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Regular paper

Clinical, biochemical and genetic risk factors for 30-day and 5-year mortality in 518 adult patients subjected to cardiopulmonary bypass during cardiac surgery – the INFLACOR study

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There is increasing evidence that genetic variability influences patients' early morbidity after cardiac surgery performed using cardiopulmonary bypass (CPB). The use of mortality as an outcome measure in cardiac surgical genetic association studies is rare. We publish the 30-day and 5-year survival analyses with focus on pre-, intra-, postoperative variables, biochemical parameters, and genetic variants in the INFLACOR (INFLAmmation in Cardiac OpeRations) cohort. In a prospectively recruited cohort of 518 adult Polish Caucasians, who underwent cardiac surgery in which CPB was used, the clinical data, biochemical parameters, IL-6, soluble ICAM-1, TNFα, soluble E-selectin, and 10 single nucleotide polymorphisms were evaluated for their association with 30-day and 5-year mortality. The 30-day mortality was associated with: pre-operative prothrombin international normalized ratio, intra-operative blood lactate, postoperative serum creatine phosphokinase, and acute kidney injury requiring renal replacement therapy (AKI-RRT) in logistic regression. Factors that determined the 5-year survival included: pre-operative NYHA class, history of peripheral artery disease and severe chronic obstructive pulmonary disease, intra-operative blood transfusion; and postoperative peripheral hypothermia, myocardial infarction, infection, and AKI-RRT in Cox regression. Serum levels of IL-6 and ICAM-1 measured three hours after the operation were associated with 30-day and 5-year mortality, respectively. The *ICAM1* rs5498 was associated with 30-day and 5-year survival with borderline significance. Different risk factors determined the early (30-day) and late (5-year) survival after adult cardiac surgery in which cardiopulmonary bypass was used. Future genetic association studies in cardiac surgical patients should account for the identified chronic and perioperative risk factors.

Key words: cardiac surgery; cardiopulmonary bypass; 30-day mortality; 5-year mortality; *ICAM1* rs5498; ICAM-1; renal replacement therapy.

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Abbreviations: AKI, acute kidney injury; VKA, vitamin K antagonist; CAD, coronary artery disease; CG, candidate gene; CPB, cardiopulmonary bypass; CPK, creatine phosphokinase; FE, Fisher's-Exact test; GFR, glomerular filtration rate; GWAS, genome-wide association study; ICAM-1, intercellular adhesion molecule-1; ICU, intensive care unit; IL-6, interleukin 6; INR, international normalized ratio; IQR, interquartile range; KW, Kruskal-Wallis test; MI, myocardial infarction; PT, prothrombin time; RRT, renal replacement therapy; SNP, single nucleotide polymorphism

INTRODUCTION

Cardiac surgery in which cardiopulmonary bypass is used elicits complex inflammatory responses that are triggered by mechanical injury of the blood cells by the pump, ischemia/reperfusion organ injury, tissue injury caused by electrocautery, allogeneic blood product transfusions, and immunological reactions to drugs and materials (Warren *et al.*, 2009). By its nature, cardiac surgery sometimes also causes a transient impairment of the heart function, which is manifested by decreased cardiac output and shock (Laffey *et al.*, 2002). Thus, organ failure that occurs after cardiac surgery may be the result of various etiologies, but is responsible for early and late postoperative mortality that limits the initial benefit of the surgery (Geissler *et al.*, 2000).

Since the development and increase in accessibility of genetic methods at the beginning of the new millennium, the search for functional genotypes that can explain postoperative morbidity in cardiac surgical patients has advanced and multiplied (Welsby *et al.*, 2004; Grocott, 2006; Podgoreanu *et al.*, 2006). Genotyping of functional genetic variants has also been recognized as a potentially useful tool for the prediction of postoperative morbidity in cardiac surgical patients (Kowalik *et al.*, 2014). After the era of candidate gene (CG) studies, the development of genome-wide sequencing technique defined new standards in genetic research (Stüber & Hoeft, 2002; Podgoreanu *et al.,* 2006; Kraft, 2009; O'Donnell & Nabel, 2011; Zeller *et al.*, 2012). Using the CG approach, functional single nucleotide polymorphisms (SNPs) have been identified that were associated with postoperative bleeding (Welsby *et al.*, 2004), myocardial infarction (MI) (Podgoreanu *et al.*, 2006), acute lung injury (Yende *et al.*, 2003; Meyer & Garcia, 2006), acute kidney injury (AKI) (Podgoreanu *et al.*, 2006), stroke (Newman *et al.*, 2006; Grocott, 2006), delirium (Newman *et al.*, 2006, Mathew *et al.*, 2007), and atrial fibrillation (Podgoreanu MV & Schwinn, 2005) in cardiac surgical patients and with sepsis in ICU patients (Henckaerts *et al.*, 2009).

However, identifying a genetic variant with a targeted statistical significance is still frequently the first and

only step in explanatory research. The underlying biological pathways behind the complex clinical phenotypes observed after cardiac surgery, i.e.: acute kidney injury, acute lung injury, atrial fibrillation, postoperative delirium, and even infection with certain bacteria, are caused by an interplay of environmental factors, treatment, and genetic variability. The complexity of these syndromes requires that the explanatory research adjust the genetic associations in concert with the specific pathological pathways and multiple clinical data, which represents a challenging task.

In 2009, the INFLACOR trial started. This was an association and integrative study with the ultimate aim of evaluating the associations between 10 SNPs and postoperative morbidity in 525 adult patients who underwent open-heart surgery on cardiopulmonary bypass. The selected 10 functional SNPs were chosen from genes that encode proteins involved in the inflammatory response to cardiopulmonary bypass or predisposing the subjects to infections in the ICU (Yende *et al.*, 2003; Stafford-Smith *et al.*, 2005; Podgoreanu MV *et al.*, 2006; Henckaerts *et al.*, 2009). In this first analysis, we publish the clinical and genetic risk factors that were identified as being associated with 30-day and 5-year mortality, including the clinical phenotypes (morbidity) that were initially chosen for the study design. The perioperative risk factors were identified from a broad list of candidate variables and postoperative serum levels of two cytokines and two adhesion molecules.

METHODS

The INFLACOR study protocol was accepted by the Independent Ethical Commission for Scientific Research at the Medical University of Gdańsk (NKEBN/358/2007) and registered at ClinicalTrials.gov (NCT01020409). All participating patients provided a written informed consent.

In the univariate analyses, a list of 154 candidate variables was examined for associations with postoperative 30-day and 5-year mortality. In addition to 55 pre-operative variables (i.e. physical data, co-morbidities, medication, and blood biochemical parameters); 43 intraoperative variables that characterized the surgery, CPB, and anesthesia and 56 variables from the postoperative course were analyzed. Among the postoperative variables, the first-day serum levels of interleukin-6 (IL-6), tumor necrosis factor α (TNFα), intercellular adhesion molecule-1 (ICAM-1), and soluble E-selectin (ESEL) and eight postoperative morbidities were included. The SNPs for 10 genes that are involved in biological pathways of postoperative morbidity after cardiac surgery or sepsis in ICU were analyzed for genotypic and allelic associations with mortality (Table 1).

Genotyping. The ten SNPs were selected from candidate gene association studies published earlier (Table 1). The blood samples for genotype and cytokine analyses were obtained 3 hours after admission to the postoperative unit. The blood cells were separated from the plasma/serum via cooled centrifugation (5 min, 3000 rpm, 4°C). The serum or plasma was aliquoted manually and stored at –80°C. Genomic DNA was extracted from the leukocytes using a Genomic Midi AX kit (A&A Biotechnology, Poland) according to the manufacturer's protocol. The SNVs were amplified in two multiplex PCRs. The genotypes were analyzed using a single base primer extension assay with a SNaPshot® Multiplex Kit (Life Technologies, Foster City, CA, USA) according to the

Table 1. Candidate variables analyzed for association with the postoperative mortality in 518 cardiac surgery patients subjected to cardiopulmonary bypass.

