RESEARCH ARTICLE

Changes in cytokine concentrations following successful ablation of atrial fibrillation

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ABSTRACT. *Aims.* **Atrial fibrillation is associated with the activation of inflammatory processes [*e.g.* higher concentrations of pro-inflammatory cytokines interleukin-6 (IL-6), C-reactive protein (CRP)], as well as a pro-thrombotic state [*e.g.* increased concentration of serum pro-thrombotic markers P-selectin and CD40 ligand (CD40L)]. The aim of the present study was to establish, whether successful epicardial ablation of AF leads to decreased concentrations of traditional inflammatory and thrombotic markers. *Methods.* Twenty-five patients with symptomatic paroxysmal or persistent AF were prospectively studied. All underwent epicardial isolation of pulmonary veins. The success of the ablation was assessed clinically and with three Holter recordings. Blood samples were drawn before, three and six months after surgery. Serum concentrations of IL-6, interleukin-10 (IL-10), CRP, CD40L and P-selectin were measured using ELISA. *Results.* AF was successfully ablated in 15 patients (SR group). In the other 10 patients (AF group), AF re-occurred during follow-up. Neither group differed with respect to age, gender, left ventricular ejection fraction, or preoperative concentrations of measured molecules. The concentrations of IL-6, CRP and CD40L decreased in successfully ablated patients; however, there was no change in the concentrations of these molecules in the AF group. The concentrations of IL-10 and P-selectin were unchanged in both groups during follow-up. *Conclusion.* Successful ablation of AF, with sinus rhythm restoration and maintenance, is associated with decreased serum levels of markers of inflammation.**

Keywords: atrial fibrillation, ablation, interleukin-6, interleukin-10, CD40L, P-selectin, C-reactive protein

Atrial fibrillation (AF) is the most common cardiac arrhythmia seen in clinical practice [1]. The treatment of AF is aimed at either restoration or maintenance of sinus rhythm (rhythm control strategy) or controlling the heart rate, and preventing thromboembolic complications (rate control strategy). Currently, rhythm control (non-pharmacological) approaches are preferred due to the poor effectiveness of pharmacological treatments in symptomatic patients [2]. In patients indicated for other cardiac surgery, parallel perioperative intervention is indicated. In others, interventional ablation is possible, and recently (since 2002) minimally invasive epicardial thoracoscopic surgery has become available [3, 4].
Frustaci *et al.* first described the high prevalence of inflammatory infiltrates in atrial biopsies compared to biopsies from atria of healthy controls. Since then, there has been growing evidence linking AF to pro-inflammatory, pro-thrombotic and hypercoagulable states. In most published studies, patients with AF have

greater circulating inflammatory marker concentrations compared to healthy controls in sinus rhythm. AF is associated with elevated, high-sensitivity C-reactive (CRP) protein and interleukin-6 (IL-6) levels compared to controls in normal sinus rhythm [5]. AF is also associated with a prothrombotic and hypercoagulable state [6]. The initiation of AF is associated with local platelet activation, within minutes of onset, and determined by the activation of surface P-selectin on platelets [7]. Furthermore, there is an apparent link between thrombogenesis and inflammation in AF [8]; whether the activation of inflammation and aggregation decreases after successful ablation of AF has not yet been well evaluated. The aim of the present study was to establish, whether successful restoration of sinus rhythm provided by AF ablation was associated with decreased inflammatory and aggregation processes. We hypothesized that sinus rhythm restoration and maintenance would be associated with decreased inflammation and aggregation.

