CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

HEPARIN-COATED CARDIOPULMONARY BYPASS EQUIPMENT. I. BIOCOMPATIBILITY MARKERS AND DEVELOPMENT OF COMPLICATIONS IN A HIGH-RISK POPULATION

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Objectives: **1. To study possible clinical benefits of heparin-coated cardiopulmonary bypass in patients with a broad range of preoperative risk factors. 2. To evaluate the correlation between the terminal complement complex and clinical outcome. 3. To identify clinical predictors of complement activation and correlates of granulocyte activation during cardiac surgery.** ***Methods:*** **Blood samples from adults undergoing elective cardiac surgery with Duraflo II heparin-coated (n = 81) or uncoated (n = 75) cardiopulmonary bypass sets (Duraflo coating surface; Baxter International, Inc, Deerfield, Ill) were analyzed for activation of complement (C3 activation products, terminal complement complex), granulocytes (myeloperoxidase, lactoferrin), and platelets (β-thromboglobulin) by enzyme immunoassays. Preoperative risk was assessed by means of the "Higgins' score." Complications (cardiac, renal, pulmonary, gastrointestinal, and central nervous system dysfunction, infections, death) were registered prospectively. Data were analyzed by analysis of variance, logistic regression, and linear regression.** ***Results and conclusions:*** **Sixty-seven percent of the patients had predefined risk factors. Complications developed in 53 patients (34%), equivalently with and without heparin-coated bypass sets (P = .44-.82), despite a significant reduction in complement and granulocyte activation by heparin coating. No clear-cut relationship between the terminal complement complex and outcome was found, even if it was significant in the models for renal and central nervous system dysfunction and infections (P = .006). The Higgins' score was significantly related to complement activation (P < .05). Approximately 50% of the variation in granulocyte activation was explained by complement ($P \leq .01$) and platelet activation (P < .05), heparin/protamine dose ratio (P = .02), duration of cardiopulmonary bypass (P < .01), and gender (P < .05). Therefore measures reducing complement activation alone will not necessarily reduce granulocyte activation sufficiently for clinical significance. (J Thorac Cardiovasc Surg 1999;117:794-802)**

Contact between blood and the foreign surfaces of a heart-lung machine evokes a systemic inflammatory reaction that may result in organ dysfunction, prolonged recovery, or death. Heparin coating of the blood-contact surfaces of cardiopulmonary bypass (CPB) equipment may reduce this inflammatory reac-

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tion. Despite reports of reduced neutrophil,[1] eosinophil,[2] monocyte,[1] platelet,[3] complement,[4] and contact activation[5] and decreased cytokine release,[6] few studies have been able to demonstrate significant clinical benefits from heparin coating. However, most studies were small, increasing the risk of drawing false negative conclusions. Furthermore, many studies included low-risk patients, rendering differences more or less impossible to detect because of low overall postoperative morbidity and mortality, as in the Duraflo II Multicentre Study.[7] The first aim of the present study was therefore to investigate possible clinical benefits of heparin coating with full systemic heparinization when including patients with a broad range of preoperative risk factors.

Complement activation is often used when comparing "biocompatibility" of CPB setups, that is, the extent of the evoked inflammatory reaction. Activated complement may directly induce tissue damage. The clinical relevance of measuring complement activation rests mainly on the study by Kirklin and coworkers[8] from 1983, showing correlations between plasma C3a concentrations and organ dysfunction. Presently, a number of sensitive immunoassays for complement activation at various levels of the cascade are available. In vitro studies have shown that quantitation of the terminal SC5b-9 complement complex (TCC) very sensitively discriminates between different CPB setups.[9] The second aim of the study was to evaluate whether TCC measurements are related to clinical outcome.

In vitro, there are great individual differences in degree of complement activation on a given stimulus, and activation during CPB also varies substantially among individuals.[4] The third aim of the study was to identify clinical predictors of increased complement activation.

Granulocyte activation is regarded as an important link between many inflammatory mediators formed during CPB and organ damage. Activated granulocytes adhere to the activated vascular endothelium and release a host of substances further damaging the endothelial cells, permitting edema formation and extravasation of granulocytes. Activated complement is a significant factor responsible for granulocyte activation during CPB. Other potential granulocyte activators are also present (eg, endotoxin, various cytokines, and platelet activation products), but their relative importance is unknown. The fourth aim of the investigation was to find correlates of granulocyte activation during cardiac surgery.

Data from a subgroup of 29 patients, in whom we explored mechanisms for the reduced complement activation observed using heparin-coated CPB circuitry, are presented separately.[10]

Patients and methods

Patients and perfusion management. A total of 156 adults admitted for elective cardiac surgery at the National Hospital were included in the study after giving informed consent. To achieve a study population including both low-risk and high-risk patients, we entered consecutive patients in the study if they belonged to one of the following groups: 51 patients (low-risk group) scheduled for coronary bypass surgery, with no major noncardiac illness and a left ventricular ejection fraction of 0.40 or more; 105 patients (high-risk group) who had significant noncardiac illness (eg, diabetes, chronic obstructive pulmonary disease, renal insufficiency, cerebrovascular disease) or a preoperative left ventricular ejection fraction less than 0.40 or who were scheduled for valve replacement or coronary bypass surgery combined with either valve replacement or carotid artery endarterectomy. Exclusion criteria were ongoing infections, liver failure, and use of steroids or nonsteroid anti-inflammatory agents except acetylsalicylic acid.

