Evidence for Development of Capillary Leak Syndrome Associated with Cardiopulmonary Bypass in Pediatric Patients with the Homozygous C4A Null Phenotype

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Background: The mechanism of postoperative capillary leak syndrome related to cardiopulmonary bypass (CPB) is unknown. The authors hypothesized that C4 gene polymorphism might be involved in the development of the syndrome because complement activation is associated with CPB and protamine administration, and the two isotypes of C4 (C4A and C4B) differ in their biochemical and functional properties after activation.

Methods: One hundred fifty-six pediatric patients referred for elective cardiac surgery with CPB were included in the study. C4 isotype studies were performed in plasma samples obtained before surgery, with use of agarose gel immunofixation and crossed immunoelectrophoresis. Five possible C4 phenotype groups were observed, which were abbreviated as follows: (1) AABB = no detectable null alleles, (2) A0BB = a single null allele (heterozygous) at the C4A locus, (3) 00BB = a homozygous C4A null allele, (4) AAB0 = a single null allele (heterozygous) at the C4B locus, and (5) AA00 = a homozygous C4B null allele. The patients were classified into five groups according to their C4 phenotypes. Before CPB and at 1 h after CPB, plasma protein was measured with a biuret test kit. Plasma colloid osmotic pressure was determined with a membrane osmometer. Evans blue dye was used to measure plasma volume, serum protein, intravenous protein pool, and transvascular escape rate of Evans blue dye.

Results: Of 156 pediatric patients enrolled, 80 were assigned to the AABB group, 28 were assigned to the A0BB group, 7 were assigned to the 00BB group, 31 were assigned to the AAB0 group, and 10 were assigned to the AA00 group, according to their C4 phenotypes. At 1 h after CPB, serum protein concentrations averaged 3.6 ± 0.4 g/dl in patients with the 00BB C4 phenotype; this value was significantly lower ($P < 0.01$) than that in patients with other C4 phenotypes. The changes of intravenous protein pool and colloid osmotic pressure were comparable with the change in serum protein concentration. At 1 h after CPB, the transvascular escape rate of Evans blue dye averaged 11.5 ± 1.3%/h in patients with the 00BB C4 phenotype; this value was significantly higher ($P < 0.01$) than that in patients with other C4 phenotypes.

Conclusions: In this study, capillary leak syndrome induced by CPB occurred only in patients with the homozygous C4A null phenotype.

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CAPILLARY leak syndrome is defined as a shift of fluid and protein from the intravascular to the interstitial space. This syndrome is characterized by generalized edema formation and ascites caused by an increase in microvascular permeability to plasma proteins (PPs), which is related to inflammatory cytokines and the activation of the complement system.[1] Cardiopulmonary bypass (CPB) produces inflammation.[2,3] The inflammation induced by CPB is a well-described systemic inflammatory response syndrome (SIRS).[4] The release of a variety of inflammatory mediators has been implicated in the pathogenesis of SIRS during CPB: tumor necrosis factor α and interleukins 1, 6, and 8.[5] Activated complement plays a crucial role in the activation of neutrophils, causing subsequent damage, particularly to the endothelium. During CPB, the activated complement system interacts with receptors on the neutrophils and induces leukocyte chemotaxis, autoaggregation, increased adherence, and superoxide generation.[6] Despite a well-described inflammatory reaction induced by CPB, the existence of capillary leak syndrome related to CPB is controversial. Capillary leak syndrome has been suggested by Seghaye et al.[7] and Plotz et al.[8] in neonates undergoing CPB. In contrast, Tassani et al.[9] did not observe capillary leak syndrome after CPB in elective, uncomplicated coronary artery bypass grafting operations.

The fourth component of human complement plays an important role not only in the classic pathway of complement activation, but also in the clearance of the activating complexes. The human C4 gene is coded at two different loci in the major histocompatibility complex on chromosome 6, and the major products of these loci are designated C4A and C4B.[10] The two isotypes of C4, C4A and C4B, differ in their binding characteristics after activation. Activated C4A binds more effectively to immune complexes and plays an important role in their clearance, whereas activated C4B has more affinity for erythrocyte membranes.[11,12] The different immune activities of activated C4A and C4B might result in different degrees of immunologic damage: One activated isotype might cause capillary leak syndrome, and another might not.

