

# Clinical Performance and Biocompatibility of Novel Hyaluronan-Based Heparin-Bonded Extracorporeal Circuits

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**Abstract:** We tested documented in vitro and ex vivo advantages of novel hyaluronan based heparin bonded extracorporeal circuits in a prospective randomized study. During the period from June until September 2005, 40 patients undergoing reoperation for coronary artery bypass grafting were allocated into two equal groups ( $n = 20$ ): Group 1 was treated with hyaluronan-based heparin-bonded circuits and group 2 was treated with uncoated control circuits. Complete blood count, fibrinogen, albumin, C3a, interleukin-2 levels, and thromboelastographic data were documented after induction of anesthesia (T1) and heparin administration before cardiopulmonary bypass (CPB) (T2), 15 minutes after initiation of CPB (T3), before cessation of CPB (T4), 15 minutes after reversal with protamine (T5), and the first postoperative day at 8:00 a.m. (T6). Hollow fibers were collected for consecutive biomaterial analysis by optical and scanning electron microscopy (SEM). Desorbed protein deposition on fibers was compared by spectrophotometry. Leukocyte counts were lower in T4-T6 in group 1 ( $p < .05$ ). Platelet counts demonstrated

significant differences at T4 and T5 in coated group ( $p < .05$ ). Albumin and fibrinogen levels were better preserved in Group 1 at T4, T5 and T4, T6, consecutively ( $p < .05$ ). C3a and IL-2 levels were lower at T3-T5 and T4-T5 in intervention group ( $p < .05$ ). Postoperative hemorrhage was  $412 \pm 50$  mL in group 1 and  $684 \pm 50$  ml in group 2 ( $p < .05$ ). Respiratory support time was shorter in group 1 versus control ( $p < .05$ ). Platelet adhesion was significantly lower in intervention group. Amount of desorbed protein was  $1.44 \pm 0.01$  mg/dL in group 1 and  $1.94 \pm 0.01$  mg/dL in control ( $p < .05$ ). SEM and spectrophotometry demonstrated better surface preservation in the hyaluronan coated group. Novel hyaluronan-based heparin-bonded circuits reduce platelet adhesion-aggregation and protein adsorption and provide better perioperative clinical parameters through platelet, albumin, and fibrinogen-sparing effects. **Keywords:** cardiopulmonary bypass, coated materials, biocompatibility; membrane oxygenators, extracorporeal circulation. JECT. 2005;37:290-295

Hyaluronan is a unique biomaterial that lends itself to crosslinking and immobilization in various ways to produce hydrophilic, lubricious, and biocompatible surfaces that the body perceives as inert when implanted (1). Interest in hyaluronan has increased dramatically since the early 1980s, with major clinical applications in ophthalmology, in the treatment of degenerative joint disease, and in adhesion prevention, combined with production of the polymer on an industrial scale (2). The ability to derivatize and complex hyaluronan with other substances makes it possible to create a range of bioactive surfaces. Device applications might include using such surfaces, for example, to

impart antithrombogenic and antibacterial properties, or to interact preferentially with certain proteins and cells (3).

Hyaluronan-coated extracorporeal circuits, commercially known as GBS™ Coating (Gish Biomedical Inc., Rancho Santa Margarita, CA), are a novel heparin-based covalent bonding consisting of a polycarbonate backbone with hydrophilic as well as hydrophobic groups. In vitro and ex vivo studies of hyaluronan coating have clearly demonstrated the advantages of this technology in various medical applications. To assess the feasibility and performance of hyaluronan-based heparin-bonded extracorporeal circuits under clinical conditions, we conducted a prospective randomized study using GBS™-coated versus uncoated (control) circuits.

## MATERIALS AND METHODS

This study was approved by the Medical Ethics Committee of the Institution. Informed consent was obtained

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from each patient included in the study. Forty patients (24 men and 16 women with a mean age of  $64.7 \pm 8$  years) undergoing reoperation for coronary artery bypass grafting with full heparin dose strategy were included in the study. Patients with history of any coagulopathy or ongoing anticoagulation, steroid therapy, nonsteroidal anti-inflammatory drugs, or aspirin within 5 days preoperatively were excluded from the study.

