Gene polymorphisms and cytokine plasma levels as predictive factors of complications after cardiopulmonary bypass

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Objective: Cardiopulmonary bypass remains associated with significant morbidity and mortality, in part caused by a systemic inflammatory response that is unpredictable and variable among patients. Several limited studies have suggested associations of cytokine plasma levels or gene polymorphisms with outcome after cardiopulmonary bypass. The present study was to determine the relationships between several circulating cytokines and their polymorphisms (single nucleotide polymorphisms), and the occurrence of postoperative clinical events in patients who underwent coronary artery bypass grafting under cardiopulmonary bypass.

Methods: Patients were genotyped for single nucleotide polymorphisms of *LTA* (Cys13Arg, +252A>G), *TNF* (-308G>A), *IL6* (-597G>A, -572G>C, -174G>C), *IL10* (-592C>A, c.*117C>T), and *APOE* (Cys112Arg, Arg158Cys). Serum samples were collected preoperatively, immediately after cardiopulmonary bypass, and at different postoperative time points to measure cytokine serum levels by enzyme-linked immunosorbent assay. The clinical end point was the composite of postoperative death, low cardiac output syndrome, myocardial infarction, sepsis, and acute renal insufficiency.

Results: Single nucleotide polymorphisms *IL6*-572GC+CC/*IL10*-592CC were associated with the clinical end point ($P = .032$ and $P = .009$, respectively). In addition to preoperative clinical conditions, the other factor associated with the clinical end point was interleukin-10 plasma levels 24 hours after surgery ($P = .017$). On the basis of these results, a predictive model of postoperative complications after coronary artery bypass grafting was created.

Conclusions: Our data suggest that focused genetic testing of the *IL6*-572G>C and *IL10*-592C>A single nucleotide polymorphisms might be a tool for identifying patients at the highest risk of poor tolerance to the inflammatory response to cardiopulmonary bypass and for implementing strategies to mitigate it, provided the generalization of these tests makes them reasonably affordable and thus favorably shifts their cost-to-benefit ratio. (J Thorac Cardiovasc Surg 2012;144:467-73)

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Despite many improvements over these last decades, cardiopulmonary bypass (CPB) still induces an acute inflammatory response associated with significant morbidity and mortality.¹ Disruption in the balance between pro- and anti-inflammatory cytokine levels has been suggested to be a key factor of a systemic inflammatory response syndrome (SIRS), resulting in organ dysfunction affecting the heart, brain, lungs, and kidneys. However, the intensity of this inflammatory response is variable among patients and remains unpredictable.

Unpredictability of the inflammatory response may be explained by genetic variability. Most studied polymorphisms are single nucleotide polymorphisms (SNPs). An SNP is a genetic variation that occurs when a nucleotide replaces another one, present in more than 1% of the population. Most commonly, these variations are found in the DNA between genes. When SNPs occur within a gene or in a regulatory region near a gene, they may play a role in disease by affecting the gene function. SNPs located in gene promoters can result in an upregulation or downregulation of the gene expression and then an increase or decrease of the protein level. Coding SNPs, which lead to an amino acid substitution, can affect the protein function.

Abbreviations and Acronyms

ARI	= acute renal insufficiency
CABG	= coronary artery bypass grafting
CE	= clinical end point
COPD	= chronic obstructive pulmonary disease
CPB	= cardiopulmonary bypass
IL	= interleukin
ITA	= internal thoracic artery
LCOS	= low cardiac output syndrome
LVEF	= left ventricular ejection fraction
MI	= myocardial infarction
SIRS	= systemic inflammatory response syndrome
SNP	= single nucleotide polymorphism
TNF	= tumor necrosis factor

Several studies have shown associations between cytokine plasma levels or gene polymorphisms and clinical outcome after CPB, but these studies often focused on a single cytokine²⁻⁵ or one clinical complication.⁶ In an attempt to overcome these limitations, we carried out a prospective study on a cohort of patients undergoing coronary artery bypass grafting (CABG) under CPB and evaluated the impact of gene polymorphisms encoding proinflammatory (interleukin [IL]-6 and tumor necrosis factor [TNF]- α) and anti-inflammatory (IL10) cytokines, as well as the corresponding plasma levels, on postoperative complications. The overall objective was to assess whether a focused genetic screening could help identify patients at high risk of developing post-CABG inflammatory complications.

