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The Activity of Protein C in Infants Undergoing Cardiopulmonary Bypass to Correct a Congenital Heart Defect – a Pilot Study*

Ocena aktywności białka C u niemowląt operowanych z powodu wrodzonej wady serca z użyciem krążenia pozaustrojowego – badania pilotażowe

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Abstract

Background. Protein C can influence postoperative outcome in infants undergoing correction of congenital heart disease.
Objectives. The aims of this study were to analyze the activity of protein C among infants undergoing cardiopulmonary bypass (CPB) and to investigate protein C activity in children with and without postoperative infection.
Material and Methods. Seventeen infants were operated on with the use of CPB in deep and moderate hypothermia. Blood samples were drawn before general anesthesia, 5 minutes after beginning CPB, 5 minutes before disconnection from CPB, 15 minutes after administering protamine, and on the second and third postoperative days. Protein C activity was measured with a Coag-Chrom 3003 coagulometer.
Results. There was decreased protein C activity 5 minutes after beginning CPB. The mean baseline activity was below the norm during CPB and gradually returned to the staring value at the end of the observation ($p < 0.0001$). Protein C activity in the pre-, peri-, and postoperative period was lower in the children with symptoms of infection, although the difference was not statistically significant between children with and without infection.
Conclusions. CPB exacerbates the preoperative deficit of protein C related with age. Protein C activity decreased during CPB. Recovery was observed in the third postoperative day. Infection in infants in the postoperative period had no impact on protein C activity (**Adv Clin Exp Med 2009, 18, 2, 163–168**).

Key words: activity, protein C, congenital heart defect, cardiopulmonary bypass, children.

Streszczenie

Wprowadzenie. Czynnikiem mającym wpływ na ciężkość przebiegu pooperacyjnego u niemowląt, poddanych korekcji kardiochirurgicznej z powodu wady wrodzonej serca w krążeniu pozaustrojowym, jest białko C.
Cel pracy. Analiza aktywności białka C podczas zabiegów kardiochirurgicznych w krążeniu pozaustrojowym, analiza przed- i okołooperacyjnych czynników ryzyka zakażenia i ich wpływ na zmiany w aktywności białka C oraz ocena profilów stężeń białka C u dzieci z objawami oraz bez objawów zakażenia uogólnionego.
Materiał i metody. Do badań włączono 17 niemowląt operowanych w krążeniu pozaustrojowym w Klinice Kardiochirurgii Dziecięcej w Poznaniu. Próbki krwi pobierano: bezpośrednio po znieczuleniu ogólnym pacjenta, 5 minut po podłączeniu i przed odłączeniem od krążenia pozaustrojowego, 15 min po podaniu protaminy, w 2. i 3. dobie po operacji. Aktywność białka C oznaczono z użyciem koagulometru Coag Chrom 3003.
Wyniki. Analiza aktywności białka C podczas zabiegów wykonywanych w krążeniu pozaustrojowym z powodu wrodzonej wady serca wskazuje na wyraźne zmniejszenie jego aktywności 5 minut po podłączeniu pacjenta do krążenia pozaustrojowego w stosunku do aktywności białka C przed operacją. Aktywność białka C podczas całego zabiegu utrzymywała się na niskim poziomie, stopniowo wzrastając, wróciła do wartości sprzed zabiegu w 3. do-

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bie po operacji ($p < 0,0001$). U pacjentów z zakażeniem uogólnionym obserwowano mniejsze wartości aktywności białka C w okresie przed-, około- i pooperacyjnym niż u pacjentów bez zakażenia. Nie stwierdzono jednak istotnych różnic statystycznych w obu populacjach.
Wnioski. Krążenie pozaustrojowe pogłębia przedoperacyjny niedobór białka C związany z wiekiem. Podczas zabiegów w krążeniu pozaustrojowym stwierdza się wyraźne zmniejszenie aktywności białka C i jej powrót do wartości sprzed zabiegu w 3. dobie po operacji. Infekcja w okresie pooperacyjnym nie ma wpływu na aktywność białka C (**Adv Clin Exp Med 2009, 18, 2, 163–168**).

Słowa kluczowe: aktywność, białko C, wada wrodzona serca, krążenie pozaustrojowe, dzieci.

Open-heart surgery carries the risk of various adverse effects, including the most threatening one, i.e. systemic inflammatory response syndrome (SIRS). The symptoms are similar to those of septicemia or sepsis and are caused by the direct contact of blood with the artificial structures of extracorporeal circulation devices. The surfaces of cannulae, oxygenator, and tubing cause the activation of complement, the coagulation cascade, and the release of cytokines, leukotrienes, and tromboxanes. All these can lead to post-perfusion syndrome refractory to conventional therapy [1].