Neonates or children with normally facilitated fluid movements across the capillary membrane and with rapid fluid shift from the intravascular into the interstitial space in case of fluid overload[13] are at particularly high risk for the development of generalized edema as a result of microvascular protein leakage during CPB. Younger age has been recognized among risk factors for an in-

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crease in total body water accumulation after cardiac operations.[14]

Therefore, the aim of our study was to analyze C4 phenotypes and the disappearance of labeled PP before and after CPB in pediatric patients to demonstrate the relation of C4 phenotypes with capillary leak syndrome.

Materials and Methods

Patients

After approval was obtained from the Ethics Committee of Union Hospital (Wuhan, China), 156 pediatric patients (aged 2–10 yr) scheduled to undergo elective cardiac surgery with CPB to repair ventricular or atrial septal defects were included in this study. Patients with hepatic or renal dysfunction and those who had received steroids in the previous 2 weeks were excluded. Written informed consent was obtained from the parents of each child before the study.

C4 Phenotypes

C4A and C4B are coded at two different loci. The presence of a null allele (*i.e.*, only a single copy of the active gene at either site) can be detected as a reduction of approximately 50% in that particular protein.[12] Therefore, null alleles (a single allele or double alleles) of C4A or C4B can be confirmed by means of protein detection. C4 isotype studies were performed in plasma samples obtained before the operation, with use of agarose gel immunofixation and crossed immunoelectrophoresis according to the techniques described by Shastri *et al.*[12] Null alleles were inferred by the reduction in the density of the corresponding C4A or C4B band. With the combinations of C4A and C4B null alleles, five possible C4 phenotype groups were observed, which were abbreviated as follows: (1) AABB = no detectable null alleles, (2) A0BB = a single null allele (heterozygous) at the C4A locus, (3) 00BB = a homozygous C4A null allele, (4) AAB0 = a single null allele (heterozygous) at the C4B locus, and (5) AA00 = a homozygous C4B null allele.[12] A and B refer to any expressed allele of the respective isotype, and 0 represents a null allele of C4A or C4B. Pediatric patients were assigned to one of these five groups according to their C4 phenotypes.

Anesthesia

Patients were premedicated with diazepam (0.2 mg/kg) and scopolamine (0.15 mg/kg) injected intramuscularly. A 22-gauge radical artery catheter, a central venous catheter (B. Braun Melsungen AG, Destruir, Germany) through the right internal jugular vein, and two intravenous catheters were placed after intramuscular injection of ketamine (8 mg/kg). Then, anesthesia was induced with fentanyl (20 μg/kg) and vecuronium (0.1 mg/kg). After tracheal intubation, mechanical ventilation with

100% oxygen was provided. Tidal volume was adjusted to achieve normoventilation and was controlled by means of blood gas analysis to maintain normal concentrations of arterial carbon dioxide. Anesthesia was maintained with inhalation of 1–1.5% isoflurane until the onset of CPB. During CPB, a bolus of diazepam (0.2 mg/kg) was given in 30-min intervals. A bolus injection of vecuronium (0.05 mg/kg) was used to maintain muscle relaxation. A preoperative volume load with hydroxyethyl starch was used to avoid a decrease in arterial blood pressure caused by induction of anesthesia. Before separation from CPB, dopamine (5 μg · kg^{-1} · min^{-1}) was infused. After termination of CPB, the infusion rate was set according to the child's circulatory state. A mean arterial pressure of 60 mmHg or greater was the target.

