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Mannose-binding lectin (MBL) insufficiency protects against the development of systemic inflammatory response after pediatric cardiac surgery

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ABSTRACT

We investigated *MBL2* and *MASP2* genotypes, serum MBL (mannose-binding lectin) levels and activities of its complexes with associated serine proteases (MASP-1, MASP -2), in relation to complications following cardiac surgery in 195 children. The incidence of SIRS was lower in patients carrying *MBL2* A/O and O/O genotypes ($p = 0.024$). Children with MBL levels <500 ng/ml had a lower risk of SIRS ($p = 0.014$) and fever ($p = 0.044$). Median MBL concentration was higher in patients who developed SIRS ($p = 0.048$) but lower in those with post-operative infections ($p = 0.046$). MBL-MASP-2 activities <100 mU/ml protected from SIRS ($p = 0.007$), low cardiac output syndrome ($p = 0.03$) and multiorgan failure ($p = 0.012$). In contrast, *MBL2* YA/YA genotypes were associated with SIRS ($p = 0.018$), low cardiac output syndrome ($p = 0.018$), fever ($p = 0.018$) and high inotropic score (VIS >30) ($p = 0.021$). Thus, low MBL concentrations and associated genotypes may protect patients from systemic inflammation while high MBL serum levels and corresponding genotypes are risk factors of postoperative complications.

1. Introduction

Recently, there has been significant progress in the treatment of congenital heart disease involving surgical and anesthetic techniques, methods of myocardial protection, blood processing, and antimicrobial prophylaxis. However, life-threatening postoperative complications, which prolong intensive care unit stay and therefore increase costs, are not uncommon (Greco et al., 2015). This problem concerns patients with postoperative hospital-acquired infections (Algra et al., 2012), low cardiac output syndrome (LCOS) and multiple organ failure which can be a consequence of both aforementioned factors. An important and often forgotten consideration is an immature or dysfunctional immune system which may cause susceptibility to infections or generate excessive systemic inflammatory response to cardiopulmonary bypass (CPB). Increasing evidence suggests that polymorphisms of genes encoding different components of the immune system influence an individual's capacity to respond to various stimuli, such as infection or extracorporeal circulation (Luo et al., 2014; Hou et al., 2010).

Complement activation is considered to be one of the main mechanisms of innate immunity involved in the response to CPB (Kozik and Tweddell, 2006; Seghaye, 2003). There are three crucial moments for complement activation by CPB: first, during contact of heparinized blood with the membranes of the circuit; second, after reperfusion of the ischemic myocardium; and third, after the production of heparin–protamin complexes. Previous studies have confirmed activation through both classical (CP) and alternative (AP) pathways. CPB membranes and myocardial reperfusion activate both CP and AP, whereas protamin–heparin complexes, endotoxins, C-reactive protein and apoptotic cells activate complement *via* the classical pathway. The last decade has also brought evidence for lectin pathway (LP) activation by CPB (Marcheix et al.,

Abbreviations: BAS, basic aristotle score; CPB, cardiopulmonary bypass; LCOS, low cardiac output syndrome; MOF, multiorgan failure; VIS, vasoactive inotropic score.

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2008). Complement activation results in anaphylatoxin (C3a, C5a) production leading to the activation, degranulation and adhesion of neutrophils, activation and degranulation of basophils and mast cells with histamine release and platelet aggregation.

An exaggerated response to contact with the CPB circuit can lead to the development of systemic inflammatory response syndrome (SIRS) and, in consequence, to multiorgan dysfunction (MODS). Younger and smaller patients are more susceptible (Seghaye, 2003). Experience from clinical studies on adults suggests that complement inhibition may be beneficial in cases of excessive activation (Smith et al., 2011). At the same time, supplementation with complement components is a promising supportive therapy for patients who are susceptible to infections because of their deficiencies (Keizer et al., 2014). In particular, there is an emerging interest in mannose-binding lectin (MBL) due to its putative role in cardiovascular disease and postoperative complications.

MBL has been extensively reviewed elsewhere (Turner, 1996; Kilpatrick, 2002; Pągowska-Klimek and Cedzyński, 2014). In brief, it recognizes carbohydrate (and other) structures through its C-type lectin domain and this leads to conformational and functional changes *via* its collagen-like domain including the activation of MBL-associated serine proteases (MASPs) and thus the complement system. Circulating plasma levels of MBL are determined genetically: three mutations in the structural gene (B, C, and D; collectively designated "O" as opposed to the normal "A") and two polymorphisms in the promoter region (H/L and X/Y) are influential (Madsen et al., 1995). Generally, individuals with the YA/YA and YA/XA genotypes have high serum MBL, while O homozygotes or compound XA/O heterozygotes have extremely low concentrations. Individuals who are XA/XA or YA/O heterozygotes have very variable but generally middling serum MBL. However, it should be stressed that there is a considerable overlap in serum levels between the different genotypes and, at the same time, a relatively wide range of concentrations among persons carrying the same genotype (Swierzko et al., 2009). MBL deficiency is considered the commonest congenital immunodeficiency. A variety of cut-off levels has been used to define deficiency or insufficiency (between undetectable protein to 500 ng/ml or higher) (Turner, 1991). A concentration of 500 ng/ml has been described as the minimum required for opsonophagocytosis (Neth et al., 2002) and Eisen et al. (2008) have argued that this cut-off is the most suitable for defining serum MBL deficiency. Certainly, 500 ng/ml is an appropriate primary cut-off when the consequences of both low and high concentrations are being investigated and therefore we have chosen that level for this study.

