same subtype was used as a negative control. The samples were incubated on ice for 30 minutes and lysed with Lyse (Beckman Coulter), washed, and suspended in PBS. Flow cytometric analysis was then performed. Gating of the cells was carried out with forward- and side-scatter parameters, and the percentage of positive cells and mean fluorescence intensity were determined.

Platelets. Platelet-rich plasma was isolated by means of centrifugation at 140*g* for 10 minutes at room temperature. Platelet-rich plasma was added to tubes containing HEPES buffer and FITC-labeled chicken polyclonal anti-P-selectin antibody (Immunsystem AB, Uppsala, Sweden) or FITC-labeled chicken polyclonal anti-whole-platelet antibody (Biopool AB, Umeå, Sweden). The samples were incubated in duplicate for 10 minutes at room temperature and were then diluted and fixed with ice-cold PBS containing 1% paraformaldehyde. No washing steps were used. For P-selectin analysis, gating of the cells in the flow cytometer was carried out by using forward- and side-scatter parameters, and for microparticle analysis, gating was performed by using forward scatter and fluorescence 1 (anti-whole-platelet antibody^{FITC}). Platelets with fluorescence of less than a preset cut-off level were identified as microparticles. The percentage of positive cells was determined.

Statistical Analysis

Statistica for Windows (StatSoft, Tulsa, Okla) was used for all statistical analyses. The results are presented as medians and 25th-75th percentile ranges. The Friedman analysis of variance test

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TABLE 1. Clinical characteristics and intraoperative and postoperative data of the patient groups

	Uncoated, ACT 480 s (group 1)	CHS coated, ACT 480 s (group 2)	CHS coated, ACT 300 s (group 3)	CHS coated, ACT 600 s (group 4)
Sex (no. of patients)				
Male	10	13	14	12
Female	4	2	1	3
Age (y)	60 (54-67)	62 (55-70)	60 (53-69)	62 (55-70)
CPB time (min)	86 (72-100)	89 (75-110)	91 (72-107)	92 (72-110)
Aortic crossclamping (min)	48 (36-58)	50 (44-66)	49 (37-62)	57 (34-62)
Surgical bleeding (mL)	700 (550-772)	575 (400-650)	600 (400-775)	550 (400-850)
Postoperative bleeding (mL)	900 (820-1025)	825 (640-1060)	925 (600-1200)	750 (650-1250)
Uncoated cannula (No. of patients)	2	3	2	2
Allogenic blood				
No. of patients	5	3	4	6
No. of units	11	7	8	14
ACT at start of CPB (s)	512*† (433-579)	536*† (497-568)	400† (352-463)	642* (557-685)
ACT at end of CPB (s)	475*† (417-521)	463*† (426-506)	343† (303-406)	568* (479-685)
Heparin (IU/mL)	31,250*† (30,000-35,000)	35,000*† (30,000-40,000)	25,000† (20,000-25,000)	40,000* (37,500-45,000)
Protamine (U/mL)	350*† (350-362)	400*†(350-450)	300† (250-300)	450* (425-475)

No significant differences existed between the groups for the clinical parameters. Data are presented as median and 25th-75th percentiles.
*Significantly different from group 3, $P < .001$.
†Significantly different from group 4, $P < .001$.

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was used for analysis of time series within each group. When significant differences were obtained, these were further evaluated by using the Wilcoxon matched pairs test for comparison between 2 samples within the series. The Kruskal-Wallis analysis of variance test was used for an overall comparison between the groups. When significant differences were obtained, these were further evaluated with the Mann-Whitney *U* test for intergroup comparison. No adjustment for multiple testing was done. Hence this study should be interpreted more as an exploratory, rather than a confirmative, study.

Results

The postoperative course and mobilization was uneventful in all but one patient, included in group 1, who died the day after the operation as a result of myocardial failure. This patient was excluded from the study and left 49 men and 10 women for statistical calculations. Three patients received Cyklokapron (tranexamic acid; Pharmacia & Upjohn Sverige AB, Stockholm, Sweden) and Octostim (desmopressinacetate; Ferring Läkemedel AB, Limhamn, Sweden) perioperatively. The 9 patients with uncoated aortacannulas and the patients receiving tranexamic acid and desmopressinacetate did not differ significantly in any clinical or laboratory parameters and were equally spread between the groups. Because there were no differences among the 4 groups in any parameters in the SMB, these results are presented as one set. There were no significant differences concerning age, CPB time, crossclamp time, or bleeding among the 4 groups (Table 1).