Children operated for congenital heart defects (CHDs) are potentially more prone to all the negative effects of extracorporeal circulation than substantially larger patients because of the relationship of the device contact surface to the body surface area and the immaturity of the immunological system. The severity of the postoperative course can be a result of multiorgan dysfunction syndrome (MODS) related to post-perfusion syndrome [2]. Therefore the latter can hardly be distinguished from infection or sepsis in CHD patients burdened with the multiple risk factor of postoperative infection. Thus one of the main aims of postoperative treatment should be to limit the systemic inflammatory response [1].

Protein C is one of the factors that may influence the condition of patients with post-perfusion syndrome. Protein C is vitamin K-dependent serine protease enzyme produced in the liver that is activated by thrombin into activated protein C (APC). Protein C is a major physiological anticoagulant. The activated form with protein S as a cofactor degrades factor Va and factor VIIIa [3, 4]. The activity of protein C is depressed in patients with SIRS. This leads to symptoms of MODS connected with disseminated intravascular coagulation (DIC) and septic shock [2].

The aim of the study was to analyze the activity of protein C in infants during and after cardiac procedures performed with the use of cardiopulmonary bypass (CPB). The second aim was to verify whether there is any difference in protein C activity between children with complicated postoperative course (manifesting symptoms of infection) and children with uneventful postoperative course.

Material and Methods

Patients

The study population consisted of 17 infants, 9 girls (53%) and 8 boys (47%) (median age: 45.5, range: 4–355 days), who underwent correction of congenital heart disease in the Department of Pediatric Cardiac Surgery, Poznan University of Medical Sciences, between January 2006 and September 2007. Their body weight ranged from 2.2 to 7.5 kg (mean: 4.5 ± 1.5 kg), height from 0.4 to 0.74 m (mean: 0.58 ± 0.09 m), and body surface area from 0.15 to 0.39 m^2 (mean: 0.26 ± 0.07 m^2). CPB time was from 59 to 224 min (median: 91 min), aorta cross-clamping (ACC) time from 21 to 95 min (mean: 46.8 ± 21.9 min), and operation time from 100 to 395 min (mean: 227.4 ± 84.9 min). Minimal CPB temperature ranged from 15 to 28°C.

The following congenital heart defects were diagnosed in the study group: ventricular septal defect (VSD) in 8 patients, VSD with pulmonary stenosis (PS) in 2, common atrioventricular canal (CAVC) in 2, atrial septal defect (ASD) in 1, common arterial trunk (CAT) in 1, hypoplastic left heart syndrome (HLHS) in 1, interrupted aortic arch (IAA) in 1, and postoperative aneurysm of the right ventricle in 1. The Ethics Committee on Human Research approved the study protocol.

Surgical Technique

All operations were performed via a median sternotomy with the use of standard CPB with uncoated tubing. The CPB apparatus was primed with blood-containing fluids. Heparin at a dose of 3 mg/kg was administered to achieve an activated clotting time (ACT) of over 400 s and was neutralized by protamine sulfate (administered in a 1 : 1 ratio). Cold crystalloid cardioplegia (St. Thomas +4°C) was administrated into the root of the aorta at 30-minute intervals. After the surgical procedure all the patients were transferred to the intensive care unit and followed according to institutional standards.

Blood Tests

Blood samples (2 ml) were collected from a central vein catheter or the CPB circuit into a tube containing sodium citrate at the following time points: after general anesthesia (A), 5 minutes after beginning CPB (B), before weaning from CPB (C), 15 minutes after administering protamine sulfate (D), on the second postoperative day (E), and on the third postoperative day (F). All the blood samples was centrifuged (6 min. at 7000 rpm) and stored at –70°C. The activity of C protein was measured with reagents and a Coag-Chrom 3003 coagulometer (Bio-Ksel Ltd., Grudziądz, Poland).

