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Genetic polymorphism in postoperative sepsis after open heart surgery in infants

Dicky Fakhri[1], Samsuridjal Djauzi[2], Tri Wahyu Murni[3], Jusuf Rachmat[4], Alida Roswita Harahap[5], Sri Endah Rahayuningsih[6], Muchtaruddin Mansyur[7] and Anwar Santoso[8]

Abstract

Background: Sepsis is one of the complications following open heart surgery. Toll-like receptor 2 and toll-interacting protein polymorphism influence the immune response after open heart surgery. This study aimed to assess the genetic distribution of toll-like receptor 2 N199N and toll-interacting protein rs5743867 polymorphism in the development of postoperative sepsis.

Methods: A prospective cohort study was conducted in 108 children <1-year old who underwent open heart surgery with a Basic Aristotle score ⩾6. Patients with an accompanying congenital anomaly, human immunodeficiency virus infection, or history of previous open heart surgery were excluded. The patients' nutritional status and genetic polymorphism were assessed prior to surgery. The results of genetic polymorphism were obtained through genotyping. Patients' ages on the day of surgery and cardiopulmonary bypass times were recorded. The diagnosis of sepsis was established according to Surviving Sepsis Campaign criteria.

Results: Postoperative sepsis was observed in 21% of patients. There were 92.6% patients with toll-like receptor 2 N199N polymorphism and 52.8% with toll-interacting protein rs5743867 polymorphism.

Conclusions: Toll-like receptor 2 N199N polymorphism tends to increase the risk of sepsis (odds ratio = 1.974; 95% confidence interval: 0.23–16.92; $p = 0.504$), while toll-interacting protein rs5743867 polymorphism tends to decrease the risk of sepsis (odds ratio = 0.496; 95% confidence interval: 0.19–1.27; $p = 0.139$) in infants <1-year old undergoing complex open heart surgery.

Keywords

Genetic predisposition to disease, Immune system, Infant, Sepsis, Toll-like receptor 2, TOLLIP protein, human

Introduction

Sepsis is one of the complications following open heart surgery in children.[1,2] In our previous study on children younger than one year of age who underwent open heart surgery, the incidence of postoperative sepsis was as high as 35%. Several factors are known to influence the immune response after open heart surgery,

[1]Pediatric and Congenital Heart Surgery Unit, Department of Surgery, National Cardiovascular Center Harapan Kita, Jakarta, Indonesia
[2]Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[3]Cardiothoracic Surgery Division, Department of Surgery, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[4]Thoracic and Cardiovascular Surgery Division, Department of Surgery, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[5]Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[6]Pediatric Cardiology Division, Department of Pediatrics, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[7]Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[8]Research and Development Division, National Cardiovascular Center Harapan Kita, Jakarta, Indonesia

Corresponding author:
Dicky Fakhri, Pediatric and Congenital Heart Surgery Unit, Department of Surgery, National Cardiovascular Center Harapan Kita, Jl. Let. Jend. S. Parman Kav. 87, Jakarta, Indonesia.
Email: difawi@yahoo.com

such as cardiopulmonary bypass (CPB) time, poor nutritional status, and younger age at the time of operation.[1,3,4] In our survey of older age groups with lower surgical complexity in the National Cardiac Center Harapan Kita, Indonesia, one of the most populous developing countries, we found a sepsis rate of 15%. Further, according to our internal database, the overall mortality rate in patients undergoing congenital heart surgery was 8.1% in 2014 (60 deaths in 742 cardiac procedures). Children younger than one year of age are more vulnerable to infection because their immune systems are still immature and mainly depend on innate immunity.[5,6] Toll-like receptor (TLR) 2 mediates the activation of innate immunity.[7] In serving its function within the immune system, the TLR 2 pathway is regulated by toll-interacting protein (TOLLIP).[8] Single nucleotide polymorphism has been identified in the TLR 2 gene and TOLLIP gene.[9,10] Currently, information regarding the role of single nucleotide polymorphism in the development of sepsis after open heart surgery in children is limited. Therefore, this study aimed to assess the role of TLR 2 polymorphism N199N and TOLLIP rs5743867 in the development of postoperative sepsis in children younger than one year of age who underwent open heart surgery.

