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Original Research Article

Interleukin 8: Changes in Paroxysmal Atrial Fibrillation

ABSTRACT

Aims: To study the levels of interleukin-8 (IL-8) in patients with paroxysmal atrial fibrillation (occurred in <48 hours) and track the changes after restoration of sinus rhythm.

Study design: Prospective

Place and Duration of Study: The study was conducted in the Intensive Cardiology Department of the First Cardiology Clinic at the University Hospital "St. Marina "- Varna for the period October 2010 – May 2012.

Methodology: We included 51 patients (26 men, 25 women; mean age 59.84±1.60 years) with paroxysmal atrial fibrillation and 52 controls (26 men, 26 women; 59.50±1.46 years) with no history of atrial fibrillation. The two groups matched by age, gender and clinical characteristics. In patients plasma concentrations of IL-8 were measured three times: immediately after admission to the ward (baseline values), twenty-four hours and twenty-eight days after rhythm restoration. In the control group the indicator was tested once. IL-8 was measured using an ELISA kit. In all patients the arrhythmia episode was discontinued by the administration of propafenone.

Results: All patients were hospitalized between the second and the twenty-fourth hour after the onset of the arrhythmia, and most frequently in the fifth hour (10 of all 51 patients). Baseline values of IL-8 were increased compared to those of the controls (77.38±3.78 vs 32.18±1.54 pg/mL, p <0.001). Twenty-four hours after restoration of sinus rhythm, IL-8 concentrations were still significantly higher (65.33±3.29 vs 32.18±1.54 pg/mL, p <0.001). On the twenty-eighth day yet there was no significant difference (28.07±1.68 vs 32.18±1.54 pg/mL, p = 0.07). **Conclusion:** Plasma concentrations of IL-8 levels are significantly elevated still in early hours of the clinical manifestation of paroxysmal atrial fibrillation as well as after the arrhythmia discontinuation. Their restoration occurs slowly over time. The established specific dynamics in IL-8 concentrations suggests a close relationship between paroxysmal atrial fibrillation and inflammation.

Keywords: interleukin-8, inflammation, atrial fibrillation, sinus rhythm

1. INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice, affecting > 1% of the general population [1]. There is an increasing interest in recent years in paroxysmal atrial fibrillation (PAF), recurrences of which are associated with electrical and structural remodeling of the atria and subsequent chronification of the rhythm disorder or in short "AF begets AF" [2]. PAF constitutes between 25 and 60% of all cases of AF, and it is even believed that the actual prevalence is higher because of the presence of asymptomatic episodes [3]. In PAF the risk of stroke and thromboembolic complications is not less than the risk of other forms of arrhythmia, including permanent AF [4].

The mechanisms involved in the appearance and clinical course of the disease are complex and still not fully understood. More and more data is being accumulated on the presence of a link between PAF and inflammation. For example, histological studies have found that the

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development of PAF is associated with local inflammatory changes in the atrial myocardium [5, 6]. It is believed that in their base lies leukocyte activation [7]. It, in turn, is a direct result of the potency and duration of action of the main inflammatory modulators, namely cytokines and chemokines [8]. In this sense their leading role is assumed in the occurrence and recurrence of PAF. That is why their research is a challenge for modern cardiology and could give an answer to a number of questions related to the treatment of the disease.

Studies have already found increased levels in certain cytokines from the inflammatory cascade such as TNF-α, IL-6 and IL-18 in patients with PAF [9, 10]. The studies conducted to date, however, are few and the indicators are predominantly determined only once. Single and to some extend discrepant data on the levels of IL-8 are present [11, 12]. There are still no clinical studies with a sufficiently long period of observation to outline the nature of the identified changes and assume a causal relationship between PAF and inflammation.

2. PURPOSE

To study the levels of IL-8 in patients with PAF (occurred in <48 hours) and track the changes after restoration of sinus rhythm.

3. MATERIAL AND METHODS

3.1 Study population

Only patients with PAF with a rhythm disorder which occurred in <48 hours prior to hospitalization were screened for the study. This allowed for an acute medication attempt for rhythm regularization.

The beginning of the rhythm disorder was accurately determined based on detailed medical history where patients determined the onset of AF as a sudden occurrence of a subjective feeling of "palpitation", continuing until hospitalization. The diagnosis "atrial fibrillation" was accepted after being objectified by electrocardiographic examination, performed immediately after the hospitalization of the patients.