Pre-operative variables:
age, sex, body mass index, logisticEUROSCORE, New York Heart Association (NYHA) grading, Canadian Cardiovascular Society (CCS) angina pectoris grading, left ventricle ejection fraction (LVEF), history of: active bacterial endocarditis (on treatment), arterial hypertension, pulmonary hypertension (PH), coronary artery sclerosis (CAS), recent (<90 days) and past myocardial infarction (MI), diabetes mellitus (DM) and daily insulin dose, chronic/paroxysmal atrial fibrillation (AF), chronic kidney disease (CKD);treatment with: immunosuppressants, antibiotics, statins, β-adrenergic blocking agents, angiotensin-converting enzyme (ACE) blockers, sartans, calcium blockers, loop diuretics, spironolactone, nitrates, acetylsalicylic acid, dual antiplatelet therapy (DAPT), vitamin K antagonist (VKA), intravenous nitroglycerin, inhaled bronchodilating agents, neurotropic agents, recent (14 days) teeth extraction, time post extraction, and eventual antibiotic prophylaxis; blood chemistry: hemoglobin (HGB), hematocrit (HCT), white blood cells (WBC), fraction of granulocytes, platelets (PLT), serum creatinine, glomerular filtration rate (GFR-MDRD formula), blood urea nitrogen, prothrombin ratio international normalized ratio (PT-INR), and C-reactive protein (CRP).
Intraoperative variables:
schedule of surgery, operation duration, aorta surgery, type of heart surgery, surgical complications, CPB duration, time of aortic cross-clamping, blood temperature on CPB, duration of arterial hypotonia on CPB (mean arterial pressure <60 mmHg), low-tidal-volume ventilation on CPB, doses of drugs used: midazolam, inhaled anesthetic agent, antibiotic, dexamethasone, heparin, protamine, tranexamic acid, antithrombin, peak doses of noradrenaline, adrenaline, dobutamine, and dopamine; transfusion of: red blood cell concentrate (RBCC), fresh frozen plasma (FFP), platelets, prothrombin complex concentrates (PCC); blood chemistry: peak lactate and glucose, nadir base excess.
Postoperative variables:
Postoperative morbidity: postoperative MI, new episode of AF, psychosis, stroke, acute lung injury (ALI), acute kidney injury (AKI) any stage, surgical re-intervention (chest re-exploration, pericardium or pleura drainage), infection; systemic inflammatory response syndrome (SIRS), highest first day axillar temperature and fever (>38.0°C), highest first day peripheral (fingertip) temperature and peripheral hypothermia (<31.0°C for 3 hours), abnormal WBC (<4.0 or >12.0 G/L), positive microbiologic culture, new antibiotic treatment, CRP >100 mg/dL, mean PF-ratio on primary mechanical ventilation, need for secondary mechanical ventilation, postoperative hours on mechanical ventilation (HOV), inflammatory opacities on chest X-ray (CXR), mean chest tube output on first day, renal replacement therapy (RRT), intra-aortic balloon pump (IABP), transfusion of: RBCC, FFP, and platelets; first-day APACHE III and SAPS II score, length of stay (LOS) in ICU. Three hours after operation serum levels: IL-6, ICAM1, soluble E-selectin, TNFα. First postoperative day: HGB, HCT, WBC and its change, PLT and its change, fraction of granulocytes, serum creatinine and its change, creatine phosphokinase and its MB-fraction, troponin I, CRP, GFR-MDRD and its change.
Gene single nucleotide polymorphisms:
IL6 rs1800796, *LBP1* rs2232582, *ICAM1* rs5498, *CRP* rs1800947, *NOD2* rs2066844, *TNF* rs1800629, *MASP2* rs2273346, *SELE* rs1805193, *NOS3* rs1799983, *TLR4* rs4986790.

manufacturer's protocol. All the primers (sequences are available upon request) were designed using Primer 3 (http://frodo.wi.mit.edu/). Capillary electrophoresis was performed using Applied Biosystems 3130 Genetic Analyzer, and the obtained electropherograms were analyzed using GeneMapper version 4.0 (Life Technologies, Foster City, CA, USA). The SNV genotypes of the selected samples were confirmed with an independent PCR followed by bidirectional sequencing using an Applied Biosystems 3130 Genetic Analyzer and the BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Foster City, CA, USA). The sequences were analyzed using Sequencher v.4.10.1 DNA (Gene Codes Corporation, Ann Arbor, MI, USA).

Serum cytokine levels. The levels of interleukin 6 (IL-6), intercellular adhesion molecule 1 (ICAM1), soluble endothelial E-selectin (ESEL), and tumor necrosis factor α (TNFα) in the deep-frozen serum samples that were obtained three hours after admission to the postoperative ICU were measured using flow cytometry bead-based assays using a Becton Dickinson FACSCanto II flow cytometer (BD Biosciences, San Diego, CA, USA) in conjunction with the following assays (CBA; Becton Dickinson Biosciences, San Diego, CA, USA): HU IL-6 CBA Flex Set Bead A7; HU TNF CBA Flex Set C4; HU SCD54 (ICAM1) CBA Flex Set 100TST A4; and HU SOLBL CD62E CBA Flex Set 100 T D9. The samples were acquired and prepared according to the manufacturer's instructions.

Statistical methods. Candidate variables were initially tested in univariate analyses for their association with 30-day mortality with either analysis of variance (ANOVA) test for continuous variables with homogeneous variance, which are reported as means and standard deviation (S.D.); or with Kruskal-Wallis (KW) test, for continuous variables for which homogenous variance was not confirmed using Bartlett's test or when the variable was categorical. The latter types of data are reported as medians and interquartile range (IQR). Continuous risk variables were converted into bipartite variables at the arithmetical middle between the means/medians and were further tested with logistic stepwise backward multiple regressions. To assess the discrimination power of the logistic regression model area under the curve of the receiver operating characteristic (AUC-ROC) was calculated. The analyses of the association with 5-year mortality were performed after conversion of the continuous variables into bipartite variables and comparison of the Kaplan-Meyer survival curves with the log-rank test. Cox regression was used to identify the risk factors that were associated with 5-year survival. The significance level of the α error for all tests was set at $p \leq 0.05$.

Genetic associations. Allelic and genotypic associations with 30-day mortality were analyzed using contingency tables. Due to the unequal proportions of the minor and major allele frequencies, to avoid eventual false negative associations, the less conservative mid-p exact test or the two-tailed Fisher-Exact test was used where appropriate. The type of genetic association: multiplicative, additive, dominant, or recessive, is reported (Clarke *et al.*, 2011). The genetic associations with 5-year mortality were analyzed using the log-rank test and were further adjusted for associations with clinical risk factors. To reject the null hypothesis of no association, a threshold of $p \leq 0.05$ was adopted.