Patients were randomized to CPB with one of the following setups:

- Heparin-coated group (n = 81): Univox oxygenator and tubing/connectors, cardiotomy suction, and a 25-mm arterial line screen filter (Bentley/Baxter, Uden, The Netherlands) with the entire blood-contact surface heparin-coated by the Duraflo II method (ionically bound heparin) (Baxter International, Inc, Deerfield, Ill)
- Uncoated group (n = 75): Uncoated, otherwise similar oxygenator, tubing, and filter set

The extracorporeal circuit was primed with 1800 mL Ringer's acetate solution containing 5000 IU heparin. Before CPB, 300 IU heparin per kilogram of body weight was administered intravenously in both groups. Additional heparin was given if needed to maintain an activated clotting time of 480 seconds or more. Cardiotomy suction and a nonpulsatile roller pump were used in all patients. Cold St Thomas' Hospital cardioplegic solution was used in addition to local cooling and moderate general hypothermia (30°C-32˚C). After CPB, 1 mg of protamine was administered for each 100 IU of heparin. Additional protamine was given if necessary to re-establish preoperative activated clotting time.

Registered variables. Age, gender, height, weight, preoperative condition, noncardiac illness, and medication were registered on admission. Type of operation, total heparin and protamine administration, duration of the operation, CPB, and aortic occlusion, chest tube drainage, and transfusions were recorded. The following postoperative complications were registered prospectively according to defined criteria:

Infective complications: Wound infection, pneumonia, mediastinitis, or sepsis

Cardiac dysfunction: Sustained need for epinephrine or intraaortic balloon pump to maintain adequate blood pressure

Renal dysfunction: Serum creatinine level greater than 200 μmol/L and/or anuria in patient with normal preoperative kidney function

Adult respiratory distress syndrome: Arterial oxygen tension less than 10 kPa with inspired oxygen fraction greater than

Table I. *Patient characteristics and variables pertaining to operation*

Operation	*CABG*	*Valve replacement*	*CABG and valve*	*CABG and vascular*	P *value*
No. of patients	82	24	41	9	
Women	6 (7%)	11 (46%)	14 (34%)	2 (22%)	.001
Age (y)	63 (61-65)	68 (64-71)	66 (62-69)	68 (64-71)	.12
Weight (kg)	80 (78-83)	76 (70-83)	71 (67-74)	77 (69-84)	.003
Higgins' score	3.1 (2.4-3.8)	3.6 (2.7-4.6)	3.8 (2.9-4.7)	4.3 (1.8-6.9)	.42
Diabetes	10 (12%)	5 (21%)	3 (7%)	1 (11%)	.46
Previous CNS symptom	10 (12%)	0 (0%)	3 (7%)	5 (56%)	.001
COPD	2 (2%)	3 (13%)	7 (17%)	0 (0%)	.02
Hypertension	15 (18%)	6 (25%)	9 (22%)	1 (11%)	.78
Renal dysfunction	5 (6%)	1 (4%)	2 (5%)	0 (0%)	.87
Compensated cardiac insufficiency	12 (15%)	11 (46%)	10 (24%)	1 (11%)	.001
Redo operation	19 (23%)	1 (4%)	6 (17%)	1 (11%)	.46
Aortic occlusion time (min)	35 (32-38)	63 (55-72)	74 (66-81)	34 (29-39)	<.001
CPB duration (min)	72 (69-77)	94 (85-103)	132 (110-154)	69 (57-80)	<.001
Time on respirator (h)	18.4 (5.8-31.1)	10.6 (3.2-18.1)	19.9 (2.9-36.9)	6.0 (4.4-7.5)	.81
Thoracic drainage (mL)	1080 (895-1266)	693 (516-871)	1223 (788-1658)	1089 (687-1491)	.20

CABG, Coronary artery bypass surgery; *CABG and valve,* coronary artery bypass surgery and valve replacement; *CABG and vascular,* coronary artery bypass surgery and carotid artery endarterectomy; *P* value was determined by the χ^2 test, ANOVA, or Kruskal-Wallis test; *CNS,* central nervous system; *COPD,* chronic obstructive pulmonary disease; *CPB,* cardiopulmonary bypass.

0.5 and bilateral chest effusions on x-ray films, without indications of cardiac failure

Gastrointestinal dysfunction: Bilirubin level greater than 50 μmol/L or acute gastrointestinal bleeding

Central nervous system dysfunction: Peripheral paralysis

Death: During primary hospital stay without recovery after operation, that is, "hospital mortality"[11]

The patient's preoperative risk status was summarized with the use of the clinical severity score published by Higgins and coworkers,[12] denoted as the "Higgins' score."

Blood samples and analyses. Samples with or without anticoagulants were obtained just before systemic heparinization, after 30 minutes of CPB, at termination of CPB, during closure of the skin over the sternum, and 3 hours after the operation. The exact sampling times were recorded. All tubes containing anticoagulants were kept on ice until centrifugation within 8 hours. Plasma or serum was stored at –70°C.