MATERIALS AND METHODS

Patient Population

A single-center prospective study was carried out in a cohort of 126 patients undergoing CABG under CPB. This study was approved by our local ethics committee. All patients provided informed consent. All patients aged more than 20 years, referred for CABG under CPB and cardiac arrest, were included. Exclusion criteria were age greater than 80 years, emergency surgery, associated surgical procedures, left ventricular ejection fraction (LVEF) less than 30%, nonsteroidal anti-inflammatory drug or corticosteroid use, preoperative dialysis, redo-surgery, single-vessel disease, hepatic insufficiency, and current infection. Chronic renal insufficiency and preoperative symptomatic cardiac insufficiency, defined by the association of LVEF less than 50% and dyspnea New York Heart Association class III/IV or recent hospitalization requirement for pulmonary edema, were not exclusion criteria.

Surgical Procedure

Patients were operated via a midline sternotomy, and CPB was performed under normothermia between 34°C and 37°C. Cardiac arrest was obtained by normothermic, intermittent antegrade or continuous retrograde, whole blood cardioplegia. All patients underwent the implantation of an internal thoracic artery (ITA) on the left descending coronary artery.

Additional bypasses were done with the use of ITA, radial artery, or saphenous vein conduits.

Definitions of the Clinical End Point

The clinical end point (CE) included postoperative death from any cause, myocardial infarction (MI), low cardiac output syndrome (LCOS), stroke, acute renal insufficiency (ARI), or sepsis. Death was reported if it occurred within 30 days or at any time during hospital admission. MI was defined as typical electrical changes on postoperative electrocardiogram or abnormal changes in wall motion on echocardiography and postoperative cardiac troponin I plasma levels greater than 15 $\mu\text{g/L}$ at any time after surgery. LCOS was defined by the requirement for mechanical assistance or prolonged inotropic support (epinephrine or dobutamine $> 5 \mu\text{g/kg/min}$ for > 30 minutes in the intensive care unit) to maintain systolic blood pressure greater than 90 mm Hg or cardiac index greater than 2.2 L/m^2 . Stroke was reported if any clinical suspicion of cerebral ischemic symptoms lasting more than 24 hours was confirmed by computed tomography. Acute renal insufficiency was defined as plasma creatinine levels greater than 200 $\mu\text{mol/L}$ or, particularly for patients having a baseline plasma creatinine level greater than 150 $\mu\text{mol/L}$, the requirement for hemodialysis at any time after surgery. Sepsis encompassed several components (pneumonia, pyelonephritis, mediastinitis, or superficial wound infection).

Cytokine Measurements

IL10 and IL6 plasma levels were measured by ELISA (Diaclone, Besançon, France). An ultrasensitive TNF α ELISA (R&D Systems, Lille, France) was used for TNF α measurement as previously described.⁷ Assays were performed at 3 time points: before anesthesia, at the end of CPB (IL10) or 4 hours thereafter (IL6 and TNF α), and 24 hours after CPB. The end-CPB and 4-hour post-CPB study points were selected on the basis of previous studies showing that they correspond to peak levels for IL10 or IL6 and TNF α , respectively.^{7,8}

Selection of Candidate Genes and Polymorphisms

Five candidate genes involved in the pathogenesis of inflammation were selected on the basis of pathway analysis and a review of association studies reported in the literature.^{2,3,9-11} The objective was not to capture a high proportion of genetic information at each locus, but rather to concentrate on SNPs previously associated with the trait, with an emphasis on their minor allele frequency ($\geq 5\%$ except for one), or those previously shown to be functional.^{4,12-14} The 10 selected SNPs were *IL10*-592C>A, *c.*117C>T*, *IL6*-597G>A, -572G>C, -174G>C, *TNF*-308G>A, *LTA*+252A>G, Cys13Arg, and the 2 polymorphisms determining $\epsilon 2$, 3, and 4 alleles of the *APOE* gene (Table E1). This limited number of SNPs also had the advantage of not weakening the power of the statistical analysis by testing many associations.