RESULTS

Characteristics of the surgical procedures

Of the 561 patients invited to participate in the INFLACOR study, the operations and genotyping were performed on 525 (100%). Of these, n=518 (98.7%) were included in current analyses. The patient flow and reasons for exclusion are presented in Fig. 1. Analysis of the Kaplan-Meier survival curve of the INFLACOR cohort showed a steep decline within the first 30 postoperative days that is typical in cardiac surgical patients, after which there was a stable decline. This provided the background for the separate analyses of the early and late mortality risk factors (Fig. 2). All patients who were included into the INFLACOR surgical cohort underwent open-heart surgery on cardiopulmonary bypass. However, they could be assigned to one of the six categories of cardiac surgical procedures, which differed significantly with respect to the main disease (aorta disease, heart valve disease, coronary artery disease, other) and pre-operative PT-INR. The surgical procedures differed substantially with regard to duration of the CPB and aorta clamping time as well as regarding the intraoperative blood transfusion and blood lactate levels and the postoperative level of the creatine phosphokinase-MB fraction (CK-MB), which are important risk factors for early and late mortality (Table 2; Zaidi *et al.*, 1999, Speziale *et al.*, 2011). Despite the obvious differences among the charac-

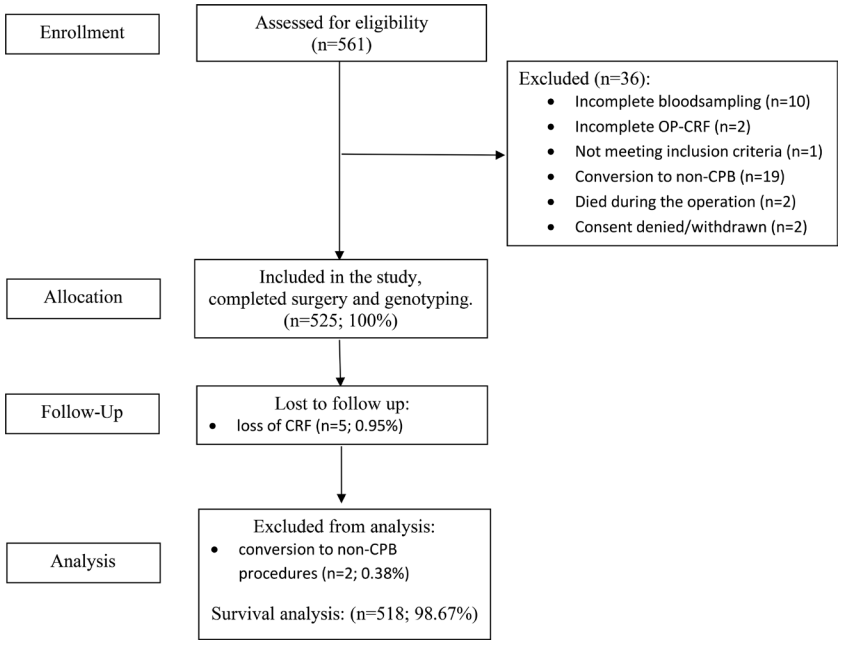

Figure 1. Patient flow diagram of the prospective, observational INFLACOR study.

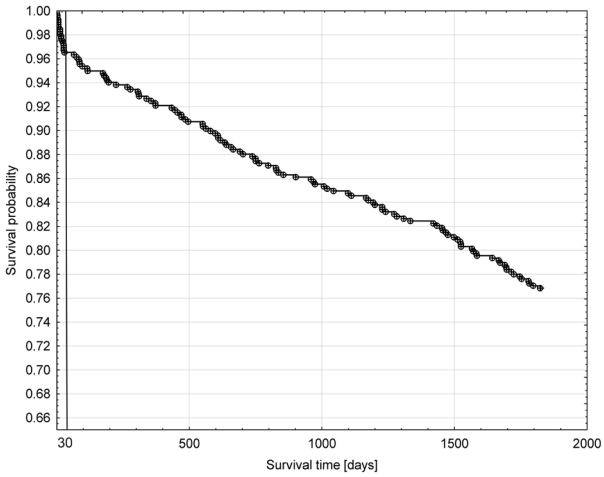

Figure 2. Kaplan-Meier survival curve of the n=518 patients from the INFLACOR cohort.
Legend: 30-day mortality: n=18 (3.5%); 5-year mortality: n=120 (23.2% or ±4.6%/year); decrease in survival was higher within the first 30-days than in the subsequent 5-years.

teristics of the surgical categories, no association with either early (chi-square p=0.59) or late (p=0.085) mortality could be confirmed in our cohort. However, other variables proved to be significant predictors of both early and late mortality.

30-day mortality risk factors

Of the 518 analyzed patients, 18 (3.5%) died within 30 days after the operation. Of the 153 analyzed candidate variables, 39 were shown to be significantly associated with the 30-day mortality in the initial univariate analysis (Suplementary Table E1 at www.actabp.pl). Of these, four remained significant in the final logistic regression model (Table 3). All four variables were either crude laboratory parameters (pre-operative prothrombin time INR, intra-operative blood lactate level, postoperative phosphokinase) or an unequivocal clinical phenotype (AKI on renal replacement therapy). Although the first-day SAPS-II score proved to be significant in the logistic regression with the four other simple variables, it was excluded from the final model as it is a compound variable and it failed to meet the significance threshold after Bonferroni correction of the p-value. The AUC-ROC for the model was 0.885. The prolonged prothrombin time was associated with vitamin K antagonist (VKA) medication (KW p=0.0000) and atrial fibrillation: 33.3% of the patients with chronic, and 8.5% with paroxysmal atrial fibrillation received VKA treatment, but these included 93.2% of all patients receiving VKA. In our cohort, the prolonged PT-INR might be used as a surrogate for advanced chronic heart failure because it correlated closely with higher NYHA classes (p=0.0015).

5-year mortality risk factors

Within the five years of follow-up, n=120 (23.2%) patients died. After a steep decline in survival within the first 30 postoperative days, the subsequent mortality rate was relatively constant at an average of 4.6% per year (range: 2.5 to 5.8% per year). In the initial univariate analysis, 55 variables were significantly associated with 5-year survival in the log-rank test (Suplementary Table E2 at www.actabp.pl). In addition to the assumed definitions of postoperative morbidities, several alternative variables, which were considered to be surrogates for the clinical phenotypes, were identified in the univariate analysis. Specifically, for 'AKI,' the tested parameters were AKI according to RIFLE, AKI requiring RRT, cre-

Table 2. Cardiac surgical procedures characteristics of 518 patients in the INFLACOR cohort.

Procedure	Aorta surgery	Valve surgery				Other
	Sole or with valve/s and/or CABG and/or other	1 valve	1 valve and/or CABG and/or other	2 or 3 valves	2 or 3 valves and/or CABG and/or other	Sole and/or CABG
No. (%)	47 (9.1)	205 (39.6)	156 (30.1)	65 (12.6)	26 (5.0)	19 (3.7)
Characteristics						
Preop PT-INR† (median; IQR)	1.02 (0.96–1.08)	1.03 (0.99–1.10)	1.03 (0.99–1.09)	1.08 (1.01–1.16)	1.08 (1.03–1.18)	1.05 (0.96–1.08)
Operation time* [h] (median; IQR)	4.9 (4.0–5.9)	3.7 (3.2–4.2)	4.6 (3.9–5.3)	4.9 (4.5–5.5)	5.5 (5.0–6.7)	3.3 (3.0–3.9)
Cardiopulmonary bypass* [min] (median; IQR)	155 (130–200)	104 (88–124)	134 (115–161)	168 (145–196)	176 (151–228)	70 (55–101)
Aortic cross-clamp* [min] (median; IQR)	105 (88–121)	68 (58–81)	86 (73–102)	120 (99–142)	125 (100–138)	35 (25–50)
RBCC* [U] (median; IQR)	3 (2-4)	2 (0–3)	2 (1–4)	2 (2–3)	2 (2–2)	1 (0–2)
Lactates* [mmol/L] (median; IQR)	2.0 (1.7–2.7)	1.6 (1.4–1.9)	1.8 (1.4–2.3)	1.8 (1.6–2.1)	2.3 (1.8–2.8)	1.7 (1.3–2.2)
CK-MB* [ng/mL] (median; IQR)	18.3 (13.8–36.4)	19.3 (12.5–29.2)	22.9 (13.4–38.1)	36.0 (28.6–61.0)	40.0 (25.5–52.9)	20.2 (14.7–34.6)