Leukocyte Activation

The number of leukocytes increased from 5.6×10^9/L ($4.6\text{-}6.4 \times 10^9$/L) before the operation to 7.6×10^9/L ($6.2\text{-}10.2 \times 10^9$/L) at the end of CPB ($P < .001$). Three hours after the operation, the number of leukocytes reached maximum levels, 13.3×10^9/L ($11.1\text{-}15.5 \times 10^9$/L). Seventy-two hours after the operation, the levels were still elevated compared with presurgical levels (11.0×10^9/L [$9.4\text{-}13.1 \times 10^9$/L], $P < .001$), although they were declining. The expression of CD11b on both monocytes and granulocytes increased during the operation ($P = .03$ and $P < .001$, respectively). At the end of the operation, the expression of CD11b on both monocytes and granulocytes was higher in group 3 (with reduced systemic heparin) compared with that in group 2 (with standard systemic heparin, $P = .008$ for both cell types). The morning after the operation, the CD11b expression on monocytes had decreased in both groups 2 and 3 ($P = .04$ and $P = .02$, respectively). The expression of CD11b on granulocytes decreased in all groups 3 hours after the operation ($P < .001$) and further the morning after the operation ($P < .001$), in group 2 to presurgical levels (Figure 1).

The release of MPO was severely increased during the

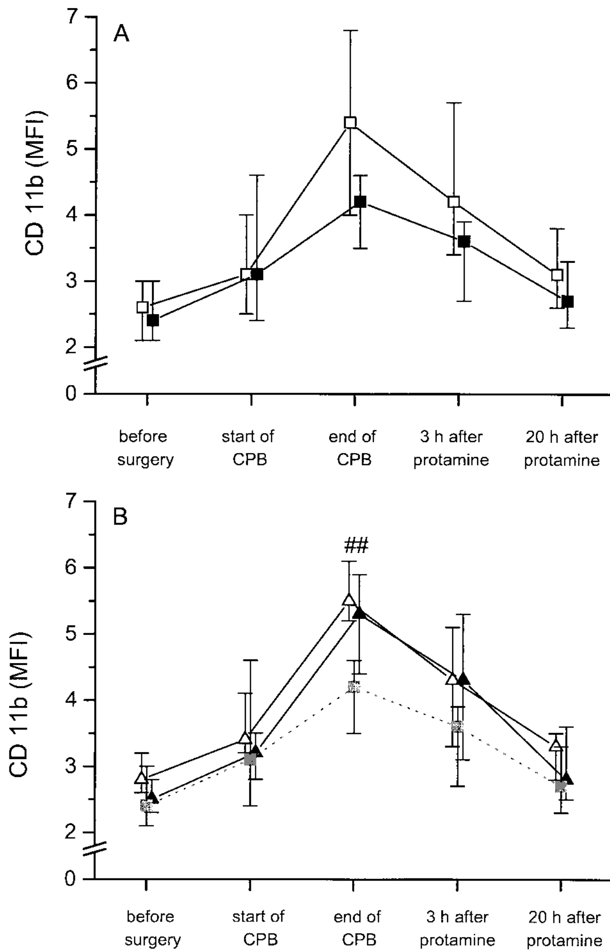

Figure 1. CD11b expression on granulocytes before, during, and after the operation in patients. A, Uncoated group with ACT of 480 seconds *(open squares)* and CHS-coated group with ACT of 480 seconds *(filled squares)*; B, CHS-coated group with low heparin dose and ACT of 300 seconds *(open triangles)*, with high heparin dose and ACT of 600 seconds *(filled triangles)*, and with standard heparin dose and ACT of 480 seconds *(filled squares, dotted line)*. ##*P* = .008, ACT of 300 seconds versus ACT of 480 seconds. Results are presented as median and 25th-75th percentile. *MFI,* Mean fluorescence intensity.

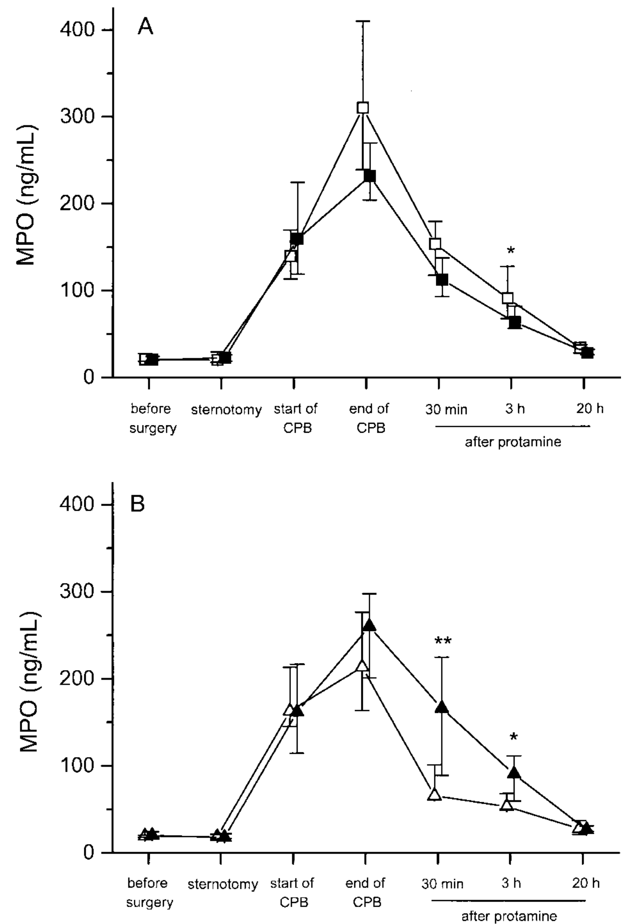

Figure 2. MPO in plasma before, during, and after the operation in patients. A, Uncoated group with ACT of 480 seconds *(open squares)* and CHS-coated group with ACT of 480 seconds (*filled squares*) **P* = .05, ***P* =.04. B, CHS-coated group with low heparin dose and ACT of 300 seconds *(open triangles)* and with high heparin dose and ACT of 600 seconds (*filled triangles*) **P* = .01, ***P* = .002. Results are presented as median and 25th-75th percentiles.