Patients and methods

We carried out a prospective cohort study on consecutive patients younger than one year of age scheduled for open heart surgery with a Basic Aristotle Score $\geqslant 6$ at the National Cardiovascular Center Harapan Kita, Jakarta from May 2014 until January 2015. The study was approved by the ethics committee of the National Cardiac Center, Harapan Kita Hospital, Jakarta, and the Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo Hospital. Informed consent was obtained from the parents of all patients. Patients with an accompanying congenital anomaly, HIV, or history of previous open heart surgery were excluded. A total of 120 patients younger than one year of age were recruited, 12 dropped out of the study (4 need extracorporeal membrane oxygenation, 6 died before postoperative day 3, one was found to have thymus aplasia, and utilization of CPB was cancelled in one); thus 108 patients were included in the analysis. The demographic characteristics of these 108 patients are summarized in Table 1; only 6.5% were neonates.

Before surgery, the patients' nutritional status was assessed according to body weight/body length Z-score. A Z-score $\leqslant -3$ was classified as severely malnourished, while a Z-score > -3 was classified as not severely malnourished. Body weight was measured using a calibrated electronic scale (Laica; Italy), and body length was measured with a length board (Seca; Seca GMbH, Germany). The patient's age on the day of the operation was also recorded; those aged $\leqslant 28$ days were classified as neonates.

Table 1. Demographic characteristics of 108 infants undergoing cardiac surgery.

Variable	No. of patients
Male	54 (50%)
Female	54 (50%)
Mean age (days) [range]	209 [4–360]
Age 28 days to <1 year	101 (93.5%)
Neonates	7 (6.5%)
Not severely malnourished	46 (42.6%)
Severely malnourished	62 (57.4%)
Cardiopulmonary bypass time (min) [range]	85 [33–265]
Cardiopulmonary bypass <90 min	62 (57.4%)
Cardiopulmonary bypass $\geqslant$90 min	46 (42.6%)

We obtained preoperative blood samples to check for genetic polymorphism. Venous blood samples (3 mL) were collected and placed in ethylenediaminetetraacetic acid. DNA was extracted using a High Pure Polymerase Chain Reaction Template Kit (Roche, Indianapolis, USA) according to the manufacturer's instructions. DNA was then examined by a real-time polymerase chain reaction using an ABI 7500 machine (Applied Biosystem, Waltham, USA). Reagents and probes for each accession number involved in the real-time polymerase chain reaction were TaqMan GTXpress (Applied Biosystem, Waltham, USA). The C/C genotype for the TLR 2 N199N gene was defined as wild type, while the C/T genotype was defined as a heterozygous mutant, and the T/T genotype was considered a homozygous mutant. The A/A genotype for the TOLLIP gene was defined as wild type, while the A/G genotype was defined as a heterozygous mutant, and the G/G genotype was considered a homozygous mutant.

All patients underwent open heart surgery under general anesthesia according to the protocol of the National Cardiovascular Center Harapan Kita. CPB was instituted using a non-coated circuit with a hard-shell microporous membrane oxygenator (Terumo Baby RX; Terumo, Japan). CPB time was recorded by the timer in the CPB machine. The surgical procedures are summarized in Table 2. Ventricular septal defect repair was the surgical procedure performed in patients with highest Basic Aristotle score. After surgery, the patients were monitored for signs of sepsis. Diagnosis of sepsis was based on the sepsis criteria of

Table 2. Surgical procedures in 108 infants.

Procedure	No. of patients
Ventricular septal defect patch repair	45 (41.7%)
Complete AVSD repair	4 (3.7%)
TAPVC repair	12 (11.1%)
TOF repair (no ventriculotomy)	5 (4.6%)
TOF repair (ventriculotomy/transannular patch)	4 (3.7%)
Tricuspid valvuloplasty	2 (1.9%)
Pulmonary artery reconstruction (main trunk)	1 (0.9%)
Pulmonary artery reconstruction*	1 (0.9%)
Aortic valvuloplasty	1 (0.9%)
Aortic stenosis (subvalvar repair)	1 (0.9%)
Valvuloplasty, mitral	2 (1.9%)
Arterial switch	9 (8.3%)
Arterial switch + ventricular septal defect repair	1 (0.9%)
Rastelli	4 (3.7%)
Coarctation repair (end-to-end)	1 (0.9%)
Aortic arch repair	1 (0.9%)
Systemic-to-pulmonary shunt + atrial septectomy	4 (3.7%)
Pulmonary artery banding + atrial septectomy	1 (0.9%)
Bidirectional cavopulmonary anastomosis	9 (8.3%)

*Peripheral branch at or beyond the hilar bifurcation. AVSD: atrioventricular septal defect; TAPVC: total anomalous pulmonary venous connection; TOF: tetralogy of Fallot.