Legend: PT-INR – prothrombin time internationalized ratio. IQR – interquartile range. RBCC – red blood cells concentrate. CK-MB – creatine kinase MB fraction. *KW p<0.0000; †KW p=0.0012. Other procedures: closure of atrial septum defect, excision of myxoma, excision of left ventricle aneurysm, myectomy of left ventricle outflow tract, ablation of atrium/pulmonary veins, resection of left atrium appendage, closure of ventricular septum defect, closure of aorto-atrial fistula, wrapping of ascending aorta, repair of intra-ventricular septum aneurysm, aortic valve annuloplasty, and transposition of pulmonary veins.

Table 3. The logistic regression model of risk factors of 30-day mortality

Variable	aOR	95%C.I.	Coefficient β	S.E.	Z-Statistic	*p*-Value
Pre-operative PT-INR >1.1	5.9	1.8-19.0	1.7694	0.6005	2.9463	0.0032
Intra-operative lactate >2.1 mMol/L	5.3	1.6-17.3	1.6601	0.6085	2.7282	0.0064
Postoperative day 1. phosphokinase >1050 [U/l]	6.2	1.8-20.9	1.8256	0.6201	2.9441	0.0032
AKI requiring RRT	24.3	6.2-95.4	3.1909	0.6977	4.5732	0.0000

Footnote: aOR – adjusted odds ratio; C.I. – confidence interval; S.E. – standard error; PT-INR – prothrombin time internationalized ratio; AKI – acute kidney injury; RRT – renal replacement therapy. Likelihood Ratio: 61.99.

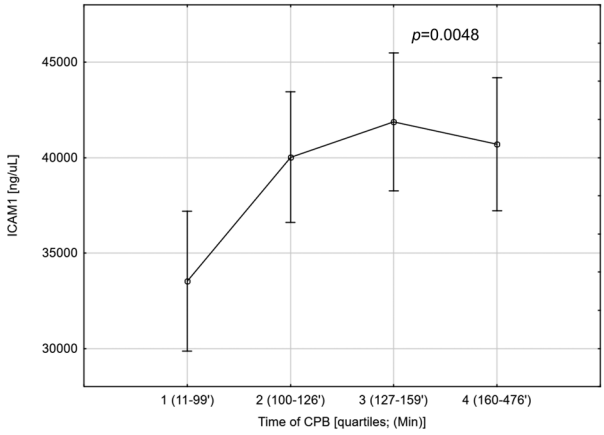

Figure 3. Mean ICAM-1 level and 95% confidence intervals in serum three hours after the operation with regard to the CPB time.

atinine in serum on day 1, and GFR calculated by the CG and MDRD formula. Before inclusion into the Cox multiple regression, such variables were ranked by significance, and only the most significant one was included in the further analysis. One of the variables identified in the univariate Kaplan-Meyer analysis was the ICAM-1 level in serum 3 hours after the operation: patients with levels >35.9 pg/μL had a higher risk of death within the five years after operation (log-rank p=0.026; Cox HR=1.5; 95% CI-1.04–2.15). However, the significance of this factor was lost in the Cox regression analysis. The ICAM-1 level was non-linearly associated with the CPB time (KW p=0.005). It was considerably higher in the second quartile of CPB time (between 100 and 126') than in the first (<100'), increased much less between the second and third quartile, but the mean ICAM-1 level was lower after procedures that showed the longest CPB time (4th quartile) (Fig. 3). Nine variables proved significant after the final iteration: four pre-operative variables associated with heart failure, COPD, and peripheral arterial sclerosis; one intra-operative variable: the transfusion of concentrated red blood cells; and four postoperative variables indicative of: infection, shock, severe AKI and perioperative myocardial infarction. Because one of the pre-operative risk factors – treatment with spironolactone – was very closely associated with the NYHA scale (chi-square p=0.0), only the NYHA was considered in the final model because of its greater significance (Table 4).

Genetic associations

Of the genetic polymorphisms whose association with 30-day mortality was tested, only *ICAM1* rs5498 demonstrated a borderline significance in the less conservative MPE allelic test (p=0.06), which suggested a higher risk

Table 4. Risk factors for 5-year mortality in the Cox regression model.

	HR (95% CI)	p
Pre-operative variables		
Peripheral artery sclerosis	2.1 (1.2–3.6)	0.0114
NYHA >2	2.1 (1.4–3.0)	0.0001
Treatment with two or more broncho-dilators	4.0 (1.9–8.4)	0.0002
Intraoperative variables:		
Transfusion of RBCC >2 U	1.7 (1.2–2.4)	0.0066
Postoperative variables:		
Peripheral hypothermia[1]	1.6 (1.1–2.4)	0.0091
Perioperative myocardial infarction[2]	2.0 (1.2–3.3)	0.0086
Infection with positive culture	2.4 (1.3–4.5)	0.0076
AKI-RRT	7.0 (3.2–15.6)	0.0000

Footnote: HR – hazard ratio; CI – confidence interval; RBCC – red blood cell concentrate; U – unit – ca. 320 mL. 1 – finger-tip temperature <31.0°C for at least three hours. 2 – when either: a) symptoms of acute myocardial infarction (acute chest pain, syncope, nausea), or b) new ECG changes when left ventricle hypertrophia or LBBB absent: 1) ST elevation in min. two leads, or 2) ST decrease and altered T wave, or 3) new LBBB, or c) new segmental impairment of heart wall contractility in echocardiography; were accompanied by rise of phosphokinase MB fraction: women >17, men >36 ng/mL, or troponin I: women >0.045, men >0.11 ng/mL. AKI-RRT – acute kidney injury requiring renal replacement therapy.

of death for carriers of the wild-type A allele (Table 5). The p-values of the allelic and genotypic dominant model log-rank tests were also slightly above the designated statistical threshold for the association with 5-year survival, p=0.06 and p=0.051, respectively (Table 6). The overall genotypic X^2 test for 5-year survival returned a p=0.13, and the X^2 for a linear trend, assuming an additive model, was shown to have p=0.07 and OR=1.14 for the mixed (AG) and 1.69 for the wild (AA) genotypes (Fig. 4). The *ICAM* rs5498 genotypes were adjusted further for associations with the risk variables identified in the Cox regression (Table 7). The *ICAM1* rs5498 genotypes were found to be significantly associated with the pre-operative NYHA class (p=0.03). The postoperative ICAM-1 levels in serum were not associated with the analyzed genotypes after adjustment for the CPB time.

DISCUSSION

The INFLACOR trial is an association and integrative study in which data from various fields of medicine including clinical, biochemical, acute phase proteins, and genetic markers were evaluated for their ability to predict postoperative morbidity and mortality within the first 30

Table 5. Genetic associations with 30-day mortality in n=518 patients of the INFLACOR study.