operation ($P < .001$) and was not completely normalized the day after the operation, although they were lower ($P < .001$) in all groups. In group 2 there were lower levels compared with those in the uncoated group, group 1, at the end of CPB ($P = .05$) and also 3 hours after the operation ($P = .04$). A reduction of the systemic heparin dose in combination with the heparin-coated surface resulted in lower levels of MPO 30 minutes after the operation compared with the standard heparin dose ($P = .03$, Figure 2).

The expression of TF on the surface of monocytes was upregulated 3 hours after the operation ($P = .003$) and remained increased the morning after the operation ($P < .001$, Table 2).

Leukocyte Activation in the Pericardial Cavity

The surface expression of CD11b on granulocytes and monocytes from the pericardial cavity was more than 3 and 4 times higher, respectively, than on cells from the circulating blood at the end of CPB ($P < .001$ for both cell types). Monocytes from the pericardial cavity expressed almost 2 times more TF than the circulating cells at the same time ($P < .001$). There were no statistically significant differences between the groups (Table 2).

Platelet Activation

The number of platelets in circulating blood was decreased during the operation in all groups, from 160 $\times$ 10^9/L (134-

TABLE 2. TF expression on monocytes before, during, and after the operation

	Before operation (n = 59)	Start of CPB (n = 58)	End of CPB (n = 58)	Pericardial cavity (n = 58)	3 h after operation (n = 59)	20 h after operation (n = 58)
TF (MFI)	0.50 (0.44-0.54)	0.51 (0.47-0.56)	0.49 (0.46-0.53)	0.84* (0.72-1.0)	0.52† (0.48-0.58)	0.54‡ (0.49-0.58)

No statistically significant differences existed between the groups. Results are presented as one set and as median and 25th-75th percentiles. *MFI*, Mean fluorescence intensity.
*Significantly different from end of CPB, $P < .001$.
†Significantly different from before the operation, $P = .003$.
‡Significantly different from before the operation, $P < .001$.

TABLE 3. Cytokines in plasma before, during, and after the operation in patients and SMB

Cytokine	Before operation (n = 59)	Sternotomy (n = 59)	Start of CPB (n = 59)	End of CPB (n = 59)	30 min after CPB (n = 59)	3 h after operation (n = 59)	8 h after operation (n = 57)	20 h after operation (n = 59)
IL-6	2 (1-4)	3† (2-4)	3‡ (2-6)	22‡ (11-40)	68‡ (44-104)	160‡ (112-220)	130‡ (104-192)	97‡ (63-147)
IL-8	4 (4-6)	5‡ (4-8)	6‡ (4-9)	15‡ (7-31)	33‡ (19-52)	28‡ (18-42)	20‡ (15-27)	12‡ (9-17)
IL-10	8 (6-12)	9 (6-15)	9* (7-17)	65‡ (34-119)	72‡ (43-120)	24‡ (17-42)	27‡ (18-40)	19‡ (14-26)
sIL-2R	760 (600-940)	710† (580-920)	710 (620-930)	560‡ (440-680)	670‡ (550-840)	760‡ (620-1010)	890‡ (750-1180)	1250‡ (1000-1620)

No statistically significant differences existed between the groups. Results are presented as one set. Data are presented in picograms per milliliter and as median and 25th-75th percentiles.
*Significantly different from before the operation, $P = .01$.
†Significantly different from before the operation, $P = .001$.
‡Significantly different from before the operation, $P < .001$.

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182 × 10^9/L) to 107 × 10^9/L (77-136 × 10^9/L, $P < .001$). In the uncoated group, group 1, and the heparin-coated group with high systemic heparin, group 4, the decrease was even more pronounced in comparison with that in the heparin-coated group with standard heparin dose, group 2 ($P = .03$ and $P = .02$, respectively), probably because of the activation and adhesion in these groups. Forty-eight hours after the operation, the platelets had returned to presurgical levels in all groups. The expression of P-selectin on circulating platelets was increased during the operation in all groups ($P < .001$); however, this expression was less in group 2 than in group 1 ($P = .004$). This positive effect of the heparin surface was not affected by the systemic heparin dose (Figure 3). The formation of platelet microparticles in the circulating blood was not significantly altered in any group during the operation (data not shown).