DONORS AND METHODS

Patients and follow-up

Twenty-five patients with symptomatic paroxysmal or persistent AF were prospectively studied. All were symptomatic and resistant to pharmacological treatment; AF was present despite treatment with at least one antiarrhythmia drug (amiodarone was used in 80% of patients). Written, informed content was obtained from each participant; the study was conducted according to the principles expressed in the declaration of Helsinki, and was approved by the local Ethics Committee. Exclusion criteria included: i) the presence of significant valve disease, ii) coronary artery disease without previous complete revascularization; iii) thyrotoxicosis; iv) systolic dysfunction of the left ventricle (i.e. ejection fraction less than 40%); v) significant pericardial effusion; vi) chronic obstructive pulmonary disease; and vii) a history of pneumothorax or history of significant thoracic surgery. Anticoagulation (warfarin, with a target INR of 2.0-3.0) and antiarrhythmia medications [amiodarone 200 mg/day, or sotalol (if amiodarone was not tolerated) 160 mg/day] were maintained for at least three months after the AF ablation. Later, warfarin treatment was given according to the CHADS$_2$ criteria [2]. The success of the ablation was assessed clinically and with three Holter recordings during the first six months following ablation. One, 24 h Holter recording was performed one month after the procedure; two, 48 h Holter recordings were performed after three and six months (Cardiette GiOtto, UK). Because paroxysmal AF immediately after ablation is quite common, its presence during the first four weeks following the procedure was not considered to be a sign of ablation failure. The ablation was considered successful, if there were no symptoms of AF more than one month after ablation (i.e. no palpitations or AF symptoms, which had been present before the procedure), and if all Holter recordings were negative for AF. Standard echocardiography evaluation was performed before the ablation (Vivid 7, GE Medical Systems, Horten, Norway).

Operative procedure

All patients underwent epicardial, microwave isolation of the pulmonary veins. The procedure was performed under general anesthesia, with selective intubation of the left bronchus and selective left lung ventilation. Three ports were inserted in the right hemithorax. After deflation of the right lung, a pericardiotomy was done above the right phrenic nerve. Next, preparation of the oblique and transverse sinus was performed, and a Flex 10 (Guidant, Santa Clara, CA, USA) catheter was inserted and encircled around the pulmonary veins. After verifying the correct position of the catheter (i.e. positioned under the auricle of the left atrium), the ablation was performed, usually in two cycles, 120 seconds each, creating a "box-lesion". After sinus rhythm was restored, perioperative testing of a conduction block between the pulmonary veins and atrial wall was carried out. In patients with fibrillating atria during surgery, electrophysiology testing could not be completed during the procedure. All procedures were performed in the Department of Cardiac Surgery of the Cardiocenter, University Hospital Kralovske Vinohrady.

Blood sampling

Blood samples were drawn before surgery, at three and at six months after surgery under standardized conditions (fasting, and 20 min rest before taking blood). Blood was drawn from an antecubital vein into 7 mL standard serum and 5 mL EDTA syringes. Syringes were centrifuged at 3,500 rpm for 15 min; serum and plasma were stored at - 70°C for batch analysis. Serum [CRP, CD40 ligand (CD40L), P-selectin] or plasma [IL-6, interleukin-10 (IL-10)] concentrations of the reported cytokines were measured using commercially available ELISA (IL-6, IL-10, P-selectin: R&D Systems, MN, USA; CD40L: Bender MedSystems, Vienna, Austria; CRP: PromoKine, Heidelberg, Germany). The ELISA Reader Elx808, Biotek, VT, USA was used. The intra-assay coefficients of variation were satisfied (< 5%).

Statistical analysis

Statistical analysis was performed by an experienced statistician using SPSS v. 12 (SPSS, TX) and Sigma STAT (Aspire Soft.Int., Ashburn, VA, USA). P-values less than 0.05 were considered to be statistically significant. Categorical variables were tested using χ^2 analysis or Fisher's exact test, as appropriate. Data were tested for normality using the Kolmogorov-Smirnov test. Data sets with a normal (Gaussian) distribution were analyzed using Student's t-test, and those with a non-Gaussian distribution using the Mann-Whitney U test. Continuous variables are reported as either mean ± standard deviation or median (interquartile range). Time-course analyses of the observed parameters were performed using analysis for repeated measurements and for > 1 between the subject's factors (including age, gender, and AF duration). Multivariate analysis used a stepwise logistic regression model.