From a total of 338 screened, only 56 participants were selected (31 men, 25 women) with restored and permanently retained sinus rhythm until the end of the study. 282 patients with PAF were dropped due to exclusion criteria (see exclusion criteria).

Two control examinations were carried out after hospital discharge – on the seventh and twenty-eighth day after discontinuation of the arrhythmia, during which the performed ECG records and detailed medical history did not reveal any recurrence of the rhythm disorder.

To balance the gender structure, 51 patients were successively selected (26 men and 25 women) with a mean age 59.84 ± 1.60 years (31-77 years).

Exclusion criteria for the study

1. Cardiovascular diseases, namely: ischemic heart disease, heart failure; inflammatory or congenital heart diseases, moderate or severe acquired valvular diseases; cardiomyopathies.

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2. Other diseases – renal, pulmonary or liver failure; diseases of the central nervous system; inflammatory and/or infectious diseases for the previous three months; neoplastic or autoimmune diseases; diseases of the endocrine nervous system (except for diabetes mellitustype 2, non-insulin dependent).
3. Intake of hormone-replacement therapy or contraceptives; pregnancy; systematic intake of analgesics, incl. non-steroidal anti-inflammatory drugs; BMI>35.
4. persistence of the rhythm disorder after propafenone application, rhythm regularization by electrical cardioversion, recurrence of the AF till the end of the study (exclusion criteria for patients).

In compiling the control group the same exclusion criteria were applied (see above), since the selection of the participants (patients and controls) aimed to a maximal degree to eliminate or equalize between the two groups the factors influencing inflammation. Thus, from a total of 169 screened, 52 were selected as controls for the study. Their mean age was 59.50±1.46 years (30-76 years) and men and women were an equal number – 26 (50%). Prior to the study the controls had no history or electrocardiographic evidence of AF.

3.2 Study design

Patients' IL-8 levels were determined three times: immediately after admission to the ward (baseline values), twenty-four hours and twenty-eight days after rhythm restoration. In the control group the indicator was tested once.

Patients were dehospitalized twenty-four hours after interruption of the rhythm disorder. All of them were monitored for a period of 28 days after rhythm regularization.

The study was conducted in the Intensive Cardiology Department of the First Cardiology Clinic at the University Hospital "St. Marina "- Varna for the period October 2010 – May 2012 after approval by the Ethics Committee of Scientific Research (№35/29.10.2010) at the same hospital and in compliance with the Declaration of Helsinki [13]. Participants were included in the study after previously signing an informed consent for participation.

3.3 Therapeutic scheme of propafenone

Propafenone was administered according to its prescribed scheme: iv 2 mg/kg bolus, followed by an infusion at a dose of 0.0078 mg/kg/min for 120 min and p.o. administration at a dose of 300 mg three times at an interval of 8 hours [14, 15]. In restored sinus rhythm the scheme was discontinued, and until the end of the study all patients received a maintenance dose of p.o. 150 mg three times daily. All patients were continuously monitored until hospital discharge.

3.4 Collection and storage of blood samples. Study of IL-8.

Venous blood was collected in a heparin vacutainer (VACUETTE/4.0 ml/Li Hep) and immediately centrifuged. Subsequently, the resulting plasma was frozen. Collection and storage of samples was carried out in full accordance with the methodology used.

Re-freezing of samples was not allowed during the conducting of the study.

Plasma concentrations of IL-8 were measured using an ELISA kit (Elabscience Biotechnology Co., Ltd, China) according to the manufacturer's protocol.

3.5 Statistical analysis

Using descriptive statistics, averages, relative shares and central tendency (Mo=mode) were calculated. The testing of the hypothesis for equality of averages and indicators of relative

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share was done using Student's t-criterion. Values of p <0.05 were considered statistically significant.

The analysis of all data was performed by a specialized statistical analysis package GraphPad PRISM, Version 5.00. The results were presented as mean±standard error of the mean (SEM) or n(%).

4. RESULTS

4.1 Clinical characteristics of patients and controls

The clinical characteristics of the group with PAF were statistically identical to that of the controls ($P > 0.05$) (Table 1).

The performed statistical analysis of the time from the onset of AF until hospitalization showed that all 51 patients were hospitalized between the second and the twenty-fourth hour after the onset of the arrhythmia, and most frequently in the fifth hour (Mo=5, 10 of all 51 patients). The mean duration of the episodes of AF prior to hospitalization was 8.14±0.76 hours (from a minimum of 2 hours to a maximum of 24 hours).