Genomic DNA Isolation and Genotype Analysis

Genomic DNA was isolated from peripheral blood leukocytes, collected preoperatively from blood samples, by proteinase K digestion and saline extraction. *TNF*-308G>A and *LTA*+252A>G were genotyped by polymerase chain reaction-restriction fragment length polymorphism with *NcoI* restriction enzyme (Promega Corp, Madison, Wis) according to the manufacturer's instructions. Other SNPs were genotyped by direct sequencing using kits and run on a 3730 DNA analyzer (Applied Biosystems, Carlsbad, Calif), and DNA sequences were analyzed by the Sequencer software (Gene Codes Corporation, Ann Arbor, Mich) (Table E2). Genotype distribution did not differ significantly from that predicted by the Hardy-Weinberg equilibrium law for each polymorphism.

Statistical Analysis

Descriptive statistics used means and standard deviations for quantitative variables and numbers and percentages for qualitative variables. Hardy-Weinberg equilibrium of genotypes was tested using chi-square tests.

The statistical analysis was performed using 2 steps. In the first step, a univariate analysis aimed at assessing risk factors of CE was performed. This analysis embedded a genotype–phenotype association study. Genotypes were coded as qualitative variables, with 3 or 2 modalities, assuming a dominant effect. The univariate analysis was based on 2-sample Wilcoxon tests for quantitative data and chi-square or Fisher exact tests, when chi-square tests were not valid, for qualitative data. A Bonferroni correction was applied for genotypes.

In the second step, variables associated with CE in the univariate analysis with a *P* value less than .20 were included in a stepwise logistic regression to build a predictive model of complications. Variables retained in the final model had a *P* value less than .05 using the Wald test and were used for building a predictive score. A score threshold was determined arbitrarily to define a high-risk group. Evaluation of the predictive power of the model was based on the area under the receiver operating characteristic curve, sensitivity, specificity, positive and negative predictive values, and relative risk of complications between the high-risk and low-risk groups. These parameters, a part area under the receiver operating characteristic curve, were first computed on the sample, and in a second step their means and 95% confidence intervals were estimated by nonparametric Bootstrap, involving 10,000 Bootstrap samples. Confidence intervals were determined using 2.5 and 97.5 percentiles of the Bootstrap sample distribution.

Relationships between cytokine log-concentrations and covariates were tested, in a univariate step, using 2-sample Wilcoxon tests for binary covariates, nonparametric analyses of variance (Kruskal–Wallis tests) for other qualitative covariates, including genotypes, and Spearman-rank correlation coefficient tests for quantitative covariates. The corresponding multivariable analyses were performed using mixed linear models with a log-transformation of the data to normalize the residuals and fit the model assumptions. The cytokine kinetics (3 log-concentrations by subject) were analyzed using mixed linear models, with log-concentrations considered as repeated data. All tests were 2-sided. Computations were performed using the SAS v9 statistical package (SAS Institute Inc, Cary, NC).

RESULTS

Clinical Events

Patient characteristics are summarized in Table 1. Procedures included 2, 3, or 4 CABGs in 35 patients (28%), 73 patients (58%), and 18 patients (14%), respectively. Revascularization was exclusively done with the 2 ITAs in 26 patients (21%). Intrathoracic arterial balloon pump or inotropic support was required for weaning from CPB in 2 patients (2%) and 16 patients (13%), respectively. Perioperative red blood cell transfusions were needed in 45 patients (36%). Mean intensive care unit length of stay was 2.2 ± 3.9 days, and mean duration under mechanical ventilation was 19.9 ± 63.9 hours.

Of the 126 patients, 18 (14.3%) experienced any one of the clinical complications included in CE. Complications included were 4 deaths (3.2%), 7 MIs (5.6%), 11 cases of LCOS (8.7%), 8 cases of ARI (6.3%), and 10 cases of sepsis (7.9%). There was no stroke. Clinical conditions associated with CE were chronic obstructive pulmonary disease (COPD) (*P* = .054), preoperative symptomatic cardiac insufficiency (*P* = 7.10^{-4}), and LVEF less than 50% (*P* = .041) (Table 2).