Patients were randomly allocated into two equal groups. The CPB circuit design of groups was identical (open system). Group 1 (study group) underwent surgery with hyaluronan-based heparin-bonded extracorporeal circuits (GBS™ Coating, GISH Biomedical Inc.;  $n = 20$ ), and group 2 (control group) underwent surgery with uncoated control extracorporeal circuits (D-708 Avant®, Dideco, Mirandola, Italy;  $n = 20$ )

### Operative Technique

Anesthesia was induced by fentanyl ( $35 \mu\text{g}/\text{kg}$ ) and muscle relaxation was established with pancronium ( $0.1 \text{ mg}/\text{kg}$ ). The patients were intubated endotracheally and ventilated with 100% oxygen. All patients were administered heparin sodium (Liquemine, Roche, Istanbul, Turkey)  $3 \text{ mg}/\text{kg}$ . Activated clotting time (ACT) was measured by Hemochron 801 (International Technodyne Corporation, Edison, NJ) and maintained greater than 480 seconds.

The ascending aorta was cannulated for arterial inflow, and the right atrium for venous return. Moderate hypothermia was induced at  $30^\circ\text{C}$ . After cross-clamping of the aorta, the heart was arrested with crystalloid potassium cardioplegia,  $10\text{--}15 \text{ mL}/\text{kg}$ . Cold blood cardioplegia was given at 20-minute intervals. Warm blood cardioplegia was administered before the aortic cross clamp was released. Bilateral internal mammary artery or radial artery grafts were used for coronary artery lesions, if not used in the first operation and saphenous vein grafts were used as alternative in other instances. Rewarming was initiated during last grafting. When  $36.5^\circ\text{C}$  was reached, cardiopulmonary bypass (CPB) was discontinued and heparin was reversed by  $3.1 \text{ mg}/\text{kg}$  protamine sulfate (Protamine, Roche, Istanbul, Turkey). The adequacy of protamine reversal was checked by ACT and corrected, when necessary.

Platelet function was evaluated by thromboelastography (TEG; ROTEG®, Pentapharm, GmbH, Germany) during the operation. Coagulation time (CT), clot formation time (CFT),  $\alpha$ -angle, mean clot firmness (MCF), and A5 were measured in samples T1 up to T5.

The CPB prime was identical for both groups: 300 mL of mannitol (60 g, 20%), 500 mL of hydroxyethyl starch, and 1000 mL of crystalloid (plasmalyte A, Eczacibasi, Turkey). Hematocrit was maintained at about 20% during

CPB with blood or fresh-frozen plasma transfusions added to circuit, when necessary. Hematocrit levels were kept similar before and after the procedure. To evaluate CPB performance, oxygen transfer rate and pressure drop in the circuits were recorded at different flows and inspired oxygen fractions, respectively (4). Cell washing was not performed. Postoperative shed blood was collected in the drainage tubes and was not autotransfused.

In the intensive care unit (ICU), red blood cells (1 unit = 300 mL) or fresh-frozen plasma was transfused to maintain hematocrit levels higher than 30% and an adequate oncotic pressure. Platelet transfusions were not needed. No anticoagulants were used.

### Perioperative Follow-Up

For each patient, hemodynamic parameters, perfusion and cross-clamp duration, intubation period, postoperative hemorrhage, the use of blood and plasma, incidence of arrhythmia, use of inotropic support, complications and infection, the duration of ICU stay and hospital stay, perioperative mortality, New York Heart Association Classification, and Doppler echocardiography were evaluated before discharge and documented. Comparison between groups was performed retrospectively.

### Blood Samples and Assays

Complete blood count (hemoglobin, hematocrit, erythrocyte, white blood cell [WBC] and platelet counts), prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen levels were recorded. Results of standard blood and urine chemistry, especially serum albumin and globulin fractions, were documented. Complement fragment, C3a anaphylatoxin levels were measured by ELISA, via turbidimetric method (Dade-Behring, Deerfield, IL). Collected samples were kept on ice. Serum interleukin 2 (IL-2) levels were measured by ELISA (Bender Medsystems, Vienna, Austria; Coefficient of variation  $<10\%$  and sensitivity  $<1.4 \text{ pg}/\text{mL}$ ).

