

INFLAMMATORY MARKERS IN PAROXYSMAL ATRIAL FIBRILLATION AND THE PROTECTIVE ROLE OF RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM INHIBITORS

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Abstract

Background. Experimental and clinical studies have shown the importance of inflammation in the pathophysiology of atrial fibrillation (AF). The renin-angiotensin-aldosterone system (RAAS) may play an important role in the pathogenesis of AF in correlation with the inflammatory process. RAAS inhibition may have important therapeutic value in limiting AF. The aim of this study was the correlation between inflammatory markers and recurrent episodes of AF in patients with known paroxysmal atrial fibrillation, with and without treatment with RAAS inhibitors.

Methods and results. We studied 82 patients with paroxysmal AF recorded at “Niculae Stancioiu” Heart Institute Cluj-Napoca, divided into two groups: group A treated with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) and group B without this medication. All patients underwent clinical examination, ECG, echocardiography and determination of plasma levels of inflammatory markers represented by high sensitivity C-reactive protein (hs-CRP) and interleukin 6 (IL-6). In the group treated with ACE inhibitors/ARBs, AF burden was significantly lower than in patients without treatment. We obtained a strong positive correlation between blood levels of high-sensitivity CRP and those of IL-6 ($r=0.64$, $p<0.001$), the number of yearly AF episodes ($r=0.570$, $p<0.001$), LA diameter ($r=0.5$, $p<0.001$) and LA volume ($r=0.5$, $p<0.001$). We found moderate positive correlations between blood levels of IL-6 and LA diameter ($r=0.305$, $p=0.01$), LA volume ($r=0.314$, $p=0.01$), the number of yearly AF episodes ($r=0.489$, $p<0.001$), the total number of AF episodes ($r=0.304$, $p<0.001$), BMI ($r=0.473$, $p<0.001$), LA area ($r=0.458$, $p<0.001$), LA area index ($r=0.334$, $p=0.007$) and LA volume index ($r=0.304$, $p=0.01$). The number of yearly AF episodes and BMI values influenced IL-6 blood levels ($t=3.46$, $p=0.001$, respectively $t=2.17$, $p=0.03$).

Conclusions. Inflammation is present in patients with AF, with or without treatment with RAAS inhibitors and is correlated with longer duration of AF, left atrial diameter and left atrial volume. ACE inhibitors and ARBs, acting on cardiac substrate and reducing the inflammatory process, may have a therapeutic protective role of decreasing AF burden.

Keywords: atrial fibrillation, inflammatory markers, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB).

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia, with the highest prevalence, affecting more than 7 million people in North America and the European Union. AF incidence increases with age, affecting 5% of people aged over 65 [1]. AF significantly decreases the quality of life and is an independent predictor of morbidity and mortality [1,2,3,4,5].

Experimental and clinical studies have shown the importance of inflammation in the pathophysiology of AF. Identification of inflammatory infiltrates, myocyte necrosis and fibrosis in biopsy samples from patients with AF undergoing surgery, support the hypothesis that AF is directly correlated with the inflammatory process [6,7,8]. Moreover, studies have evidenced elevated levels of C-reactive protein (CRP) and interleukin 6 (IL6) in patients with persistent or permanent AF [9]. Some studies have also shown that the renin-angiotensin-aldosterone system (RAAS) may play an important role in the pathogenesis of AF in correlation with the inflammatory process [9].

RAAS release of angiotensin II (AG II) can have a direct impact on the atrial electrophysiological properties [11], fibrosis, the extent of the left atrium (LA) and the autonomic tone, all contributing to the electrical and structural remodeling involved in the initiation and perpetuation of AF [6,11,12,13,14].

Previous studies and meta-analyses [15] have suggested that RAAS inhibition by administration of angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) may have important therapeutic value in limiting AF, especially in patients with hypertension (HTN) [16] or with heart failure (HF) [17,18].

Therefore, our purpose was to study the correlation between inflammatory markers and recurrent episodes of AF in patients with known paroxysmal atrial fibrillation, with and without treatment with ACE inhibitors or ARBs.

Material and methods

Selection of patients

We have identified a total of 868 patients with AF in the emergency department records of "Niculae Stancioiu" Heart Institute Cluj-Napoca, between January 2007 and December 2012. We excluded 101 patients coming from other counties, expecting a low compliance in the study. Of the 767 patients from Cluj-Napoca and Cluj county, we selected those with paroxysmal AF.