SNP Survivors *n*=500 Nonsurvivors *n*=18	Alleles		allelic test; *p*-value; RR (95%CI)	Genotypes			type of association	γ	genotypic test; *p*-value; RR (95%CI)
	A (n)	*a* (n)		*AA* (n)	*Aa* (n)	*aa* (n)			
MASP2 rs2273346	T 987 35	C 13 1	FE; 0.39; 2.1 (0.3–14.2)	TT 487 17	TC 13 1	CC 0 0	additive?/ multiplicative?	2.1	FE; 0.32 –
CRP rs1800947	G 912 31	C 88 5	MPE; 0.16; 1.6 (0.7–4.1)	GG 412 13	GC 88 5	CC 0 0	additive?/ multiplicative?	1.8	X^2; 0.27 –
SELE rs1805193	G 887 31	T 113 5	MPE; 0.31; 1.3; (0.5–3.2)	GG 395 13	GT 97 5	TT 8 0	dominant?	1.5	X^2; 0.60; X^2; 0.49; 1.4 (0.5-3.9)
TNF rs1800629	G 854 29	A 146 7	MPE; 0.21; 1.4; (0.6–3.1)	GG 362 12	GA 130 5	AA 8 1	recessive	3.2	X^2; 0.43; FE; 0.27; 3.3 (0.5-22.4)
IL6 rs1800796	G 943 32	C 57 4	FE; 0.16; 2.0; (0.7–5.5)	GG 447 15	GC 49 2	CC 4 1	recessive	5.6	X^2; 0.12; FE; 0.16;6.0 (0.98-37.0)
NOS3 rs1799983	G 673 25	T 327 11	MPE; 0.40; 0.91 (0.5–1.8)	GG 225 9	GT 223 7	TT 52 2	none	1.0	X^2; 0.89 –
TLR4 rs4986790	A 939 33	G 61 3	FE; 0.48; 1.4; (0.4–4.4)	AA 440 15	AG 59 3	GG 1 0	dominant?	1.5	X^2; 0.81; FE; 0.47; 1.4 (0.4-4.9)
NOD2 rs2066844	C 967 36	T 33 –	FE; 0.63; –	CC 467 18	CT 33 0	TT 0 0	additive?/ multiplicative?	3.7	FE; 0.62 –
ICAM1 rs5498	A 560 25	G 440 11	MPE; 0.06; 0.6 (0.3–1.1)	AA 153 8	AG 254 9	GG 93 1	additive	0.5	X^2; 0.26; trend X^2; 0.07
LBP1 rs2232582	T 862 30	C 138 6	MPE; 0.30; 1.2 (0.5–2.9)	TT 374 13	TC 114 4	CC 12 1	recessive	2.3	X^2; 0.70; FE; 0.37; 2.3 (0.3-15.9)

Footnote: The data and tests of allelic and genotypic associations are provided. The allele numbers are doubled, because any individual had two alleles of the genotyped SNP in his/her genome. *A* – major allele; *a* – minor allele. Genotypes: *AA* – homozygous major, *Aa* – heterozygous, *aa* – homozygous minor. Statistical tests: MPE – mid-*p* exact for 2x2 table in the allelic analysis; FE – Fisher-Exact test for 2x2 table; RR – risk ratio, CI – confidence interval. γ – genetic penetrance parameter – the factor, by which the penetrance of the disease (in this study the 30-day mortality) changes with regard to the genotypes.

days and 5 years after cardiac surgical treatment. In this study, we documented various risk factors for early and late mortality after cardiac surgery. Because of the relatively low early mortality, the numbers in the INFLACOR cohort were too low to demonstrate differences among the types of surgery. Of the 55 pre-operative candidate variables, four demonstrated significant association with 30-day mortality in the univariate analysis, but, unexpectedly, only one remained in the final multiple regression model: the pre-operative prothrombin time international normalized ratio (PT-INR). In our patients, the prolonged PT characterized patients with atrial fibrillation and severe chronic circulatory failure. Additionally, only one intraoperative variable, an elevated blood lactate level, increased the risk of death within the first postoperative month by approximately five-fold.

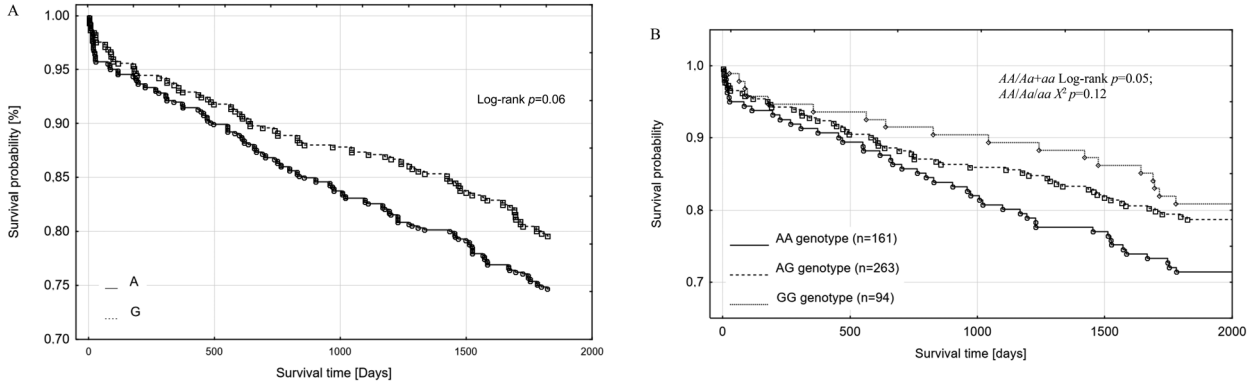

Figure 4. The 5-year Kaplan-Meier survival curves for the alleles and genotypes of *ICAM1* rs5498 in n=518 patients in the INFLACOR trial – panel A – alleles, panel B – genotypes.

Table 6. Genetic associations with 5-year mortality in n=518 patients of the INFLACOR study.

SNP Survivors *n*=398 Nonsurvivors *n*=120	Alleles		allelic test; *p*-value; RR (95%CI)	allelic log-rank test *p*-value	Genotypes			type of association	γ	genotypic test; *p*-value; RR (95%CI)	genotypic log-rank test *p*-value (genotypes)
	A (n)	*A* (n)			*AA* (n)	*Aa* (n)	*Aa* (n)				
MASP2 rs2273346	T 783 239	C 13 1	FE; 0.21; 0.3 (0.05–2.0)	0.17	TT 385 119	TC 13 1	CC 0 0	multiplicative?/ additive?/ dominant?	0.3	FE; 0.21; 0.3 (0.05–2.0)	0.17 (*AA* / *Aa*)
CRP rs1800947	G 729 214	C 67 26	MPE; 0.13; 1.2 (0.9-–1.7)	0.26	GG 331 94	GC 67 26	CC 0 0	additive?/ recessive?	1.3	FE; 0.22; 1.3 (0.9–1.8)	0.23 (*AA* / *Aa*)
SELE rs1805193	G 709 209	T 87 31	MPE; 0.20; 1.2 (0.8–1.6)	0.42	GG 317 91	GT 75 27	TT 6 2	dominant?	1.2	FE; 0.37; 1.2 (0.8–1.7)	0.38 (*AA* / *Aa*+*aa*)
TNF rs1800629	G 676 207	A 120 33	MPE; 0.31; 0.9 (0.7–1.2)	0.69	GG 284 90	GA 108 27	AA 6 3	recessive?	1.5	FE; 0.44; 1.5 (0.6–3.7)	0.37 (*AA*+*Aa* / *aa*)
IL6 rs1800796	G 751 224	C 45 16	MPE; 0,28; 1.1 (0.7–1.8)	0.52	GG 357 105	GC 37 14	CC 4 1	additive?/ dominant?/ multiplicative?	1.2?	FE; 0.50; 1.2 (0.7–1.9)	0.48 (*AA* / *Aa*+*aa*)
NOS3 rs1799983	G 534 164	T 262 76	MPE; 0.36; 1.0 (0.8–1.2)	0.68	GG 176 58	GT 182 48	TT 40 14	undetermined/ none?	≈ 1	X^2; 0.53 –	0.57 (*AA* / *Aa* / *aa*)
TLR4 rs4986790	A 751 221	G 45 19	MPE; 0.11; 1.3 (0.9–1.9)	0.19	AA 353 102	AG 45 17	GG 0 1	additive?/ multiplicative?	1.2	FE; 0.27 1.2 (0.8–1.9)	0.34 (*AA* / *Aa*+*aa*)
NOD2 rs2066844	C 771 232	T 25 8	MPE; 0.43; 1.0 (0.6–1.9)	0.88	CC 373 112	CT 25 8	TT 0 0	undetermined/ none?	≈ 1	FE; 0.83 –	0.89 (*AA* / *Aa*)
ICAM1 rs5498	A 437 148	G 359 92	MPE; 0.03; 0.8 (0.6–1.01)	0.06	AA 115 46	AG 207 56	GG 76 18	dominant?	0,8	FE; 0.06 0.7 (0.5–0.99)	0.051 (*AA* / *Aa*+*aa*)
LBP1 rs2232582	T 685 207	C 111 33	MPE; 0.48; 1.0 (0.7–1.4)	0.94	TT 296 91	TC 93 25	CC 9 4	recessive?	1.4	FE; 0.51 1.3 (0.6–3.1)	0.46 (*AA*+*Aa* / *aa*)

