Toll-like receptor polymorphisms (*TLR2* and *TLR4*) association with the risk of infectious complications in cardiac surgery patients

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D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(1):57–63

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Funding sources
None declared

Conflict of interest
None declared

Received on May 30, 2022
Reviewed on August 4, 2022
Accepted on August 18, 2022

Published online on September 22, 2022

Abstract

Background. Postoperative infection is a common healthcare-associated problem, and unfortunately, a serious complication in cardiac surgery patients. Toll-like receptors (TLRs) are crucial in activating non-specific immunity mechanisms and integrating elements of the immune system, due to interactions between specific and non-specific responses.

Objectives. In this study, the association of *TLR2* or *TLR4* with the risk of postoperative infections in cardiac surgery patients undergoing a coronary artery bypass grafting (CABG) procedures was investigated.

Materials and methods. Our research was carried out on a cohort of 299 consecutive adult patients with ischemic heart disease (IHD) who underwent a planned CABG procedure. These patients were monitored for the presence of a postoperative infection over a 30-day observation period. All patients were investigated for 2 *TLR2* gene mutations – R753Q (rs5743708) and T16934A (rs4696482), and 2 polymorphisms of the *TLR4* gene – D299G (rs4986790) and T399I (rs4986791). The final stage of the study was an evaluation of the hypothetical association between *TLR2* and *TLR4* gene variances and postoperative infections in patients undergoing CAGB procedures.

Results. The prevalence of infections in the final cohort was 15.3% (46/299). The most common infections were surgical site infections, which were diagnosed in 21 patients (45.6%), bloodstream infections in 15 patients (32.6%) and pneumonia in 10 patients (21.8%). Logistic regression demonstrated that the presence of the AG+GG of D299G (rs4986790) and CT+TT of T399I (rs4986791) variants was related to a higher incidence of infection in patients undergoing CAGB procedures.

Conclusions. To our knowledge, this is the first study of its kind to demonstrate that *TLR2* and *TLR4* mutations affect the risk of post-CABG infections. Being a carrier of the AG+GG of D299G (rs4986790) or CT+TT of T399I (rs4986791), *TLR4* variants constitute a postoperative risk factor for infection in patients undergoing CAGB procedures.

Key words: infection, *TLR4*, *TLR2*, CABG, postoperative

Cite as
Żukowska A, Ciechanowicz A, Kaczmarczyk M, Brykczyński M, Żukowski M. Toll-like receptor polymorphisms (*TLR2* and *TLR4*) association with the risk of infectious complications in cardiac surgery patients. *Adv Clin Exp Med*. 2023;32(1):57–63. doi: 10.17219/acem/152885

DOI
10.17219/acem/152885

Background

Cardiac surgery carries a high likelihood of postoperative infections due to the invasive nature of the surgery and the subsequent inflammatory response. Postoperative infections can have serious repercussions for the patient and lead to prolonged hospitalization, as well as increased hospitalization costs. A reduction of the prevalence of infections requires not only an augmentation in surgical behavior but also the implementation of a competent epidemiological and microbiological approach.[1,2]

In accordance with current publications, the occurrence of infectious complications after cardiac surgery is between 3.3% and 26.8%.[1–3] The mortality coefficient among cardiac surgery patients with an infection during the postoperative period is approx. 16.8% compared to 2.9% in patients without an infection.[4] A multicenter research study has revealed an infection incidence after open heart surgery using cardiopulmonary bypass (CPB) of approx. 26.8%.[1] Among the most common complications, pneumonia can occur in approx. 57.1% of all postoperative infections.[5] This appears to be a relevant matter, as the mortality in cases of pneumonia associated with mechanical ventilation (ventilator-associated pneumonia (VAP)) reaches 46%. Infectious events occurring postoperatively substantially increase hospitalization costs and prolong hospitalizations. The increased frequency of infections after cardiac surgery procedures is multifactorial. The patient's preoperative condition, underlying diseases and perioperative course are all crucial components in regard to the development of an infection.[3,5,6]

