Predictors of Postoperative Atrial Fibrillation in Patients With Coronary Artery Disease Undergoing Cardiopulmonary Bypass: A Possible Role for Myocardial Ischemia and Atrial Inflammation

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Objective: **To evaluate the preoperative presence of C-reactive protein (CRP) and troponin T(hs-TnT) in patients with coronary artery disease (CAD) undergoing cardiopulmonary bypass (CPB) in order to better clarify the role of atrial inflammation and/or myocardial ischemia in the development of postoperative atrial fibrillation (POAF).**

Design: **Prospective, nonrandomized study.**

Setting: **University hospital.**

Participants: **Thirty-eight consecutive ischemic patients admitted to the authors' hospital for CAD undergoing elective on-pump coronary artery bypass grafting (CABG).**

Intervention: **Elective on-pump CABG.**

Measurements and Main Results: **Peripheral blood samples were collected from all patients before and 24 hours after CABG to assess high sensitive (hs)-CRP and troponin T (hs-TnT) levels. The patients' heart rhythm was monitored by continuous ECG telemetry. Biopsies from the right atrial appendage were obtained at the beginning of the CABG procedure in order to perform immunohistochemistry for CRP and reverse transcription polymerase chain reaction for CRP mRNA expression.**

Fourteen patients out of 38 (36%) developed POAF. Atrial CRP was found in 31 patients (82%), 10 with POAF and 21 with sinus rhythm (71% *v* 87% respectively, p = ns). None of the atrial samples was positive for CRP mRNA. Atrial CRP did not correlate with serum hs-CRP levels and with occurrence of POAF, but with the incidence of diabetes (p = 0.010). Postoperative hs-TnT levels, but not hs-CRP levels, were identified as the only predictor of POAF occurrence (p = 0.016).

Conclusions: **In patients undergoing CABG, neither peripheral nor tissue preoperative CRP levels, but only postoperative hs-TnT levels, correlated with POAF, suggesting the primary role of an ischemic trigger of atrial fibrillation.**

KEY WORDS: atrial fibrillation, inflammatory mediators, coronary artery bypass grafts, myocardial ischemia, immunochemistry

DESPITE MEDICAL AND SURGICAL developments, the incidence of atrial fibrillation (AF) after coronary artery bypass graft surgery (CABG) is 16% to 50%,[1–7] with consequent prolonged hospitalization and increased risk of stroke and mortality (5% at 1 year and 16% at 10 years).[8–11] The mechanisms responsible for postoperative atrial fibrillation (POAF) still are debated.[12,13] In particular, a number of studies suggest a link between oxidative stress and inflammation associated with cardiopulmonary bypass (CPB) and POAF.[14–21]

It is well known that CPB causes an acute systemic inflammatory response[22,23] characterized by the increase of serum C-reactive protein (CRP) levels, peaking on the second postoperative day with a time course similar to that of POAF.[17]

In addition to systemic inflammation caused by cardiopulmonary bypass, atrial inflammation might contribute to the occurrence of POAF. Chen at al found a higher number of CD45-positive cells in the right atrial appendage with AF as compared to that in patients in sinus rhythm undergoing valve surgery.[24] Accordingly, the authors found CRP in atrial cardiomyocytes more frequently in patients with AF than in those with Wolff-Parkinson-White syndrome undergoing catheter-based ablation procedures.[25]

Interestingly, other studies have highlighted a potential role for perioperative myocardial injury in the development of POAF.[26–30] In order to better elucidate the potential role of preoperative atrial inflammation and/or myocardial ischemic injury in the development of POAF, the authors evaluated right atrial appendage specimens taken at the early phases of the surgical procedure for the presence of CRP and serum hs-TnT levels in the perioperative period in a selected population of patients undergoing CABG. The authors also investigated the time course of systemic markers of inflammation, such as hs-CRP levels, white blood cell (WBC) count, and fibrinogen levels in this setting of cardiac surgery.