Footnote: The data and tests for allelic and genotypic associations are provided. The allele numbers are doubled, because any analyzed individual had two alleles of the genotyped SNP in his/her genome. *A* – major allele; *a* – minor allele. Genotypes: *AA* – homozygous major, *Aa* – heterozygous, *aa* – homozygous minor. All the analyses treated the minor allele as the 'risk'-allele. Statistical tests: MPE – mid-*p* exact test; FE – Fisher-Exact test for 2x2 table; RR – risk ratio, CI – 95% confidence interval. γ – genetic penetrance parameter - the factor, by which the penetrance of the disease (in this analysis the 5-year mortality) changes with regard to the genotypes. The type of association was estimated by analysis of mortality in 3x2 contingency tables and determined the merging of data for the 2x2 genotypic test: additive/multiplicative – *aa* / *AA* or if *aa* not available *aA* / *AA*; dominant – *aa* + *aA* / *AA*; recessive – *aa* / *aA* + *AA*.

Table 7. Adjustment of *ICAM* rs5498 genotypes against the risk variables for 5-year mortality.

Risk variables for 5-year mortality	Genotype prevalence [%] (AA / AG / GG)	chi-square *p*	Trend analysis *p*; OR*
Peripheral artery sclerosis	8.1 / 6.8 / 4.3	0.50	0.26
NYHA >2	50.3 / 41.1 / 34.0	0.031	0.0086; 1.0/1.35/1.96
Treatment with two or more bronchodilators	3.7 / 3.0 / 1.1	0.46	0.25
Transfusion of RBCC >2 U	36.7 / 39.9 / 31.9	0.37	0.61
Peripheral hypothermia	42.9 / 44.5 / 44.7	0.94	0.75
Perioperative myocardial infarction	9.9 / 8.0 / 6.4	0.59	0.31
Infection with positive culture	5.6 / 5.7 / 7.5	0.80	0.59
AKI-RRT	4.4 / 1.1 / 3.2	0.11	0.35

Legend: * OR – odds ratios calculated against the basal odd for the wild AA genotype are provided only when the trend was significant ($p \leq 0.05$).

This finding corresponds with a recent Brazilian study, which identified the same variable in association with early mortality after CPB cardiac surgery (Hajjar *et al.*, 2013). The two postoperative variables associated with 30-day mortality, elevated creatine phosphokinase, which is indicative of peri-operative MI (Zaidi *et al.*, 1999; Thygesen *et al.*, 2007), and acute kidney failure with renal replacement therapy (Kowalik *et al.*, 2011), are already well-known risk factors for early postoperative mortality after cardiac surgery.

Other variables were associated with greater risk of death within the first five years after a successful cardiac surgery. Whereas peripheral arteriosclerosis and chronic heart failure increased the risk of death two-fold, severe chronic obstructive pulmonary disease increased it four-fold, which was similar to the data reported by others (Speziale *et* al., 2011; Nicolini *et al.*, 2016). Interestingly, of the intraoperative variables, only greater blood transfusion increased the risk of death by approximately 70% within the first 5 years. We also identified four postoperative morbidities that were important long-term mortality predictors: shock, myocardial infarction, sepsis, and severe acute kidney injury, all of which are well-known risk factors for postoperative mortality in cardiac surgical patients (Gummert *et al.*, 2004; Speziale *et al.*, 2011). These data suggest that any possible genetic association should be evaluated in the pathophysiological context of the identified pre- or postoperative morbidities. It is noteworthy, that risks of early and late mortality in cardiac surgery, are both strongly influenced not only by patients' preoperative status, but also by perioperative variables and postoperative morbidities.

Of the analyzed cytokines and adhesion molecules, only IL-6 and ICAM1 were found in univariate analysis to be associated with 30-day and 5-year mortality, respectively. High ICAM1 level in serum was shown to be a stable risk variable up to the final iterations in the Cox regression, but none of these novel markers proved to be useful in the final regression models for 30-day mortality or for the 5-year mortality prediction. Although the ICAM-1 levels in the INFLACOR patients 3 hours post-surgery depended on the duration of CPB, they were not associated with the analyzed *ICAM1* rs5498 genotypes, even after adjustment for the CPB duration. However, while one study confirmed an association between rs5498 and the soluble ICAM-1 levels in healthy women (Bielinski *et al.*, 2011), another provided evidence that ICAM-1 levels in serum are also determined by an interplay among the *NFKBIK*, *PNPLA3*, *RELA*, and *SH2B3* genetic loci (Pare *et al.*, 2011).

Of the ten genetic variants analyzed in the INFLACOR cohort, only *ICAM1* rs5498 was shown to have a borderline significance for the association with both the 30-day and 5-year mortality, with the wild A allele being the risk allele. Patients with both copies of the minor G allele had a lower risk of death than patients with one copy, and the risk for those with the mixed genotype was lower than for those with two copies of the wild A allele. The minor allele was also weakly associated with a lower prevalence of peripheral arteriosclerosis in the INFLACOR cohort. ICAM-1 is a cell surface glycoprotein that belongs to the superfamily of adhesion immunoglobulins. This molecule has five extracellular domains and is typically expressed on endothelial cells and cells of the immune system (Anbarasan *et al.*, 2015). It is crucially involved in neutrophil migration into tissues during acute inflammation and in arteriosclerotic plaque formation (Lyck & Enzmann, 2015). It is broadly accepted to be a marker of endothelial injury, a process that is considered to be an undesirable side-effect of the use of cardiopulmonary bypass in cardiac surgery. The rs5498 A>G SNP studied in the INFLACOR trial is a missense upstream variant in the Exon 6 region and is one of the seven known functional polymorphisms of the *ICAM1* gene. This variant causes the Lys469Glu substitution in domain 5 (Anbarasan *et al.*, 2015). The *ICAM1* rs5498 SNP was extensively examined in multiple studies for associations with several cardiovascular phenotypes with conflicting results regarding the risk allele. Whereas some studies and meta-analyses found that the major A allele was associated with MI (Hu *et al.*, 2017), diabetic microvascular complications and diabetic nephropathy (Su *et al.*, 2013); others found that the minor G allele was the risk allele for coronary artery disease (Luo *et* al., 2014), for postoperative MI in a cohort of 434 patients undergoing CABG surgery (Podgoreanu *et al.*, 2006), and for an increased risk of MI in smokers (Sarecka-Hujar *et al.*, 2009), and that it was associated with higher levels of serum ICAM1 in white non-surgical patients (Bielinski *et al.*, 2011).