Hemoglobin, hematocrit, and blood cell counts were determined in an automated analyzer (Technicon H-1, Miles, Tarrytown, NY).

Complement activation measured as C3 activation products (C3bc), C5a-desArg, and TCC was quantitated in enzyme immunoassays in ethylenediaminetetraacetic acid plasma.[13]

Granulocyte activation was analyzed with the use of enzyme immunoassays specific for the degranulation products myeloperoxidase (MPO) and lactoferrin (LF) in ethylenediaminetetraacetic acid plasma.[14,15]

Platelet degranulation was assessed by determination of β-thromboglobulin (BTG) in citrate-theophylline-adenosine-dipyridamole–containing plasma (Diatube-H, Diagnostica Stago, Asnieres-sûr-Seine, France) in a novel competitive enzyme immunoassay (see appendix).

Endotoxin was analyzed in heparin-anticoagulated samples drawn into pyrogen-free tubes at termination of CBP (n = 136) by *Limulus* amebocyte lysate assay (Chromogenix, Endosafe, Charleston, SC).

To obtain a preoperative complement profile of each patient, we quantitated C3 and C4 antigen in the baseline serum samples by nephelometry (Behring laser nephelometer, Behringwerke AG, Marburg, Germany) according to the manufacturer's instructions. Classical (CH_{50}-c) and alternative (CH_{50}-a) total hemolytic complement activity were measured by means of microwell techniques.[16]

No measurements were corrected for hemodilution.

Statistics. Data are given as mean with 95% confidence intervals in parenthesis. For the complement, granulocyte, and platelet activation data, the highest concentration for each patient irrespective of time of occurrence was identified and denoted "maximal C3bc," "maximal TCC," and so on, in the following. As a summary measure, the area under the time curve for the activation parameters was calculated for each patient and is denoted "C3bc area," "TCC area," and so on.[17]

The activation parameters were analyzed with 2-way repeated-measures analysis of variance (ANOVA) after logarithmic transformation, using duration of CPB as a covariate. Other comparisons of variables between groups were performed with the χ^2 test, Fisher's exact test, 2-tailed *t* test, or ANOVA, or with the Mann-Whitney *U* test or Kruskal-Wallis test if not normally distributed.

Factors correlated to each complication were identified with standard methods for logistic regression using the SPSS program package (SPSS, Inc, Chicago, Ill). Linear regression identifying clinical predictors of complement activation and correlates of granulocyte activation was performed by means of standard methods in SPSS (details in the appendix).

The study was approved by the regional ethical committee on February 25, 1993.

Table II. *Postoperative complications**

	All patients	*Uncoated CPB set*	*Heparin-coated CPB set*	P *value†*
Cardiac dysfunction	20 (13%)	9 (12%)	11 (14%)	.82
CNS dysfunction	8 (5%)	3 (4%)	5 (6%)	.56
Renal dysfunction	11 (7%)	4 (5%)	7 (9%)	.44
Infection	21 (14%)	12 (16%)	9 (11%)	.56
Wound infection		3 (4%)	5 (6%)	.56
Pneumonia		7 (10%)	6 (7%)	.57
Sepsis		0 (0%)	2 (2%)	.50
Mediastinitis		4 (5%)	1 (1%)	.14
Gastrointestinal dysfunction	3 (2%)	2 (3%)	1 (1%)	.50
Reoperation	25 (16%)	13 (18%)	12 (15%)	.55
Death	9 (6%)	5 (7%)	4 (5%)	.64

CPB, Cardiopulmonary bypass; *CNS,* central nervous system.
*Two patients who died during the operation were not included in analysis of other complications.
†χ^2 Test or Fisher's exact test.

Table III. *Maximal and summary measures of activation parameters*

Parameter	*Uncoated CPB set*	*Heparin-coated CPB set*	P *value**
Maximal MPO concentration ($\mu g \times L^{-1}$)	872 (802-942)	783 (730-837)	.09
Area under MPO curve ($\mu g \times L^{-1} \times min \times 10^3$)	205 (178-232)	183 (165-200)	.22
Maximal LF concentration ($\mu g \times L^{-1}$)	1101 (963-1239)	902 (810-994)	.03
Area under LF curve ($\mu g \times L^{-1} \times min \times 10^3$)	250 (206-294)	198 (173-223)	.14
Maximal C3bc concentration ($AU \times mL^{-1}$)	103 (93-114)	93 (81-105)	.07
Area under C3bc curve ($AU \times mL^{-1} \times min \times 10^3$)	23.9 (21.5-26.4)	19.3 (16.9-21.8)	.01
Maximal C5a-desArg concentration ($ng \times mL^{-1}$)	14.5 (12.3-16.6)	16.3 (12.9-19.8)	.22
Area under C5a-desArg curve ($ng \times mL^{-1} \times min \times 10^2$)	39.6 (33.6-45.6)	44.6 (34.3-54.9)	.54
Maximal TCC concentration ($AU \times mL^{-1}$)	6.4 (5.8-7.0)	3.9 (3.5-4.4)	.001
Area under TCC curve ($AU \times mL^{-1} \times min \times 10^3$)	1.3 (1.1-1.4)	0.8 (0.7-0.9)	.001
Maximal BTG concentration ($ng \times mL^{-1}$)	875 (617-1132)	828 (646-1009)	.79
Area under BTG curve ($ng \times mL^{-1} \times min \times 10^3$)	159 (122-195)	149 (121-178)	.99

CPB, Cardiopulmonary bypass; *MPO,* myeloperoxidase; *LF,* lactoferrin; *AU,* arbitrary units; *TCC,* terminal compliment complex; *BTG,* β-thromboglobulin.
*Mann-Whitney *U* test.