METHODS

Thirty-eight consecutive patients with coronary artery disease (CAD) (without previous history of AF admitted to the authors' department for elective CABG from January 2009 to February 2010) were enrolled in this prospective study. Exclusion criteria included liver or kidney failure, recent infections or inflammatory disease in the last 6 months, autoimmune diseases, neoplasm, recent trauma, or surgery in the last 6 months. Demographic data, cardiovascular risk factors such as hypertension, diabetes, dyslipidemia, smoking, familial history of CAD, history of chronic kidney disease, CAD clinical features, medical therapy, ECG findings, and echocardiographic parameters, such as left ventricular ejection fraction (LVEF) and left atrial size, were collected. In particular, chronic kidney disease was defined as estimated glomerular filtration rate, determined with Modification of Diet for

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Renal Disease formula, less than 60 mL/min/1.73 m^2 in the last 3 months as described elsewhere.[31] The study was approved by the ethics committee of the authors' university, and all patients gave written informed consent.

Following median sternotomy and administration of an IV bolus of heparin (300 IU/kg) to obtain an activated coagulation times (ACT) >480s, normothermic cardiopulmonary bypass was instituted by cannulation of the right atrium and ascending aorta. Right atrial appendage biopsies were performed at the early phase of the intervention at the cannulation site.

Then, myocardial protection was accomplished by anterograde intermittent blood cardioplegia isothermic to the systemic perfusion temperature. Additional boluses of heparin (100 IU) were administered if needed to maintain ACT in the range. Whenever possible, the left internal mammary artery was used to graft the left anterior descending coronary artery and saphenous vein grafts for the remaining coronary arteries. At the end of CPB and achievement of stable hemodynamic conditions, anticoagulation was reversed by administration of protamine sulphate (1-1.5 mg per 100 IU of heparin administered in the previous hour). Surgical parameters, such as CPB time and temperature, aortic clamping time, and body temperature during the intervention, were collected for each patient.

The formalin-fixed, paraffin-embedded specimens, obtained by right atrial appendage biopsy during CPB, were sectioned at 5μm and stained with hematoxylin and eosin and with hematoxylin and Van Gieson to confirm the presence of atrial cardiomyocytes. Myocardial infiltration of inflammatory cells was examined histologically. Sections from each specimen displaying atrial cardiomyocytes were cut at 5μm, mounted on glass, and dried overnight at 37°C. All sections then were deparaffinized in xylene, rehydrated through a graded alcohol series, and washed in PBS, which also was used for all subsequent washes and for antibody dilution. Endogenous peroxidase activity was blocked by 5% hydrogen peroxide. For immunohistochemistry, tissue sections were heated twice in a microwave oven for 5 minutes each at 700 W in citrate buffer (pH = 6). The murine monoclonal antibody (clone CRP-8, IgG1, Sigma, St. Louis, MO) directed against human CRP was used at 1:500 dilution and incubated overnight at 4°C. Then, the sections were processed with the standard streptavidin-biotin-immunoperoxidase method (DAKO Universal Kit, DAKO Corp, Carpinteria, CA). Diaminobenzidine was used as the final chromogen, and hematoxylin as the nuclear counterstain. The monoclonal antibody CRP-8 displayed its reactivity against native and denatured CRP without cross-reaction with human serum, amyloid P component, human haptoglobin, human alpha-1-acid glycoprotein and human IgG. All specimens were analyzed to assess cell number and the immunoreactivity to CRP by a single investigator who was blinded to the patients' characteristics. Atrial specimens were scored as positive or negative for the presence or absence of CRP.