Paroxysmal AF was defined, according to current guidelines, as having the following characteristics: onset in less than seven days, self-limited, spontaneously reversible or reversible by medication. The exclusion criteria were the following: acute coronary syndrome, dilated cardiomyopathy in advanced stages, NYHA III and IV functional class heart failure, known valvular heart disease, inflammatory diseases and cancer. The application of the

exclusion criteria resulted in the selection of 162 patients. They were contacted by phone or mail, but only 82 of them accepted the invitation to participate in the study. Patients were divided into two groups: group A with ACE inhibitor or ARB therapy and group B without ACE inhibitor or ARB therapy.

All patients signed the informed consent form regarding the confidentiality of medical records and the informed consent form to participate in the research study, approved by the hospital's Ethics Committee.

Clinical assessment and laboratory analysis

All patients underwent clinical examination, ECG, echocardiography (Vivid S6, General Electric) and blood samples were taken from the forearm vein. Patients with body mass index (BMI) >25 were considered overweight (BMI between 25 and 29.9 kg/m²) and obese (>30 kg/m²). We determined plasma levels of total cholesterol, HDL-cholesterol, triglycerides and inflammatory markers represented by high sensitivity C-reactive protein (hs-CRP) and interleukin 6 (IL-6). For the latter ones, we used Vacuette tubes with clot activator (producer Grainer). Hs-CRP was determined using the immunoturbidimetric assay with Cobas Integra 400 analyzer and IL-6 by enzyme immunoassay technique with immuno-chemiluminescent assay on a Beckman Coulter Access analyzer. The lower limits of detection were 0.15 mg/L for hs-CRP and 0.5 pg/ml for IL-6.

Antiarrhythmic medication included beta-blockers (71.87%), amiodarone (18.75%) and propafenone (28.12%) without significant differences between groups. A small number of patients were treated with statins (25%).

After the results, patients from both groups with hs-CRP and IL-6 levels higher than 10 mg/L and 10 pg/ml were excluded from the final analysis, as these levels would have altered the final results, most likely indicating general subclinical inflammatory processes. Thus, 64 patients (33 in group A and 31 in group B) remained for the final analysis.

Statistical analysis

Statistical analysis was performed using MedCalc Software, version 12.5.

Variables were characterized by frequency, mean±SD and median (25th and 75th percentile), depending on the type of variables. The normality of continuous variables distribution was checked using the Kolmogorov-Smirnov test. To check for significant differences between the different variables in the two groups we used the t test for continuous variables with normal distribution, the Mann-Whitney test for non-normal distribution and the χ^2 test for categorical variables. We applied Spearman correlations to analyze a possible association between inflammation and the studied parameters. Multivariate analysis was performed using binary logistic regression and linear regression.

Results

Clinical characteristics of patients

Demographic and clinical characteristics of patients are presented for both groups in Table I. There were no statistically significant differences between the two groups in terms of age, gender, body mass index. In group A, hypertension was present in all subjects, and obesity, ischemic heart disease and diabetes mellitus were more common. The oldness of AF was significantly higher in group B. AF burden was assessed based on clinical symptoms as the number of yearly episodes and their duration.

There was no statistically significant difference between the two groups in terms of echocardiographic characteristics. There were no significant differences in the treatment associated with beta-blockers, amiodarone, propafenone, statins. ACE inhibitors used in patients in group A were perindopril (24.24%), ramipril (6.06%) and quinalapril (3.03%). ARBs used were: telmisartan (42.42%), candesartan (9.09%), irbesartan (9.09%) and valsartan (6.06%).

In the group treated with ACE inhibitors/ARBs, the AF burden was significantly lower than in patients without treatment.

The levels of inflammatory markers

The oldness of AF, hs-CRP and IL-6 had an abnormal distribution.

We obtained a strong positive correlation between blood levels of high-sensitivity CRP and those of IL-6 ($r=0.64$, $p<0.001$), the number of yearly AF episodes ($r=0.570$, $p<0.001$), LA diameter ($r=0.5$, $p<0.001$) and LA volume ($r=0.5$, $p<0.001$). We found moderate positive

correlations between blood levels of high-sensitivity CRP and BMI values ($r=0.482$, $p<0.001$), the total number of episodes of AF ($r=0.440$, $p<0.001$), LA area ($r=0.458$, $p<0.001$), LA area index ($r=0.369$, $p=0.003$) and LA volume index ($r=0.443$, $p<0.001$). Correlations were also preserved when considering the treatment with ACE inhibitors/ARBs or statins.