Results

Patient and operative variables (Table I). Four groups of operative procedures were considered: coronary bypass surgery (CABG, n = 82), valve replacement (valve, n = 24), coronary bypass surgery combined with valve replacement (CABG + valve, n = 41), and coronary bypass surgery combined with carotid artery surgery (CABG + vascular, n = 9). As expected, there were significant differences among these groups with respect to gender, body weight, aortic occlusion times, and duration of CPB.

The patients in the heparin-coated and uncoated groups were comparable with respect to gender, age, Higgins' score, type of operation, diabetes, previous central nervous system symptoms, chronic obstructive pulmonary disease, hypertension, renal dysfunction, compensated cardiac insufficiency, redo operation, aortic occlusion, CPB duration, and heparin and protamine doses (P = .18-.93).

The mean Higgins' score was 3.4 (3.0-3.9). Sixty-nine patients (44%) had scores of more than 3, and 34 patients (22%) had scores of more than 5, confirming that our study included a large proportion of high-risk patients.

Complications (Table II). One hundred three patients (66%) had an uneventful recovery. Fifty-three patients (34%) experienced one or more complications, including revisions for bleeding. Twenty-four reoperations were necessitated by surgical bleeding (n = 12), bleeding caused by generalized oozing (n = 6), mediastinitis (n = 4), paravalvular leakage (n = 1), and endocarditis (n = 1). No patients fulfilled the criteria for adult respiratory distress syndrome. Seven patients died before the tenth postoperative day, 1 on day 20, and 1 on day 36. There were no significant differences

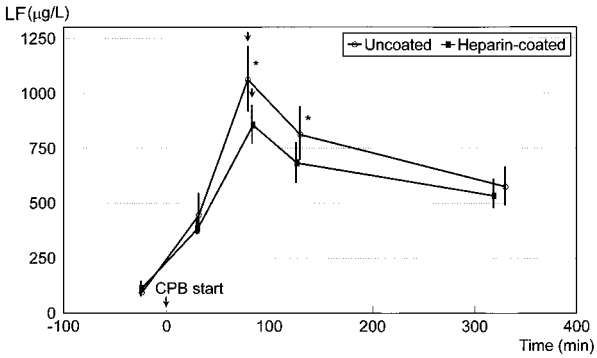

Fig 1. Granulocyte activation measured as plasma lactoferrin (*LF,* μg/L) during cardiac surgery in 156 patients using heparin-coated (n = 81) or uncoated (n = 75) CPB circuits. Data are mean with 95% confidence interval by mean sampling time in each treatment group. *Arrows* show start and termination of CPB in each group. Statistical comparisons by ANOVA after logarithmic transformation: Intergroup differences: $P = .32$; LF changes by time: $P < .0005$; group by LF change interaction: $P < .0005$. LF concentrations were significantly lower with heparin coating at termination of CPB and at closure of the wound over the sternum (*$P < .05$).

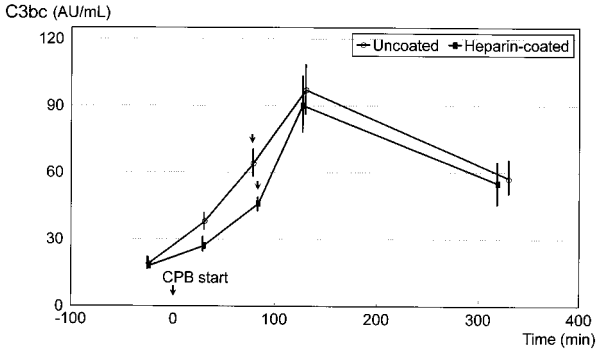

Fig 2. Complement C3 activation measured as plasma C3bc (arbitrary units per milliliter) during cardiac surgery in 156 patients using heparin-coated (n = 81) or uncoated (n = 75) CPB circuits. Data are mean with 95% confidence intervals by mean sampling time in each treatment group. *Arrows* show start and termination of CPB in each group. Statistical comparisons by ANOVA after logarithmic transformation: Intergroup differences: $P < .0005$; C3bc changes by time: $P < .0005$; group by C3bc change interaction: $P = .01$.

between the heparin-coated and uncoated groups for any complication (Table II) or in total thoracic drainage (uncoated, 1109 mL [895-1324 mL]; heparin-coated, 1020 mL [798-1242 mL]; $P = .57$) or duration of respiratory support (uncoated, 18 hours [7-28 hours]; heparin-coated, 16 hours [4-29 hours]; $P = .88$).

