Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2015; 3(8D):3013-3019 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2015.v03i08.053

Research Article

Predictive Significance of Interleukin-6, Interleukin-8 and Tumor Necrosis Factor-Alpha in Paroxysmal Atrial Fibrillation

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Abstract: Our previous studies presented significant increases in plasma concentrations of Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Tumor Necrosis Factor-alpha (TNF-α) as early as before the twenty-fourth hour after the clinical manifestation of paroxysmal atrial fibrillation (PAF). The early changes in pro-inflammatory cytokines give serious reasons to suppose that inflammatory process participates directly in the disease initiating mechanisms. In this sense, it is quite appropriate to seek in these changes a predictive value for PAF manifestation. IL-6, IL-8 and TNF-α were studied in 51 patients (26 men, 25 women; mean age 59.84±1.60 years) with PAF (time of the occurrence<48 hours) and 52 controls (26 men, 26 women; mean age 59.50±1.46 years) matched by gender, age and comorbidities. In the present study we used a logistic regression model with a single explanatory variable and a multivariable logistic model to determine the prognostic value of the indicators. The logistic regression model with a single explanatory variable showed that IL-6, IL-8 and TNF-α were statistically significant indicators of the clinical manifestations of PAF ($p<0.001$; $p=0.045$; $p=0.014$, respectively). With the increase in their values, the probability of developing the disease also increased($B_i=0.33>0$; $B_i=0.34>0$; $B_i=0.44>0$, respectively). The multivariate logistic model confirmed the results ($p=0.017$; $p=0.049$; $p=0.012$, respectively). Plasma concentrations of IL-6, IL-8 and TNF-α provide prognostic information about the clinical manifestation of PAF. They could be used in the overall clinical risk assessment, which in turn would affect the prophylactic and therapeutic approach to the disease.
Keywords: Interleukin-6 (IL-6), Interleukin-8 (IL-8), Tumor Necrosis Factor-alpha (TNF-α), paroxysmal atrial fibrillation

INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice, affecting >1% of the general population [1]. In Europe, more than 6 million people suffer from the disease and it is expected their number to double in the next 50 years, and in the US it is expected to affect 15.9 million [2, 3]. Data on the growing incidence give reasons to define AF as the "new epidemic" [4].

A series of studies demonstrated the negative impact of the arrhythmia on quality of life. It is a significant risk factor for thromboembolic events and is considered to cause one in five ischemic strokes [5].AF increases cardiovascular morbidity and mortality, reduces physical capacity and cognitive function of patients [6, 7].

Paroxysmal atrial fibrillation (PAF) represents between 25% and 60% of all cases of AF and the risk of stroke in it is not less than that in persistent and permanent AF [8]. The asymptomatic forms of the disease often remain undiagnosed, and therefore it is believed that about 30% of cryptogenic strokes are a consequence of PAF [9]. In this sense, introducing predictive for the expression of PAF biomarkers into clinical practice would contribute significantly to the assessment of embologenic risk and would be an important addition to the already well-established CHA_2DS_2-VAScscore. Undoubtedly the predictive biomarkers will be also relevant in anti-relapse treatment.

Our previous studies present a significant increase in plasma concentrations of Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Tumor Necrosis Factor-alpha (TNF-α) in patients with PAF compared to controls with no episodes of the arrhythmia to date ($p<0.001$) (Table 1) [10, 11, 12]. The changes were established as early as before the twenty-fourth hour

after the clinical manifestation of paroxysmal atrial fibrillation (PAF). The patient and control group were free from accompanying diseases that affect the levels of studied cytokines. Precisely the formation of clear groups and the early changes give us a serious reason to assume that inflammation participates directly in the development of the arrhythmia. The idea of the involvement of the inflammatory process in the pathogenesis of AF is not new, but there is still uncertainty about its contribution to the clinical manifestation and course of the disease.

It is well known that the examined by us cytokines are one of the key regulatory molecules in the inflammatory response both on a local and systemic level. They are characterized by high sensitivity and fast dynamics in the presence of a stimulus [13, 14]. Therefore, cytokines allow for good monitoring of the inflammatory process. This was a prerequisite for our research.