Blood samples were collected, using a radial or femoral artery catheter, in tubes containing potassium-ethylene diamine tetraacetic acid at the following intervals:

Baseline: After induction of anesthesia (before administration of heparin) (T1)

TEG control: After heparin administration before CPB (T2).

On CPB: 15 min. after initiation of CPB (T3)

Off CPB: Before cessation of CPB (T4)

Protamine: 15 min. after reversal with protamine (T5)

ICU: First postoperative day at 8:00 a.m. (T6)

### Microscopy and Spectrophotometry

At the termination of CPB, the complete circuit was

rinsed with saline solution. The oxygenator was removed, treated with glutaraldehyde solution at room temperature and dismantled by a saw under sterile conditions. Hollow fibers were collected for later microscopic and protein desorption studies (5). A mean number of 300 fibers (6 cm) were put into a 15-mL plastic tube with 1% sodium dodecyl sulfate (Pharmacia Biotechnology, Sweden) and 1% Triton X-100 solution (Bio-Rad, Cambridge, MA). The tube was then placed in a 38-kHz, 80-W ultrasonic washer (Kaijyo, Japan) for 1 hour and treated in phosphate-buffered saline buffer solution at pH 7.4 under constant temperature of 25°C for 6 hours. The sample was passed through a filter (Milipore Corporation, Bedford, MA) and prepared for further laboratory study by optical microscopic and analytic techniques.

Blood cells adhesion and aggregation were analyzed using optical microscopy and the amount of desorbed protein in each specimen for every patient was evaluated quantitatively with a COBAS MIRA Spectrophotometer (Roche Diagnostics Systems Inc., Branchburg, NJ) with its range adjusted to greater than 0.01. Fiber contents were also examined under scanning electron microscopy (SEM).

### Statistical Analysis

Data were expressed as mean  $\pm$  standard error of mean. Two-way analysis of variance was used to analyze differences over time in each group (Repeated-measures analysis of variance) and *t* test for differences between groups. A *p* value less than .05 was considered significant. Data were analyzed using SPSS program (version 12.0, Chicago, IL).

## RESULTS

Preoperative physical and laboratory findings for all patients were within normal ranges. No significant differences were observed between two groups with respect to hemodynamic performance and blood gases.

### CPB Performance

Pressure drop and oxygen transfer rates in circuits did not demonstrate any significant differences. In group 1 (study group), pressure changes were  $59 \pm 5$  mmHg,  $76 \pm 6$  mmHg, and  $92 \pm 6$  mmHg and in group 2 (control group), they were  $65 \pm 5$  mmHg,  $83 \pm 6$  mmHg, and  $98 \pm 6$  mmHg at a pump flow of 3, 4, and 5 L/min, respectively. The oxygen transfer rate was  $104 \pm 8$  mL/min,  $148 \pm 9$  mL/min,  $195 \pm 10$  mL/min in hyaluronan group, and  $96 \pm 8$  mL/min,  $138 \pm 9$  mL/min,  $187 \pm 10$  mL/min in control group at an inspired oxygen fraction of 0.7, 0.8, and 0.9, respectively. Other perioperative data for the two groups are summarized in Table 1.