Blood tests. By ANOVA, there were no differences

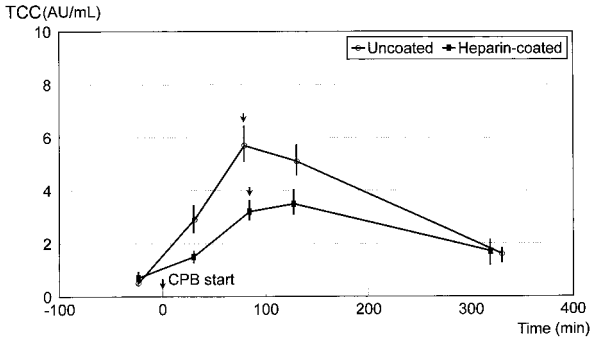

Fig 3. Complement activation measured as plasma TCC (arbitrary units per milliliter) during cardiac surgery in 156 patients using heparin-coated (n = 81) or uncoated (n = 75) CPB circuits. Data are mean with 95% confidence intervals by mean sampling time in each treatment group. *Arrows* show start and termination of CPB in each group. Statistical comparisons by ANOVA after logarithmic transformation: Intergroup differences: $P < .0005$; TCC changes by time: $P < .0005$; group by TCC change interaction: $P < .0005$.

in hemoglobin concentration ($P = .52$), but leukocytes tended to be fewer in the uncoated group ($P = .07$) (data not shown). Platelet numbers decreased from 181×10^9/L ($171\text{-}191 \times 10^9$/L) to 113×10^9/L ($104\text{-}121 \times 10^9$/L (uncoated) and 180×10^9/L ($170\text{-}190 \times 10^9$/L) to 123×10^9/L ($116\text{-}130 \times 10^9$/L) (heparin-coated) after 30 minutes of CPB, and there were no differences in platelet numbers by ANOVA ($P = .50$).

Activation parameters are shown in Table III. By ANOVA using CPB duration as covariate, differences in LF ($P < .05$), C3bc ($P < .0005$), and TCC ($P < .0005$) between the heparin-coated and uncoated groups were significant (Figs 1 to 3).

Baseline C3bc, TCC, C3 and C4 antigen, CH_{50}-c, and CH_{50}-a were within normal ranges (data not shown).

Logistic regression models (Table IV). No model was fitted for gastrointestinal dysfunction (n = 3). For death, only maximal concentrations of the activation parameters were used during model fitting, because some patients died before all samples were drawn. For all models, fit was good by the Hosmer-Lemeshow test. If only the MPO, TCC, and BTG variables were included instead of all the activation parameters, a substantial loss of model significance and fit was observed (data not shown).

Regression models for complement and granulocyte activation. All complement activation parameters were significantly correlated to the Higgins' score ($P < .001\text{-}.05$) and use of uncoated CPB set ($P < .0005\text{-}.02$), but the percentage of variation explained by the models (R^2) was less than 35% (data not shown). The models

Table IV. *Logistic regression models for complications*

Complication	*Variable*	P *value**	*Coefficient*	*SE*
Death	Maximal BTG	.0003	0.0010	0.0003
	Maximal C5a-desArg	.02	–0.23	0.12
Infections	Area under LF curve	.003	0.0079	0.0028
	Area under TCC curve†	.006	–3.0	0.94
Cardiac dysfunction	Re-instituted CPB‡	.0001	4.6	1.5
	Age	.04	0.065	0.034
	Area under TCC curve	.08	0.66	0.36
Renal dysfunction	Area under LF curve§	.004	0.0072	0.0055
	Maximal TCC	.006	–0.073	0.043
	Maximal BTG	.05	1.3	0.7
CNS dysfunction	CABG + vascular operations‖	.0003	3.0	0.96
	Area under TCC curve	.006	1.1	0.39

SE, Standard error; *BTG,* β-thromboglobulin; *LF,* lactoferrin; *TCC,* terminal complement complex; *CPB,* cardiopulmonary bypass; *CNS,* central nervous system; *CABG,* coronary artery bypass surgery.
*Likelihood ratio test.
†Coded as above or below the median concentration.
‡CPB re-instituted during weaning because of cardiac failure.
§Transformations: LF and TCC, squared; BTG, logarithmic.
‖Combined CABG and vascular operations compared with all other operations.

Table V. *Linear regression models for granulocyte activation*

*Dependent variable**	*Explanatory variables*	P *value*	*Coefficient*	*SE*	R^2 *for model*
Maximal MPO concentration	Area under TCC curve*	.001	0.15	0.042	0.17
	Female gender	.02	0.15	0.061	
	Area under BTG curve	.04	0.039	0.019	
MPO area	Area under TCC curve	<.0005	0.23	0.04	0.49
	CPB duration $\times 10^{-1}$	<.0005	0.040	0.008	
	Area under BTG curve*	.01	0.088	0.035	
	Female gender	.03	0.14	0.064	
Maximal LF concentration	Area under C3bc curve	<.0005	0.14	0.033	0.32
	Area under BTG curve*	<.0005	0.18	0.044	
	Female gender	.004	0.25	0.085	
	Heparin/protamine dose ratio	.02	0.72	0.32	
LF area	Area under BTG curve*	<.0005	0.20	0.047	0.48
	Female gender	.003	0.28	0.089	
	Area under TCC curve	.009	0.19	0.070	
	Area under C3bc curve	.01	0.12	0.044	
	CPB duration*	.01	0.31	0.12	

SE, Standard error; *MPO,* myeloperoxidase; *TCC,* terminal complement complex; *BTG,* β-thromboglobulin; *LF,* lactoferrin; *CPB,* cardiopulmonary bypass.
*Transformation: logarithmic.

for C3bc area, maximal TCC, and TCC area were slightly improved by including duration of CPB or aortic occlusion, whereas inclusion of other intraoperative variables (heparin or protamine doses, or whether the patient required more than one attempt at weaning from CPB) gave no improvement.