Cytokine Circulating Levels and Their Association With Genetic Polymorphisms

Plasma levels of IL10, IL6, and TNF α were elevated at the end of CPB (IL10) or 4 hours thereafter (IL6 and

TABLE 1. Preoperative and perioperative patient characteristics

	CE	No CE	<i>P</i>
n (%)	18 (14)	108 (86)	
Preoperative characteristics			
Age	67.3 $\pm$ 9.5	63.4 $\pm$ 9.0	.11
Gender M/F (% male)	16/2 (89)	95/13 (88)	NS
BMI	25.7 $\pm$ 3.6	27.0 $\pm$ 3.7	NS
Cardiovascular risk factors			
Diabetes mellitus	4 (22)	31 (29)	NS
Hypertension	13 (72)	69 (64)	NS
Hypercholesterolemia	15 (83)	88 (81)	NS
Tobacco consumption	4 (22)	29 (27)	NS
COPD	5 (28)	11 (10)	.06
Previous MI	8 (44)	39 (36)	NS
Preoperative symptomatic cardiac insufficiency	5 (28)	2 (2)	7.10^{-4}
Treated atrial fibrillation	2 (11)	4 (4)	NS
LVEF 30%–50%	5 (28)	10 (9)	.05
Intraoperative characteristics			
Operative duration (h)	4.2 $\pm$ 0.8	4.0 $\pm$ 0.8	NS
CPB duration (h)	1.6 $\pm$ 0.4	1.5 $\pm$ 0.5	NS
Aortic clamp duration (h)	0.9 $\pm$ 0.4	0.9 $\pm$ 0.3	NS
Lowest temperature under CPB ($^{\circ}$ C)	35.1 $\pm$ 0.6	35.1 $\pm$ 0.9	NS

BMI, Body mass index; NS, not significant.

TNF α) and decreased 24 hours after CPB (*P* < 10^{-4}) (Figure 1). Cytokine plasma levels 24 hours after CPB remained higher (IL6, *P* < 10^{-4}) or were not significantly higher (IL10 and TNF α) than baseline levels. IL10 plasma levels 24 hours after CPB were found to be significantly associated with CE (*P* = .017) (Figure 2, Table 2).

TABLE 2. Risk factors for clinical complications according to clinical end point

	CE n (%) or median (Q1–Q3)	No CE n (%) or median (Q1–Q3)	Univariate <i>P</i> (Bonferroni <i>P</i>)
Age (/10)	6.80 (6.10–7.60)	6.30 (5.65–7.00)	.106
COPD	5 (27.8)	11 (10.2)	.054
Preoperative symptomatic cardiac insufficiency	5 (27.8)	2 (1.9)	7.10^{-4}
LVEF 30%–50%	5 (27.8)	10 (9.3)	.041
<i>IL6</i> –572G>C*			
GG	12 (66.7)	96 (88.9)	.032
GC	6 (33.3)	10 (9.3)	(.32)
CC	0 (0.0)	2 (1.9)	
<i>IL10</i> –592C>A*			
CC	16 (88.9)	54 (50.0)	.009
CA	2 (11.1)	44 (40.7)	(.09)
AA	0 (0.0)	10 (9.3)	
24-h IL10 concentration	11.0 (2.0–37.0)	2.0 (0.0–9.0)	.017

*For multivariable analysis, *IL6*–572G>C is coded (GC or CC = 1, GG = 0) and *IL10*–592C>A is coded (CC = 1, CA or AA = 0).

FIGURE 1. Perioperative cytokine plasma levels. *CPB*, Cardiopulmonary bypass; *IL*, interleukin; *TNF*, tumor necrosis factor.