CPB Technique

The circuit of the CPB comprised a roller pump (Sarns 9000; Sarns/3M, Ann Arbor, MI) and a membrane oxygenator. The circuit was primed with lactated Ringer's solution, albumin, mannitol, and leukocyte-depleted packed erythrocytes. Anticoagulation was accomplished by means of intravenous administration of heparin sulfate (300 U/kg), which was neutralized with protamine sulfate at the end of CPB. A nonpulsatile flow of 150 ml · kg^{-1} · min^{-1} was maintained throughout CPB. All patients were cooled to moderate hypothermia ranging from 30° to 33°C (nasopharyngeal). Cardiac arrest was accomplished by means of aortic cross clamping coupled with infusion of high-potassium (20 mEq/l) blood cardioplegic solution (20 ml/kg) through the aortic root. Blood gas management during CPB was directed toward maintenance of pH at 7.35–7.40 and arterial carbon dioxide tension (Paco$_2$) at 35–40 mmHg. Arterial oxygen tension (Pao$_2$) was maintained higher than 200 mmHg. Blood gas management was conducted according to the principle of alpha-stat. Hemofiltration of the perfusate with a hemoconcentrator with or without supplemental transfusion of leukocyte-depleted packed erythrocytes was performed to keep the hematocrit greater than 25%. Pediatric patients were rewarmed to 36°C before weaning from bypass. After CPB, protamine (heparin and protamine in a ratio of approximately 1:1.5) was injected for approximately 7 min through a peripheral vein to neutralize heparin until the preoperative activated clotting time was achieved. Mean blood pressure (MBP) and central venous blood pressure (CVP) were recorded before CPB and at 1 h after CPB, respectively.

Escape of Plasma Albumin, Plasma Volume, and Intravascular Protein Pool

Plasma protein concentrations were measured with a biuret test kit (Jianwei Co., Nanjing, China). Plasma colloid osmotic pressure was determined with a membrane osmometer (Unico Co., Shanghai, China). The transvas-

cular escape rates (TERs) of PP from the intravascular compartment before CPB and 1 h after CPB were assessed by means of measuring the disappearance of intravenously injected Evans blue dye. The method has been described in detail.[15] Evans blue dye is safe to use in infants and children.[16] Briefly, blood samples were taken before the injection of Evans blue dye to determine the blank absorbance of plasma (seven samples within 0.5 h). After weaning from CPB, Evans blue dye (0.2 mg/kg) was intravenously injected. Blood samples were obtained every 10 min (at 10, 20, 30, 40, 50, and 60 min) as described by Tassani *et al.*[9] The absorbance of plasma was determined in a spectrophotometer (Unico Co.) at λ values of 620 and 740 nm. Linear regression was calculated from the absorbance of undyed plasma (A) at these two wavelengths:

$$A_{620} = a + b \times A_{740} \tag{1}$$

The absorbance of dyed plasma at 620 nm (Evans blue dye [EB_{620}]) was corrected for blank absorbance calculated from equation 1 and the following:

$$EB_{620corr} = EB_{620} - (a + b \times EB_{740}) \tag{2}$$

Specific protein dyeing (sEB) was calculated from equation 2 and the PP concentration:

$$sEB = EB_{620corr}/PP \tag{3}$$

The decay of sEB with time was fitted as follows,

$$sEB_t = sEB_0 \times e^{-k \times t} \tag{4}$$

using the following transformation,

$$\ln(sEB_t) = [\ln(sEB_0)] - k \times t \tag{5}$$

with sEB_0 indicating theoretic sEB at the time of dye injection and immediate complete mixing with plasma volume (PV), sEB_t indicating sEB at any time t, and k indicating the disappearance rate constant. sEB_0 and k were obtained by calculating the linear regression from the sampling times t and the corresponding sEB_t values.

The TER was calculated as follows:

$$TER = (1 - e^{-k \times 60}) \times 100[\%/h] \tag{6}$$

Plasma volume was calculated from the injected dose of EB (EB_i) and EB_0,

$$PV = EB_i/EB_0 \tag{7}$$

with EB_0 indicating $sEB_0 \times PV$ (see equation 3).

The intravascular protein pool (IVP) was calculated from the PP concentration and the PV as follows:

$$IVP = PP \times PV \tag{8}$$

Statistical Analysis

The data are presented as mean $\pm$ SD. Intergroup and intragroup values were compared by means of two-way analysis of variance to analyze the combined effect of time and group. The Student-Newman-Keuls comparison test was used to evaluate any differences in the results.