Unfortunately, the genetic analyses in this study failed to confirm any genetic association at the defined level of statistical significance. There are three possible reasons for this. First, the INFLACOR cohort was non-homogeneous with regard to the surgical interventions, in contrast to other studies – i.e., the PEGASUS trial, in which a cohort of only CABG patients was studied. On the one hand, this would seem to require adjustment for the different surgical categories in the genetic analyses, but on the other, the type of surgical procedure was not a determining factor for either early or late postoperative mortality. This is an important observation that confirms our initial assumption that pooling different cardiac surgical procedures including aortic valve replacement with mitral valve replacement and/or with coronary surgery was reasonable. CPB time was not found to be a stable variable in the analyses for either the short- or long-term survival. Second, we initially assumed that the chosen genotypic variants that were shown to be associated with postoperative morbidities including acute kidney injury, sepsis, atrial fibrillation, and myocardial infarction would also remain associated with postoperative mortality. This assumption could not be proven, most likely due to the relatively small number of patients in our sample. However, our study confirmed four postoperative morbidities as important predictors of 5-year survival: shock, sepsis, myocardial infarction, and AKI-RRT. Additional genetic analyses using these morbidities as the outcome measures appear reasonable (Chanock & Manolio, 2007; Clarke *et al.*, 2011). Third, we assumed that the SNVs selected for this study were 'functional' genetic variants. However, of the SNPs studied in the INFLACOR study, currently, only *ICAM1* rs5498 (Pare *et* al., 2011; Anbarasan *et al.*, 2015) and *TNF* rs1800629 (Lee *et al.*, 2012*)* were verified using GWAS. It therefore remains possible that the other analyzed SNPs are not the functional genetic variants. The INFLACOR study has also several other important limitations. It does not fully comply with the STREGA guidelines, particularly with regard to replication of source studies, rationale for the choice of genes and variants, treatment effects in studying quantitative traits, relatedness, and volume of the data (Little J *et al.*, 2009). Nevertheless, the suggested associations between *ICAM1* rs5498 variants, a history of peripheral arterial sclerosis, and postoperative 5-year survival might be true, as ICAM-1 molecule plays an important role in the pathogenesis of arterial sclerosis (Lyck & Enzmann, 2015). The positive aspects of this study include low re-

fusal rate of participants and the good representation of adult patients undergoing cardiac surgery in the INFLACOR sample and quality of genotyping, reflected by remaining of all the results in HWE.

Despite the listed shortcomings, the current analyses provided useful information on clinical risk factors for short- and long-term mortality after mixed cardiac surgical procedures. This study highlights how the risk of early and late death is the result of complex interplay between patient's related factors – inherited and acquired, with intraoperative variables, and postoperative morbidity in cardiac surgical patients operated on cardiopulmonary bypass. These data may be useful for assessment of perioperative risk in cardiac surgery, for planning risk reduction strategies, and future genetic association studies. As adjusting for clinical data in genetic association studies is nowadays pivotal, future genetic analyses should account for the currently evaluated risk factors.

CONCLUSIONS

The INFLACOR study demonstrated four significant risk factors for 30-day mortality after various types of cardiac surgical procedures that were performed under cardiopulmonary bypass conditions: pre-operative prothrombin time INR, intraoperative blood lactate, postoperative creatine phosphokinase-MB fraction, and acute kidney failure that required renal replacement therapy. The risk factors for 5-year mortality were as follows: a history of peripheral arterial sclerosis, severe COPD, and advanced heart failure; intraoperative red blood cell transfusion; and postoperative shock, sepsis, myocardial infarction, and acute renal failure that required renal replacement therapy. The serum level of IL-6 three hours after the operation was associated with 30-day mortality, and the serum level of ICAM-1 was associated with 5-year mortality in univariate analyses. Our data also suggested a better 5-year survival after cardiac surgery performed under CPB for carriers of the minor A allele of the *ICAM1* rs5498.

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REFERENCES

Anbarasan C, Bavanilatha M, Latchumanadhas K, Mullasari SA (2015) ICAM-1 molecular mechanism and genome wide SNP's association studies. *Indian Heart J* **67**: 282–287. doi: 10.1016/j.ihj.2015.03.005

Bielinski SJ, Reiner AP, Nickerson D, Carlson C, Bailey KR, Thyagrajan B, Lange LA, Boerwinkle EA, Jacobs DR, Gross M (2011) Polymorphisms in the *ICAM1* gene predict circulating soluble intercellular adhesion molecule-1 (sICAM-1) *Atherosclerosis* **216**: 390–394. doi: 10.1016/j.atherosclerosis.2011.02.018

Chanock SJ, Manolio T (2007) Replicating genotype-phenotype associations. *Nature* **447**: 655–660

Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT (2011) Basic statistical analysis in genetic case-control studies *Nat Prot* **6**: 121–133. doi: 10.1038/nprot.2010.182

Geissler HJ, Hölzl P, Marohl S, Kuhn-Regnier F, Mehlhorn U, Südkamp M, de Vivie ER (2000) Risk stratification in heart surgery: comparison of six score systems. *Eur J Cardiothorac Surg* **17**: 400–406.

Grocott HP (2006) Genetic influences on cerebral outcome after cardiac surgery. *Sem Cardiothor Vasc Anesth* **10**: 291–296. doi: 10.1177/1089253206294344

Gummert JF, Bucerius J, Walther T, Doll N, Falk V, Schmitt DV, Mohr FW (2004) Requirement for renal replacement therapy in patients undergoing cardiac surgery. *Thorac Cardiovasc Surg* **52**: 70–76. doi: 10.1055/s-2004-817806

Hajjar LA, Almeida JP, Fukushima JT, Rhodes A, Vincent J-L, Osawa EA, Galas RBG (2013) High lactate levels are predictors of major complications after cardiac surgery. *J Thor Cardivasc Surg* **146**: 450–460. doi:10.1016/j.jtcvs.2013.02.003

Henckaerts L, Nielsen KR, Steffensen R, Van Steen K, Mathieu C, Giulietti A, Wouters PJ, Milants I, Vanhorebeek I, Langouche L, Vermeire S, Rutgeerts P, Thiel S, Wilmer A, Hansen TK, Van den Berghe G (2009) Polymorphisms in innate immunity genes predispose to bacteremia and death in the medical intensive care unit. *Crit Care Med* **37**: 192–201. doi: 10.1097/CCM.0b013e31819263dB

Hu P, Dai T, Yu W, Luo Y, Huang S (2017) Intercellular adhesion molecule 1 rs5498 polymorphism is associated with the risk of myocardial infarction. *Oncotarget* **32**: 52594–52603. doi: 10.18632/oncotarget.17529.