Table 1. Characteristics of patients' and control group.

	Patients with PAF	**Control group**	***P* values**
Number of participants in the group	51	52	*P*= .89
Mean age (years)	59.84±1.60	59.50±1.46	*P*= .87
Men/Women	26/25	26/26	*P*=1/*P*= .93
Accompanying diseases			
Hypertension	37 (72.54%)	34 (65.38%)	*P*= .44
Diabetes mellitus type 2	3 (5.88%)	2 (3.84%)	*P*= .62
Chronic ulcer disease	2 (3.92%)	0	*P*= .15
Status after hysterectomy	2 (3.92%)	1 (1.92%)	*P*= .54
Benign prostatic hyperthrophy	1 (1.96%)	0	*P*= .32
Dyslipidemia	4 (7.84%)	3 (5.77%)	*P*= .69
Medicaments for Hypertension and Dyslipidemia			

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Beta blockers	19 (37.25%)	17 (32.69%)	*P*= .62
ACE inhibitors	15 (29.41%)	14 (26.92%)	*P*= .78
Sartans	11 (21.57%)	9 (17.31%)	*P*= .58
Statins	4 (7.84%)	3 (5.77%)	*P*= .69
Deleterious habits			
Smoking	8(15.69%)	7(13.46%)	*P*= .75
Alcohol intake	7(13.72%)	6(11.53%)	*P*= .74
BMI (kg/m^2)	23.85±0.46	24.95±0.45	*P*= .09
Echocardiographic measurements			
LVEDD (mm)	52.57±0.58	52.29±0.57	*P*= .73
LVESD (mm)	34.43±0.56	34.73±0.48	*P*= .69
EF (%)	62.98±0.70	61.54±0.58	*P*= .12
IVS (mm)	10.37±0.23	9.92±0.26	*P*= .20
PW (mm)	10.24±0.21	9.73±0.28	*P*= .16
LA обем (ml/m^2)	22.81±0.45	23.82±0.48	*P*= .13
RVEDD (mm)	30.54±1.58	29.17±1.52	*P*= .18

4.2 Concentrations of IL-8

From Figure 1 it is seen that baseline plasma concentrations of IL-8 in patients were increased compared to those of controls (77.38±3.78 vs 32.18±1.54 pg/mL, *P*<0.001). Twenty-four hours after restoration of sinus rhythm, the measured values of IL-8 were still significantly higher than in controls (65.33±3.29 vs 32.18±1.54 pg/mL, *P*<0.001). On the twenty-eighth day there was no significant difference in the values in patients and controls (28.07±1.68 vs 32.18±1.54 pg/mL, *P*= .07).

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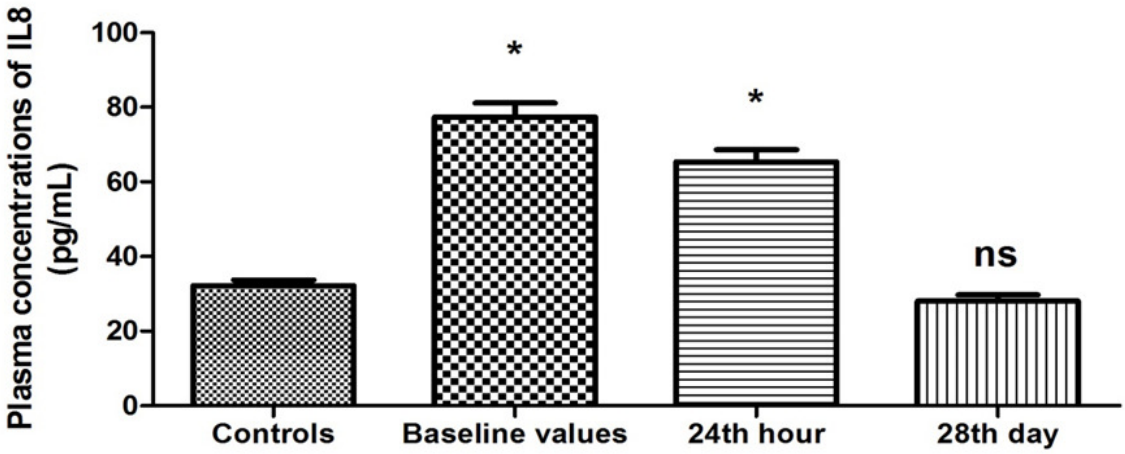

Figure 1. Changes in plasma concentrations of IL-8 (pg/mL) in patients with PAF. (baseline values – values upon patients' hospitalization; 24th hour – values 24 hours after rhythm regularization; 28th day - values 28 days after rhythm regularization; *- $P<0.001$; ns – statistically insignificant difference).

