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Serum levels of interleukin-2 predict the recurrence of atrial fibrillation after pulmonary vein ablation

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ABSTRACT

Aims: Interleukin-2 has a significant antitumor activity in some types of cancer, and has been associated with the development of atrial fibrillation (AF). In addition, IL-2 serum levels in recent onset AF have been related with pharmaceutical cardioversion outcomes. We evaluated the hypothesis that a relationship exists between inflammation and the outcome of catheter ablation of AF.
Methods: We studied 44 patients with paroxysmal AF who underwent catheter ablation. Patients with structural heart disease, coronary artery or valve disease, active inflammatory disease, known or suspected neoplasm, endocrinopathies, or exposure to anti-inflammatory drugs were excluded. All study participants underwent evaluation with a standardized protocol, including echocardiography, and cytokine levels of interleukin-2, interleukin-4, interleukin-6, interleukin-10, tumour necrosis factor-alpha, and gamma-interferon determination before procedure. Clinical and electrocardiographic follow-up were performed with Holter-ECG at 3, 6 and 12 months in order to know if sinus rhythm was maintained.
Results: After catheter ablation of the 44 patients included (53 ± 10 years, 27.3% female), all patients returned to sinus rhythm. During the first year of follow-up seven patients (15.9%) experienced recurrence of AF. The demographics, clinical and echocardiographic features, and pharmacological treatments of these patients were similar to those who maintained sinus rhythm. The only independent factor predictive of recurrence of AF was an elevated level of IL-2 (OR 1.18, 95% CI 1.12–1.38).
Conclusions: High serum levels of interleukin-2, a pro-inflammatory non-vascular cytokine, are associated with the recurrence of AF in patients undergoing catheter ablation.

1. Introduction

The physiopathology of atrial fibrillation (AF) is complex and not fully known. There is plausible evidence associating inflammation with the onset and perpetuation of atrial fibrillation [1], and with the thrombosis related to this arrhythmia [2].

Interleukin-2 (IL-2) was the first human interleukin to be identified, typified and purified. It is an indicator of inflammation, chiefly produced by activated T lymphocytes, which may activate T cells and NK cells [3], and present significant antitumor activity in some types of cancer, such as metastatic renal cells and malignant melanoma [4,5].

Thus, prolonged treatment of metastatic renal cancer patients with IL-2 has been associated with the incidence of atrial fibrillation [6]. In addition, high baseline levels of this interleukin have been associated with a lower effectiveness of cardioversion in patients with recent onset AF [7], showing it to be a powerful independent predictive factor for failure of cardioversion. Finally, low levels of IL-2 in patients undergoing coronary revascularisation surgery have been linked to a lower incidence of postoperative AF [8].

This study was designed to investigate the potential association between serum levels of IL-2 and the results of catheter ablation in patients with AF.

2. Methods

2.1. Population

We designed a prospective study of patients referred to our centre for ablation for atrial fibrillation between June 2010 and December 2011. We included paroxysmal atrial fibrillation

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patients with a normal ejection fraction (EF) who had not been admitted for heart failure or experienced changes in medication in the previous three months, who were to undergo pulmonary vein ablation and who gave their signed informed consent. AHA/ACC/ESC guidelines defining paroxysmal AF were followed [9].

All of the patients included were in sinus rhythm at the time of sampling, which was taken before the procedure in order to avoid potential greater inflammatory activity in patients who could have been included in persistent AF [10].

We excluded patients who had undergone heart surgery or prior percutaneous ablation, had a history of infection or an active inflammatory process in the previous 90 days, and patients with structural cardiopathy, endocrinopathies including thyroid disorders, diabetes mellitus, known or suspected neoplasia, patients exposed to anti-inflammatory or corticosteroid medication, or those undergoing a hypolipidemic treatment.

Ten controls were included, consisting of volunteers, in sinus rhythm, with similar demographic and clinical characteristics to the patients being studied, with the objective of checking whether interleukin levels were higher in patients with atrial fibrillation.

This study was performed in compliance with the Declaration of Helsinki and was approved by the centre's Ethics Committee; all patients gave written informed consent to their participation in the study.

2.2. Clinical protocol

2.2.1. Echocardiography, ablation and follow-up

All of the patients were assessed using a standard protocol that included a medical history, physical examination, chest radiograph, 12-lead electrocardiogram, and an elemental analysis (cell count and basic biochemistry). In the 48 h prior to the procedure, peripheral venous blood was extracted in order to measure interleukin-2, interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), tumour necrosis factor alpha (TNF-alpha) and interferon gamma (IFNG).