Kowalik MM, Lango R, Klajbor K, Musial-Świątkiewicz V, Kołaczkowska M, Pawlaczyk R, Rogowski J (2011) Incidence, mortality and mortality related risk factors of acute kidney injury requiring hemofiltration treatment in patients undergoing cardiac surgery – a single centre 6 year experience. *J Cardiothorac Vasc Anesth* **25**: 619–624. doi: 10.1053/j.jvca.2010.12.011

Kowalik MM, Lango R (2014) Genotype assessment as a tool for improved risk prediction in cardiac surgery. *J Cardiothorac Vasc Anesth* **28**: 163–168. doi: 10.1053/j.jvca.2013.01.002

Kraft P, Hunter DJ (2009) Genetic risk prediction – are we there yet? *N Engl J Med* **360**: 1701–1703: doi: 10.1056/NEJMp0810107

Laffey JG, Boylan JF, Cheng DCH (2002) The systemic inflammatory response to cardiac surgery. *Anesthesiology* **97**: 215–252

Lee YH, Bae SC, Choi SJ, Ji JD, Song GG (2012) Genome-wide pathway analysis of genome-wide association studies on systemic lupus erythematosus and rheumatoid arthritis. *Mol Biol Rep* **39**: 10627–10635. doi: 10.1007/s11033-012-1952-x

Little J, Higgins JPT, Ioannidis JPA, Moher D, Gagnon F, von Elm E, Khoury MJ, Cohen B, Davey-Smith G, Grimshaw J, Scheet P, Gwinn M, Williamson RE, Zou GY, Hutchings K, Johnson CY, Tait V, Wiens M, Golding J, van Duijn C, McLaughlin J, Paterson A, Wells G, Fortier I, Freedman M, Zecevic M, King R, Infante-Rivard C, Stewart A, Birkett N. STrenghtening the Reporting of Genetic Association Studies (STREGA) – an extension of the STROBE statement. *PLos Med* **6**: e1000022. doi: 10.1371/journal.pmed.1000022

Luo JY, Ma YT, Xie X, Yang YN, Li XM, Ma X, Yu Z, Chen BD, Liu F (2014) Association of intercellular adhesion molecule-1 gene polymorphism with coronary artery disease. *Mol Med Rep* **10**: 1343–1348. doi: 10.3892/mmr.2014.2360

Lyck R & Enzmann G (2014) The physiological roles of ICAM-1 and ICAM-2 in neutrophil migration into tissues. *Curr Opin Hematol* **22**: 53–59. doi: 10.1097/MOH.0000000000000103

Mathew JP, Podgoreanu MV, Grocott HP, White WD, Morris RW, Stafford-Smith M, Mackensen GB, Rinder CS, Blumenthal JA, Schwinn DA, Newman MF (2007) Genetic variants in P-selectin and C-reactive protein influence susceptibility to cognitive decline after cardiac surgery. *J Am Coll Cardiol* **49**: 1934–1942. doi: 10.1016/j.jacc.2007.01.080

Meyer NJ, Garcia JG (2007) Wading into the genomic pool to unravel acute lung injury genetics. *Proc Am Thorac Soc* **4**: 69–76. doi: 10.1513/pats.200609-157JG

Newman MF, Mathew JP, Grocot HP, Mckensen GB, Monk T, Welsh-Bohmer KA, Blumenthal JA, Laskowitz DT, Mark DB (2006) Central nervous system injury associated with cardiac surgery. *Lancet* **368**: 694–703

Nicolini F, Fortuna D, Contini GA, Pacini D, Gabbieri D, De Palma R, Gherli T (2016) Long-term outcomes of conventional aortic valve replacement in high-risk patients: where do we stand? *Ann Thorac Cardiovasc Surg* **22**: 304–311. doi: 10.5761/atcs.oa.16-00165

O'Donnell CJ & Nabel EG (2011) Genomics of cardiovascular disease. *N Engl J Med* **365**: 2098–2109. doi: 10.1056/NEJMra1105239

Pare G, Ridker PM, Rose L, Barbalic M, Dupuis J, Dehghan A, Bis JC, Benjamin EJ, Shiffman D, Parker AN, Chasman DI (2011) Genome-wide association analysis of soluble ICAM-1 concentration reveals novel association at the *NFKBIK*, *PNPLA3*, *RELA*

and *SH2B3* loci. *PLoS Genet* **7**: e1001374. doi: 10.1371/journal.pgen.1001374

Podgoreanu MV, Schwinn DA (2005) New paradigms in cardiovascular medicine. *J Am Coll Cardiol* **46**: 1965–1977. doi: 10.1016/j.jacc.2005.08.040

Podgoreanu MV, White WD, Morris RW, Mathew JP, Stafford-Smith M, Welsby IJ, Grocott HP, Milano CA, Newman MF, Schwinn DA (2006) Inflammatory gene polymorphisms and risk of postoperative myocardial infarction after cardiac surgery. *Circulation* **114**: I275–I281

Sarecka-Hujar B, Zak I, Krauze J (2009) Interactions between rs5498 polymorphism in the *ICAM1* gene and traditional risk factors influence susceptibility to coronary artery disease. *Clin Exp Med* **9**: 117–124. doi: 10.1007/s10238-008-0022-0

Speziale G, Nasso G, Barattoni MC, Esposito G, Popoff G, Argano V, Greco E, Scorcin M, Zussa C, Cristell D, Coppola R, Chierchia S, Marchese A, Caldarola P, Fattouch K, Tavazzi L (2011) Short-term and long-term results of cardiac surgery in elderly and very elderly patients. *J Thorac Cardiovasc Surg* **141**: 725–731. doi: 10.1016/j.jtcvs.2010.05.010

Stafford-Smith M, Podgoreanu MV, Swaminathan M, Phillips-Bute B, Mathew JP, Hauser EH, Winn MP, Milano C, Nielsen DM, Smith M, Morris R, Newman MF, Schwinn DA (2005) Association of genetic polymorphisms with risk of renal injury after coronary bypass graft surgery. *Am J Kidney Dis* **45**: 519–530

Stüber F, Hoeft A (2002) The influence of genomics on outcome after cardiovascular surgery. *Curr Opin Anaesthesiol* **15**: 3–8

Su X, Chen X, Liu L, Chang X, Yu X, Sun K (2013) Intracellular adhesion molecule – 1 K469E gene polymorphism and risk of diabetic microvascular complications: a meta-analysis. *PLoS ONE* **8**: e69940. doi: 10.1371/journal.pone.0069940

Thygesen K, Alpert JS, White HD. (2007) Universal Definition of Myocardial Infarction. *Circulation* **116**: 2634–2653. doi: 10.1161/CIRCULATIONAHA.107.187397

Warren OJ, Smith AJ, Alexiou C, Rogers PLB, Jawad N, Vincent C, Darzi AW, Athanasiou T (2009) The inflammatory response to cardiopulmonary bypass: part 1 – mechanisms of pathogenesis. *J Cardiothorac Vasc Anesth* **23**: 223–231

Welsby IJ, Podgoreanu MV, Phillips-Bute B, Mathew JP, Smith PK, Newman MF, Schwinn DA, Stafford-Smith M (2005) Genetic factors contribute to bleeding after cardiac surgery. *J Thromb Haemost* **3**: 1206–1212

Yende S, Quasney MW, Tolley E, Zhang Q, Wunderink RG (2003) Association of tumor necrosis factor gene polymorphisms and prolonged mechanical ventilation after coronary artery bypass surgery. *Crit Care Med* **31**: 133–140. doi: 10.1097/01.CCM.000004507.15614.D2

Zaidi AM, Fitzpatrick AP, Keenan DJM, Odom NJ, Grotte GJ (1999) Good outcomes from cardiac surgery in the over 70s. *Heart* **82**: 134–137

Zeller T, Blankenberg S, Diemert P (2012) Genomwide association studies in cardiovascular disease – an update 2011. *Clin Chem* **58**: 92–103. doi: 10.1373/clinchem.2011.170431