When cytokine plasma levels were assessed in relation with gene polymorphisms (Table E3), IL10 plasma levels 24 hours after CPB were found to be associated with SNP *IL6-572G>C*; patients with *IL6-572CC* genotype had the highest IL10 levels ($P = .040$). IL6 plasma levels 24 hours after CPB were associated with *APOE* polymorphism; patients with *APOE3/3* genotype had lower IL6 levels than those with *APOE3/4* or *3/2* genotypes ($P = .011$ and $P = .027$ in univariate and multivariable analyses, respectively). A weak association was observed between $TNF\alpha$ plasma levels before anaesthesia and SNP *TNF-308G>A*; patients with *TNF-308AA* genotype had the lowest $TNF\alpha$ levels ($P = .073$ and $P = .039$ in univariate and multivariable analyses, respectively). When analyzed over the 24-hour kinetics, $TNF\alpha$ plasma levels were associated with SNP *LTA Cys13Arg* ($P = .019$); patients with *LTA 13ArgArg* genotype had the highest $TNF\alpha$ plasma levels before

A

B

FIGURE 3. Relations between SNPs and CE. A, SNP *IL6-572G>C*. B, SNP *IL10-592C>A*. CE, Clinical end point.

anaesthesia and 24 hours after CPB, but $TNF\alpha$ levels 4 hours after CPB were similar for the 3 genotypes.

Genetic Polymorphisms and Postoperative Adverse Events

Genetic polymorphisms associated with CE were SNPs *IL6-572G>C* and *IL10-592C>A*, because a higher number of complications included in this end point was seen in patients with *IL6-572GC* or *CC* genotypes (OR, 8.5; $P = .004$) or the *IL10-592CC* genotype (OR, 11.4; $P = .009$). However, these observations were not supported after Bonferroni correction (Figure 3, A and B, Table 2).

Predictive Model

We defined a prediction score for patients at high risk of complications. The predictive score formula was

FIGURE 2. Perioperative IL10 plasma levels and the occurrence of clinical complications according to the CE. Error bars represent the standard error of the mean. *CPB*, Cardiopulmonary bypass.

score = $-7.2 + 0.69 \times (\text{age}/10) - 2.1 \times (IL6-572GG) + 2.4 \times (IL10-592CC) + 2 \times (COPD) + 4.3 \times (\text{treated cardiac insufficiency})$.

A score greater than -0.65 had a 68% predictive positive value, a 95% predictive negative value, a 72% sensitivity, a 94% specificity, and a relative risk of 14.64. These predictive parameters were estimated by bootstrap ($n = 10,000$). Bootstrap parameters had a mean and 95% confidence interval of 68 (45–88), 95 (91–99), 71 (48–92), 94 (89–98), and 20.71 (5.73–96.25), respectively.

DISCUSSION

The CPB-induced SIRS has been widely studied for many years. It has now been convincingly demonstrated that this SIRS involves at least 3 interconnected plasma protease pathways: the complement cascade, cellular responses, and cytokine secretion.⁸ More recently, several studies have shown conflicting results concerning the influence of genetic polymorphisms on cytokine release and the related magnitude of this inflammatory response.^{2,15} By using a relatively large group of patients who underwent CABG, a strict standardized procedure, and definition of the CE, we show that 2 cytokine polymorphisms (*IL6-572G>C* and *IL10-592C>A*) and IL10 serum level 24 hours after CPB seem to influence the occurrence of adverse outcomes after CABG surgery under CPB.

Death has now become rare after CABG surgery (1%–3%), but the rate of postoperative complications still varies between 10% and 30%. In our study, death occurred in 4 patients (3.1%) and CE occurred in 18 patients (14.3%), with ARI in 6.4% and MI in 5.6%. Our data stand above the median rate of complications reported in a meta-analysis including more than 200,000 patients among 176 series of patients undergoing CABG.¹⁶ These differences can be explained by the definitions of complications. Actually, ARI after CABG varies from 0% to 6.2% and MI varies from 0% to 29%. Our definitions of ARI and MI were among the most sensitive in the literature. Cardiac troponin I plasma levels have been shown to be the most relevant markers to assess MI after revascularization, over electrocardiogram changes and creatine kinase-MB.¹⁷ In addition, recent cohort studies suffer from the lack of patients displaying a dysmetabolic syndrome, including various metabolic risk factors such as abdominal obesity, dyslipidemia, hypertension, and hyperglycemia, which are thought to be linked to insulin resistance.¹⁸ These patients are now increasingly referred for CABG and are prone to develop more postoperative ARIs and MIs.¹⁹