**Table 1.** Perioperative data for the two groups.

	Hyaluronan	Uncoated	<i>p</i> Value
Duration of CPB (min)	86.2 $\pm$ 7	84.2 $\pm$ 8	NS
Duration of x-clamp (min)	62.4 $\pm$ 4	64.2 $\pm$ 4	NS
t-intub (h)	8.2 $\pm$ 2	12.1 $\pm$ 2.5	<.05
Postoperative hemorrhage (mL)	412 $\pm$ 50	684 $\pm$ 50	<.05
Arrhythmia (n)	0	AF:2	NS
Blood transfusion (unit)	2.1 $\pm$ 0.5	2.6 $\pm$ 0.5	NS
Blood products (unit)	2.5 $\pm$ 0.5	3.1 $\pm$ 0.5	NS
Inotropic support (n)	0	2	NS
ICU stay (day)	2 $\pm$ 0.1	2.5 $\pm$ 0.1	NS
Postoperative NYHA class	2.4 $\pm$ 0.1	2.2 $\pm$ 0.1	NS
Postoperative ejection fraction (%)	51.7 $\pm$ 8	50.6 $\pm$ 7	NS
Hospital stay (day)	6.7 $\pm$ 1	7.8 $\pm$ 1	NS
Mortality rate (%)	0	0	NS

### Blood Samples and Assays

There were no significant differences in PT or aPTT between the two groups throughout the procedure.

### Intergroup Comparisons Over the Course of Time

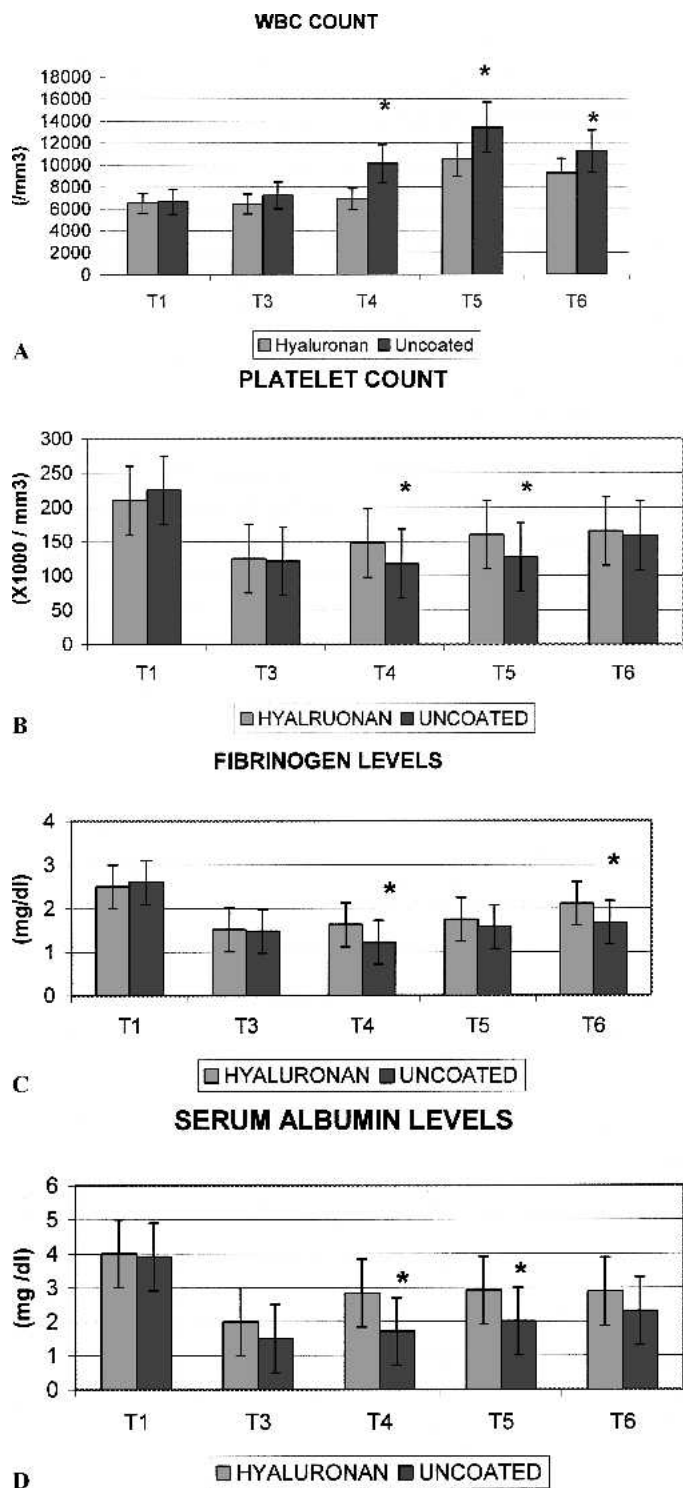
In the hyaluronan group, WBC counts were significantly lower than control group at T4, T5, and T6 (*p* < .05; Figure 1A). Platelet counts demonstrated significant preservation in favor of the study group at T4 and T5 (*p* < .05; Figure 1B). Fibrinogen levels were significantly better preserved in study group at T4, T6 and serum albumin levels at T4 and T5 (*p* < .05; Figure 1C and D). In the hyaluronan group, serum IL-2 levels were significantly lower at T4 and T5 and C3a levels at T3, T4, and T5 with respect to control (*p* < .05; Figure 2A and B).