Results

C4 Phenotypes

Of the 156 pediatric patients included in the study, 80 had no null alleles (AABB; 51.28%), 28 had one C4A null allele (A0BB; 17.95%), 7 had the homozygous C4A null phenotype (00BB; 4.49%), 31 had one C4B null allele (AAB0; 19.87%), and 10 had the homozygous C4B null phenotype (AA00; 6.41%). According to their C4 phenotypes, 80 children were assigned to the AABB group, 28 were assigned to the A0BB group, 7 were assigned to the 00BB group, 31 were assigned to the AAB0 group, and 10 were assigned to the AA00 group.

Surgical Data

The preoperative and perioperative data of 156 pediatric patients with different C4 phenotypes are summarized in table 1. Except for 2 children in the 00BB group, the other patients were weaned from CPB without problems. Two children in the 00BB group had a heavy decrease in arterial blood pressure and decreased lung compliance after protamine administration. They were given CPB support immediately. After 15 min of CPB, they were weaned from CPB uneventfully. All pediatric patients had an uneventful postoperative recovery. No child required mechanical ventilation for more than 24 h. No patient died. Age, sex, left ventricular ejection fraction, ventricular septal defect number, atrial septal defect number, aortic cross clamp time, minimal nasopharyngeal temperature, dopamine dosage at 1 h after CPB, diuresis dosage at 1 h after CPB, and volume of fresh frozen plasma at 1 h after CPB were not significantly different in patients among the five groups. The duration of CPB in patients of the 00BB group was significantly longer than that of the other four groups because the two patients who needed repeated CPB belonged to this group.

PP-related Data

Capillary leak syndrome was defined as the increased TER of Evans blue dye from plasma. At 1 h after CPB, every patient with the 00BB phenotype had an increased TER value compared with the values before CPB. PV was similar in these patients before and at 1 h after CPB. Decreased MBP, serum protein (SP), IVP, and plasma colloid osmotic pressure (COP) and increased CVP were observed in these patients at 1 h after CPB compared with the values before CPB. MAP, CVP, PV, SP, IVP, TER, and COP values of every patient in the 00BB group before and at 1 h after CPB are shown in table 2.

Before CPB, MBP, CVP, PV, SP, IVP, TER, and COP values were similar in patients with different C4 pheno-

Table 1. Preoperative and Perioperative Data

Variable	AABB (n = 80)	A0BB (n = 28)	00BB (n = 7)	AAB0 (n = 31)	AA00 (n = 10)
Age, yr	5 (2–10)	6 (2–9)	5 (3–8)	6 (2–10)	5 (2–7)
Sex (M/F)	52/28	20/8	5/2	23/8	5/5
LVEF, %	58 ± 6	54 ± 5	57 ± 6	60 ± 8	54 ± 4
Weight, kg	18 ± 7	18 ± 6	19 ± 7	18 ± 6	17 ± 5
No. of VSDs	61	20	5	20	6
No. of ASDs	19	8	2	11	4
CPB duration, min	58 ± 10	60 ± 9	72 ± 13*	55 ± 11	54 ± 8
Cross clamping duration, min	21 ± 8	22 ± 7	23 ± 5	20 ± 8	22 ± 7
Minimal nasopharyngeal temperature, °C	32 ± 2	33 ± 1	32 ± 2	33 ± 1	32 ± 1
Dopamine at 1 h after CPB, $\mu g \cdot kg^{-1} \cdot min^{-1}$	5 ± 2	5 ± 2	8 ± 4	5 ± 2	5 ± 2
Diuresis dosage at 1 h after CPB, $ml \cdot kg^{-1} \cdot h^{-1}$	7 ± 2	7 ± 3	8 ± 3	7 ± 2	7 ± 2
FFP transfusion at 1 h after CPB, ml/kg	5 ± 2	5 ± 2	5 ± 3	5 ± 2	5 ± 2

Two patients in the 00BB group needed repeated cardiopulmonary bypass (CPB). Values are presented as n, mean ± SD, or median (range).

* $P < 0.01$ *vs.* the other four groups.