Cytokines and Clinical Outcomes

In this study, proinflammatory (IL6 and $TNF\alpha$) and anti-inflammatory (IL10) plasma levels increased after CPB and decreased 24 hours later, as described in previous studies.²⁰ IL6 and $TNF\alpha$ have been shown to be key mediators of

SIRS after CPB.⁸ IL10 is an anti-inflammatory cytokine, which, in contrast, is expected to exert a protective effect on the outcome after CPB.²¹ However, several studies have suggested that high IL10 plasma levels could also result in an immune “hyporesponsiveness,” leading to a higher risk of postoperative infectious complications.⁵ Indeed, we observed more clinical complications in patients with highest IL10 plasma levels 24 hours after CPB.

In 1996, Rao and colleagues²² reported a 9.1% rate of LCOS after CABG in a large adult population. Our series confirm that LCOS remains one of the major severe complications after surgical coronary revascularization. Although LCOS and MI may be related to periprocedural factors, such as inadequate myocardial protection, a correlation between postoperative IL6 and IL10 plasma levels and postoperative myocardial injury has been noted by Giomarelli and colleagues.²³ Our data could not confirm this specific finding because LCOS and MIs were not separately analyzed from the other components of our CE. However, it is conceivable that a higher IL10 response reflects the occurrence of a greater inflammatory insult to counteract, which would be consistent with the observation that these high IL10 levels were associated with severe complications.

Single Nucleotide Polymorphisms and Clinical Outcomes

In this study, we observed an association between clinical complications after CPB and 2 cytokine gene polymorphisms, SNPs *IL6-572G>C* and *IL10-592C>A*. The SNP *IL6-572G>C* has been associated with several cardiovascular disorders, such as coronary heart disease¹⁰ and abdominal aortic aneurysms.²⁴ Furthermore, Jia and colleagues¹⁰ demonstrated a correlation between this SNP and postoperative MI after cardiac surgery under CPB. Our data suggest that *IL6-572G>C* is more generally associated with an adverse postoperative course; patients bearing the *IL6-572GC* and *IL6-572CC* genotypes have a 9-fold increased risk of experiencing one of the complications included in our CE. In contrast with the study by Brull and colleagues,² a significant association between SNP *IL6-572G>C* and IL6 plasma levels was not observed in our study. The study published in 2000 by Terry and colleagues⁴ could explain this discordance. These authors showed a cooperative influence of various genetic polymorphisms on *IL6* transcriptional regulation. These polymorphisms were located in the *IL6* gene promoter and included SNP *IL6-572G>C*. In addition, cytokines are most often present in the serum complexed to their soluble receptors. We measured the total production of IL6, because both the free form and that complexed to its receptor are active, whereas in other studies the dosage of IL6 only detected the free form of IL6.²⁵ This technical variation in the dosage of IL6 may explain discrepancies in the reported correlation between IL6 serum levels and SNPs for this cytokine. Nevertheless, we did

observe an association between SNP *IL6-572G>C* and IL10 plasma levels 24 hours after CPB, and these 2 factors were also, in this study, independently associated with clinical complications.

The SNP *IL10-592C>A* is in strong linkage disequilibrium with 2 other SNPs located in the *IL10* gene promoter, *IL10-1082G>A* and *-819C>T*. Haplotype *IL10-1082G/-819C/-592C* has been associated with higher IL10 plasma levels after pediatric cardiac surgery with CPB, and higher IL10 levels were associated with clinical immunoparalysis, suggesting a higher risk of postoperative infectious complications.⁵ These data are consistent with the association that we observed between the *IL10-592CC* genotype and a higher risk of clinical complications according to our CE, which included sepsis. No association was found between SNP *IL10-592C>A* and IL10 plasma levels, but this SNP was associated with clinical complications, as were IL10 plasma levels 24 hours after CPB.