Table 3. Genotype distributions in 108 infants.

Genotype	No. of patients
Toll-like receptor 2 N199N (rs3804099)	
• Wild type (allele C/C)	8 (7.4%)
• Heterozygous mutant (allele C/T)	36 (33.3%)
• Homozygous mutant (allele T/T)	64 (59.3%)
Toll-interacting protein polymorphism (rs5743867)	
• Wild type (allele A/A)	51 (47.2%)
• Heterozygous mutant (allele A/G)	47 (43.5%)
• Homozygous mutant (allele G/G)	10 (9.3%)

the Surviving Sepsis Campaign.[11] Cytokine balance after CPB is normally achieved on postoperative day 3.[12] Therefore, the incidence of sepsis was considered only from the third postoperative day onwards. Hemodynamic monitoring was carried out every 24 h in the first 3 days and every 8 h from day 3 onwards.

Our cardiac center combines and utilizes 3 guidelines for prevention and control of infection: World Health Organization,[13] Centers for Disease Control and Prevention (available at: http://www.cdc.gov/hicpac/pubs.html#a1), and the Indonesian Ministry of Health. We maintain environmental temperature, humidity, and air pressure in our cardiac operating rooms as required by Decree of the Indonesian Minister of Health no. 1204/MENKES/SK/X/2004. For the surgical aseptic and antisepsis solution, we utilized 2.0% chlorhexidine gluconate and 0.5% chlorhexidine gluconate +70% ethanol for the first and second washes of the surgical area. Other measures of infection control and prevention, such as hand decontamination, personal hygiene, clothing, masks, gloves, safe injection practices, and sterilization, were performed according to the guidelines and monitored by the hospital's infection control and prevention committee.

Statistical analysis were performed using SPSS version 20 software package (SPSS, Inc., Chicago, IL, USA). The chi-square test was used for categorical variables. The relationship between categorical variables (CPB time, nutritional status, age group, genotypes) and sepsis were assessed in bivariate as well as multivariate analysis. A p value <0.05 was regarded as statistically significant.

Results

Of the 108 patients included in this study, 23 (21.29%) developed postoperative sepsis and 4 of these 23 patients died. In genetic polymorphism, both heterozygous and homozygous mutants were classified in one category, which was mutant. Only 7.4% of the patients had a TLR2 N199N wild type genotype, while the remaining 92.6% were mutant. In terms of the TOLLIP gene, 47.2% of the patients had a wild type genotype, while the remaining 52.8% were mutant. Genotype distributions are presented in Table 3, and the allelic discrimination plots for each polymorphism are presented in Figure 1 and Figure 2. In bivariate analysis, TLR 2 N199N mutant genotype tended to increased the risk of sepsis (odds ratio = 1.974; 95% confidence interval: 0.23–16.92; $p = 0.504$), while TOLLIP rs5743867 mutant genotype tended to decreased the risk of sepsis (odds ratio = 0.496; 95% confidence interval: 0.19–1.27; $p = 0.139$).

Discussion

The percentage of patients who developed postoperative sepsis in this study (21.29%) was far higher than that reported in other centers such as the Cincinnati Children's Hospital (3%) and Society of Thoracic Surgeons Congenital Heart Surgery (2.6%).[2] Patients

Table 4. Bivariate analysis of genetic polymorphism and occurrence of postoperative sepsis.

Variable	Category	Postoperative sepsis		Odds ratio (95% confidence interval)	*p* value
		Yes ($n=23$)	No ($n=85$)		
Toll-like receptor 2 N199N polymorphism	Mutant	22 (95.7%)	78 (91.8%)	1.974 (0.23–16.92)	0.504
	Wild type	1 (4.3%)	7 (8.2%)		
Toll-interacting protein rs5743867 polymorphism	Mutant	9 (39.1%)	48 (56.5%)	0.496 (0.19–1.27)	0.139
	Wild type	14 (60.9%)	37 (43.5%)		