Release of Cytokines

The proinflammatory cytokines IL-6 and IL-8 were increased during the operation ($P < .001$) in all groups. There were no statistically significant differences between the groups. IL-6 continually increased until 3 hours after the operation, whereas IL-8 reached the maximum level at 30 minutes after protamine administration. The day after the operation, both IL-6 and IL-8 were still increased ($P < .001$ for both). The release of the anti-inflammatory cytokine IL-10 was 8-fold increased in all groups during the operation ($P < .001$). Three hours after the operation, the levels were lower ($P < .001$), although increased, and remained increased the following morning. sIL-2R decreased during the operation ($P < .001$), with minimum levels at the end of CPB. The morning after the operation, sIL-2R was elevated compared with levels before the operation ($P < .001$). All fluctuations were within the normal range for sIL-2R. There were no statistical differences among the groups (Table 3).

Activation of the Coagulation Cascade

Thrombin generation measured as F_{1+2} and TAT (TAT data not shown, consistent with F_{1+2}) increased early during the operation in all groups ($P < .001$). A further increase of F_{1+2} was recorded during ($P < .001$) and 30 minutes after ($P < .001$) CPB, although levels were lower in group 2 compared with those in all other groups ($P = .01$). Eight and 20 hours after the operation, the generation of thrombin was lower in all groups compared with levels at the end of the operation ($P < .001$), although not reaching presurgical levels (Figure 4). The levels of sF, reflecting thrombin activity, were increased in all groups during the operation, from 17 mg/L (14-22 mg/L) before the operation to 28 mg/L (22-33 mg/L) at the end of CPB ($P < .001$), with remaining high levels the morning after the operation.

Fibrinolysis

PAP was increased during CPB ($P < .001$), with a further increase in all groups until 3 hours after the operation ($P < .001$), although it was less in group 2 ($P = .01$). Eight hours

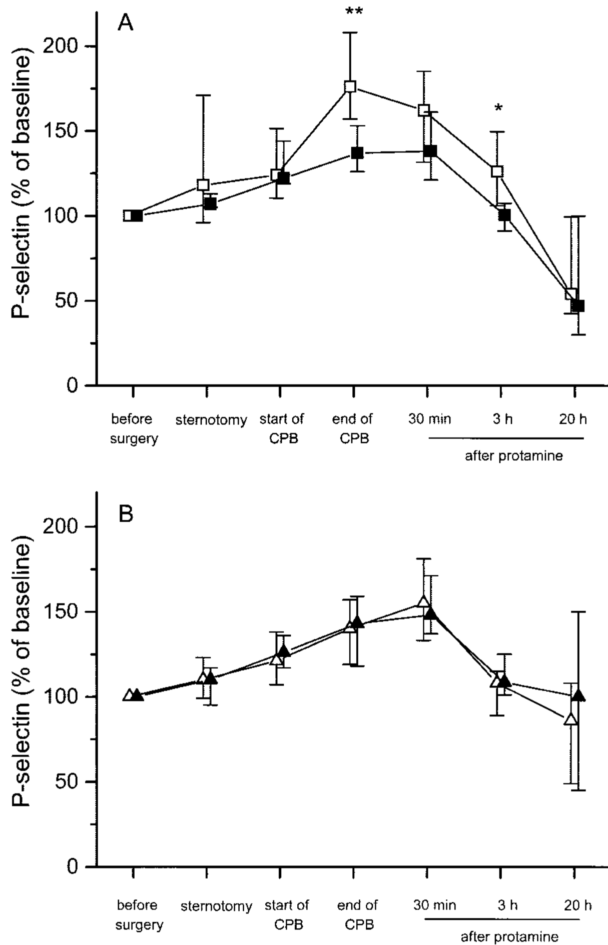

Figure 3. P-selectin expression on platelets before, during, and after the operation in patients. A, Uncoated group with ACT of 480 seconds *(open squares)* and CHS-coated group with ACT of 480 seconds (*filled squares)* *P = .005, **P = .004. B, CHS-coated group with low heparin dose and ACT of 300 seconds *(open triangles)* and with high heparin dose and ACT of 600 seconds *(filled triangle)*. Results are presented as median and 25th-75th percentiles.

