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## **Stepwise improvement of cardiopulmonary bypass for neonates and infants**

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## chapter 5

# **Phosphorylcholine Coating of Bypass Systems Used for Young Infants Does Not Attenuate the Inflammatory Response**

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## **Abstract**

**Background.** Contact of blood with the artificial surfaces of the cardiopulmonary bypass (CPB) system is considered to be a main cause of complement activation. Improving the biocompatibility of the system by reduction of contact activation of blood elements and thereby producing less inflammatory response is evidently desired, especially for neonates and infants who are more susceptible to the deleterious effects of CPB. A phosphorylcholine coating, PHISIO<sup>®</sup>, is designed to mimic the natural interfaces of blood. The aim of this study is to compare the influence of a phosphorylcholine-coated CPB system versus an uncoated CPB system on complement activation and clinical outcomes.

**Methods.** In this prospective, randomized, blind, one center study, 28 neonates and infants with a bodyweight between 3 and 6 kg who were undergoing cardiopulmonary bypass were divided in two groups, the phosphorylcholine group and the control group. Thirteen patients were assigned to the phosphorylcholine group and 15 patients to the control group. Patients with Down syndrome, prematurity, cyanosis, or reoperation were excluded. Complement factor C3b/c, human neutrophil elastase (HNE), interleukin-6, and C-reactive protein were measured before, during, and after CPB. Duration of intensive care stay, ventilation time, highest body temperature, and inotropic medication were the clinical variables.

**Results.** No significant differences were found between the groups for complement factor C3b/c, HNE, interleukin-6, or C-reactive protein during and after CPB. No clinical differences were observed between the groups.

**Conclusions.** Phosphorylcholine coating does not attenuate the complement activation during CPB in neonates and infants.

## **Introduction**

Seghaye and colleagues reported that the fullterm neonate shows significant complement activation and leukocytes stimulation when undergoing cardiopulmonary bypass (CPB) [1]. Complement activation and leukocytes stimulation may result in postoperative organ dysfunction. Contact of blood with the nonbiologic surfaces of the CPB system has been designated as the main cause of complement activation. Improving the biocompatibility of CPB systems by means of less contact activation of blood elements and thereby less inflammatory response is evidently desirable [2].

A phosphorylcholine coating, PHISIO<sup>®</sup> (Dideco, Mirandola, Italy), is designed to mimic natural cellular surfaces and thereby to avoid recognition by the blood as foreign material. The outer cell surface is composed of predominantly phosphorylcholine polar groups that largely contribute to the nonthrombogenic properties exhibited by blood cells. Because of the hydrophilic character of the polar head group, there is less protein adsorption [3]. Furthermore, phosphorylcholine is a regular component of the outer cell membrane of all human cells [4]. In this prospective, randomized, blind, one-center study, we aimed to compare the effects of phosphorylcholine coating versus noncoating of the CPB systems on complement activation, its effect on leukocytes stimulation, and clinical outcomes in neonates and infants with a bodyweight between 3 and 6 kg. For this purpose, we measured C3b/c, a stable complement activation product, human neutrophil elastase (HNE), as a marker of neutrophils degranulation [1], the cytokine interleukin-6 (IL-6), which is a good predictor of clinical outcome [5], and C-reactive protein (CRP), an acute phase protein, as a marker for inflammatory response. Clinical variables were intensive care unit stay, ventilation time, the highest body temperature in the first 24 postoperative hours, and use of inotropic medication.

## **Material and Methods**

### *Patients*

Twenty-eight patients with a bodyweight between 3 and 6 kg who were undergoing surgical repair of their congenital heart defects with the use of CPB and moderate (28°C) hypothermia were included in this prospective, randomized, blind study. The Medical and

Ethical Committee of our institution approved the study in January 2002. Written informed consent from parents or guardians was obtained for all patients.

Patients were randomly assigned into the phosphorylcholine-coated group (PC group; n=13) or into the noncoated control group (NC group; n=15). Patients with Down syndrome, other syndromes or chromosomal abnormalities, prematurity, cyanosis (defined as oxygen saturation lower than 75%), use of circulatory arrest, and cardiac reoperation were excluded from this study. Postoperatively, inotropic support was used if necessary. No steroids, aprotinin, and other medication that might affect the inflammatory response were used throughout the study.