Significant variables explaining the granulocyte activation parameters included complement and platelet activation, heparin/protamine dose ratio, duration of CPB, and gender (Table V). The models explained approximately 50% of the variation in MPO area and LF area, but less than 35% of the variation in maximal MPO and LF. The models were not improved by including the other intraoperative variables mentioned above, and plasma endotoxin concentrations at termination of CPB were not correlated to granulocyte activation.

Discussion

Clinical benefit of heparin coating. Our investigation included a substantial proportion of patients having elevated Higgins' scores, and we observed a number of postoperative complications. Even so, there were no differences ($P > .44$) in number of complications

between the patients treated with Duraflo II heparin-coated and uncoated CPB circuits. This is in keeping with the relatively few previous reports of improved clinical outcome with heparin-coated CBP devices.[18-20] Reduced systemic heparinization may increase the benefits of heparin coating,[21] pointing at heparin- and protamine-induced effects on the body's defense systems as more harmful than previously acknowledged. However, not all agree that reduction of systemic heparin is sufficiently safe. We used full systemic heparinization in both groups to study only one variable—heparin coating.

Our study demonstrates that significant differences in biochemical markers cannot directly be interpreted into clinical relevance, even in a study including more than 150 patients and a fair number of outcome "events." Obviously, very many different factors may contribute to post-CPB morbidity and mortality in each patient, not all of which are related to biocompatibility. The present knowledge about pathogenesis is probably fragmentary, rendering it difficult to find efficient measures to reduce risk.

TCC and clinical outcome. No clear-cut relationship between TCC and clinical outcome was found, even if TCC was significant in the logistic regression models for infections, renal dysfunction, and central nervous system dysfunction. The logistic regression model coefficients should be interpreted cautiously in a study of 156 patients. The TCC concentration had a negative coefficient in the models for infections and renal dysfunction. Thus the patients with less TCC formation were at higher risk for the development of these complications. The explanation for this finding is unknown. Maybe the shape of the activation curve is of importance, for example, whether the patient responds with a rapid, intense production of TCC that peaks early or tends to produce less TCC per unit of time, but over a longer period.

For central nervous system dysfunction and cardiac dysfunction, the activation parameters significantly improved models including widely known risk factors such as carotid artery surgery and difficult weaning from CPB, respectively. Most variables that were tested other than the activation parameters had *P* values of much more than .20 (data not shown). Thus, as a whole, the indicators of complement, granulocyte, and platelet activation were all clinically relevant parameters of biocompatibility, but each marker including TCC had varying significance with respect to the different complications. Whether inclusion of activation markers of other cellular and humoral defense systems such as endothelial cells, monocytes, coagulation, and fibrinolysis improves assessment of biocompatibility, warrants further study.

Clinical predictors of complement activation. Our study clearly showed that the present knowledge of individual factors influencing complement activation during cardiac surgery is insufficient. The Higgins' score was a significant explanatory variable for all complement activation parameters. To our knowledge, this is a new observation. The Higgins' score was developed for patients undergoing CABG.[12] We used it for all patient groups because it is a well-established risk score predicting postoperative morbidity, not only mortality, in cardiac surgical patients, and testing of each potential risk factor itself would necessitate a much larger study population. The risk factors included in the Higgins' score were picked because of their proven relationship with postoperative complications and mortality. Perhaps one reason that these factors carry such an increased risk is their tendency to influence complement activation. Six of the 13 variables in the Higgins' score (ie, reduced left ventricular ejection fraction, prior vascular surgery, chronic obstructive pulmonary disease, anemia, operative aortic stenosis, and diabetes) are related to an increased risk of reduced peripheral oxygenation. Ischemia is known to induce complement activation,[22] and patients with these conditions may have a relative tissue ischemia during CPB, increasing complement activation. Furthermore, patients with reduced organ function in the preoperative period may be more susceptible to complement-related damage, giving them an additional disadvantage.

Correlates of granulocyte activation. Inclusion of markers of contact activation might have further improved the regression models. An important explanatory variable for granulocyte activation in addition to complement activation was duration of CPB. Ischemia in the lower part of the body, including the abdomen, is likely during crossclamping. Release of mediators from ischemic tissues on reperfusion activates the coagulation and fibrinolytic systems[23] and may also activate granulocytes.[24]

Granulocyte activation and release of BTG from platelets were significantly correlated. These cells may both activate and inhibit each other, but in general they tend to positively stimulate activation of one another during CPB.[25] The present study demonstrates that platelet-induced granulocyte activation is of practical importance.