RESULTS

Clinical results

Twenty-five patients with atrial fibrillation were enrolled in the study. The mean age of the study population was 59.5 ± 8.2 years, there were 19 men and 6 women, and the mean BMI was 26.4 ± 1.3. AF was paroxysmal in nine patients and persistent in the other 16 patients. The mean ejection fraction of the left ventricle was 55.8 ± 10.4. In 11 patients, a small mitral insufficiency (1/4) was present. Two patients had undergone percutaneous coronary intervention before the ablation; the other 23 patients had undergone coronary angiography, which revealed no significant stenosis in the coronary arteries. The clinical characteristics are summarized in table 1. In all patients, antiarrhythmia medication was used before the ablation (amiodarone was used in 20 of 25 patients). However, due to lack of efficacy, anti-arrhythmics were withdrawn from the majority of patients during

Table 1
Clinical characteristics of the two groups

	SR group	AF group	P-values
Age (years)	58.2 ± 6.6	61.4 ± 10.7	n.s.
Gender (male, %)	14 (93%)	5 (50%)	< 0.05
BMI	26.6 ± 1.7	26.1 ± 0.1	n.s.
Duration of AF	40.3 ± 61.5	27.8 ± 25.4	n.s.
Hypertension	9 (60%)	5 (50%)	n.s.
Diabetes mellitus	0 (0%)	1 (10%)	n.s.
EF of LV	55.3 ± 11.3	56.5 ± 11.1	n.s.
LVEDd	53.3 ± 4.5	52.6 ± 5.8	n.s.
LA size	43.0 ± 4.8	44.5 ± 3.7	n.s.
MI insufficiency (I of IV)	6 (40%)	5 (50%)	n.s.

BMI: body mass index; EF of LV: ejection fraction of left ventricle; LVEDd: left ventricle end diastolic dimension; LA size: left atrial size; MI insufficiency: mitral insufficiency (all only grade 1 of 4).

pre-operative monitoring and treatment with beta-blockers was initiated. The actual preoperative antiarrhythmia treatment of study participants is summarized in *table 2*.

All patients underwent AF ablation between April 2007 and April 2008. During the six months of outpatient follow-up, 15 patients remained in sinus rhythm (SR group), i.e. they were without clinical symptoms and with no AF during Holter monitoring. AF re-occurred in 10 patients (AF group). The clinical characteristics and differences between the SR and AF groups are summarized in *tables 1* and *2*. Both groups were comparable with respect to basic clinical characteristics, except for gender (more men in the SR group). The effect of ablation was assessed starting at the end of the first month following ablation. During the first month after ablation, reoccurrence of AF is not unusual. Of the 15 patients, where ablations were ultimately viewed as successful, there were occasional paroxysms of AF during the first month, however after the first month and throughout the remainder of the study there were no other episodes of AF. During the first month, six patients had episodes of AF and three had to undergo direct current version; although all were AF-free during the final five months of the follow-up.

Table 2
Description of preoperative treatment of the two groups

	SR group	AF group	P-value
PCI	1 (7%)	1 (10%)	n.s.
DC version	7 (47%)	3 (30%)	n.s.
ACE inhibitors	9 (60%)	4 (40%)	n.s.
ARB	1 (7%)	0 (0%)	n.s.
BB	13 (87%)	7 (70%)	n.s.
Ca channel blockers	4 (27%)	1 (10%)	n.s.
Statin	5 (33%)	2 (20%)	n.s.
Spironolactone	1 (10%)	0 (0%)	n.s.
Amiodarone	3 (20%)	1 (10%)	n.s.
Propafenone	3 (20%)	2 (20%)	n.s.
Sotalol	1 (7%)	1 (10%)	n.s.

PCI: percutaneous coronary intervention; DC: direct current; ACE: angiotensin-converting enzyme; ARB: angiotensin II receptor blockers; BB: beta blockers; Ca: calcium.

Among the 15 successfully ablated patients, five (33%) suffered preoperatively from paroxysmal and 10 (66%) from persistent AF. Among the 10 unsuccessfully ablated patients, four suffered from paroxysmal and six from persistent AF. The type of AF had no effect on the success of the ablation.