The immune system can identify intruding pathogens through a group of receptors known as pattern-recognition receptors (PRRs). These receptors identify molecular patterns associated with pathogens (pathogen-associated molecular patterns (PAMPS)) and subsequently activate a cascade of signals to generate an immune response. The PRRs are comprised of various groups of molecules such as retinoic acid-inducible gene I (RIG-I-like) receptors, toll-like receptors (TLRs), absent in melanoma-2 (AIM-2)-like receptors (ALRs), Nod-like receptors (NLRs), and others.[7] The presence of TLRs has been confirmed in most immune cells, especially in those responsible for the recognition of pathogens, but also on the surface of neutrophils, eosinophils and lymphocytes. In addition to the cells of the immune system, TLRs are also present on the surface and inside cells that can come into contact with pathogens, such as respiratory epithelial cells, gastrointestinal tract cells, endothelial cells, skin cells, urogenital tract epithelial cells, adipocytes, and myocytes.[8] Ten TLRs with different ligand binding specificities have been identified in humans. The TLRs are key in activating non-specific immune mechanisms and are an important integrating element in the immune system due to interactions between specific and non-specific responses. The binding of PAMPs to TLRs leads to the activation of cells that recognize foreign antigens by inducing the expression of genes responsible for the synthesis of pro-inflammatory cytokines, chemotactic proteins and defensins.[9,10]

For instance, *TLR2* has been shown to be associated with an intrinsic response to Gram-positive pathogens through the recognition of bacterial cell wall components, while *TLR4* plays an important role in activating a host response against Gram-negative bacteria.[7–9]

A vast amount of research on the relationship between various gene polymorphisms and inflammatory responses associated with infections has been conducted. The discovery of the role genetic variants and gene mutations play in the encoding of immunological response proteins could be used to predict those with an increased risk of postoperative infections and lead to an improvement in perioperative care.

Objectives

The purpose of this research was to estimate whether *TLR2* or *TLR4* polymorphisms are related to the probability of postoperative infections in cardiac surgery patients who have undergone a coronary artery bypass grafting (CABG) procedure.

Materials and methods

Study group

Our research was carried out on a cohort of 299 consecutive adult patients with ischemic heart disease (IHD) undergoing a planned CABG procedure at the Department of Cardiac Surgery (Pomeranian Medical University, Szczecin, Poland). Exclusion criteria included having previously undergone thoracic surgery, off-pump CABG procedures, the presence of an active infection, valvular operations, and emergency procedures (Fig. 1). The presented research was approved by the Pomeranian Medical University Ethics Committee (approval No. KB-0080/176/09). Informed written consent was obtained from all patients.

Standard operating procedures and antibiotic prophylaxis were performed in all participants. The patients were observed for the presence of a postoperative infection during a 30-day follow-up period. All patients were evaluated for 2 *TLR2* gene mutations: R753Q (rs5743708) and T16934A (rs4696482), and 2 polymorphisms of the *TLR4* gene: D299G (rs4986790) and T399I (rs4986791).

In addition, clinical data such as New York Heart Association score (NYHA), Canadian Cardiovascular Society score (CCS), logistic EuroSCORE (ESlog), smoking, length of coronary artery disease (CAD), blood loss volume, blood transfusion requirement, serum creatinine level, and length of CBP, as well as demographic data were recorded.