After Bonferroni correction, which is usually required in case of SNP statistical analyses, these 2 associations seemed to be weakened. Nevertheless, our results showed that the association of SNPs *IL6-572G>C* and *IL10-592C>A*, along with IL10 plasma level at 24 hours, preexisting COPD or cardiac insufficiency, and age could be predictive of complications after CABG surgery. If confirmed, this predictive model could have implications in the pre- and postoperative management of specific high-risk patients.

Study Limitations

We acknowledge that this study has some limitations. First, small sample sizes conducted us to correlate the various SNPs with the composite end point, thus making it difficult to assess the specific impact of the genetic pattern on a given postoperative complication. This was unavoidable because the low incidence of individual events required them to be grouped into a composite criterion to provide an adequate statistical power. However, from a merely clinical standpoint, the conclusions remain relevant by showing that the genetic profile allowed the identification of patients at risk of poor bypass-related outcomes, even though the specific component driving the composite end point remains elusive. Second, SIRS may not be always advocated as the cause for some complications of our composite end point. For example, inadequate myocardial protection could lead to postoperative MI or LCOS. However, warm blood cardioplegia (antegrade or retrograde) was consistently used in all patients, which should have contributed to optimize myocardial protection.²⁶ Third, this genetic profiling was restricted to a limited number of SNPs. This choice may look oversimplistic in view of the multiplicity of intermingling pathways involved in the genesis of the post-CPB inflammatory response; however, it was dictated by previously published data that identified the selected markers as

key players in this response but clearly do not exclude that other SNPs may have a greater predictive value.

CONCLUSIONS

Overall, our data suggest that focused genetic testing of the *IL6-572G>C* and *IL10-592C>A* SNPs might be a tool for identifying patients at the highest risk of poor tolerance to the inflammatory response to CPB and for possibly implementing strategies to mitigate it, such as off-pump surgery, drugs, or device-based interventions.²⁷ However, this first requires that the generalization of these genetic diagnostic tests makes them reasonably affordable and thus favorably shifts their cost-to-benefit ratio.

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TABLE E1. Genetic polymorphisms evaluated in the study

Gene (symbol)	Nucleotide substitution	Genomic context	SNP identification*	Cytogenetic locus	Minor allele frequency	Hardy-Weinberg <i>P</i>
Interleukin 6 (<i>IL6</i>)	-597G>A	5'UTR	rs1800797	7p15.3	0.306	.246
	-572G>C	5'UTR	rs1800796		0.079	.141
	-174G>C	5'UTR	rs1800795		0.310	.655
Interleukin 10 (<i>IL10</i>)	-592C>A	5'UTR	rs1800872	1p32.1	0.262	.532
	*117C>T	3'UTR	rs3024496		0.437	.467
Tumor necrosis factor- α (<i>TNFα</i>)	-308G>A	5'UTR	rs1800629	6p21.33	0.135	.823
Lymphotoxin alpha (<i>LTA</i>)	+252A>G†	intron 1	rs909253	6p21.33	0.333	.423
	37T>C	Cys13Arg	rs2229094		0.254	.596
Apolipoprotein E (<i>APOE</i>)	388T>C	Cys130Arg	rs429358	19q13.32	e2: 0.044	.433
	526C>T	Arg176Cys	rs7412		e4: 0.115	

UTR, Untranslated region. *From the National Center for Biotechnology Information's dbSNP public database (<http://www.ncbi.nlm.nih.gov/SNP/>). †Notation most used in association studies. The HGVS nomenclature is c.-10+90A>G.

TABLE E2. Primers used for genotype analysis

Primer	Sequence 5' → 3'	Amplicon length (bp)	Annealing T (°C)
IL6-F	TGGCAAAAAGGAGTCACACA	659	64
IL6-R	GCCTCAGACATCTCCAGTCC		
IL6-Fint	CAAGACATGCCAAAGTGCTG		
IL6-Rint	GAGTTTCCTCTGACTCCATCG	487	61
IL10-592-F	CTGTGCCTCAGTTG CTCAC		
IL10-592-R	CCTCAGCTGTGGGTTCAT		
IL10*117-F	GGAGAACAAGCAACCTCTGC	592	61
IL10*117-R	AGCTAG AAAGCGTGGTCAGG		
LTACys13Arg-F	TGTCTCCCTCTGCTCACCTT	338	67
LTACys13Arg-R	GGCTCTAGGGCTCAAGGTTT		
APOE-F	TGAAGGAGTTGAAGGCCTAC	502	58
APOE-R	TGCTCCTTCACCTCGTCCAG		
TNF-F	AGGCAATAGGTTTTGAGGGCCAT	107	60
TNF-R	TCCTCCCTGC TCCGATTCCG		
LTA+252-F	CCGTGCTTCGTGCTTTGGACTA	782	54
LTA+252-R	AGAGGGGTGGATGCTTGGGTTC		