The concentrations of cytokines

The time-courses of the concentrations of inflammatory markers CRP, IL-6, IL-10 are shown in *figure 1*. The time-course of the concentrations of CRP was significantly different between groups (ANOVA, p = 0.001). Baseline CRP concentrations did not differ between groups. While the concentrations of CRP decreased in the SR group from 2.52 (1.55-3.78) ng/mL before ablation to 0.84 (0.34-1.7) ng/mL at six months, the concentrations of CRP in the AF group remained unchanged [2.87 (0.86-4.02) ng/mL before *vs* 2.29 (1.65-3.13) ng/mL at six months]. The time-course of IL-6 concentrations was significantly different between groups (ANOVA, p = 0.034). The concentration of IL-6 decreased significantly in the SR group (2.34 (1.36-3.46) ng/mL before *versus* 1.78 (1.27-2.18) ng/mL at six months, p < 0.05), but did not change in the AF group [2.85 (1.78-4.54) ng/mL before *vs* 3.14 (2.42-3.21) ng/mL at six months, p = n.s.]. Baseline IL-6 concentrations did not differ between groups. The baseline IL-10 concentrations did not differ between groups, and did not change during the follow-up *(figure 1C)*. The time-course of the concentrations of markers of aggregation, CD40L and P-selectin, are shown in *figure 2*. The time-course of the concentrations of CD40L was significantly different between groups (ANOVA, p < 0.05). Baseline CD40L concentrations did not differ between groups. In the SR group, CD40L decreased after ablation [2.36 (1.42-3.62) ng/mL before *vs* 1.13 (0.41-1.56) ng/mL at six months, p < 0.05], the concentration in the AF group did not change [1.56 (0.86-2.8) ng/mL *versus* 2.43 (1.82-2.68) ng/mL, p = n.s.]. The concentrations of P-selectin were similar in both groups at baseline, and did not change in ether group during follow-up.

Multivariate analysis

In a multivariate analysis, only male gender was associated with a decrease in serum concentration of measured markers. No other clinical or laboratory variables were associated with the decrease in measured cytokines in the multivariate analysis.

DISCUSSION

The major finding of our study was that the concentration of markers of inflammation and aggregation decreased after successful AF ablation. In cases where ablation was unsuccessful and AF persisted, marker values remained unchanged.