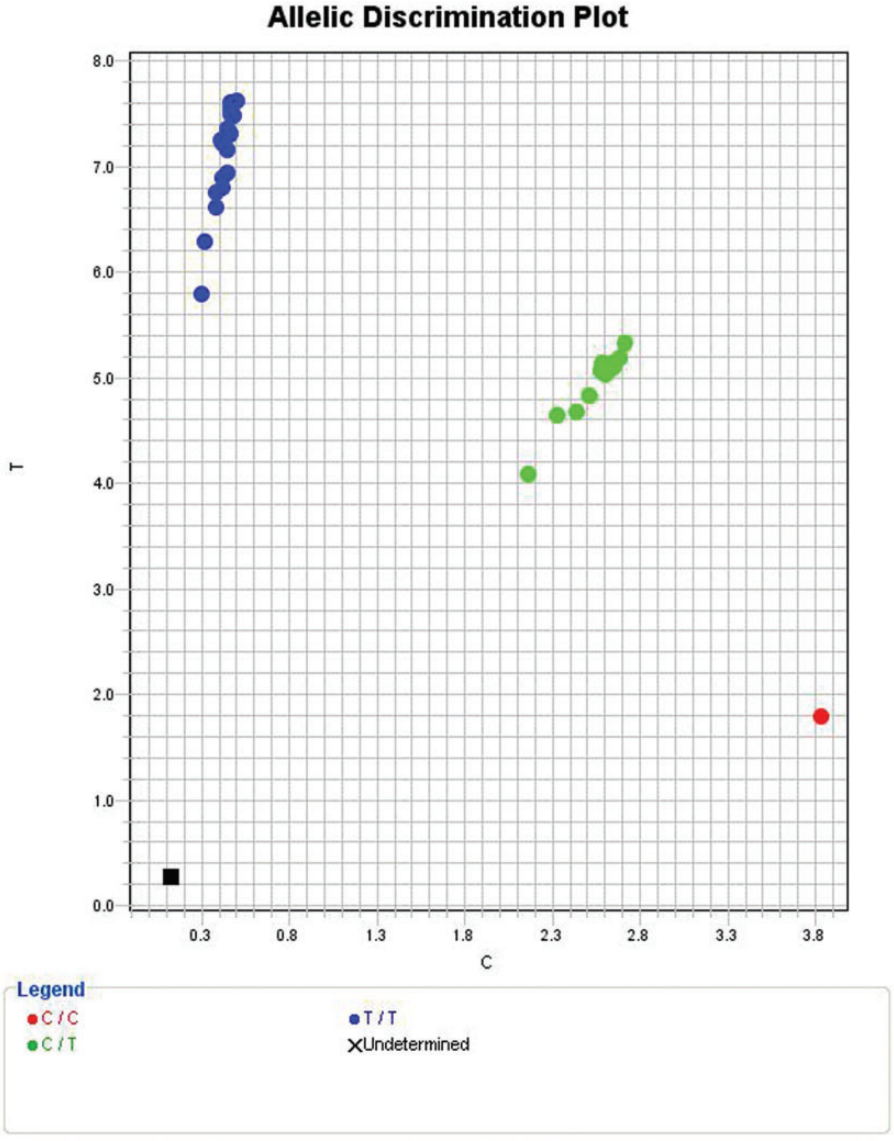

Figure 1. Allelic discrimination plot for TLR2 N199N polymorphism. Red dot: wild type (allele C/C); green dot: heterozygous mutant (allele C/T); blue dot: homozygous mutant (allele T/T); C: cysteine; T: threonine.

who developed postoperative sepsis in this study had a younger age with a median of 111 days (range 4–360 days), lower Z-score (median −3.39, range −5.81 to 0.56), longer CPB time (median 149 min, range 37–265 min), and lower Basic Aristotle score (median 9, range 6–11).

In genotype distribution, TLR2 N199N mutant genotype were shown to increase the odds of