bp, Base pair; T, temperature; F, forward; R, reverse; int, internal sequencing primer.

TABLE E3. Cytokine plasma levels and polymorphism associations Cytokine levels and polymorphism associations (at each cytokine measurement)

Cytokine measure	SNP	Median (Q1–Q3) per genotype			Univariate <i>P</i>	Multivariable <i>P</i>
(TNFα) before CPB	<i>LTA</i> Cys13Arg	CysCys	CysArg	ArgArg	.097	NS
		1.0 (0.0–2.0)	1.0 (0.0–2.0)	2.0 (1.0–4.0)		
	<i>TNF</i> –308G>A	GG	GA	AA	.073	.039
		1.0 (1.0–2.0)	1.0 (0.0–1.0)	0.5 (0.0–1.0)		
(IL6) 4 h after CPB	<i>IL6</i> –174G>C	GG	GC	CC	.056	NS
		91 (68–159)	106 (73–165)	63 (48–105)		
	<i>TNF</i> –308G>A	GG	GA	AA	.099	NS
		93 (68–145)	139 (73–171)	169 (155; 183)		
(IL6) 24 h after CPB	<i>IL6</i> –597G>A	GG	GA	AA	.054	NS
		58.5 (41.5–81)	67 (51–115)	49.5 (37–63)		
	<i>IL6</i> –174G>C	GG	GC	CC	.059	NS
		58 (42–81)	68 (50–114)	53 (43–62)		
	<i>APOE</i> alleles 3	0	1	2	.011	.027
		46 (38–53)	91 (53–121)	59 (38–74)		
(IL10) 24 h after CPB	<i>IL6</i> –572G>C	GG	GC	CC	.040	NS
		2 (0–10)	7 (2–16)	78 (34–122)		
(TNFα) 24 h after CPB	<i>LTA</i> Cys13Arg	CysCys	CysArg	ArgArg	.088	NS
		1.0 (0.0–3.0)	2.0 (1.0–2.0)	3.0 (2.0–4.0)		
Cytokine kinetic and polymorphism associations						
(IL6) (mean ± SD)						
SNP	Genotype	Before CPB	4 h after CPB	24 h after CPB	<i>P</i> ₁	<i>P</i> ₂
<i>APOE</i> alleles 3	0	3.0 ± 2.8	52.0 ± 15.6	45.5 ± 10.6	.048	NS
	1	3.8 ± 9.7	122.2 ± 61.0	99.2 ± 68.5		
	2	4.6 ± 23.8	131.5 ± 160.5	64.9 ± 39.6		
(TNFα) (mean ± SD)						
SNP	Genotype	before CPB	4 h after CPB	24 h after CPB	<i>P</i> ₁	<i>P</i> ₂
<i>TNF</i> –308G>A	GG	1.7 ± 2.6	4.4 ± 4.0	2.1 ± 3.3	.056	NS
	GA	1.0 ± 1.3	4.3 ± 4.7	2.6 ± 6.7		
	AA	0.5 ± 0.7	5.0 ± 1.4	1.0 ± 1.4		
<i>LTA</i> Cys13Arg	CysCys	1.2 ± 1.2	4.4 ± 4.1	2.2 ± 4.6	.019	NS
	CysArg	1.7 ± 3.4	4.4 ± 4.3	2.1 ± 4.1		
	ArgArg	3.0 ± 2.9	4.3 ± 3.5	3.4 ± 2.6		

NS, Not significant; *P*₁, genotype effect; *P*₂, genotype × kinetics interaction; SD, standard deviation. Concentration unit is pg/mL.