Definitions

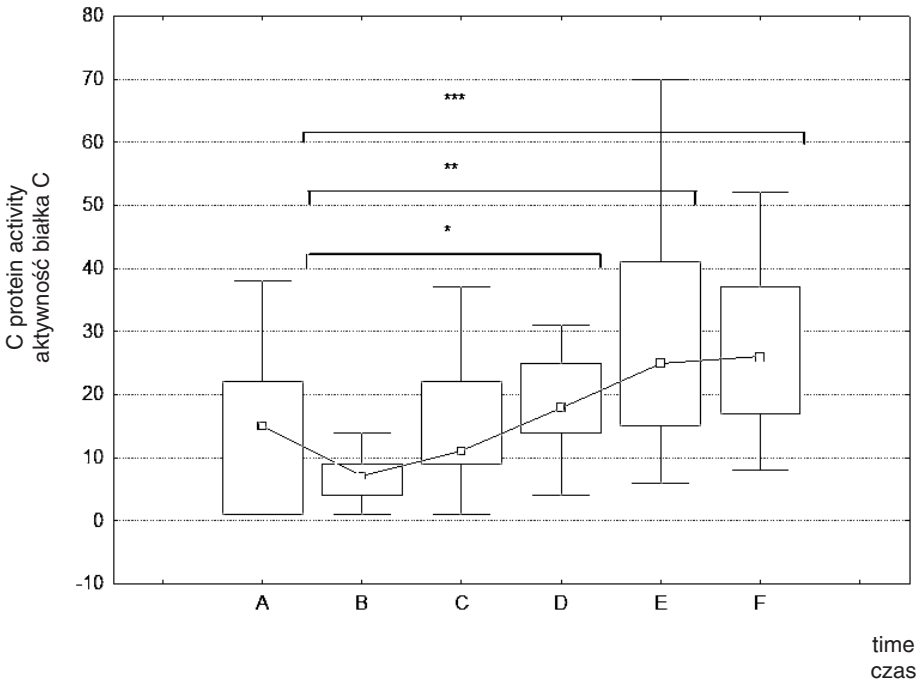
Increased C-reactive protein level (CRP) in the postoperative period and one of the following symptoms: increased white blood count (WBC), decreased platelet count (PLT), tachypnea, body temperature above 38 C, or positive blood culture, were regarded as infection. The normal ranges of the parameters in this population of children below one year of age were protein C activity > 70%, WBC 5–17 × 10^3/mm^3, PLT 220–520 × 10^3/mm^3, and CRP 0–0.5 mg/l.

Statistic Analysis

The statistical analysis was performed using GraphPad Instat 3 and STATISTICA 6.0. The normality of distributions was assessed by the Shapiro-Wilk test. Mean and standard deviation or median and range describe the variables accordingly. All variables in the figures are shown as medians and quartiles. The patients were divided into groups of children younger and older 45.5 days (the median of age of the population) and children with and without symptoms of infection. Intergroup comparisons were performed using Student's *t* tests or the Mann-Whitney test (according to the variable distribution). Multiple repeated intra-group comparisons were performed with the Friedman test with the post-hoc Dunn's test. A *p*-value less than 0.05 was considered significant in all tests.

Results

Mean protein C activity was statistically different at all time points ($p < 0.0001$). A decrease was observed after the initiation of CPB (between time points A and B), although it was not significant. Protein C activity subsequently began to rise. Post-hoc comparisons showed significant increases between time point B and the time points D ($p < 0.01$), E ($p < 0.001$), and F ($p < 0.001$) (Fig. 1).



*B vs. D ($p < 0.01$).
**B vs. E ($p < 0.001$).
***B vs. F ($p < 0.001$).