5. DISCUSSION

The statistical processing of our data showed significant differences in plasma concentrations of IL-8 in patients and controls (Figure 1). In the samples taken immediately after hospitalization, the levels of IL-8 in patients with PAF were much higher ($P<0.001$). As noted in the "Results" section, they were tested in the first hours of the clinical manifestation of the disease (up to the twenty fourth hour). The early and significant increase of IL-8 gives serious grounds to assume that the identified changes are closely related and specific to PAF, and not an accidental laboratory finding. In this sense, the clinical characteristics of the participants are essential for the results. The low burden of diseases (Table 1) eliminates their potential effect on IL-8 plasma concentrations. Moreover, the identity of the patient and control group in terms of the following indicators: age, sex, BMI, bad habits, comorbidities and their treatment, gives an accurate chance to reflect the net effect of PAF on the studied indicator. At the same time this makes the comparison between the groups as objective as possible.

Chemokines are key components of the inflammatory response due to their ability to attract and activate various subpopulations of white blood cells [16]. IL-8 is the prototypical chemokine of the CXC subfamily, which is characterized by a single amino acid separating the two amino-terminal cysteine residues of the protein [17]. It plays an important role in inflammation and its function is associated with attraction and activation of the fundamental for the inflammatory response cells, namely monocytes and neutrophils [18]. This establishes IL-8 as a leading pro-inflammatory cytokine [19]. Its elevated concentrations are a sign for activation of the inflammatory process. In this sense, the high baseline concentrations of IL-8 measured in our study show increased pro-inflammatory processes even in the early hours of the clinical expression of the disease.

Despite the key role of IL-8 in the inflammatory cascade, studies on IL-8 are single. Li et al establish changes which are unidirectional to our study [20]. In their study, the once

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166 measured levels of IL-8 in patients with PAF were significantly elevated relative to controls.
167 Conversely, the results of Luiba et al. did not show a statistically significant difference in the
168 levels of IL-8 in patients with PAF and controls [12]. The contradictory results confirm the
169 necessity of the conducted by us study.

170 Twenty-four hours after restoration of sinus rhythm, the established changes in the levels of
171 IL-8 during hospitalization were retained (Figure 1). PAF patients showed again increased
172 levels of the studied indicator. This result is largely expected, given the specific
173 characteristics of interleukin. It is known that it is synthesized very early in the inflammatory
174 response and remains active at the site of inflammation over a long period of time, for days
175 and weeks [21]. Thus, the inflammatory process appears to be activated not only during the
176 paroxysmal episodes of AF but also after them. This fact gives us ground to assume that
177 inflammatory changes in the myocardium cumulate even in sinus rhythm. Elimination of the
178 arrhythmia is not an equivalent to elimination of the pathophysiological processes associated
179 in AF clinical course. Decreased IL-8 levels, statistically insignificant from controls, were
180 measured yet on the twenty-eighth day after restoration of sinus rhythm. The inflammatory
181 activity was reduced, though this was reported late after the rhythm restoration.

182

183 ## 6. CONCLUSION

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185 IL-8 is an important pro-inflammatory marker. Its levels are significantly elevated still in early
186 hours of the clinical manifestation of the disease and persist after rhythm regularization.
187 Their restoration occurs slowly over time. The established specific dynamics in IL-8
188 concentrations suggests a close relationship between the intimate mechanisms of PAF
189 appearance and the inflammatory process.
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192 ## CONSENT

193 All authors declare that written informed consent was obtained from all participants of the
194 study. A copy of the written consent is available for review by the Editorial office/Chief
195 Editor/Editorial Board members of this journal.

196 ## ETHICAL APPROVAL

197 The study was approved by the Ethics Committee of Scientific Research (№35/29.10.2010)
198 at “St. Marina” hospital Varna and was also performed in compliance with the Declaration of
199 Helsinki.