ASD = atrial septal defect; EF = ejection fraction; FFP = fresh frozen plasma; LV = left ventricle; VSD = ventricular septal defect.

types (table 3). MBP and CVP values at 1 h after CPB were similar in patients with different C4 phenotypes. At 1 h after CPB, in all patients, CVP was significantly higher and MBP was significantly lower than before CPB. A slightly decreased PV at 1 h after CPB was detected, with no statistical significance ($P > 0.05$), compared with PV before CPB in all groups.

At 1 h after CPB, SP decreased significantly ($P < 0.05$) compared with that before CPB in patients with the AABB, A0BB, AAB0, and AA00 C4 phenotypes (table 3). At 1 h after CPB, SP averaged 3.6 ± 0.4 g/dl in patients with the 00BB C4 phenotype; the value was significantly lower ($P < 0.01$) than that in patients with other C4 phenotypes.

The changes in IVP and COP were comparable with the change in SP (table 3).

At 1 h after CPB, TER increased slightly ($P > 0.05$) compared with that before CPB in patients with the AABB, A0BB, AAB0, and AA00 C4 phenotypes (table 3). At 1 h after CPB, TER averaged 11.5 ± 1.3%/h in patients with the 00BB C4 phenotype; the value was significantly higher ($P < 0.01$) than that in patients with other C4 phenotypes.

Discussion

Capillary leak syndrome is defined as the increased TER of Evans blue dye from plasma. According to this definition, the current study shows that CPB-associated capillary leakage develops in every pediatric patient with the homozygous C4A null phenotype (00BB group). Al-

Table 2. Data of Seven Pediatric Patients in 00BB Group before CPB and at 1 h after CPB

Variable	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
MBP, mmHg							
Before CPB	62	60	61	67	65	62	61
1 h after CPB	58	54	51	50	65	56	63
CVP, cm H_2O							
Before CPB	7	8	6	6	7	7	6
1 h after CPB	10	13	9	10	10	12	11
PV, ml/kg							
Before CPB	56	54	55	57	60	55	61
1 h after CPB	57	50	51	66	52	53	55
SP, g/dl							
Before CPB	6.7	6.6	6.5	6.8	6.7	6.6	6.6
1 h after CPB	3.1	3.9	4.1	3.8	3.0	3.5	4.0
IVP, g/kg							
Before CPB	3.7	3.6	3.6	3.9	4.0	3.6	4.0
1 h after CPB	1.8	2.0	2.1	2.5	1.6	1.9	2.2
TER, %/h							
Before CPB	7.7	7.8	7.7	7.2	7.5	7.4	7.8
1 h after CPB	9.9	10.5	11.6	11.9	13.2	13.0	10.2
COP, mmHg							
Before CPB	23.4	22.6	22.4	23.7	23.6	22.0	23.9
1 h after CPB	19.6	18.9	18.8	19.1	18.7	18.6	18.9

COP = plasma colloid osmotic pressure; CPB = cardiopulmonary bypass; CVP = central venous pressure; IVP = intravascular protein pool; MBP = mean blood pressure; PV = plasma volume; SP = serum protein; TER = transvascular escape rate of Evans blue dye from plasma.

Table 3. Data of Pediatric Patients in Five Groups before CPB and at 1 h after CPB