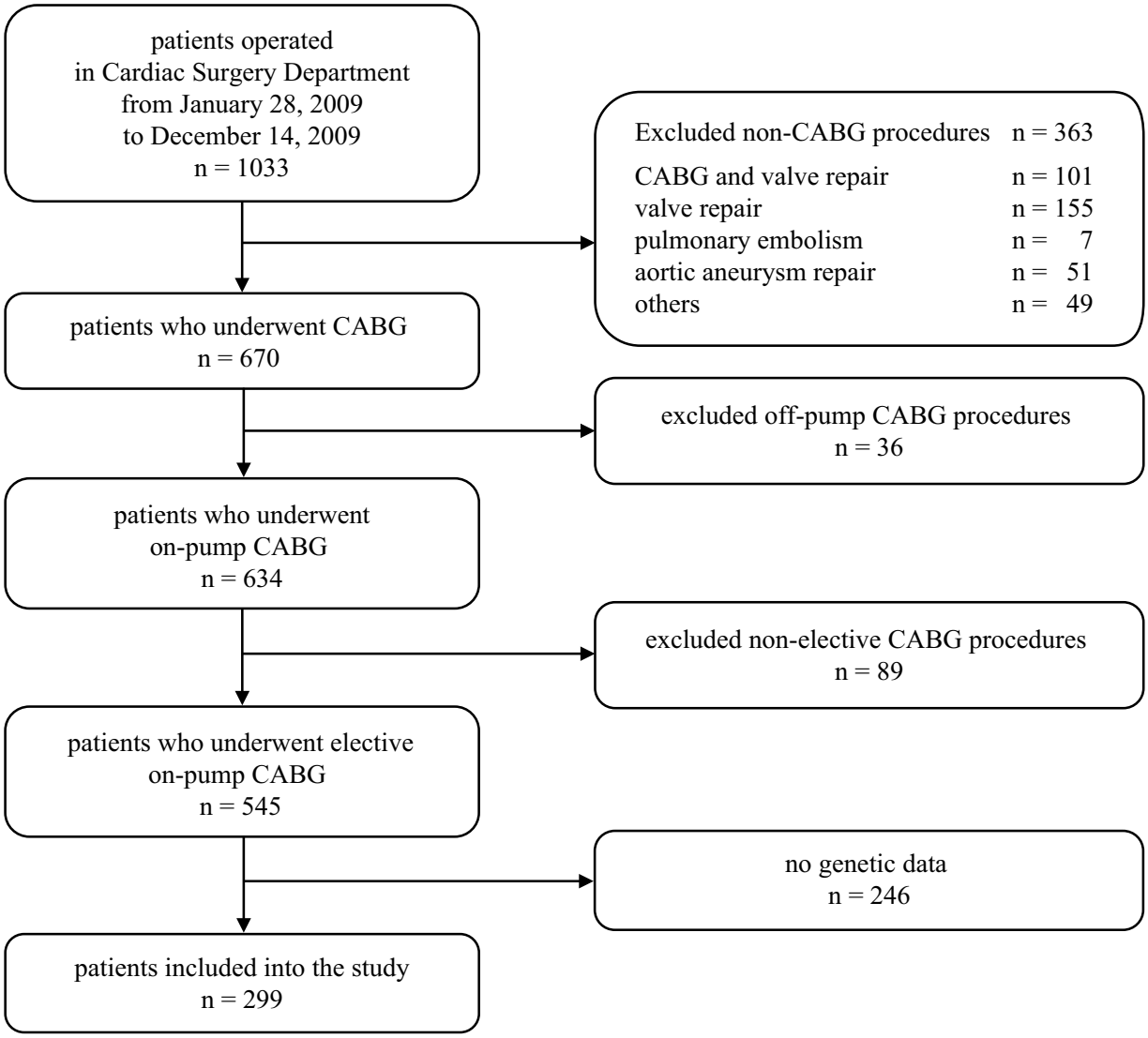

Fig. 1. Study flowchart

CABG – coronary artery bypass grafting.

Criteria for postoperative infection

We identified surgical site infections (SSIs) based on the presence of soreness or vulnerability, regional edema, redness or warmth, and the existence of purulent drainage from the incision site or bacterial growth in wound cultures that appeared at the incision site up to 30 days postoperatively. These criteria are concordant with the European Centre for Disease Prevention and Control (ECDC) definitions. In each case, the wounds were swabbed for microbiological examination. If systemic features of infection, such as fever, chills, hypotension, and elevated C-reactive protein (CRP), white blood cell (WBC) counts or serum procalcitonin (PCT) levels were present, blood cultures were drawn.[11]