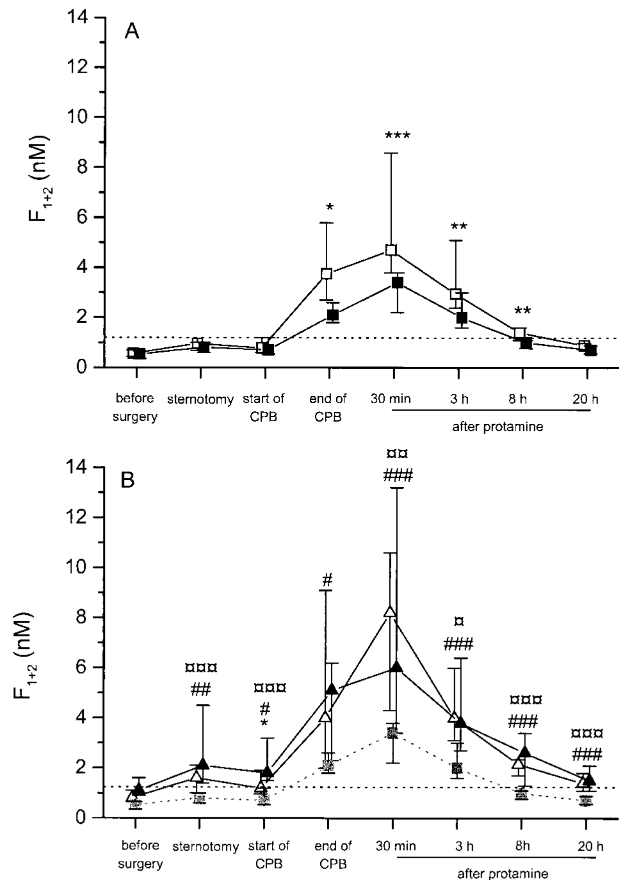

Figure 4. F_{1+2} in plasma before, during, and after the operation in patients. A, Uncoated group with ACT of 480 seconds *(open squares)* and CHS-coated group with ACT of 480 seconds (*filled squares)*. *P = .05, **P = .02, ***P = .008. B, CHS-coated group with low heparin dose and ACT of 300 seconds *(open triangles)*, high heparin dose and ACT of 600 seconds *(filled triangles)*, and standard heparin dose and ACT of 480 seconds (*filled squares, dotted line*. *P = .02, ACT of 300 seconds versus ACT of 600 seconds; #P = .03, ACT of 300 seconds versus ACT of 480 seconds; ##P = .005; ###P < .001; ¤P = .004, ACT of 600 seconds versus ACT of 480 seconds; ¤¤P = .008; ¤¤¤P < .001. Results are presented as median and 25th-75th percentiles.

after the operation, the levels of PAP were lower ($P < .001$) and even normalized in group 2. The next morning, PAP levels were normalized in all groups (Figure 5).

Intrinsic Pathway

The formations of FXIa-AT and FXIIa-AT increased during the operation and returned to presurgical levels the following morning. FXIa-AT reached maximum level at the end of CPB in all groups ($P < .001$); however, this was lower in group 2 compared with that in group 4 ($P = .05$, data not shown). The increase of FXIIa-AT was, however, earlier, starting before CPB. Groups 1 and 2 reached maximum levels at the start of CPB, whereas group 3 and 4 levels increased further during CPB ($P < .001$, Figure 6). The formation of FXIIa-C1 INH increased during CPB, although significantly only in group 3 ($P = .003$). There was a decrease after CPB in all groups ($P < .001$) except group 1 ($P = .8$), followed by an increase in all groups until 20 hours after the operation ($P < .001$, data not shown).

Shed Mediastinal Blood

In the SMB the number of leukocytes was 4-fold lower ($P < .001$) in all groups compared with circulating blood 3 hours after the operation. Both granulocytes and monocytes showed 5 times higher expression of CD11b compared with the circulating cells ($P < .001$ for both). The release of

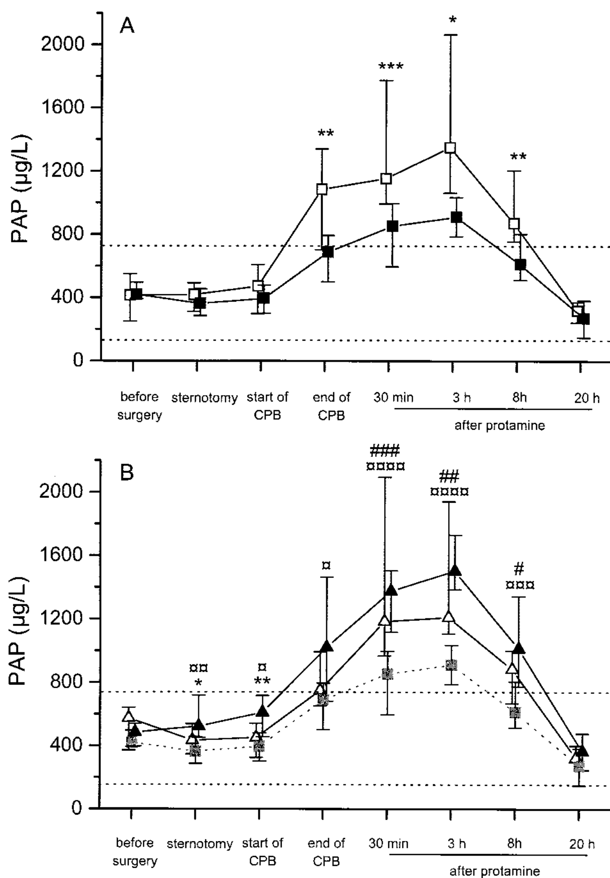

Figure 5. PAP in plasma before, during, and after the operation in patients. A, Uncoated group with ACT of 480 seconds *(open squares)* and CHS-coated group with ACT of 480 seconds *(filled squares)*. **P* = .02, ***P* =.01, ****P* = .006. B, CHS-coated group with low heparin dose and ACT of 300 seconds *(open triangles)*, with high heparin dose and ACT of 600 seconds *(filled triangles)*, and with standard heparin dose and ACT of 480 seconds *(filled squares, dotted line)*. **P* = .04, ACT of 300 seconds versus ACT of 600 seconds; ***P* =.01; #*P* = .03, ACT of 300 seconds versus ACT of 480 seconds; ##*P* = .01; ###*P* = .004; ¤*P* = .01, ACT of 600 seconds versus ACT of 480 seconds; ¤¤*P* = .004; ¤¤¤*P* = .001; ¤¤¤¤*P* < .001. Results are presented as median and 25th-75th percentiles.