Protamine neutralizes heparin by reversible complex formation. Heparin may later be released, because protamine has a shorter half-life than heparin.[26] In vitro, heparin preincubation results in more extensive granu-

locyte activation on later stimulation with the complement-analog *N*-formyl-met-leu-phe.[14] Granulocyte activation was correlated to the heparin/protamine dose ratio, with relatively more heparin increasing activation. The clinical significance of heparin-induced granulocyte activation is supported by previous observation of reduced granulocyte activation with heparin-coated CPB only accompanied by reduced systemic heparin, whereas complement activation decreased also with full systemic heparinization.[27]

Female gender was an additional significant variable for granulocyte activation. Women carry a higher complication risk after cardiac surgery, in part because they tend to be sicker before the operation.[28] On the basis of our investigation, increased granulocyte activation may be one factor increasing the risk of complications in women.

Our study demonstrates that granulocyte activation during cardiac surgery is multifactorial. Therefore measures reducing complement activation alone may not be sufficient to achieve a clinically relevant reduction in postoperative organ dysfunction.

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REFERENCES

1. Moen O, Høgåsen K, Fosse E, Degrelid E, Brockmeier V, Venge P, et al. Attenuation of changes in leukocyte surface markers and complement activation with heparin-coated cardiopulmonary bypass. Ann Thorac Surg 1997;63:105-11.
2. Nilsson L, Peterson C, Venge P, Borowiec JW, Thelin S. Eosinophil granule proteins in cardiopulmonary bypass with and without heparin coating. Ann Thorac Surg 1995;59:713-6.
3. Fukutomi M, Kobayashi S, Niwaya K, Hamada Y, Kitamura S. Changes in platelet, granulocyte, and complement activation during cardiopulmonary bypass using heparin-coated equipment. Artif Organs 1996;20:767-76.
4. Videm V, Svennevig JL, Fosse E, Semb G, Østerud A, Mollnes TE. Reduced complement activation with heparin-coated oxygenator and tubings in coronary bypass operations. J Thorac Cardiovasc Surg 1992;103:806-13.
5. te Velthuis, Baufreton C, Jansen PG, Thijs CM, Hack CE, Sturk A, et al. Heparin coating of extracorporeal circuits inhibits contact activation during cardiac operations. J Thorac Cardiovasc Surg 1997;114:117-22.
6. Yamada H, Kudoh I, Hirose Y, Toyoshima M, Abe H, Kurahashi K. Heparin-coated circuits reduce the formation of TNF alpha during cardiopulmonary bypass. Acta Anaesthesiol Scand 1996; 40:311-7.
7. Wildevuur CR, Jansen PG, Bezemer PD, et al. Clinical evaluation of Duraflo II heparin treated extracorporeal circulation circuits (2nd version). The European Working Group on heparin coated extracorporeal circulation circuits. Eur J Cardiothorac Surg 1997; 11:616-23.
8. Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenoweth DE, Pacifico AD. Complement and the damaging effects of cardiopulmonary bypass. J Thorac Cardiovasc Surg 1983;86:845-57.
9. Videm V, Mollnes TE, Garred P, Svennevig JL. Biocompatibility of extracorporeal circulation: in vitro comparison of heparin-coated and uncoated oxygenator circuits. J Thorac Cardiovasc Surg 1991;101:654-60.
10. Videm V, Mollnes TE, Bergh K, Fosse E, Mohr B, Hagve TA, et al. Heparin-coated cardiopulmonary bypass equipment. II. Mechanisms for reduced complement activation in vivo. J Thorac Cardiovasc Surg 1999;117:803-9.
11. Edmunds LH, Clark RE, Cohn LH, Grunkemeier GL, Miller DC, Weisel RD. Guidelines for reporting morbidity and mortality after cardiac valvular operations. Ann Thorac Surg 1996;62:932-5.
12. Higgins TL, Estafanous FG, Loop FD, Beck GJ, Blum JM, Paranandi L. Stratification of morbidity and mortality outcome by preoperative risk factors in coronary artery bypass patients. JAMA 1992;265:2344-8.
13. Mollnes TE. Analysis of in vivo complement activation. In: Herzenberg LA, Weir DM, Herzenberg LA, Blackwell C, editors. Weir's handbook of experimental immunology. Volume 78, 5th ed. Boston: Blackwell Science; 1997. p. 78.1-78.8.
14. Videm V. Heparin in clinical doses "primes" granulocytes to subsequent activation as measured by myeloperoxidase release. Scand J Immunol 1996;43:385-90.
15. Hegnhøj J, Schaffalitzky de Muckadell OB. An enzyme-linked immunosorbent assay for measurement of lactoferrin in duodenal aspirates and other biological fluids. Scand J Clin Lab Invest 1985;45:489-95.
16. Mollnes TE, Høgåsen K, Hoaas BF, Michaelsen TE, Garred P, Harboe M. Inhibition of complement-mediated red cell lysis by immunoglobulins is dependent on the Ig isotype and its C1q binding properties. Scand J Immunol 1995;41:449-56.
17. Altman DG. Practical statistics for medical research. London: Chapman & Hall; 1996. p. 430.
18. Ranucci M, Cirri S, Conti D, Ditta A, Boncilli A, Frigiola A, et al. Beneficial effects of Duraflo II heparin-coated circuits on postperfusion lung dysfunction. Ann Thorac Surg 1996;61:76-81.
19. Watanabe H, Miyamura H, Hayashi J, Ohzeki H, Sugawara M, Takahashi Y, et al. The influence of a heparin-coated oxygenator during cardiopulmonary bypass on postoperative lung oxygenation capacity in pediatric patients with congenital heart anomalies. J Card Surg 1996;11:396-401.
20. Baufreton C, Le Besnerais P, Jansen P, Mazzucotelli JP, Wildevuur CR, Loisance DY. Clinical outcome after coronary surgery with heparin-coated extracorporeal circuits for cardiopulmonary bypass. Perfusion 1996;11:437-43.
21. Saenz A, Larranaga G, Alvarez L, Greco E, Marrero A, Lunar M, et al. Heparin-coated circuit in coronary surgery: a clinical study. Eur J Cardiothorac Surg 1996;10:48-53.
22. Mollnes TE, Fosse E. The complement system in traumarelated and ischemic tissue damage: a brief review. Shock 1994;2:301-10.
23. Wagner WR, Johnson PC, Thompson KA, Marrone GC. Heparin-coated cardiopulmonary bypass circuits: hemostatic alterations and postoperative blood loss. Ann Thorac Surg 1994;58:734-40.
24. Ceriana P. Effect of myocardial ischaemia-reperfusion on granulocyte elastase release. Anaesth Intensive Care 1992;20:187-90.