### *Anesthesia*

All patients were premedicated with oral midazolam (0.5 mg/kg). Anesthesia was induced with sevoflurane and continued with midazolam (0.10 mg · kg<sup>-1</sup> · h<sup>-1</sup>), sufentanil (0.04 ug · kg<sup>-1</sup> · min<sup>-1</sup>), and pancuronium (0.15 mg/kg).

### *Cardiopulmonary Bypass*

Before aortic cannulation, heparin (300 IU/kg) was given, and the activated clotting time was maintained above 480 seconds during CPB. For all patients, a Dideco Lilliput D901 (Dideco, Mirandola, Italy) closed system was used, with the difference of the phosphorylcholine coating. The coating was from arterial canula to venous canula, from “tip to tip.” A Stockert roller pump (Stockert Instrumente GMBH, Munich, Germany) was used, inducing a nonpulsatile flow. The priming solution of 270 mL consisted of 100 mL fresh frozen plasma, 50 mL 20%, w/v, human albumin (Sanquin, Amsterdam, Netherlands), Mannitol (0.5 g/kg), Ringers’ solution, and packed red blood cells if necessary to maintain a hematocrit of 25%. The CPB flow was maintained at 2.4 L · m<sup>-2</sup> · min<sup>-1</sup> at 37<sup>0</sup>C and 1.6 L · m<sup>-2</sup> · min<sup>-1</sup> at 25<sup>0</sup>C nasopharyngeal. The alpha-stat method was used for blood gas management. For myocardial protection, St Thomas cardioplegia solution was given and repeated every 30 minutes. Ultrafiltration, conventional or modified, was not used throughout the study.

### *Samples*

Samples of whole blood (2 mL) were collected in tubes containing 10 mmol/L ethylenediamine tetraacetic acid, 10 mmol/L benzamidine, and 100 mg/mL soy bean trypsin inhibitor, final concentrations. Sample moments were after induction of anesthesia but before sternotomy (baseline), 10 minutes after start of CPB, 5 minutes before end of CPB, and 15 minutes and 6 hours after protamine administration. Samples were centrifuged (3,000 rpm, 10 minutes) and stored at  $-70^{\circ}\text{C}$  until analysis.

### *Laboratory Measurements*

Plasma levels of activated C3 were measured with enzyme-linked immunosorbent assay (ELISA) in which specific monoclonal antibody against a neoepitope on activated C3 was used to catch the activation fragments, and biotinylated polyclonal sheep antibodies against C3 to detect bound complement fragments [6]. As the assay does not discriminate between C3b, C3bi, or C3c, the activation products detected in the assay are further referred to as C3b/c. Results were expressed as nmol/L C3b/c, referring to an in-house standard with known levels of activation products. Elastase- $\alpha$ 1-antitrypsin complexes were measured with an ELISA in which antielastase antibodies were coated onto an ELISA plate, and bound complexes detected with biotinylated monoclonal antibody against complexed- $\alpha$ 1-antitrypsin. Purified human neutrophilic elastase added to pooled plasma was used as a standard [6]. Results were expressed as ng/mL elastase (HNE). Interleukin 6 and CRP were measured with sandwich-type ELISAs (CLB; Department Immune Reagents, Amsterdam, Netherlands).

### *Collection of Clinical Data*

Data on intensive care unit stay, ventilation time, highest body temperature in the first 24 hours, and use of inotropic medication were collected in a retrograde fashion.

### *Statistical Analyses*

Statistical analyses were performed using the statistical computing package SPSS12.01 (SPSS, Chicago, Illinois). Patients' data were compared with unpaired t test. Data

corresponding to cytokines values were not normally distributed. After logarithmic transformation of the raw data, we used repeated measures analysis of variance with the Greenhouse-Geisser correction to test for differences within the groups throughout the five time periods and between the two groups. Clinical data were also not normally distributed and were analyzed with the Mann-Whitney U test. Raw data are expressed as mean  $\pm$  SD values. All *p* values less than 0.05 were considered statistically significant.

## Results

There was no difference between the two groups in patients' age ( $p=0.80$ ) bodyweight ( $p=0.97$ ), and diagnoses. The CPB time ( $p=0.60$ ) and aortic cross clamp time ( $p=0.94$ ) showed no differences between the groups (Table 1). Four patients in the PC group and 3 patients in the NC group were preoperatively treated with prostaglandin E1 (PGE1). No deaths or major complications occurred in either group.