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201 ## REFERENCES

202 1. Potpara TS, Lip GY. Lone atrial fibrillation: what is known and what is to come. Int J
203 Clin Pract. 2011;65(4):446-57.
204 2. Wijffels MC, Kirchhof CJ, Dorland R, Allessie MA. Atrial fibrillation begets atrial
205 fibrillation. A study in awake chronically instrumented goats. Circulation.
206 1995;92(7):1954-68.
207 3. Seet RC, Friedman PA, Rabinstein AA. Prolonged rhythm monitoring for the
208 detection of occult paroxysmal atrial fibrillation in ischemic stroke of unknown cause.
209 Circulation. 2011;124(4):477-86.
210 4. Lip GY, Hee FL. Paroxysmal atrial fibrillation. QJM. 2001;94(12):665-78.

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5. Frustaci A, Chimenti C, Bellocci F, Morgante E, Russo MA, Maseri A. Histological substrate of atrial biopsies in patients with lone atrial fibrillation. Circulation. 1997;96(4):1180-4.
6. Narducci ML, Pelargonio G, Dello Russo A, Casella M, Biasucci LM, La Torre G. et al. Role of tissue C-reactive protein in atrial cardiomyocytes of patients undergoing catheter ablation of atrial fibrillation: pathogenetic implications. Europace. 2011;13(8):1133–40.
7. Friedrichs K, Klinke A, Baldus S. Inflammatory pathways underlying atrial fibrillation. Trend Molec Med. 2011;17:556-563.
8. Commins SP, Borish L, Steinke JW. Immunologic messenger molecules: cytokines, interferons, and chemokines. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S53-72.
9. Sata N, Hamada N, Horinouchi T, Amitani S, Yamashita T, Moriyama Y, Miyahara. C-reactive protein and atrial fibrillation. Is inflammation a consequence or a cause of atrial fibrillation? Jpn Heart J. 2004;45:441–445.
10. Luan Y, Guo Y, Li S, Yu B, Zhu S, Li S, Li N, Tian Z, Peng C, Cheng J, Li Q, Cui J, Tian Y. Interleukin-18 among atrial fibrillation patients in the absence of structural heart disease. Europace. 2010;12(12):1713-8.
11. De Gennaro L, Brunetti ND, Montrone D, De Rosa F, Cuculo A, Di Biase M. Inflammatory activation and carbohydrate antigen-125 levels in subjects with atrial fibrillation. Eur J Clin Invest. 2012 Apr;42(4):371-5.
12. Liuba I, Ahlmroth H, Jonasson L, Englund A, Jönsson A, Säfström K, Walfridsson H. Source of inflammatory markers in patients with atrial fibrillation. Europace. 2008;10(7):848-53.
13. World Medical Association Declaration of Helsinki (2008). Ethical principles for medical research involving human subjects. 59th WMA General Assembly. Seoul.
14. Bellandi F, Cantini F, Pedone T, Palchetti R, Bamoshmoosh M, Dabizzi RP. Effectiveness of Intravenous Propafenone for Conversion of Recent-Onset Atrial Fibrillation: A Placebo-Controlled Study. Clin Cardiol. 1995;18: 631-634.
15. Bianconi L, Mennuni M. Comparison Between Propafenone and Digoxin Administered Intravenously to Patients With Acute Atrial Fibrillation. Am J Cardiol. 1998;82:584-588.
16. Skelton NJ, Quan C, Reilly D, Lowman H. Structure of a CXC chemokine-receptor fragment in complex with interleukin-8. Structure. 1999;7(2):157-68.
17. Remick DG. Interleukin-8. Crit Care Med. 2005;33(12 Suppl):S466-7.
18. Apostolakis S, Vogiatzi K, Amanatidou V, Spandidos DA. Interleukin 8 and cardiovascular disease. Cardiovasc Res. 2009;84(3):353-60.
19. Guo Y, Lip GY, Apostolakis S. Inflammation in atrial fibrillation. J Am Coll Cardiol. 2012;60(22):2263-70. doi: 10.1016/j.jacc.2012.04.063.
20. Li J, Solus J, Chen Q, Rho YH, Milne G, Stein CM, Darbar D. Role of inflammation and oxidative stress in atrial fibrillation. Heart Rhythm. 2010;7(4):438-44.
21. DeForge LE, Fantone JC, Kenney JS, Remick DG. Oxygen radical scavengers selectively inhibit interleukin 8 production in human whole blood. J Clin Invest. 199;90(5):2123-9.