According to the ECDC, patients can be diagnosed with pneumonia based on radiological findings of at least 2 chest examinations along with a minimum of 1 of the following: novel or increasing/sustained infiltrates, consolidations or cavitations, as well as an increase in temperature or leukocyte count (<4000 WBC/mm^3 or ≥12,000 WBC/mm^3). The criteria also included individuals with age ≥70 years, presenting with confusion without an identifiable cause, as well as at least 2 of the following: new onset of purulent sputum, change in sputum character, increased respiratory secretions, increased suctioning requirements, new-onset or worsening cough, dyspnea, tachypnea, rales or bronchial tone, worsening gas exchange (e.g., deteriorating oxygenation (e.g., $PaO_2/FiO_2 \leq 240$), increased oxygen demands, or extended (increased) ventilator support. In each case, sputum or bronchoalveolar lavage (BAL) samples were obtained for microbial culture.[12]

Bloodstream infection (BSI) was diagnosed in patients with one of the following findings: pyrexia (>38°C), shivers or low blood pressure, and identified bacterial growth on one or more blood samples, or had the usual commensal bacteria (i.e., co-negative staph., diphtheroids, *Propionibacterium* spp., *Bacillus* spp.) present in more than 1 positive blood culture obtained from different sites.[13]

Genetic analysis

All patients were investigated for 2 well-known functional *TLR2* and *TLR4* gene mutations. During the course of hospitalization, 2 mL-specimens of whole blood were taken from all participants via a typical venipuncture technique. Peripheral blood leukocytes were used for genomic DNA isolation using a Mini Kit QIA DNA (Qiagen, Hilden, Germany). Afterwards, the polymerase chain reaction (PCR)-restriction fragments length polymorphism technique was applied to examine the rs5743708 and rs4696482 of *TLR2* mutations, as well as for rs4986790 and rs4986791 of *TLR4* mutations. All details of the genetic investigation (primers, PCR conditions, amplifications, electrophoresis, and cleaving) were thoroughly described in our previous publication.[14]

The final step of the study was to investigate the hypothetical association between *TLR2* and *TLR4* gene variances and postoperative infections in individuals undergoing CAGB procedures.

Statistical analyses

Compatibility with the Hardy–Weinberg equilibrium model was calculated using a χ^2 test. The composition of individual genotypes with and without postoperative infections was tested using Fisher's exact method. Unpaired and paired Student's t-tests and Mann–Whitney U tests were used to evaluate a correlation among the groups. Adjusted and unadjusted models of multivariate analysis were used to evaluate the connection between TLR polymorphisms and the frequency of post-CABG septic complications. The p-values <0.05 were treated as statistically significant. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were reported when logistic regression analysis was performed. All tests were performed using Statistica v. 8.0 (StatSoft, Inc., Tulsa, USA).

Results

The mean age in the analyzed cohort was 63 years. The study population consisted of 77 (25.7%) females, 42% smokers at the time of admission, and 152 (50.8%) individuals in class 0 of the NYHA scoring. Baseline characteristics were not significantly different between the infected and non-infected groups except for the duration of CAD, CABG, perioperative blood loss volume, and ESlog (Table 1).

The prevalence of infections in the final cohort was 15.3% (46/299). Surgical site infections were the most common, diagnosed in 21 patients (45.6%), followed by bloodstream