Variable	AABB (n = 80)	A0BB (n = 28)	00BB (n = 7)	AAB0 (n = 31)	AA00 (n = 10)
MBP, mmHg					
Before CPB	61.6 ± 5.5	62.8 ± 3.1	62.6 ± 2.5	62.5 ± 2.9	62.6 ± 3.1
1 h after CPB	58.9 ± 6.7*	59.2 ± 5.9*	56.7 ± 5.7*	59.0 ± 6.2*	60.2 ± 7.1
CVP, cm H_2O					
Before CPB	6.8 ± 1.2	6.7 ± 1.1	6.7 ± 0.8	7.0 ± 2.1	6.9 ± 1.3
1 h after CPB	10.4 ± 1.5†	10.5 ± 1.2†	10.7 ± 1.4†	10.4 ± 1.7†	10.0 ± 1.6†
PV, ml/kg					
Before CPB	56.5 ± 3.0	57.0 ± 2.8	56.9 ± 2.7	56.4 ± 2.6	56.7 ± 2.9
1 h after CPB	55.8 ± 5.9	57.1 ± 5.0	54.9 ± 5.5	54.5 ± 5.0	56.1 ± 3.8
SP, g/dl					
Before CPB	6.6 ± 0.2	6.7 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.6 ± 0.1
1 h after CPB	6.2 ± 0.6*	6.3 ± 0.7*	3.6 ± 0.4†‡	6.3 ± 0.7*	6.3 ± 0.4*
IVP, g/kg					
Before CPB	3.8 ± 0.3	3.8 ± 0.2	3.8 ± 0.2	3.8 ± 0.3	3.8 ± 0.2
1 h after CPB	3.6 ± 0.8*	3.5 ± 0.6*	2.0 ± 0.3†‡	3.5 ± 0.7*	3.5 ± 0.6*
TER, %/h					
Before CPB	7.7 ± 0.2	7.6 ± 0.3	7.6 ± 0.2	7.6 ± 0.2	7.6 ± 0.2
1 h after CPB	7.9 ± 1.5	7.8 ± 1.3	11.5 ± 1.3†‡	7.8 ± 1.7	7.7 ± 1.5
COP, mmHg					
Before CPB	23.3 ± 0.8	23.1 ± 0.8	23.1 ± 0.7	23.2 ± 0.6	23.0 ± 0.7
1 h after CPB	23.0 ± 0.8*	22.7 ± 0.9*	18.9 ± 0.3†‡	22.8 ± 0.7*	22.6 ± 0.4*

Values are presented as mean ± SD.

* $P < 0.05$.   † $P < 0.01$ *vs.* pre-CPB values.   ‡ $P < 0.01$ *vs.* values of the other four groups.

COP = plasma colloid osmotic pressure; CPB = cardiopulmonary bypass; CVP = central venous pressure; IVP = intravascular protein pool; MBP = mean blood pressure; PV = plasma volume; SP = serum protein; TER = transvascular escape rate of Evans blue dye from plasma.

though CPB caused PP-related values such as SP, IVP, and COP to change markedly in patients with the AABB, A0BB, AAB0, and AA00 C4 phenotypes, we cannot conclude that capillary leak syndrome developed in any patient in these four groups because their TERs of Evans blue dye from plasma did not increase significantly.[9]

This study also shows a higher frequency of the homozygous C4A null phenotype in Chinese patients than in white patients. Awdeh and Alper,[10] in their study of 100 randomly selected white patients, found 1 subject (1%) with the homozygous C4A null phenotype. Shastri et al.,[12] in their study of 79 patients (they did not state whether the patients were white or not), found no subject with the homozygous C4A null phenotype. Their not finding any patient with the homozygous C4A null phenotype might be because of the small number of patients included in their study. In the current study of 156 Chinese children, the number was large enough to include all C4 phenotypes, and we found 7 patients (4.49%) with the homozygous C4A null phenotype. Our finding shows that a relatively higher incidence of adverse reactions to CPB may occur in Chinese patients than in white patients. Therefore, detection of C4 phenotype before surgery can warn us of the occurrence of serious adverse reactions to CPB in some patients. Because the frequency of the homozygous C4A null phenotype is low and capillary leak syndrome develops after CPB only in patients with the homozygous C4A null phenotype, capillary leak syndrome may not be detected because of a lack of patients with the homozygous C4A null phenotype if a small number of patients is selected.

The failure of Tassani et al.[9] to observe capillary leak syndrome after CPB in their study might be because of the small number of patients (only 16) included. According to the previous studies,[10,12] it is almost impossible to include a patient with the homozygous C4A null phenotype in such a small number of patients. In contrast, Seghaye et al.[7] found that capillary leak syndrome developed in 13 of 24 neonates. There might be two reasons for their findings. One reason might be that neonates with the homozygous C4A null phenotype were included in their study. Another reason might be the different criteria they used to define capillary leak syndrome. They defined the occurrence of capillary leak syndrome from a decrease in PP concentration soon after the onset of CPB.[7] PP concentration, however, reflects not only the intravascular protein pool, but also changes in plasma water, which may be increased after institution of CPB, depending on the priming volume of the extracorporeal circuit, the type of cardioplegia, and subsequent fluid substitution.[9]