It is well known that AF is associated with pro-inflammatory and pro-thrombotic states [6, 5, 9]. Elevated CRP can predict an increased risk of developing AF [10]. On the other hand, patients with AF have higher CRP
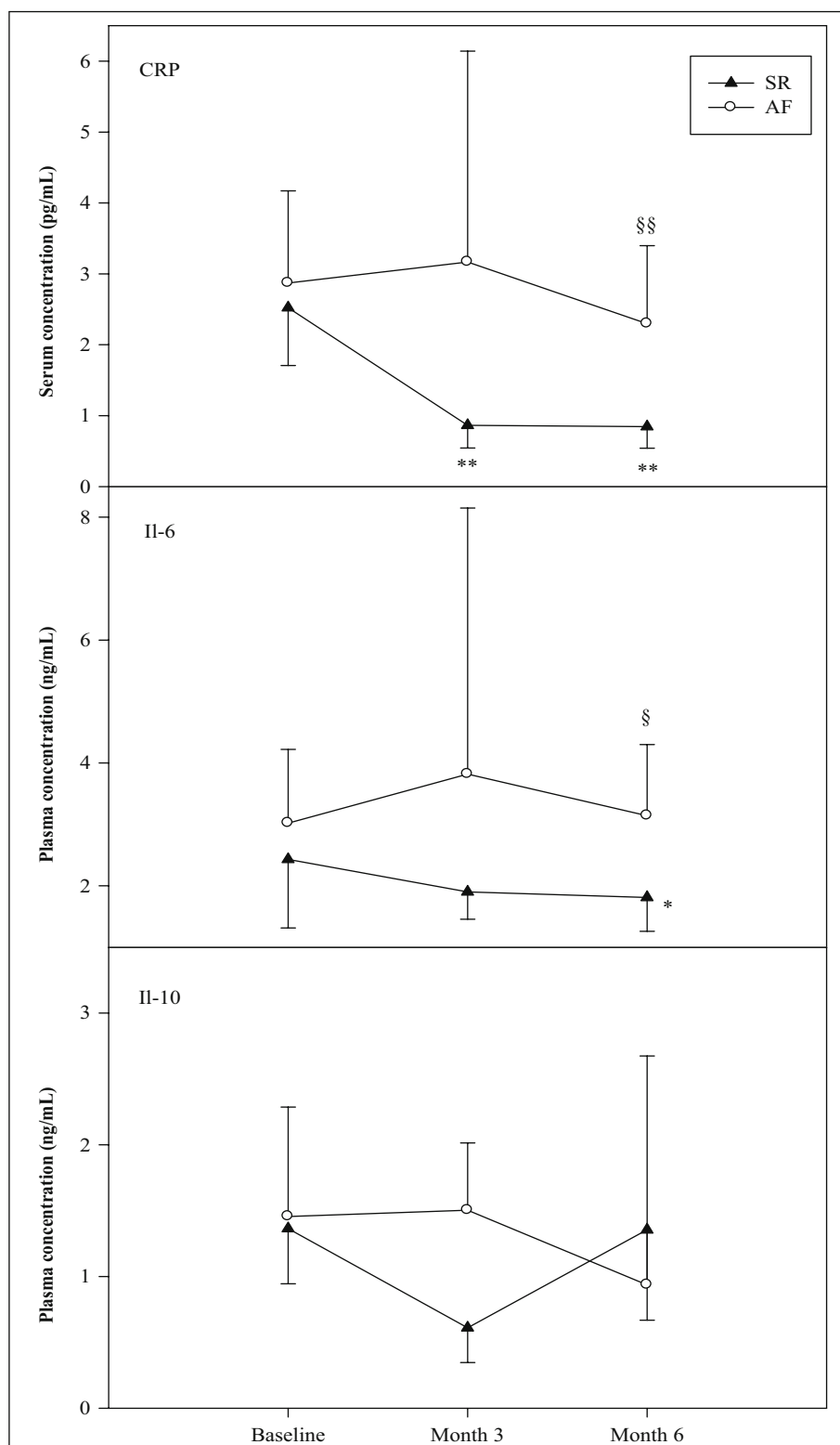
Figure 1

The time-course of the concentrations of CRP, IL-6 and IL-10. Data are presented as median and interquartile range, and are shown as closed triangles (SR group, n = 15) or open circles (FS group, n = 10). Data sets were analyzed by repeated measures ANOVA and the Mann-Whitney U test. Significance within the SR group (the comparison with baseline preoperative value) is shown by * (* $p < 0.05$), significance between the SR and the AF groups (the samples for the same time point) by § (§ $p < 0.05$, §§ $p < 0.01$).