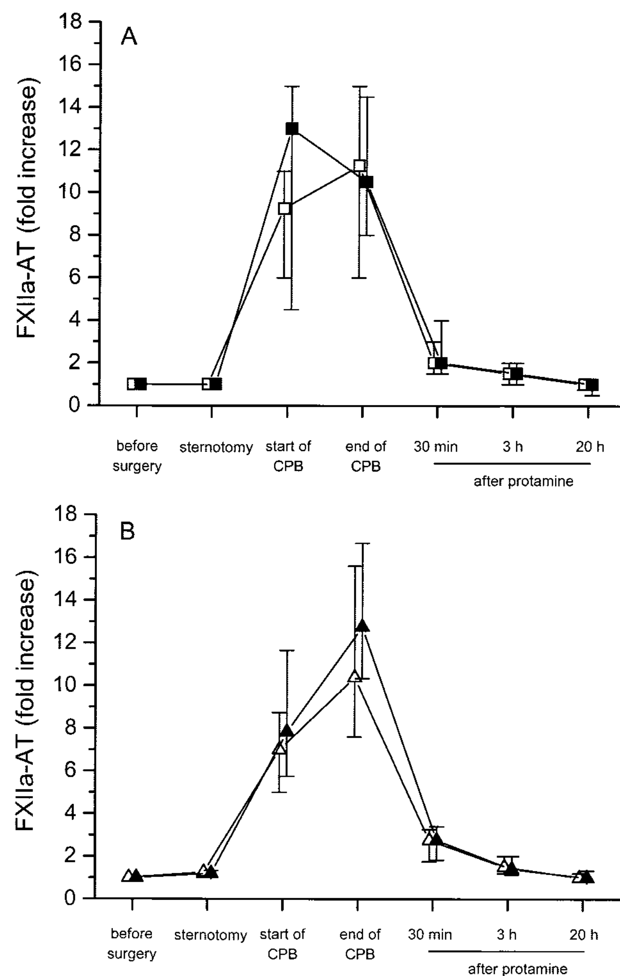

Figure 6. FXIIa-AT in plasma before, during, and after the operation in patients. A, Uncoated group with ACT of 480 seconds *(open squares)* and CHS-coated group with ACT of 480 seconds *(filled squares)*. B, CHS-coated group with low heparin dose and ACT of 300 seconds *(open triangles)* and with high heparin dose and ACT of 600 seconds *(filled triangles)*. Results are presented as median and 25th-75th percentiles.

MPO was 15 times higher than in the circulating blood at the same time ($P < .001$). Monocytes in the SMB showed almost 2-fold higher expression of TF ($P < .001$) compared with systemic cells. The number of platelets were 2 times lower ($P < .001$) in SMB. Although few, the shed platelets were activated and expressed 2 times more P-selectin compared with that in circulating cells ($P < .001$). Moreover, the amount of platelet microparticles was 7-fold higher in the SMB than in systemic blood ($P < .001$). The release of IL-6 and IL-8 was more than 40 and 36 times higher, respectively, than in systemic blood ($P < .001$). However, the level of IL-10 in SMB was not different from that in systemic blood 3 hours after the operation ($P = .2$). The level of sIL-2R was 2 times lower ($P < .001$) in SMB compared with that in systemic blood. The formation of thrombin was dramatic in the SMB, with F_{1+2} levels almost 200-fold the levels in systemic blood ($P < .001$). The level of TAT was higher than 100 times the highest standard (ie, >6000 μg/L), which was more than 280 times that in the systemic blood ($P < .001$). The thrombin activity reflected by sF was 13-fold higher ($P < .001$), and the fibrinolysis was 6-fold greater than in circulating blood ($P < .001$), as measured by PAP levels. The activation of the intrinsic pathway was also increased in the shed blood, the level of FXIa-AT was 130 times higher, and the level of FXIIa-AT was more than 180 times higher ($P < .001$ for both)

compared with that in systemic blood. The formation of FXIIa-C1 INH in SMB was half the level in systemic blood 3 hours after the operation ($P = .0014$, Table 4).