Table 1: Plasma concentrations of IL-6, IL-8 and TNF-α in the control subjects and patients with PAF

	Control subjects	**Patients**	**P value**
IL-6 (pg/mL)	14.21 ± 0.50	29.88 ± 1.68	p<0.001
IL-8 (pg/mL)	32.18±1.54	77.38 ± 3.78	p<0.001
TNF-α (pg/mL)	8.20 ± 0.29	15.06 ± 0.81	p<0.001

PURPOSE

The purpose of this study was to analyze the established by us changes in IL-6, IL-8 and TNF-α in patients with PAF in view oftheir predictive value for the manifestation of the disease.

MATERIALS AND METHODS

Study population

Significant increases of plasma concentrations of IL-6, IL-8 and TNF-α were established in a study of 51 patients (26 men, 25 women; mean age 59.84±1.60 years) with PAF (time of occurrence of the episodes <48 hours). They were selected from a total of 338 patients (see excl. criteria), hospitalized on grounds of a subjective sensation of "palpitations". The diagnosis "atrial fibrillation" was objectified on the basis of ECG immediately after the hospitalization of the patients.

Patients with PAF and the following diseases and conditions were excluded from the study:

1. Cardiovascular diseases: coronary artery disease; chronic heart failure; uncontrolled hypertension; inflammatory diseases of the heart; congenital heart defects; moderate or severe acquired valvular diseases; cardiomyopathies.
2. Other diseases: kidney or liver failure; diseases of the central nervous system; inflammatory and/or infectious diseases in the past three months; neoplastic or autoimmune diseases; chronic lung disease; diseases of the endocrine system (with the exception of type 2 diabetes mellitus, non-insulin dependent, with a good control).
3. Intake of hormone replacement therapy or oral contraceptives, pregnancy, systemic administration of analgesics including NSAIDs; obesity with BMI>35.
4. Persistent rhythm disorder after administration of propafenone; restoration of sinus rhythm by electrical cardioversion (*only for patients*).

Mandatory inclusion criteria were sustained recovery and maintenance of sinus rhythm after pharmacoversion with propafenone. In the absence of contraindications, the drug was administered in the prescribed for it scheme with a maximum duration of 24 hours [15, 16].

52 participants (26 men, 26 women; mean age 59.50±1.46 years) from a total of 169 screened without a history or ECG data for AF to date were selected for controls. The same exclusion criteria, used for the patient group, were applied to controls.

The study was conducted in the Intensive Coronary Care unit of First Cardiology Clinic at the University Hospital "St. Marina"- Varna for the period October 2010 – May 2012 after approval by the Ethics Committee of Research (№35/29.10.2010) at the same hospital and in accordance with the Declaration of Helsinki [17]. Participants were included in the study after previously signing the informed consent for participation.

Sample collection and laboratory procedures

Plasma concentrations of IL-6, IL-8 and TNF-α were tested immediately after hospitalization of the patients. Collection and storage of blood samples and the tests used are described in detail in our articles [10, 11, 12]. All procedures related to the study of the indicators were carried out in full compliance with the requirements of laboratory methods.

Statistical analysis

In the present study we used a logistic regression model with a single explanatory variable and a multivariable logistic model. This allowed us to seek predictors for the manifestation of PAF among the studied cytokines. Besides, this model made it possible to calculate the prognostic probability with which for a

certain value of the indicator it was expected for the complication to occur.

The mean values, standard error of the mean (SEM) and relative shares, cited by our previous studies, were presented using descriptive statistics. The testing of the equality hypothesis was done using Student's t-criterion.

Data analysis was performed with the specialized statistical analysis package STATISTICA, Version 10.0, 2010 (Stat Soft, Inc., Tulsa, OK, USA). The results were presented as mean ± SEM or n (%).Values of p <0.05 were considered statistically significant.

RESULTS

The logistic regression model with a single explanatory variable showed that plasma concentrations of IL-6, IL-8 and TNF-α were statistically significant indicators of clinical manifestations of PAF (p <0.001; p=0.045; p=0.014, respectively). With the increase in the values of each of these indicators, the probability of developing the disease also increased (B_i=0.33 >0; B_i=0.34 >0; B_i=0.44 >0, respectively).