A two-dimensional echocardiogram was carried out in the 48 h prior to the procedure using Vivid S6 equipment (General Electric Co). Left atrium volume was estimated by applying Simpson's formula, taking two and four chamber images from the apical plane. Although the reference method for estimating the left atrium dimensions is a multi-cut computerised tomography (CT), several studies have shown a high correlation between this method and measurement of left atrium volume by means of echocardiography [11,12].

The atrial fibrillation ablation protocol was carried out under conscious sedation using fentanyl and midazolam. All patients were anticoagulated with acenocumarol for a minimum of one month prior to the ablation. The left femoral vein was punctured once in order to position a decapolar catheter in the coronary sinus; a double puncture of the right femoral vein was performed to advance an irrigated tip ablation catheter to the His; a Mullins sheath was used to carry out a single transseptal puncture with a Brockenbrough needle, using only fluoroscopic guidance, with the His and the coronary sinus as references. A guide wire was passed to the left atrium and the sheath was removed to advance the ablation catheter through the transseptal orifice to the left atrium. The Mullins sheath was again advanced along the guide to the left atrium and a LASSO Xp (Biosense Webster) circular catheter was introduced through the sheath to map pulmonary veins. As soon as the transseptal puncture was performed, a dose of 100 units per kilo of heparin sodium was administered and an uninterrupted perfusion of heparin was continued via the Mullins sheath, maintaining an ACT between 250 and 350 ms throughout the whole procedure. A three-dimensional map of the left atrium and the pulmonary veins was made using CARTO (Johnson & Johnson) or Ensite NAVx (St Jude Medical) navigation systems indistinctly, under guidance from images obtained via the multi-cut computerised tomography carried out in the days before the procedure. In all cases, a circumferential pulmonary vein ablation was performed using an irrigated tip ablation catheter until electrical isolation of the veins was achieved and confirmed by means of circular catheter (entry and exit block in all 4 veins). In the cases where there had been a previously documented typical atrial flutter, or if one was induced during the procedure, an ablation of the cave-tricuspid isthmus was carried out. Anti-coagulation and anti-arrhythmia medication was maintained for at least the 3 months subsequent to the procedure (the "blanking period"). After this period, anti-coagulants were stopped only if the $CHADS_2$ score was lower than 2.

Clinical follow-up was carried out at 3, 6 and 12 months, and included a physical examination with ECG and a 24 Holter-ECG, with the aim of detecting relapses in atrial fibrillation after the procedure. It was considered recurrence to be when the patient presented clinical AF, in the ECG at check-up or when the Holter-ECG provided evidence of AF of at least 30 s, from the third month after procedure.

2.3. Processing of samples

The samples of peripheral venous blood were collected in BD Vacutainer EDTA tubes and immediately stored at 4 °C for no longer that one hour before processing the plasma. The plasma was centrifuged at 3000 rpm for 15 min at 4 °C. Subsequently, the samples were stored at −80 °C awaiting analysis. All samples were stored and analysed at the end of the study in the Biomedical Research Laboratory of the Hospital.

Quantification of Cytokines: IL-2, IL4, IL-6, IL-10, TNF alpha, and Interferon-gamma using flow cytometry.

In order to quantify cytokines, the *Human* Cytometric Bead Array (CBA kits; BD Pharmingen, CA, USA) was used, following the manufacturer's recommendations supplied by the distributor. Total concentration of plasma proteins was determined with the Bradford (BioRad, CA USA) quantification method. Readings of the total concentration of proteins were carried out at 595 nm on Versamax (GE, CT, USA) equipment. BSA in PBS (Sigma–Aldrich, St Lois MO, USA) was used as the calibration curve for the protein samples. To determine cytokines, 40 μg/ml total protein for were used for each sample of each patient. After incubation of antibodies and the respective washings, data acquisition was made with the Culter-Dako Cytomation cytometer (Dako cytomation, Inc, Chicago, IL, USA). The calibration curves of each cytokine were used to make the calculation of final concentrations, considering in each case the detection limit established (Table 1) by the manufacturer (BD Pharmingen, CA, USA). For each cytokine analysed, a specific profile corresponding to the phycoerythrin (PE) spectrum was obtained.

Table 1
Cytokines detection limit established (pg/ml).

Cytokines	pg/ml
IL-2	2.6
IL-4	4.9
IL-6	2.4
IL-10	4.5
TNF	3.8
IFN-γ	3.7

IL indicates interleukin; INFγ indicates interferon gamma; TNFα indicates tumour necrosis factor alpha.