Discussion

The foreign surfaces of extracorporeal equipment are procoagulant and do activate the inflammation system. Heparin coating improves biocompatibility of the surfaces, as reflected by reduced complement and granulocyte activation and reduced production of proinflammatory cytokines.[11,25,26]

Despite the fact that a number of experimental studies have demonstrated inhibition of FXa and thrombin as a result of immobilized heparin, many clinical studies using heparin-coated bypass circuits have failed to demonstrate reduced thrombin formation.[18,27] To our knowledge, only one study[28] with heparin coating (Duraflo II surface) showed reduced formation of thrombin. The seeming discrepancy between the experimental and clinical results with respect to activation of coagulation might reflect that a number of factors in addition to the artificial surfaces, such as pumping of blood, rheologic imperfections, surgical trauma, and retransfusion of pericardial and SMB come into play in the clinical setting.

The aim of the first part of this study was to investigate the biocompatibility of a newly developed heparin surface, the CHS. It should be emphasized that the CHS is a stable heparin coating with no demonstrable release of heparin and a high capacity to bind antithrombin. Judged by these characteristics, the CHS is comparable with the Carmeda Bioactive Surface but different from the Duraflo II, which is based on an ionic complex with sustained release of heparin and the cationic surfactant used in the complex with no demonstrable capacity to bind antithrombin.

Markers of inflammation, coagulation, and fibrinolysis were included in the analysis to obtain an overall view of processes activated at blood contact with biomaterials. The CHS significantly reduced the activation of coagulation, fibrinolysis, platelets, and inflammation in comparison with that on the uncoated surface in combination with a standard dose of systemic heparin. These results indicate that the CHS is more biocompatible than an uncoated surface, not only in terms of inflammatory parameters but also in terms of reduced thrombin generation.

The improved biocompatibility achieved by coating the surface of the extracorporeal device with heparin has initiated studies in which the dose of systemic heparin has been reduced. However, these studies have presented conflicting results. Some have shown a reduction of granulocyte activation[11] and a lower incidence of homologous transfusions,[12] whereas others found it clinically safe but not in favor to standard heparin levels in combination with available heparin surfaces.[10,29] Yet others have concluded that a reduction of systemic heparin should not be made because of an increased formation of thrombin.[30] An important difference between various clinical studies is the use of different surfaces and heparin doses at the same time.[30,31] This design makes it hard to distinguish between the effects of heparin coating and those induced by means of reduced anticoagulation. Thus in the second part of the present study, we investigated the effect of the CHS in combination with a reduced or increased systemic heparin dose compared with the standard systemic heparin dose used in part 1 in respect to inflammation, coagulation, and fibrinolysis.

In this study the reduced heparin dose was aimed at an ACT at 300 seconds, although this was not quite accomplished. The results were 400 seconds before and 343 seconds after CPB; however, these were significantly different from that seen in the coated group with a standard heparin level. The ratio between these 2 groups was constant during CPB (Table 1). The decrease of systemic heparin in combination with this new heparin-coated surface resulted in a slightly beneficial effect on inflammation reflected by the inflammatory marker MPO analyzed in this study. CD11b expression on the surface of leukocytes and data on coagulation and fibrinolysis, however, indicated elevated activation in the group with reduced heparin. These results might be explained by a requirement of a certain amount of

TABLE 4. Markers of inflammation and coagulation in SMB

	3 h after operation in patients	3 h after operation in SMB
Leukocytes (10^9/L)	13.3 (11.1-15.5)	3.5 (2.6-4.7)†
CD11b granulocytes (MFI)	3.9 (3.2-5.0)	18.3 (15.7-21.8)†
CD11b monocytes (MFI)	4.4 (3.7-5.6)	21.7 (18.6-24.8)†
MPO (ng/mL)	70.3 (55.1-96.2)	1064 (909-1160)†
TF (MFI)	0.52 (0.48-0.58)	0.90 (0.82-1.11)†
Platelets (10^9/L)	133 (103-164)	47 (37-67)†
P-selectin (% of baseline)	112 (99-125)	248 (174-342)†
Platelet μ-particles (% of baseline)	113 (85-133)	739 (488-970)†
IL-6 (pg/L)	160 (112-220)	6900 (5000-9600)†
IL-8 (pg/L)	28 (18-42)	1010 (770-1720)†
IL-10 (pg/L)	38 (17-42)	42 (21-44)
sIL-2R (pg/L)	760 (620-1010)	300 (260-380)†
F_{1+2} (nmol/L)	3.1 (2.3-4.7)	595 (472-932)†
TAT (μg/L)	21.6 (14.0-31.3)	$>$6000 ($>$6000–$>$6000)†
sF (mg/L)	22 (18-28)	285 (243-300)†
PAP (μg/L)	1211 (921-1730)	7480 (5220-12,175)†
FXIa-AT	0.05 (0.03-0.07)	6.46 (4.49-10.0)†
FXIIa-AT	0.05 (0.02-0.08)	8.8 (5.52-16.0)†
FXIIa-CI INH	1.04 (0.63-1.74)	0.42 (0.23-0.71)*

No significant differences existed between the groups for the SMB. Results are presented as one set and as median and 25th-75th percentiles. *MFI*, Mean fluorescence intensity.
*Significantly different from values in patients 3 hours after the operation, $P = .0014$.
†Significantly different from values in patients 3 hours after the operation, $P < .001$.