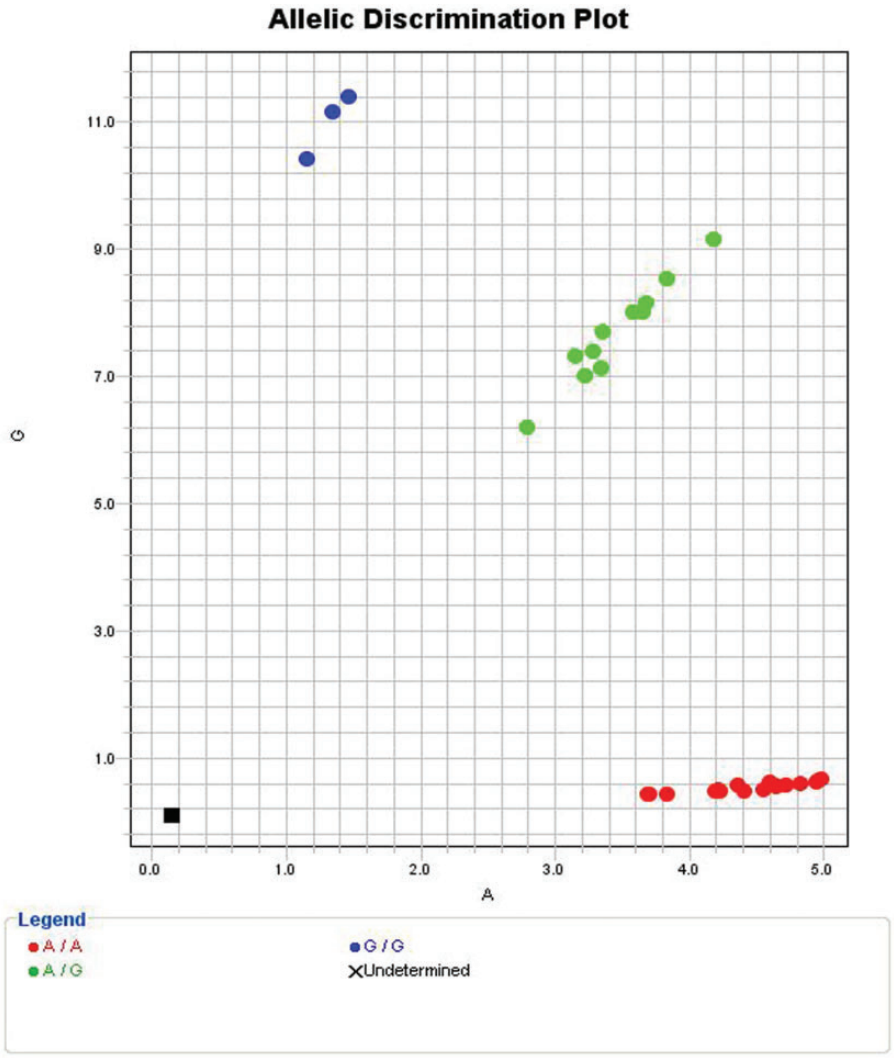

Figure 2. Allelic discrimination plot for TOLLIP rs5743867 polymorphism. Red dot: wild type (allele A/A); green dot: heterozygous mutant (allele A/G); blue dot: homozygous mutant (allele G/G); A: alanine; G: glycine.

postoperative sepsis while TOLLIP rs5743867 mutant genotype reduced the odds of postoperative sepsis although these results were not statistically significant. A previous study by Abu-Maziad and colleagues[9] showed that TLR2 N199N polymorphism was associated with a higher risk of sepsis in neonates, and the results of our study agree with this. In bivariate analysis, the TLR2 N199N mutant genotype increased the risk of sepsis. but this result was not statistically significant. This might be due to the difference in age groups of the subjects involved in both studies: Abu-Maziad and colleagues[9] studied neonates only, whereas our study involved children younger than one year of age, which might have diluted the risk of sepsis in the study population. Nevertheless, when we investigated further in the neonate age group, all neonates involved had TLR2 N199N mutant genotype and 6 out of 7 neonates (85.7%) developed postoperative sepsis.

TOLLIP works as a regulator in the TLR2 pathway by inhibiting phosphorylation of interleukin-1 receptor-associated kinase. Therefore, activation of nuclear factor-kappa B was disrupted.[8] In the TOLLIP mutant genotype, the aforementioned regulatory function was disrupted. A previous study in the Chinese Han population showed that TOLLIP rs5743867 polymorphism reduced the risk of sepsis in patients in the intensive care unit.[10] This finding is consistent with our result. TOLLIP polymorphism displayed a protective role in the development of sepsis in this study.

We concluded that in infants younger than one year of age undergoing complex open heart surgery, TLR2 N199N tends to increased the risk of postoperative sepsis, while TOLLIP rs5743867 polymorphism tends

to reduced the risk of postoperative sepsis. Further studies on TLR2 N199N and TOLLIP polymorphism are needed to fully understand the role and comprehensive mechanism of these two factors in the development of postoperative sepsis.

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Declaration of conflicting interests

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