Table 1. The baseline characteristics

Variable	Whole group (n = 299)	Non-infected (n = 253)	Infected (n = 46)	p-value
Age [years], M ±SD	63.6 ±9.8	63.2 ±9.9	65.8 ±9.5	0.2837#
Male sex, n (%)	222 (74.2)	187 (73.9)	35 (76.1)	0.8556*
Height [cm], M ±SD	166.9 ±9.1	166.9 ±9.2	166.9 ±8.9	0.8361#
Weight [kg], M ±SD	78.6 ±13.6	78.6 ±13.8	78.5 ±12.3	0.3521#
BMI [kg/m^2], M ±SD	28.2 ±4.2	28.2 ±4.1	28.1 ±3.6	0.3011#
NYHA class, n (%)				
0	152 (50.4)	127 (50.2)	25 (54.3)	0.1921*
I	23 (7.8)	21 (8.3)	2 (4.5)	
II	49 (16.6)	42 (16.6)	7 (15.2)	
III	65 (21.8)	57 (22.5)	8 (17.4)	
IV	10 (3.4)	6 (2.4)	4 (8.6)	
CAD [months], M ±SD	60.3 ±112.2	64.9 ±130.5	61.2 ±114.8	0.0001#
Smoking, n (%)	103 (34.4)	90 (35.6)	13 (28.3)	0.2564*
ESlog	6.4 ±7.5	6.2 ±9.8	6.5 ±7.6	0.0064#
CSS class, n (%)				
I	9 (3.7)	8 (3.9)	1 (2.6)	0.8632$
II	58 (23.9)	50 (24.4)	8 (21.1)	
III	132 (54.3)	109 (53.2)	23 (60.5)	
IV	44 (18.1)	38 (18.5)	6 (15.8)	
Costs [PLN]	7969 ±10770	6953 ±7093	13556 ±21181	0.014#
Hospital stay [days]	8.61 ±6.2	8.06 ±5.1	11.65 ±10.2	0.021$
In-hospital mortality, n (%)	8 (2.67)	6 (2.01)	2 (4.34)	0.524*

M ±SD – mean ± standard deviation; CAD – coronary artery disease; BMI – body mass index; CSS – Canadian Cardiovascular Society; NYHA – New York Heart Association; ESlog – logistic EUROScore; * Fisher's exact test; # Mann–Whitney U test; $ χ^2 Pearson's test.

infection in 15 patients (32.6%) and pneumonia in 10 patients (21.8%). The occurrence of infections did not affect hospital mortality, but it significantly extended the hospitalization time (p = 0.021) and significantly increased the overall cost of treatment (p = 0.014) (Table 1). Vacuum-assisted closure (VAC) therapy was not used in any of the cases studied. Of the patients in the study group, 8 died during hospitalization.

Hospital mortality in the study group was 2.67% and did not differ statistically between the groups of patients with and without infections. The univariate analysis showed that older age (p = 0.018), clamping time (p = 0.002), longer duration of CAD (p = 0.001), concentration of creatine kinase-MB (CKMB) >100 during the postoperative period (p = 0.001), increased drainage during the postoperative period (p = 0.001), and a higher NYHA classification score before surgery (p = 0.001) significantly increased the risk of hospital death. Due to the small number of deaths in the study group, it was not possible to demonstrate significance in the multivariate analysis. When analyzing the causes of death, in 2 cases it was possible to unequivocally indicate an infection as the direct cause of death, and in both cases, the patients died of sepsis. Of the remaining 6 cases, 4 suffered a perioperative infarction, while the remaining 2 died of multiple organ failure.

The compositions of genotypes were coherent with the Hardy–Weinberg equilibrium model for all *TLR2* and *TLR4* single-nucleotide polymorphisms (SNPs) in patients with and without infection.

Of the 299 patients, 158 (52.8%) were TA heterozygous with *TLR2* T-16934A (rs4696480) SNPs. The AA variant was identified in 68 cases (22.8%) and the TT variant was identified in 74 cases (24.8%). There were no statistically significant differences among the infected and non-infected groups in relation to SNPs of the T-16934A *TLR2* polymorphisms. Approximately 93% of cases were GG wild-type homozygous for the *TLR2* Arg753Gln (rs5743708). Regarding the *TLR2* Arg753Gln (rs5743708) polymorphism, 21 (7.1%) of the participants were GA heterozygotes, and there were no homozygous patients in the whole study group.