The equations of the fitted models were respectively:

ln[*p*/(1-p)] = -6.08 + 0.33IL-6

ln[*p*/(1-p)] = -15.51 + 0.34IL-8

ln[*p*/(1-p)] = -4.59 + 0.44 TNF-α,

Where *p* was the probability for the manifestation of PAF (Figure 1, 2, 3).They allow to determine the probability for the manifestation of the disease for each measured value of the indicator. The models we used correctly classified 77.88%, 85.44% and 72.82%, respectively, from the observed in our sample cases.

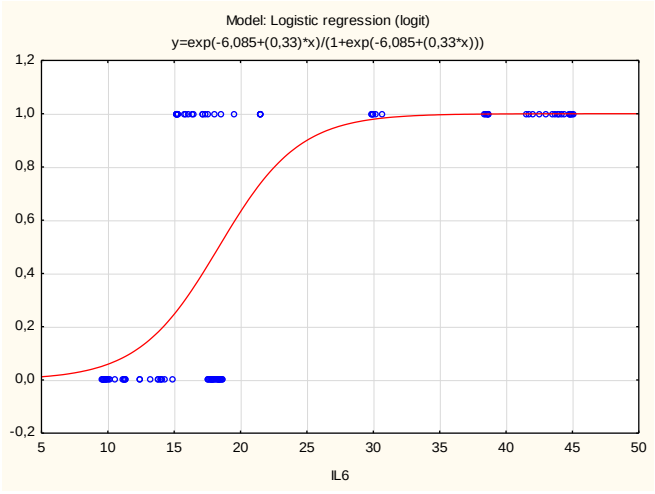

Fig. 1: Probability distribution of IL-6 estimated using logistic model.

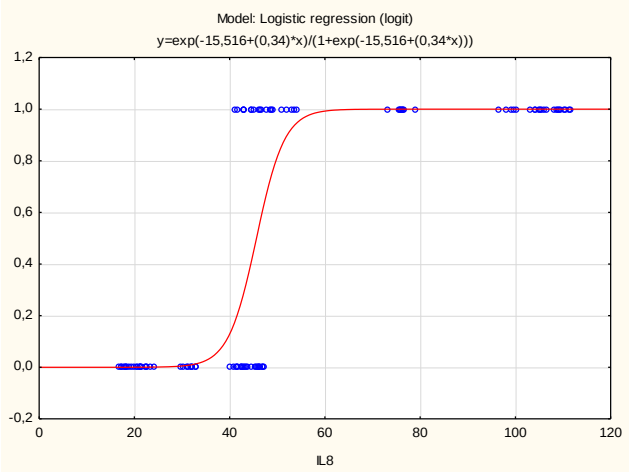

Fig. 2: Probability distribution of IL-8 estimated using logistic model.

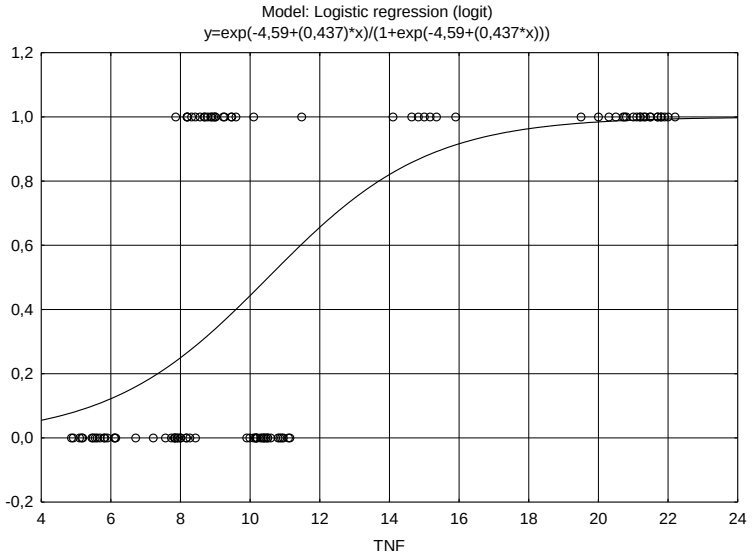

Fig. 3: Probability distribution of TNF-*α* estimated using logistic model.