Table 1. Demographic and CPB Data

	PC group		NC group		<i>p</i> value
	mean	SD	mean	SD	
Number of patients	13		15		
<b>Demographic data</b>					
Age (days) <sup>a</sup>	79.4	84.6	87.5	80.4	0.80
Body weight (kg) <sup>a</sup>	4.2	0.98	4.2	0.96	0.97
<b>Diagnosis</b>					
AVSD	0		3		
VSD	5		2		
TAPVC	1		2		
Truncus arteriosus	0		1		
TGA	5		5		
TOF	0		1		
DORV	1		1		
ASD	1		0		
<b>CPB data</b>					
CPB time (min) <sup>a</sup>	117.5	48.6	108.6	38.0	0.60
Clamp time (min) <sup>a</sup>	68.9	32.1	69.7	26.3	0.94

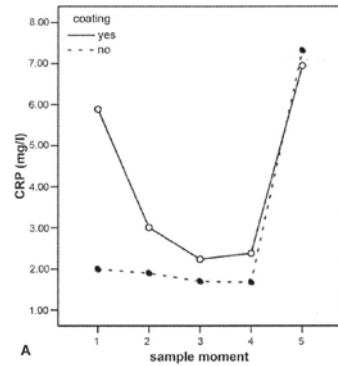
<sup>a</sup> Values expressed as mean ASD = atrial septal defect; AVSD = atrioventricular septum defect; CPB = cardiopulmonary bypass; DORV = double outlet right ventricle; min = minutes; NC = noncoated control group; PC = phosphorylcholine-coated group; *p* value = student *t* test; TAPVC = totally abnormal pulmonary connection; TGA = transposition of the great arteries; TOF = tetralogy of Fallot; VSD = ventricular septal defect.

There was a significant rise in complement factor C3b/c for both groups, but there was no significant difference between the groups. The C3b/c increased rapidly in both groups after the start of CPB and reached the highest point after the protamine administration. Six hours after CPB, complement factor C3b/c values had almost returned to baseline in both groups (Table 2). The HNE peaked at the end of the CPB procedure. The values decreased rapidly and had almost returned to baseline after 6 hours. Also, for HNE, there was a significant time difference in the groups, but over time, both groups showed equal behavior (Table 2). Interleukin-6 started to increase slightly during CPB and rose rapidly after the CPB procedure and protamine administration. After 6 hours, IL-6 was still increased (Table 2).

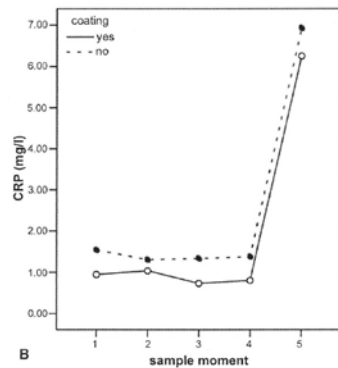
Table 2 laboratory measurement results

	group	t=1	<i>p</i> value	t=2	<i>p</i> value	t=3	<i>p</i> value	t=4	<i>p</i> value	t=5	<i>p</i> value
Complement factor C3b/c (nmol/L) (SD)	PC	49.1 (36.28)	0.15	94.08(42.10)	0.82	184.62(57.94)	0.26	269.46(126.02)	0.46	80.31(52.79)	0.12
	NC	32.47(12.12)		93.07(43.63)		164.47(64.70)		234.40(98.32)		52.47(32.40)	
Human neutrophil elastase (ng/mL) (SD)	PC	33.23(23.80)	0.58	29.23(12.95)	0.76	150.15(91.46)	0.48	130.62(69.42)	0.53	96.23(70.59)	0.09
	NC	43.86(79.03)		37.60(54.95)		137.20(109.50)		124.07(101.82)		62.73(25.87)	
Interleukin-6 (pg/mL) (SD)	PC	5.91 (2.49)	0.56	6.07 (3.16)	0.77	15.85 (14.56)	0.16	32.95 (24.09)	0.36	247.84(230.43)	0.48
	NC	5.42 (1.14)		6.16 (2.46)		25.67 (19.66)		40.29 (21.75)		231.71(117.56)	
C-Reactive protein (mg/L) (SD)	PC	0.95 (1.58)	0.22	1.04 (0.96)	0.25	0.74 (0.57)	0.36	0.81 (0.82)	0.26	6.25 (2.69)	0.78
	NC	1.54 (4.21)		1.31 (3.01)		1.34 (2.97)		1.38 (3.29)		6.39 (4.06)	

The mean baseline of CRP of the PC group is higher ( $5.8 \pm 8.8$  mg/L versus  $2.0 \pm 4.1$  mg/L) compared with that of the control group. When the patients who received preoperative PGE1 were left out, no difference was observed anymore between the groups at baseline point ( $0.9 \pm 1.5$  mg/L versus  $1.5 \pm 4.2$  mg/L). The CRP level did not increase during the CPB procedure but rapidly increased after the protamine administration. No significant differences were found between the groups (Fig 1A and B; Table 2).