levels than controls in sinus rhythm [5]. The relationship between AF and inflammation seems to be a vicious circle, where inflammation begets AF and *vice versa*. Moreover, markers of inflammation can sometimes predict the future of AF [8]. Conway reported a significant association between CRP and the success of cardioversion. CRP was found to be elevated in AF patients compared to controls in sinus rhythm, and CRP levels were seen as predictors of initial (short-term) cardioversion success [8].
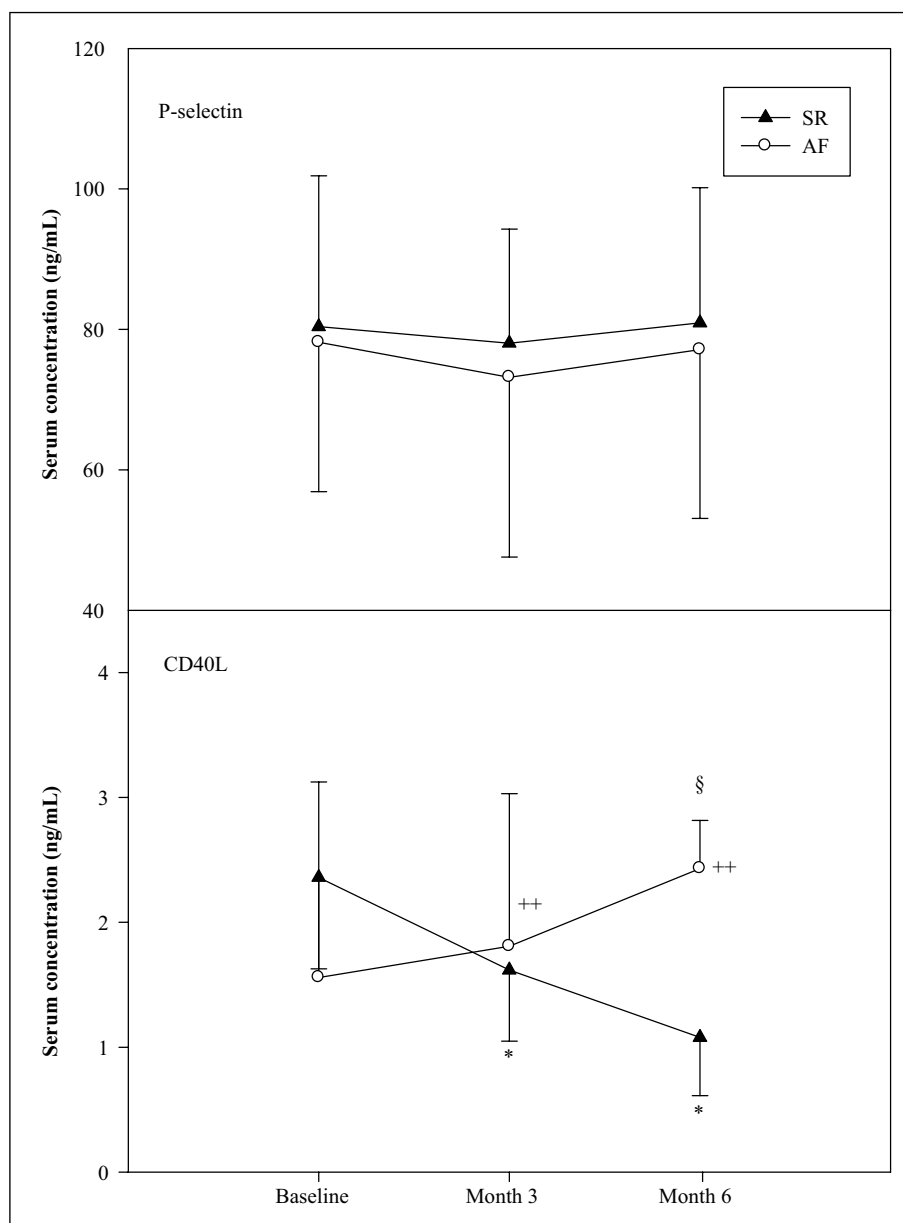
Figure 2

The time-course of the concentrations of P-selectin and CD40L. Data are presented as median and interquartile range, and are shown as closed triangles (SR group, n = 15) or open circles (FS group, n = 10). Data sets were analyzed by repeated measures ANOVA and the Mann-Whitney U test. Significance within the SR group (the comparison with baseline preoperative value) is shown by * (* $p < 0.05$), significance within the AF group (the comparison with baseline preoperative value) by + (++ $p < 0.01$), significance between the SR and the AF groups (the samples for the same time point) by § (§ $p < 0.05$, §§ $p < 0.01$).