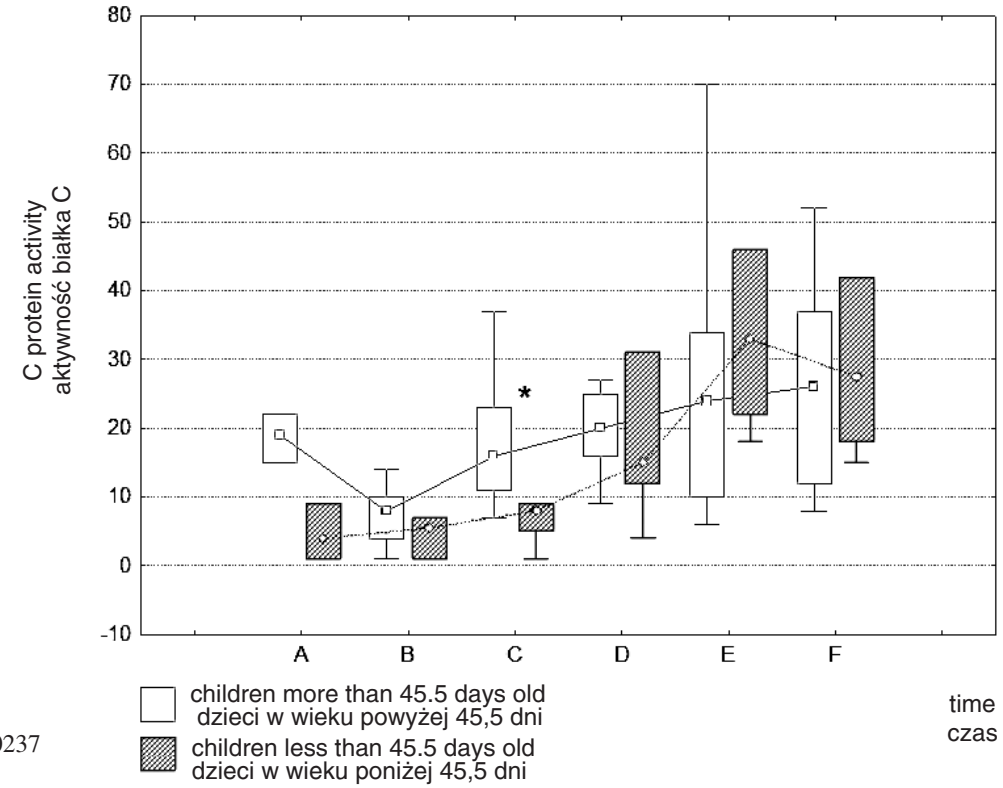
*B *vs* D ($p < 0,01$).
**B *vs* E ($p < 0,001$).
***B *vs* F ($p < 0,001$).

Fig. 1. Mean protein C activity over time

Ryc. 1. Średnie wartości aktywności białka C w czasie

Time points in Fig. 1–3: A – after general anesthesia, B – 5 minutes after beginning CPB, C – before weaning from CPB, D – 15 minutes after administering protamine sulfate, E – on the second postoperative day, F – on the third postoperative day.

Punkty czasowe na ryc. 1–3: A – bezpośrednio po znieczuleniu ogólnym pacjenta, B – 5 minut po podłączeniu do krążenia pozaustrojowego, C – przed odłączeniem od krążenia pozaustrojowego, D – 15 min po podaniu siarczanu protaminy, E – w 2. dobie po operacji, F – w 3. dobie po operacji.



* $p = 0.0237$

Fig 2. Median age and statistical differences between protein C activity at the time points in children less than and more than 45.5 days

Ryc. 2. Mediana wieku oraz różnice statystyczne między aktywnościami białka C w poszczególnych punktach czasowych wśród dzieci w wieku poniżej oraz powyżej 45 dni