25. Bazzoni G, Dejana E, de Machio A. Platelet-neutrophil interactions: possible relevance in the pathogenesis of thrombosis and inflammation. Haematologica 1991;76:491-9.
26. Shanberge JN, Murato M, Quattrociocchi-Longe T, van Neste L. Heparin-protamine complexes in the production of heparin rebound and other complications of extracorporeal bypass procedures. Am J Clin Pathol 1987;87:210-17.
27. Øvrum E, Mollnes TE, Fosse E, Holen EÅ, Tangen G, Ringdal MAL, et al. High and low heparin dose with heparin-coated cardiopulmonary bypass: activation of complement and granulocytes. Ann Thorac Surg 1995;60:1755-61.
28. Wenger NK, Speroff L, Packard B. Cardiovascular health and disease in women. N Engl J Med 1993;329:247-56.

Appendix

BTG immunoassay. The plates were coated with partly purified BTG from activated human platelets. Samples and biotinylated (Biotinylation kit, Sigma Chemical Co, St Louis, Mo) antihuman BTG antibody (Biogenesis, Poole, Dorset, United Kingdom) were added, and peroxidase-conjugated avidin (Zymed Laboratories, San Francisco, Calif) was added in the final step. The substrate was *o*-phenylenediamine dihydrochloride (Sigma), and optical density was read at 490 nm in a microtiter plate reader (ELX800, Bio-Tec Instruments, Winooski, VT). Purified BTG from human platelets (Celsus Laboratories, Cincinnati, Ohio) was used as standard.

Logistic regression modeling. For each defined complication, type of operation, age, gender, use of heparin-coated or uncoated CPB circuit, Higgins' score, body weight, aortic occlusion time, duration of CPB, and the activation parameters were entered into multivariate logistic regression model fittings, as well as possibly relevant variables identified in univariate logistic regression. Near-significant variables (*P* values between .05 and .10) were kept in the model if removal substantially reduced model fit, and goodness-of-fit was assessed by the Hosmer-Lemeshow test. Linearity of the logits for all continuous variables was checked by plotting and, if necessary, transformations were applied to achieve linearity. The variables were scaled by the following factors to achieve a reasonable size of the coefficients: LF area in model for infections: 10^{-3}, TCC area in models for cardiac and central nervous system dysfunction: 10^{-3}, square of LF area in model for renal dysfunction: 10^{-6}.

Linear regression modeling. The dependent variables for complement activation were maximal C3bc, C3bc area, maximal TCC, and TCC area after logarithmic transformation. The independent variables were age, gender, scheduled type of operation, height, weight, Higgins' score, heparin-coated or uncoated CPB set, and the intraoperative variables heparin and protamine doses, duration of aortic occlusion and CPB, and whether the patient required more than one attempt at weaning from CPB. After fitting the best model, we tested whether the model was significantly improved by inclusion of antigen C3, antigen C4, CH_{50}-c, and CH_{50}-a. The dependent variables for granulocyte activation were maximal MPO, MPO area, maximal LF, and LF area after logarithmic transformation. The independent variables were age, gender, scheduled type of operation, height, weight, Higgins' score, heparin-coated or uncoated CPB set, aortic occlusion time, duration of CPB, duration of CPB after release of aortic crossclamp, doses of heparin and protamine, relationship of heparin/protamine, C3bc area, TCC area, BTG area, and endotoxin concentration at termination of CPB. For all models, residual plots were examined and, if necessary, explanatory variables were transformed. Some variables were scaled by a factor of 10^{-1} to 10^{-5} to achieve a reasonable size of the coefficients.