### Microscopy and Spectrophotometry

Microscopic evaluation of blood cells demonstrated significantly increased adhesion and aggregation of platelets in uncoated groups. Light microscopy of Giemsa stained samples and optical micrographs demonstrated increased aggregation of platelets in the fibers of uncoated circuits. Similarly, blood protein (microalbumin) adsorption analysis demonstrated a significantly increased amount of microalbumin on uncoated fibers (*p* < .05; Figure 3). On SEM evaluation, hyaluronan based heparin bonded circuits were almost completely free from debris, protein and cells in contrast to uncoated circuits (Figure 4A and B).

## DISCUSSION

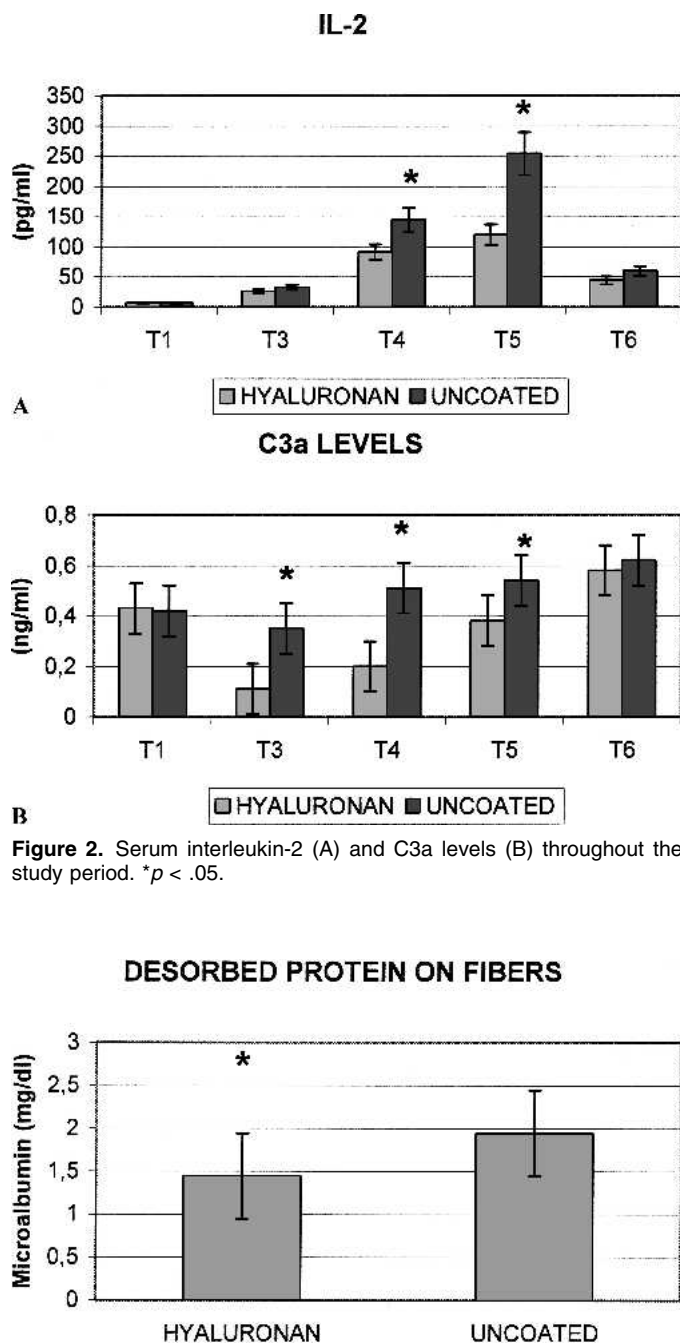
Hyaluronan is a polysaccharide of the glycosaminoglycans class. It is a specific biologic polymer that is found in all tissues and body fluids in every mammalian species as well as in microorganisms in a similar chemical form (6). As one might expect for a material that is ubiquitous in the body, the biocompatibility of hyaluronan-modified surfaces has been well established. The only requirement for medically usable preparations is to remove inflammatory