ACD

soluble heparin to downregulate the procoagulant activity caused by the CPB and not accomplished by the surface per se. Heparin induces secretion of TF pathway inhibitor from endothelial cells in a dose-dependent manner, thus inhibiting TF/FVIIa.[32,33] Moreover, heparin can inhibit binding of fibrinogen, factor X, iC3b, and intercellular adhesion molecule 1 to Cd11b/CD18.[34] This might result in alteration of leukocyte functions involved in the activation of coagulation and inflammation.

The higher dose of systemic heparin (ACT of 600 seconds) in combination with the heparin surface was designed to clarify whether modulation of inflammation and coagulation by means of heparin could be further improved. However, the results from this group demonstrated the opposite, with increased activation of coagulation. Heparin is known to exert a variety of effects on cells in the vessel walls and circulation. Heparin potentiates platelet aggregation, recently shown to be caused by decreased nitric oxide production, an important endogenous inhibitor of platelet-mediated thrombosis.[35] This observation might in part explain the prothrombotic effects of the high dose of heparin.

Contact activation was reflected distinctly differently by the appearance of FXIIa-AT and FXIa-AT complexes, respectively. Whereas FXIa was reduced in group 2 compared with that seen in groups 3 and 4, there were no differences among the groups with respect to FXIIa. Quite interestingly, the generation of FXIIa started earlier, after sternotomy, with no further increase during CPB in groups 1 and 2. It has been reported that the capacity of the natural endothelium to inhibit FXIIa is impaired after systemic administration of heparin,[36] which might explain the early appearance of FXIIa, irrespective of the type of extracorporeal circuit, after heparinization of the patient. Because the generation of FXIa is greatly influenced by a thrombin feedback loop,[37] it seems plausible that the time course and relative concentrations of FXIa would coincide with those of thrombin formation. Reduced contact activation in connection with the use of Duraflo II has previously been reported.[7] However, this conclusion was based on reduced formation of kallekrein-CI INH complexes, with no direct measurements of FXIIA or FXIa or any signs of decreased thrombin formation.

TF is not normally expressed in the circulation. Monocytes in samples taken from the pericardial cavity have been shown to express approximately 2-fold increased TF on the cell surface[3] in a study performed with an uncoated extracorporeal device. Our results are confirming, with no differences between the groups. The use of cardiotomysuction in this study with recirculation of the blood might contribute to the increased platelet activity and thrombin generation seen directly after the operation in this study. However, all groups in the study have been treated according to the same routine, and we therefore believe that comparisons between the groups can be made.

Activation of coagulation and inflammation during CPB is a complex interplay involving both the intrinsic and, to a larger extent, the TF pathways. In experimental studies with simulated extracorporeal circulation, thrombin generation occurs before TF expression on monocytes, the only blood cell able to express TF, indicating a role of the intrinsic pathway.[38] In vivo, the surgical trauma with exposure of TF and involvement of inflammation makes the picture more complex.

Autotransfusion of shed blood after cardiac surgery is an often-used method to reduce the exposure of patients to homologous blood,[14,15] however debated.[17,22,39] Differences of threshold levels for transfusion between countries and clinics further complicates the possibility to evaluate and compare these studies.[40] In our study, in which no autotransfusion was performed, 30% of the patients needed homologous blood and received 2.2 units per patient, or a total of 0.67 units per patient. Furthermore, the quality of shed blood has been questioned,[39,41] with indications of defective hemostasis[19,22,42] and reports of systemic blood activation[21] and more febrile patients[39] after autotransfusion of this blood.

We found, in agreement with previous studies, that the SMB after CPB was characterized by activated cells, extraordinary activated coagulation, and inflammatory processes. The shed blood contained few cells but with a high expression of surface markers, indicating elevated activation. The low number of cells might be caused by adhesion, lysing, or dilution by means of exsudation.

In this study autotransfusion was not performed, and the duration of increased inflammatory and coagulant activity appeared to be shorter compared with that seen in previous studies in which shed blood was retransfused.[20,27,41,42] The high postsurgical levels of thrombin formation may be a consequence of the autotransfusion. Thrombin has been shown to induce several cell responses involved in inflammation, including, among others, cytokine release in monocytes and endothelial cells.[43] In contrast, the levels of IL-10, an anti-inflammatory cytokine and a potent inhibitor of monocyte activation and with anticoagulant effect,[44] did not differ between systemic blood and shed blood. Consequently, the imbalance between the cytokines in shed blood may reflect ongoing activity with subsequent effects on the coagulation and inflammatory systems.

During part 1 of the present study, we could conclude that the newly developed heparin surface is more biocompatible than an uncoated surface. The differences seen between parts 1 and 2 of the study are merely reflecting the importance of the systemic heparin level than the effect of the surface. The low dose of systemic heparin may not be sufficient to maintain the antithrombotic activity at the same

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level as a standard heparin dose during this kind of operation. The high dose, on the other hand, resulted in a direct cell-activating situation rather than a further anti-inflammatory and anticoagulatory effect.

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