The involvement of MBL and complement lectin pathway activation in the pathogenesis of cardiovascular disease and ischemia-reperfusion injury in general is well documented (Pągowska-Klimek and Cedzyński, 2014). However, this topic has not been addressed in the pediatric population, in which innate immunity, including the lectin pathway of complement activation, is particularly important as adaptive immunity is immature. Surprisingly, we know little about the incidence of immune disorders among children with congenital heart disease and about the role of lectin pathway activation in the development of complications after pediatric cardiac surgery. The aim of our study was to investigate whether the pre-operative concentrations or activities of MBL and/or the patients' genotypes (*MBL2*, *MASP2*) are associated with post-operative complications.

2. Material and methods

2.1. Patients

In total, 195 children, aged 3 months to 17 years (mean: 3 years and 4 months; 80 girls/115 boys) with congenital heart disease on whom cardiac surgery with the use of cardiopulmonary bypass was performed, were recruited to this prospective study. Mean basic Aristotle score (BAS) was 7.59 (SD: 2.06; median: 8), while mean hospital length of stay was 14.4 ± 12.1 days. Mean CPB time was 85.9 min ($\pm$42.40), mean aorta cross-clamping time was 42.9 min ($\pm$27.24). The study was approved by the local ethics committee. The written informed consent of the parents of the patients was obtained.

The procedures performed were: atrial septal defect repair (16 cases), atrioventricular septal defect repair (10 cases), bidirectional cavopulmonary anastomosis (bidirectional Glenn) (26 cases), pulmonary atresia correction with homograft (11 cases), hybrid approach stage II procedure (3 cases), double outlet of right ventricle–intraventricular repair (2 cases), Rastelli procedure (3 cases), Ross procedure (8 cases), definitive correction of tetralogy of Fallot (19 cases), ventricular septal defect repair (25 cases), mitral valvuloplasty (5 cases), valve replacement (6 cases), and Fontan operation-external conduit (50 patients).

The postoperative course was observed and documented until hospital discharge. Patients were screened for symptoms of infection, respiratory failure, sepsis (according to consensus definition 2005) (Goldstein et al., 2005), non-infectious systemic inflammatory response (SIRS) (Goldstein et al., 2005), low cardiac output syndrome (LCOS, according to criteria proposed by Hoffman et al. (2002)), and renal insufficiency requiring renal replacement therapy. Hepatic dysfunction was defined as prothrombin time at least 2× normal and ALT > 100 IU/l. The maximum VIS (vasoactive inotropic score) level over the first 72 h was recorded (Wernovsky et al., 1995; Gaies et al., 2010). Symptoms of non-infectious postoperative SIRS were observed during the first 3 postoperative days; tachycardia due to anxiety, increased doses of inotropic agents and tachyarythmias were not included. Only patients with no evidence of precocious infection were included; the final decision whether a patient qualified for the "non-infectious" group was made after assembling all laboratory tests, supported by clinical evaluation or post mortem diagnosis. Multiorgan dysfunction (MODS) was diagnosed when dysfunction of at least two organs was observed. ICU length of stay (ICULOS) and total hospitalization time were recorded.

Exclusion criteria were: age less than 3 months, preoperative mechanical ventilation, preoperative infection or organ dysfunction, and death during surgery.

2.2. Genotyping

Blood samples for DNA preparation were taken into tubes containing sodium citrate and stored at −20 °C. DNA was extracted from blood samples with the use of Gene MATRIX quick blood purification kit (EURx Ltd., Poland), according to the manufacturer's protocol.

Single nucleotide polymorphisms in the promoter region, at positions −550 (H/L) and −221 (Y/X) were investigated with the use of allele-specific PCR (Bak-Romaniszyn et al., 2011) while those located in codons 52 (A/D), 54 (A/B) and 57 (A/C) of exon 1 of the *MBL2* gene were investigated with the use of PCR-RFLP procedures (Cedzynski et al., 2004). The XA/O and O/O genotypes were considered to constitute MBL deficiency while YA/YA genotypes were presumed to confer high MBL serum concentration/activity. The missense mutation within the *MASP2* gene, c.359 A > G, leading to the exchange of aspartic acid for glycine residue 120 (D120G) in the MASP-2 protein (and in consequence to the total functional deficiency of complement lectin pathway in a homozygous state) was determined by the PCR-RFLP method as reported by Swierzko et al. (2009).