A



B

Fig 1. (A) Values of C-reactive protein (CRP), with patients who used preoperative prostaglandin E1 included for statistical analysis. (Solid line = with phosphorylcholine coating; dashed line = no coating.) (B) Values of C-reactive protein (CRP), with patients who used preoperative prostaglandin E1 excluded from statistical analysis.

(Solid line = with phosphorylcholine coating; dashed line = no coating.)

The mean length of stay in the pediatric intensive care unit was not different between the two groups. Also ventilation time, body temperature, and inotropic dosage were not significantly different in both groups (Table 3).

## Discussion

The present study was performed to compare the effects of CPB on the inflammatory response and clinical outcomes of a phosphorylcholine-coated CPB system versus an uncoated CPB system in pediatric patients with a bodyweight of 3 to 6 kg. We were not able to show any differences between the two groups. Yu and colleagues showed an improved biocompatibility during an in vitro study with reduced protein adsorption and complement activation [3]. The results during in vivo studies are controversial. De Somer and colleagues showed lower complement activation and a better preservation of platelets

in a study with 10 patients [7]. Böning and coworkers demonstrated that phosphorylcholine coating or heparin coating of the CPB system used for pediatric surgery causes the same biologic effects [8]. They also demonstrated that the group with a larger prime volume shows higher values of IL-6 and higher tumor necrosis factor- $\alpha$  [8]. Horten and coworkers were not able to show any differences between a heparin-coated system and an uncoated system with a study population of 200 patients [9]. Interestingly, Tárnok and colleagues demonstrated that children undergoing major cardiovascular surgery without CPB show almost the same complement activation as the children undergoing major cardiovascular surgery with the use of CPB [10]. These findings are also reported in adults [11, 12]. The inflammatory response after CPB is not only initiated by the artificial surface of the CPB system, although it is seen by many as being the main cause [13]. The response is also triggered by the gas-blood interfaces, ischemia-reperfusion injury, and other proinflammatory stimuli [10, 11]. The duration of the CPB procedure and the condition of the patient play roles in the extent of the inflammation after CPB [14]. It is also known that suction and retransfusion of mediastinal shed blood contribute to the inflammatory response [15]. In our study, suction and retransfusion was performed equally in both groups. The pediatric population is a widely spread population regarding to the differences in age, bodyweight, diagnoses, and syndromes. To achieve a homogeneous group of patients for this study, we used strong exclusion criteria.

Radical oxygen species (ROS) activates nuclear factor- $\kappa$ B (NF- $\kappa$ B), which is an important protein in the regulation of the acute phase response of inflammation. Nuclear factor- $\kappa$ B stimulates the production of, among others, IL-1, IL-6, and tumor necrosis factor- $\alpha$  [16]. Down syndrome is a genetic disorder associated with ROS, and patients with Down syndrome show a wide range of defects regarding either specific or nonspecific immunity [17, 18]. Body and blood values of cyanotic patients are severely affected by the chronic hypoxia, and uncontrolled reoxygenation is associated with ROS [19]. Circulatory arrest with deep hypothermia gives a higher postoperative leukocyte count, probably due to a higher  $\beta$ -adrenergic stimulation [20]. Complement levels correlate with gestational age, particularly in the late prenatal period. Preterm infants have lower complement activity and complement component levels than full-term infants

[21]. Also, neutrophil counts are lower in preterm infants [22]. Patients indicated for reoperations were excluded because of the expected longer surgery time.

In conclusion, phosphorylcholine coating does not attenuate the complement activation during CPB, nor does it have an influence on clinical outcomes. As mentioned above, the inflammation after CPB is not only triggered by the artificial surface but is dependent upon many factors. Coating alone will not affect the inflammation as desired. Other agents should be considered for this purpose, such as ultrafiltration during or after CPB and the use of corticosteroids and protease inhibitors.

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