The total RNA was extracted using Trizol (Invitrogen, Grand Island, NY) as described previously, blinded to histologic results. All atrial specimens were evaluated for CRP mRNA expression. cDNA was synthesized from 1 lg total RNA in 20 lL reaction using oligo (dT) 18 Primer and RevertAid™ M-MuLV Reverse Transcriptase (Fermentas, St. Leon-Rot, Germany). mRNA expressions of CRP, AT1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were examined by RT-PCR. Sequence-specific PCR primers used were CRP, forward 50-TCGTATGCCACCAAGAGACAAGACA-30, reverse 50-AACACTTCGCCTTGCACTTCATACT-30 [11], giving 440 bp PCR product; AT1, forward 50-ACTAGGCATCAT ACGTGACTGTAG-30, reverse 50-TGTTGAAAGGTTTG AGTGGG-30, giving 196 bp PCR product; GAPDH, forward 50-AACATCATCCCTGCCTCTACTGG-30, reverse 50-CTCCGACGCCTGCTTCACC-30, giving 189 bp PCR product. PCR products were separated by electrophoresis on 2% agarose gel. Expression of mRNA was quantified as relative to internal control GAPDH.

Immediately before surgery and 24 hours after, blood samples were drawn from the radial artery. White blood cell count, serum fibrinogen, and serum high sensitivity (hs)-CRP levels were assessed as inflammatory markers. Serum hs-TnT levels were assessed as myocardial necrosis marker. Serum hs-CRP was assayed using a latex-enhanced immunonephelometric assay (BN II, Siemens Diagnostic Glasgow, DE). Cardiac hs-TnT was quantitatively determined using a 1-step electroimmunoassay based on electrochemilumiscence technology (Elecsys 2010, Roche), with a lower detection limit of 0.01 ng/mL.

All patients were monitored by ECG telemetry until discharge. Patients who developed POAF were subjected to electrical or pharmacologic cardioversion.

Continuous variables are expressed as median accompanied by interquartile range, as Shapiro-Wilk test showed that the data were not normally distributed, whereas categoric variables are expressed as absolute frequencies and percentages. Comparisons between groups were performed with Mann-Whitney U test, Wilcoxon test for paired samples, or chi-square test, as appropriate. Univariate logistic regression analysis was performed, including all clinical features, surgical variables, and laboratory measurements (both inflammatory and myocardial damage markers) possibly related to POAF or atrial CRP localization. Then, variables showing a significant association ($p < 0.10$) were included in multivariate logistic regression. All tests were 2-sided, and a value of $p < 0.05$ was settled for statistical significance. Statistical analysis was performed using SPSS 19 (SPSS, Inc Chicago, IL).

RESULTS

Thirty-eight patients were enrolled in the study (mean age 61 years, male gender 73%). Clinical presentation was an acute coronary syndrome in 20 patients (54%), without patients with ST-elevation myocardial infarction. Eighteen patients (46%) presented with stable angina. Thirty patients (79%) had severe 3-vessel disease. All 38 (100%) patients were in sinus rhythm on admission. Postoperative monitoring by ECG telemetry in the authors' department was obtained for mean time intervals of 9 ± 3 days. Fourteen patients out of 38 (36%) developed AF during the postoperative period (POAF group) POAF occurred early (within 72 hours from CABG) in 12 (86%) and late (after 72 hours from CABG) in only 2 (14%) patients. The remaining 24 patients (64%) exhibited constant sinus rhythm in the postoperative period (SR group).

Clinical, surgical features and laboratory findings of POAF and SR patients are shown in Table 1. No significant difference was observed between the 2 groups with regard to age, gender, prevalence of cardiovascular risk factors, clinical presentation of CAD, medical therapy, or surgical parameters. Of note, all patients with severe left ventricular dysfunction (LVEF <35%) developed POAF ($p = 0.018$); furthermore, POAF patients had a higher rate of 3-vessels coronary disease (100% *v* 67%, respectively; $p = 0.015$) and of chronic kidney disease ($p = 0.012$) than the SR group.

Preoperative hs-CRP levels, WBC count, fibrinogen, and hs-TnT levels did not differ significantly between the 2 groups. After CABG, serum levels of hs-CRP and hs-TnT increased significantly in both RS and POAF groups as compared to baseline levels (hs-CRP: $p = 0.013$ for POAF group and $p < 0.001$ for SR group, [Fig 1]; hs-TnT: $p < 0.001$ for both groups, [Fig 2]). Of note, serum postoperative hs-TnT levels were significantly higher in the POAF group than in the SR