We found moderate positive correlations between blood levels of IL-6 and LA diameter ($r=0.305$, $p=0.01$), LA volume ($r=0.314$, $p=0.01$), the number of yearly AF episodes ($r=0.489$, $p<0.001$), the total number of AF episodes ($r=0.304$, $p<0.001$), BMI ($r=0.473$, $p<0.001$), LA area ($r=0.458$, $p<0.001$), LA area index ($r=0.334$, $p=0.007$) and LA volume index ($r=0.304$, $p=0.01$). Correlations were also preserved when considering the treatment with ACE inhibitors/ARBs or statins.

We obtained significant differences between hs-CRP median levels in patients with hypertension and in those without hypertension ($p=0.003$). Obese patients had statistically significantly higher levels of hs-CRP and IL-6 compared to those with normal weight ($p=0.002$, $p=0.002$, respectively $p=0.004$). Patients who were treated with statins did not have different levels of hs-CRP or IL-6, compared with those who did not undergo lipid lowering therapy ($p=0.21$, respectively $p=0.3$).

In order to assess the independent effect of the parameters studied on interleukin 6 levels we used multiple linear regression considering the levels of IL-6 as dependent variable (Table II). The number of yearly AF episodes and BMI values influenced IL-6 blood levels ($t=3.46$, $p=0.001$, respectively $t=2.17$, $p=0.03$).

Table I. Demographic and clinical characteristics of patients.

Variable	Group with ACE inhibitors/ARBs	Group without ACE inhibitors/ARBs	p
Age (mean, SD)	60.82±7.39	58.45±7.86	0.22
Men (number)	12	14	0.64
BMI (mean, SD)	29.17±3.65	27.65±4.54	0.14
Overweight or obese (number)	29	20	0.05
AF age (median; 25 th , 75 th percentile)	2 (1; 6)	5 (3; 10)	0.02
Ischemic heart disease	5	0	0.07
CVA (number)	2	2	1
DM (number)	9	2	0.06
HTN (number)	33	24	0.01
LA diameter (mean, SD)	40±5.56	37.58±5.33	0.08
LA volume (mean, SD)	54.67±11.03	50.94±14.08	0.24
LA volume index (mean, SD)	28.61±5.5	26.39±6.81	0.15
Hs-CRP (median; 25 th , 75 th percentile)	2.2(1.14; 4.29)	2.1(1.13; 4.05)	0.96
IL-6 (median; 25 th , 75 th percentile)	2.32(1.32; 3.02)	2.57(1.51; 3.34)	0.66
Beta-blocker (number)	26	20	0.32
Amiodarone (number)	7	5	0.84
Propafenone (number)	7	11	0.32
Statin (number)	12	4	0.06

Table II. The results of multiple linear regression regarding IL-6.

Variable	Standardized coefficients		p	CI 95%	
	beta			Min	Max
Number of yearly AF episodes	0.398		0.001	0.200	0.747
Left atrial area index related to BMI	0.027		0.808	0.378	0.483
BMI	0.247		0.034	0.018	0.444

Discussion

The data of this study show that patients treated with ACE inhibitors/ARBs had a significantly lower AF burden than those without treatment. However, the result should be cautiously interpreted, given that there was no long-term Holter monitoring in order to identify arrhythmic episodes, but only an analysis based on the patients' symptoms. A first estimation shows no significant differences between the two groups in terms of hs-CRP and IL-6 levels.

Inflammatory markers, RAAS and AF

Experimental studies have shown that both inflammation [19,20] and atrial fibrosis [21] are pathogenetic mechanisms involved in the onset and persistence of AF. There are demonstrated pathophysiological correlations between inflammation and fibrosis explaining this interrelation, with detrimental effect causing electrical and structural atrial remodeling, AF being a progressive disease. Atrial structural remodeling was evidenced by several imaging methods, such as nuclear magnetic resonance imaging, the ground of Utah classification of left atrial wall injury [22]. The degree of fibrosis is directly correlated with the percentage of paroxysmal or permanent AF and thus, with the degree of AF.