Chung *et al.* found, in a case-control study, that patients with AF had increased CRP levels; additionally they found that patients with persistent AF had significantly higher CRP levels than those with paroxysmal AF [11]. Although not statistically significant, markers of inflammation (CRP) decreased 14 days after cardioversion. Acevedo measured CRP and thrombin-antithrombin complexes in 130 patients with newly diagnosed AF over a period of one year following cardioversion. At the one-year follow-up, mean CRP levels were still significantly elevated in patients that remained in AF compared to those who converted to sinus rhythm [12]. Marcus *et al.* measured inflammatory markers in 26 patients with atrial flutter (arrhythmia that is also associated with higher inflammatory status), before and up to six

months after ablation. Successful ablation of atrial flutter was associated with a rapid (within 1.5 month) decrease in CRP and a later decrease in IL-6 levels [13]. This is in full agreement with our findings, where SR patients showed significantly decreased concentrations of pro-inflammatory markers six months following ablation, whereas the concentration of these markers in patients who remained in AF were unchanged.

Recently, McCabe *et al.* observed 38 patients with AF after radiofrequency ablation [14]. The concentration of inflammatory markers (CRP and IL-6), increased six weeks following ablation (but only in patients with a recurrence of AF), with a later decline to preoperative values. Although the decline at six months did not reach statistical significance (in comparison to pre-ablation

values), the authors concluded that the inflammatory response is likely declining by that time. Unfortunately, they did not report any differences in levels of inflammatory cytokines between patients with and without recurrence of AF at six months following ablation.

In our cohort, baseline cytokine concentrations did not differ between AF and SR patients, and thus failed to predict the recurrence of arrhythmia. There are some reports that suggest a link between cytokine concentrations and successful cardioversion rates in AF. CRP levels were reported to be a predictor of initial cardioversion success (although they failed to predict long-term outcomes) [8]. However, considering our small sample size, our negative finding must be weighted accordingly, although sample size may not be the only explanation. In studies where cytokines were predictive, cardioversion was accomplished using direct current cardioversion, so all patients were treated uniformly. Our study involved a different procedure, and failure to note predictive links associated with cytokines could have easily been related to procedural failure (incomplete pulmonary vein isolation). Additionally, not all reports have confirmed the findings of Conway and others. Schnabel *et al.* in a very large cohort of 2,863 patients, reviewing 12 cytokines (such as IL-6, CRP and CD40L) did not find any predictive power regarding AF. Only osteoprotegerin, which was not measured in our study, was independently associated with future arrhythmia [15].

The concentration of IL-10 did not change over time in either group. The role of IL-10 has been studied in patients following cardiac surgery. Hak reported elevated levels of IL-10 in patients who having undergone cardiac surgery went on to develop post-operative AF [16]. However, different surgeries present substantially different kinds of trauma and it is not possible to compare such data with data from patients with non-surgical AF. There are few publications regarding the role of IL-10 in patients with non-surgical AF. In light of the unchanged concentration between patients in AF and SR, before and after ablation, non-surgical AF must not present a stimulus which is sufficiently strong to elevate IL-10 levels.

While the concentrations of CD40L decreased over time, the concentrations of P-selectin did not change. A progressive decrease in CD40L in AF patients, following SR restoration and maintenance, has been described by Hammwohner *et al.* [17]. This is in agreement with our data, although the maintenance of SR was achieved using another technique. While P-selectin and CD40L are markers of elevated platelet activity, there was no correlation between them, not only in our patient cohort, but also in previous studies [18]. While CD40L seems to be more involved in the inflammatory status [17], previous studies [17-20] have shown no correlation between the concentrations of P-selectin and either CD40L or other pro-inflammatory cytokines. The lack of a direct correlation between CD40L and P-selectin, along with the different time-courses following ablation in our patients, might reflect different aspects of platelet biology, and more pronounced role of inflammation in those patients with atrial fibrillation.

In conclusion, in patients with successfully ablated AF, there were decreases in inflammatory as well as pro-thrombotic markers. These decreases were not seen in cases where ablations were unsuccessful.

The most important limitations of our study were the small number of patients and the absence of a control group of patients without AF. Furthermore, some patients suffered from other co-morbidities, which may have influenced the concentration of cytokines. Relative to the multivariate analysis, the small group size may have resulted in some statistical correlations being missed. To the best of our knowledge, there are limited reports regarding the time-course of inflammatory and thrombotic markers following AF ablation.

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