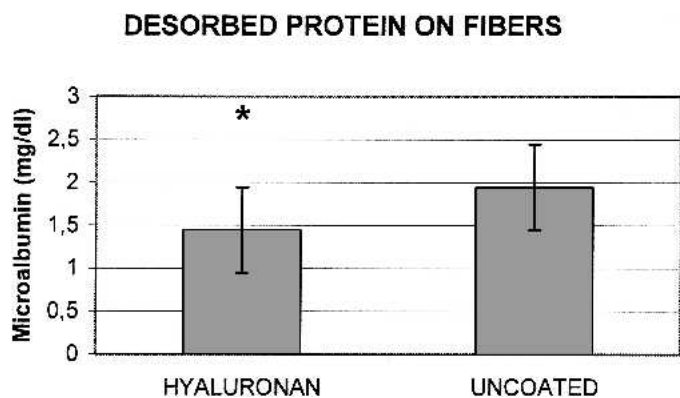
**Figure 1.** Trend in hematologic and biochemical variables throughout the study period. White blood cell count (A), platelet count (B), fibrinogen levels (C), and albumin levels (D). \* $p < .05$ .

fractions, for which processes and techniques have been developed over the years (7–9).

It has been demonstrated that the use of heparin-coated extracorporeal circuits may improve biocompatibility with



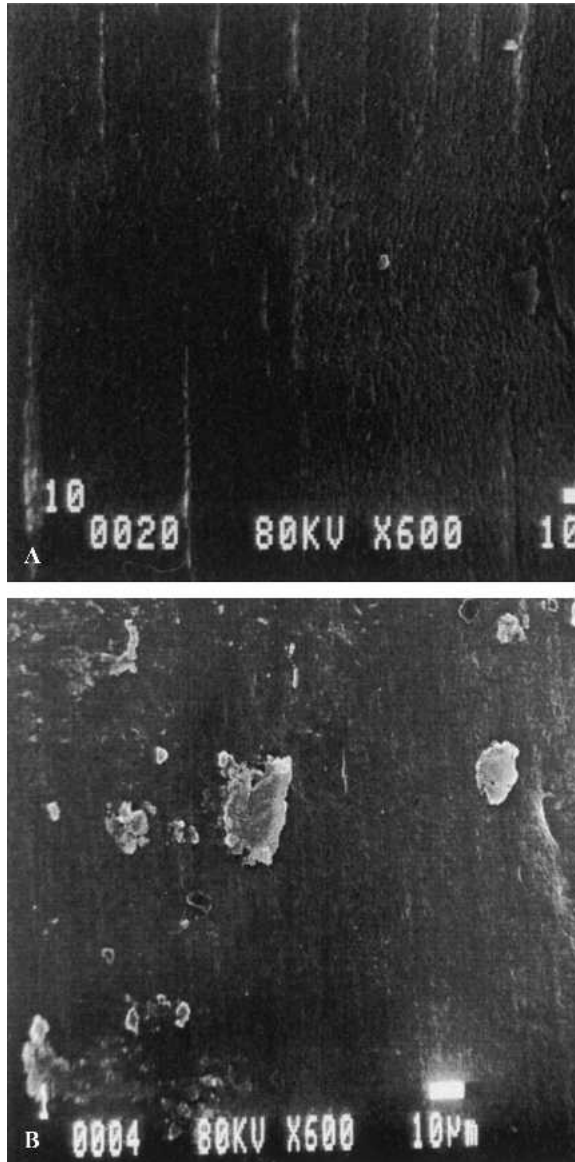
**Figure 2.** Serum interleukin-2 (A) and C3a levels (B) throughout the study period. \* $p < .05$ .