Experimental and clinical studies have demonstrated the influence of RAAS on the initiation and maintenance of atrial inflammation by complex pathophysiological mechanisms such as: increased production of proinflammatory cytokines (IL-6, IL-8, TNF-alpha, IFN-gamma), increased production of chemoattractant proteins and selectins (P and S) [23].

A recently published study that aimed to identify the site of inflammation in patients with paroxysmal or persistent AF by analyzing multiple inflammatory markers harvested at different levels did not show significant differences in terms of CRP, IL-8 and TGF- β 1 from patients in SR. However, the relatively low level of inflammatory markers in paroxysmal and persistent AF suggests that the inflammatory process is low and appears as a transient event in the natural history of AF [24].

These results are inconsistent with those of another recently published article showing that in patients with short-term persistent atrial fibrillation, early AF recurrence seems to be associated with inflammation, represented by elevated levels of IL-6. TGF-beta-1, LVEF and early recurrence of atrial fibrillation are independently associated with progression to permanent AF [25].

Spearman's rank correlation was used in our study in order to analyze a possible association between inflammation and studied parameters and thus we obtained a strong positive correlation between hs-CRP and IL-6 blood levels and the number of yearly AF episodes, LA diameter and LA volume, data which seem largely consistent with that of the last study cited.

Cardiac substrate, treatment with RAAS blockers and AF

The potential benefit of RAAS modulation has been demonstrated in hypertensive patients with heart failure and multiple cardiovascular risk factors [26,27]. Former or recent clinical trials have revealed the protective role of the treatment with ACE inhibitors/ARBs in patients with paroxysmal AF [28,29]. In the LIFE study, hypertensive patients treated with Losartan showed a 33% reduction of new AF episodes than those treated with atenolol for a follow-up period of 4.8 years [27]. In the SOLVD study, patients with LV dysfunction treated with enalapril had a significantly lower rate of developing AF than the placebo group [18]. In the CHARM study, patients with heart failure treated with candesartan had a significant reduction in AF development than the placebo group [30]. A meta-analysis published in 2005 demonstrated a favorable effect of RAAS inhibition in primary and secondary prevention of AF [15].

However, there are studies supporting the contrary, including a recently published one [31], showing no differences in the occurrence of new AF episodes in patients with ischemic heart disease regardless of treatment with ACE inhibitors or ARBs. Trying to identify the absence of the benefit of RAAS modulation in this study, the authors studied elderly subjects (mean 78 years of age) and concluded that the treatment with ACE inhibitors or ARBs would have little effect on extended atrial fibrosis in elderly patients.

We can compare the degree of LA fibrosis assessed by delayed enhancement MRI (DE-MRI) and the recurrence of AF after ablation, as resulting from data published by the research team in Salt Lake City in 2011 [22]. UTAH I patients (less than 5% fibrosis) had no AF recurrence, UTAH II patients (5-20% fibrosis) had a 28% recurrence rate, UTAH III patients (20-35% fibrosis) had a 34.7% recurrence rate, and UTAH IV patients (over 35% fibrosis) had 56.3% recurrence.

We could make an intuitive and speculative assessment that the effectiveness of ACE inhibitors/ARBs is validated in early stages of AF, in paroxysmal and short-term persistent AF, where there are more inflammatory processes and where fibrosis is not over-expressed compared to long-term persistent AF, and in permanent AF, where extensive and well expressed fibrosis, with significant structural remodeling, determines, by multiple re-entry circuits, the perpetuation and self-support of arrhythmia and reduces the effectiveness of RAAS blockers.

In our study, the mean age of patients was 60.82 and 58.45 years respectively, without statistical difference between groups. Hypertension was an associated predominant pathology, present in both groups, but 100% in group A (which explains their previous treatment with ACE inhibitors or ARBs), while ischemic heart disease, stroke or diabetes mellitus were less common.

Regarding this pathology, patients treated with ACE inhibitors/ARBs (group A) had a lower load and lower age of AF), which could be explained by the protective effect of RAAS blockers.

Conclusions

Inflammation is present in patients with AF, with or without treatment with RAAS inhibitors and is correlated with a longer duration of AF, left atrial diameter and left atrial volume. ACE inhibitors and ARBs, acting on cardiac substrate and reducing the inflammatory process, may have a therapeutic protective role of decreasing the AF load.

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