It was also found that in the 299 patients who were investigated for the inherence of *TLR4* D299G SNP (rs4986790), nearly 90% (260/299) were AA homozygous, with the absence of this mutation. The AG polymorphism was observed in 38 individuals (12.7%), whereas the GG variant was observed in only 1 individual. In further evaluations, both AG and GG subgroups were treated as one. In addition, of the 299 patients who were screened for the presence of the *TLR4* T399I SNP (rs4986791), 86.9% of the patients (260/299) were CC homozygous. The CT genotype was seen in 38 cases (12.7%), whereas the TT variant was found in only 1 individual. In further evaluations, both CT and TT subgroups were treated as one. The *TLR4* genotyping showed that the AG+GG variants of D299G (rs4986790) and CT+TT variants of T399I (rs4986791) were more often observed in patients with postoperative infections (Table 2). Logistic regression demonstrated that the presence of the AG+GG variants of D299G (rs4986790) and CT+TT variants of T399I (rs4986791) were related to a higher incidence of infection in patients undergoing CAGB procedures (Table 3).

Table 2. The relationship between analyzed genotypes and postoperative infections

TLR polymorphism	Hardy–Weinberg equilibrium p-value	Infection status, n (%)		p-value*	Total, n (%)
		non-infected	infected		
TLR2 R753Q					
GG (wild-type)	0.549	238 (93.0)	41 (95.3)	0.749	279 (93.3)
GA		18 (7.0)	2 (4.7)		20 (6.7)
AA		0	0		0
Total		256 (100)	43 (100)		299 (100)
TLR2 T16934A					
TA	0.288	130 (50.8)	28 (65.2)	0.184	158 (52.8)
TT		64 (25.0)	9 (20.9)		73 (24.4)
AA		62 (24.2)	6 (13.9)		68 (22.8)
Total		256 (100)	43 (100)		299 (100)
TLR4 D299G					
AA	0.288	227 (89.7)	33 (71.7)	0.003	260 (86.9)
AG+GG		26 (10.3)	13 (28.3)		39 (13.1)
Total		253 (100)	46 (100)		299 (100)
TLR4 T399I					
CC	0.288	226 (89.3)	33 (71.7)	0.003	259 (86.6)
CT+TT		27 (10.7)	13 (28.3)		40 (23.4)
Total		253 (100)	233 (100)		299 (100)

Table 3. Logistic regression

Variable	HR	95% CI	SE	p-value
TLR4 D299G AA variant	3.665	1.655–8.114	0.403	0.001
TLR4 T399I CC variant	3.532	1.601–7.790	0.401	0.002
Blood loss	2.119	1.351–3.581	0.247	0.002

HR – hazard risk; 95% CI – 95% confidence interval; SE – standard error.

Discussion

Postoperative infections are common healthcare-associated problems, and, unfortunately, are serious complications in patients undergoing cardiac surgery. This group of patients differs substantially from patients undergoing surgery in other surgical departments due to the detrimental impact of the cardiopulmonary pump circuit. This leads to a widespread initiation of the innate immune system and subsequently affects the response to pathogens.[15] Previous research has demonstrated a prevalence of postoperative infections ranging from 4.9% to approx. 22% in cardiac surgery wards.[1–3] Gelinjs et al. showed a prevalence of 4.9% in a cohort of 5158 patients.[1] In contrast, Kollef et al. demonstrated a higher prevalence of 21.7%.[3] These variations could be in part due to a discrepancy in the diagnostic criteria used, materials and methods, the inclusion criteria applied, infection control guidelines used, or the types of cardiac surgery interventions performed. This study demonstrated an overall prevalence of hospital-acquired infections (HAI) that was relatively high at 15.3%, which was surprising for the authors. The most likely cause of this phenomenon may be an over-diagnosis of infections by the attending physicians and, consequently, the excess use of antibiotics. Regardless of the infection criteria used, in the case of SSIs, the existence of purulent exudates from the incision site requires subjective assessment. Similarly, in the case of pneumonia, the presence of novel or increasing/sustained infiltrates, consolidations or cavitations was also assessed subjectively. Moreover, the authors were not able to state whether all of the abovementioned criteria were applied in these cases. Our thesis was indirectly confirmed by the fact that there was no need for VAC therapy in patients with surgical site infections. On the other hand, the reported prevalence of hospital-acquired infections is similar to the results of research conducted by van Klarenbosch et al. in 2020,[16] which was reported to be 14.5%. The similarities in both studies can be attributed to several factors such as BMI, ESlog, transfusions requirements, and CPB time. In our study, surgical site infections were the most common and were present in 21 patients (45.6%), followed by bloodstream infection in 15 patients (32.6%), and pneumonia, which was diagnosed in 10 patients (21.8%). The incidence of pneumonia was comparable to other authors' studies.[17,18] The mortality rate observed in our study group did not differ from that reported in other studies, and only slightly exceeded 2%, which was not related to the occurrence or lack of postoperative infections.[19]