Cardiopulmonary bypass is associated with a wide variety of adverse physiologic and immunologic effects. Ischemia–reperfusion, endotoxemia, and activation of monocytes by artificial surfaces are the predominant mechanisms that trigger SIRS.[17,18] SIRS commonly causes capillary leak syndrome. However, according to the investigation by Tassani et al.,[9] the duration of SIRS caused by CPB is too short for capillary syndrome to develop. Tassani et al. did not consider the effect of C4 genetic polymorphism on the development of capillary leak syndrome, for SIRS might be serious enough to

induce capillary leak syndrome in patients with the homozygous C4A null phenotype. Our results confirmed the effect of C4 genetic polymorphism on the development of capillary leak syndrome. After patients are exposed to short CPB, capillary leak syndrome is likely to develop in patients with the homozygous C4A null phenotype but not in those with other phenotypes. We can conclude from our findings that the primary cause of capillary leak syndrome is the C4A null gene; CPB is just one of its inducing factors. CPB did not induce development of the syndrome in patients with other homozygous C4 phenotypes in this study. If CPB were the primary cause, it would induce development of the syndrome in all patients, regardless of their C4 phenotypes.

Capillary leak syndrome is an overall result of inflammation. Numerous investigations have been performed to measure inflammatory mediators released as a result of CPB.[19-21] However, no inflammatory mediator alone is substantially responsible for the development of capillary leak syndrome. For this reason, we did not measure any inflammatory mediator in the current study.

One possible mechanism involved in C4 genetic polymorphism and capillary leak syndrome lies in reduced clearance of heparin–protamine complexes.[12] Heparin–protamine complexes can be regarded as immune complexes,[22] and they activate complement by means of the classic pathway.[23] Among the inflammatory cascades, activated complements of the complement system contribute to all phases of the inflammatory response.[24] Animal studies have shown clearance of heparin–protamine complexes in the liver.[25] The two isotypes of C4, C4A and C4B, differ in their binding characteristics after activation. Activated C4A binds more effectively to immune complexes and plays an important role in their clearance, whereas activated C4B has more affinity for erythrocyte membranes.[11] Patients with the homozygous C4A null phenotype lack the C4A isotype of C4. Without the C4A subcomponent, less activated C4 may be bound to the heparin–protamine complexes. Thus, their clearance is reduced. This reduced clearance may allow the heparin–protamine complexes to stay in circulation, thereby activating more complement and generating more C4a and C3a.[12] This paradox of increased binding of activated complements has been shown to occur with some immune complexes.[26] We observed that two patients in the 00BB group needed repeated CPB because of a heavy decrease in arterial blood pressure and decreased lung compliance after protamine administration in this study. The finding shows that protamine itself or heparin–protamine complex may be another inducing factor of capillary leak syndrome.

Activated complement of the complement system contributes to all phases of the inflammatory response.[24] Although C3a and C4a are useful markers of *in vivo* complement activation, the mediation of inflammation occurs mainly by C5a, a complement split product generated further along the complement cascade.[24,27] C5a rapidly binds to receptors on neutrophils and monocytes, especially polymorphonuclear neutrophils.[28] Polymorphonuclear neutrophils are the key cells in SIRS. When activated, these cells release various inflammatory mediators, such as eicosanoids, proteolytic enzymes, oxygen radicals, and cytokines. The reactivity of polymorphonuclear neutrophils is modulated by diverse cytokines. It has been shown that proinflammatory and antiinflammatory cytokines are released during CPB.[19-21]

From this study, we conclude that capillary leak syndrome induced by CPB or heparin–protamine complex occurs only in patients with the homozygous C4A null phenotype. It implies that interindividual variation in the incidence of adverse effects of CPB exists in patients undergoing open heart surgery. Detection of C4 phenotypes before surgery will warn of the occurrence of serious systemic inflammatory response and subsequent development of capillary leak syndrome secondary to CPB in some patients.

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