**Figure 3.** Desorbed amount of protein on hollow fibers \* $p < .05$ .

reduction in activation of blood components and therefore mitigate the subsequent whole body inflammatory response (10,11). In our previous studies (as well as those of other investigators) with various surface-modifying additives, we have demonstrated that heparin coatings are predominantly effective in alleviating systemic inflammatory response (SIRS) and polymer based coatings in platelet and protein preservation (12–14).

Polymer-based heparin bonded circuits are new in the market, and scientific literature is limited (15). We have



**Figure 4.** Scanning electron micrographs of hollow fibers: coated (A) and uncoated circuits (B).

published results only on polyethylene-based heparin coating (14).

In our study on hyaluronan-based heparin-bonded circuits, the preventive effect on SIRS dominated. WBC counts, IL-2, and C3a levels were significantly lower in coated group. Additional advantage of platelet preservation also was apparent as demonstrated by platelet counts.

One of the fundamental problems that also should be noted in a clinical evaluation is the difficulty of calculating hemodilution. In our study, we used absolute values of blood and cellular elements and did not account for hemodilution. This is because the hematocrit values of groups remained about the same before and after the operation. Moreover, as erythrocyte suspensions, fresh fro-

zen plasma, or both were infused during bypass grafting, the hematocrits and the red cell counts were changed artificially, thus making it difficult to calculate the correct factor for hemodilution. Using this methodology and working with the absolute values reflected the changes in trends between study and control groups. Also, coronary suction was used in both groups not to change the standard protocol.

Optical microscopy, SEM, and spectrophotometer showed significantly protective contribution of hyaluronan coating over uncoated circuits. Nevertheless, it is difficult to correlate the benefits of a novel device demonstrated in laboratory studies with significant clinical benefits in patients. Studies with similar objectives and methods have often yielded contradictory results, which suggest that the specific response mechanisms of CPB may vary between patients (16). With regard to clinical outcomes in our study, positive results in laboratory parameters in the study group translated into less postoperative hemorrhage and shorter respiratory support time.

The high standard of current CPB systems has made it increasingly difficult to test technical improvements in clinical studies involving relatively small patient groups. In most cases, the statistical power of such studies will not suffice to show a significant clinical benefit associated with changes in the CPB circuit. The significance of showing biocompatibility through reduced biologic markers has not correlated with measurable clinical benefit of surface modification during CPB.

We have tested 20 patients for each group and have also chosen reoperation for coronary artery bypass grafting, which is a more challenging clinical situation to manage. Greater homogeneity may be achieved by identifying those patients at greater risk of exaggerated responses to CPB in larger study groups.

As with most technologic advances, patients who benefit the most will be those who are in most need. It is likely that somewhere within these subpopulations surface-modified circuits will find their most significant utility. This is not to say others will not benefit from it. It is only a matter of time until the costs for incorporating this technology are reduced to that point that it becomes a standard of care for the conduct of CPB (17).

The primary endpoint of current technological advances, at least for the present, would be to improve the biocompatibility of the CPB circuit to a level that matches the low systemic inflammatory response and hemocompatibility evoked when, for instance, coronary artery bypass grafting is performed off-pump. Also inflammatory response during CPB is multifactorial and combined therapies may be more efficient than a single intervention to improve outcome. Both pharmacologic interventions and modification of techniques or mechanical devices may have clinical implications (18).

On the basis of our data, we conclude that hyaluronan-based heparin-bonded circuits reduced platelet adhesion and aggregation and protein adsorption. Coated surfaces resulted in a better perioperative clinical status for the patients through positive modulation of SIRS, including platelet, albumin and fibrinogen sparing effects.

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