In our research, we raised the issue of whether *TLR2* or *TLR4* polymorphisms are related to the occurrence of postoperative infections in cardiac surgery patients undergoing a CABG procedure. Genetic analysis of 2 *TLR2* SNPs (rs4696480 and rs5743708) and 2 *TLR4* SNPs (rs4986790 and rs4986791) led to each of the 4 variants being identified. The allele prevalence of the examined *TLR2* and *TLR4* polymorphisms strongly match the results reported by other authors.[20,21] We were not able to demonstrate any significant disparity in *TLR2* SNPs variants among patients with and without postoperative infections. However, we managed to show a significant correlation between *TLR4* (AG+GG of D299G) and (CT+TT of T399I) SNPs in comparison to wild-type carriers and the incidence of post-CABG infections in cardiac surgery patients. The relationship between carriers of the TLR polymorphisms and the development of infection has been studied in various groups of patients. In a group of 155 acute myeloid leukemia cases, Schnetzke et al. reported a relationship between *TLR4* and *TLR2* polymorphisms and the incidence of sepsis in their study group.[22] Other authors also demonstrated that the Arg753Gln of the *TLR2* mutation was significantly correlated with a greater incidence of pneumonia in a group of patients with acute myeloid leukemia (AML) undergoing a chemotherapy induction protocol. Papadopoulos et al. were able to demonstrate an association between the 2 *TLR4* polymorphisms and the occurrence of serious infections in a cohort of 199 human immunodeficiency virus (HIV)-positive patients.[23] However, other authors failed to establish a significant relationship between *TLR2* and *TLR4* polymorphisms and the occurrence of infections.[24]

In our research, the significant influence of *TLR4* polymorphisms on the predisposition of CABG individuals to postoperative infections could partly be explained by the expression of *TLR4* on enteric epithelium cells, pulmonary epithelial cells and other cells playing the role in recognizing infectious particles and initiating as well as managing a major immune reaction against various groups of pathogens.[25] While *TLR4* primarily identifies Gram-negative bacterial molecules, *TLR2* is able to identify a range of molecules, such as Gram-positive and Gram-negative bacterial components.[26,27] The TLRs play an extremely important role, not only as PRRs, but also through their effect on damage-associated molecular patterns (DAMPs) as well as high-mobility group proteins, heat shock proteins, phospholipids, and others. The SNPs alone are not sufficient to explain the entire genetic component involved

in the development of infections.[26,27] However, the importance of polymorphisms in TLR alleles and the interaction of SNPs in infections requires further investigation.

Limitations of the study

The main limitation of our study is its retrospective nature. Another limitation is the probable over-diagnosis of infections due to the subjective assessment of clinical symptoms and radiological images. Finally, the group of patients was relatively small. However, multivariable evaluation was still possible.

Conclusions

In summary, this research is the first of its kind to demonstrate that *TLR2* and *TLR4* mutations affect the risk of post-CABG infections. The frequency of the various alleles in our study population is in accordance with the current literature. Our study did not show an association between the analyzed SNPs in *TLR2* and susceptibility to infections. Carriers of the AG+GG of D299G (rs4986790) or CT+TT of T399I (rs4986791) *TLR4* variants can contribute to the prevalence of infections during the perioperative period in patients undergoing CAGB procedures.

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