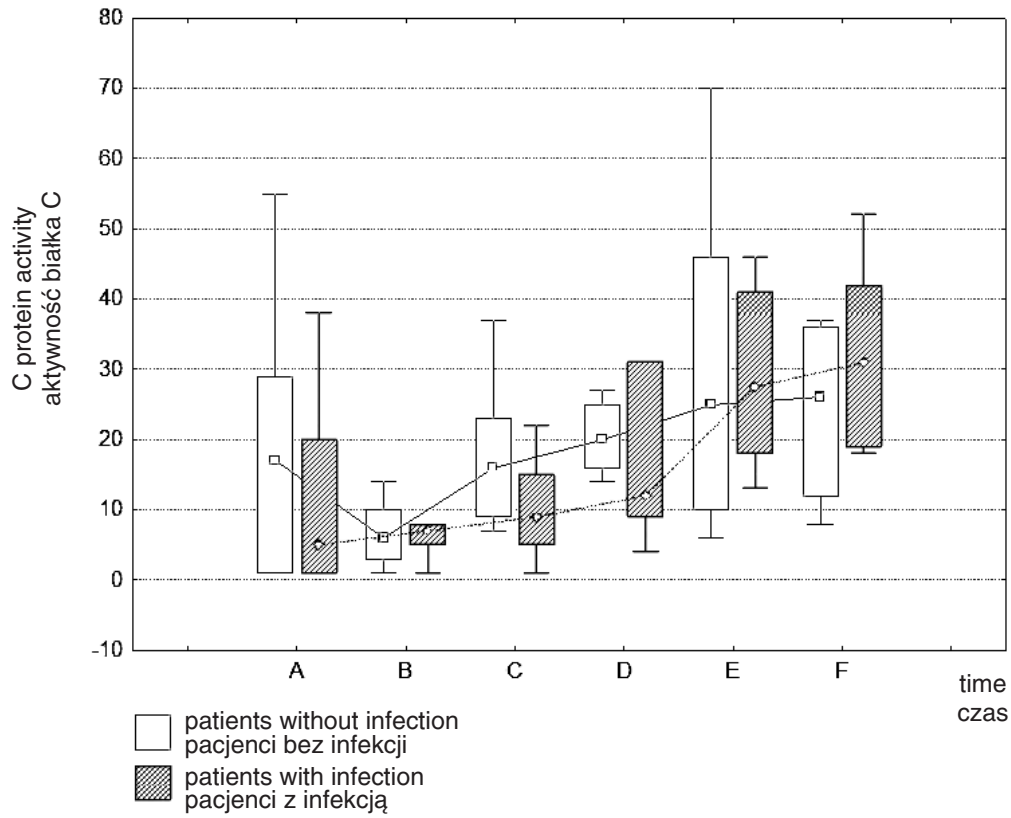


Fig. 3. Mean protein C activity in patients with and without infection

Ryc. 3. Średnia aktywność białka C u dzieci z i bez zakażenia uogólnionego

The mean activity of protein C among the children over 45.5 days old was higher than in those less than 45.5 days old. However, statistically significant differences were found only at time point C (Fig. 2).

In the patients with symptoms of postoperative infection, the mean activities of protein C at time points A, C, and D were lower than in the patients without infection, without reaching statistical significance. No relevant intergroup differences were observed at time points E and F. However, at these time points the mean activity of protein C was higher in the patients with infection (Fig. 3).

Discussion

Normal ranges of preoperative protein C activity were observed in just one patient (6%), the oldest individual in the study group (age: 255 days), who underwent a correction of VSD. The literature data indicate that protein C concentration and activity below the normal range might be observed in neonates suffering from congenital heart defects with congestive heart failure. This can be related to diminished liver perfusion and consequential liver insufficiency [3]. The immaturity of the coagulation system in neonates and infants might also be a cause [4].

The analysis of protein C activity in the presented cohort revealed decreased protein C activity 5 minutes after beginning CPB. Petaja and coworkers observed the same phenomenon [5]. This cannot be attributed to the administration of heparin during CPB since this drug does not influence the metabolism of protein C [6–8]. However, it can be related to protein C activation by CPB-damaged endothelium during CPB [9]. Free and endothelial forms of thrombomodulin appear to play an important role in protein C activation under conditions of increased thrombin activity during CPB [9]. Finally, the settling of protein C on CPB tubing walls [10] and hemodilution may concomitantly explain the decrease in protein C activity [5, 11].

The activity of protein C returned to the preoperative levels three days after surgery in 13 patients (76.5%). The time required for the restoration of protein C activity to preoperative levels is associated with congenital heart defect, the performance of the left ventricle before and after the operation [3], the intensity of coagulation system activation by CPB [5], and the use of hypothermia [9]. Boldt studied the influence of hypothermia in patients undergoing cardiac operations on changes in the coagulation system. Protein C concentrations were significantly lower in hypothermic than in normothermic patients and also remained decreased in the period after bypass [9]. Petaja observed that CPB in neonates caused rapid and profound alterations in the coagulation systems and initiated consumptive coagulopathy lasting at least three days after surgery [5]. This is in accord with the present results.

The mean activity of protein C at time point C was significantly higher among the children over 45.5 days old. These findings might be explained by the immaturity of the hemostatic system in the neonates compared with infants and older children [5] and the major capability in infants to restore protein C level in response to a decreased level after beginning CPB.

Protein C activity in the pre-, peri-, and postoperative period was not statistically significantly different in the children with and without symptoms of infection in the postoperative period. It is documented that the activity of protein C in patients with infection is significantly lower than in those without infection [12, 13]. A low activity of protein C is usually correlated with a high risk of infection. Thus supplementation with recombinant human protein C (rhAPC) in both active and inactive forms among high-risk neonates and infants undergoing CPB could potentially alleviate its chronic and acute deficiencies. However, there are still insufficient data to support the use of rhAPC in the management of severe infection in infants [12–14].

The small number of patients and the nonhomogeneous population constitute study limitations. The study failed to reveal differences between protein C activity in patients with and without infection. Further assessment of the activity of protein C during the postoperative period in children with congenital heart disease in CPB is warranted.

The authors concluded that CPB exacerbates the preoperative deficit of protein C related with age. Protein C activity decreased during CPB and recovered on the third postoperative day. Infection in infants in the postoperative period had no impact on protein C activity.

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