The multivariate logistic model confirmed the predictive value of IL-6, IL-8 and TNF-α (p=0.017; p=0.049; p=0.012, respectively). The fitted equation showed that with the increasein the values of IL-6 ($\hat{\beta}_1 = 0.21 > 0$**),** IL-8 ($\hat{\beta}_2 = 0.31 > 0$ **)** and TNF-α ($\hat{\beta}_3 = 0.5 > 0$ **)** there was an increase in the probability *p*of the manifestation of the arrhythmia. The multivariate logistic model we created looked like this:

$$\ln[p/(1-p)] = -23.09 + 0.21\text{IL-6} + 0.31\text{IL-8} + 0.5\text{TNF-}\alpha$$

The statistical analysis in our previous studies showed that the patient group did not differ statistically from the control one in terms of number of participants in the group, mean age, gender structure, accompanying diseases, dyslipidemia and conducted treatment (*prior to hospitalization*). There was no significant difference between the two groups in terms of frequency of harmful habits and body mass index (BMI) (Table 2). The equalization of the two groups made it possible to compare them objectively and is a good prerequisite to seek predictive values of the established changes.

Table 2: Clinical characteristics of patients with PAF and the control group

	Patients with PAF	**Control group**	**P value**
Number of participants	51	52	0.89
Age (years)	59.84±1.60	59.50±1.46	0.87
Men/Women	26/25	26/26	1/ 0.93
Accompanying diseases			
Hypertension	37 (72.54%)	34 (65.38%)	0.44
Diabete mellitus type 2	3 (5.88%)	2 (3.84%)	0.62
Dyslipidemia	4 (7.84%)	3 (5.77%)	0.69
Medicaments for Hypertension and Dyslipidemia			
Beta blockers	19 (37.35%)	17 (32.69%)	0.62
ACE inhibitors	15 (29.41%)	14 (26.92%)	0.78
Sartans	11 (21.57%)	9 (17.31%)	0.58
Statins	4 (7.84%)	3 (5.77%)	0.69
Metformin	3 (5.88%)	2 (3.84%)	0.62
Deleterious habits			
Smoking	8 (15.69%)	7 (13.46%)	0.75
Drinking alcohol	7 (13.72%)	6 (11.53%)	0.74
Body mass index (BMI) (kg/m²)	23.85±0.46	24.95±0.45	0.09

DISCUSSION

Inflammatory changes in AF are described as early as the 90s of the last century. The first results in this direction were presented by Frustaci et al. from histological examinations of the atrial myocardium [18]. Later, inflammatory activity was established also on a systemic level. Elevated plasma concentrations were measured in a number of inflammatory biomarkers such as chemokines, interleukine, acute phase proteins and others [19, 20]. Most often studied from them are CRP and IL-6, for which it is well known that they are the most unambiguous and definitive indicators of systemic inflammation. A series of studies also have proven that the values of inflammatory biomarkers increase with the accumulation of clinical risk factors for the development of AF [21, 22]. Inflammatory activity showed prognostic significance in patients with AF, and elevated levels are associated with increased mortality and thromboembolic events [23, 24, 25].

IL-6 is determined as the primary cytokine of the inflammatory cascade that is involved in both the acute phase and chronic inflammation [26]. Similarly, IL-8 is a key pro-inflammatory molecule and its levels are a good indicator of the activity of the inflammatory process [27]. TNF-α regulates the inflammatory response by triggering IL-6 and IL-1 production and stimulates metalloproteinase and neutrophil migration [28].

AF is often defined as an inflammatory disease. Therefore the predictive value of these key inflammatory molecules represents significant clinical interest in the AF population.

Several studies have shown that elevated serum levels of IL-6 are associated with the onset of AF after radiofrequency catheter ablation and electrical cardioversion [29, 30, 31]. Similar results were presented in patients after coronary artery by-pass operation. The increase in the values of the cytokine correlated with post-CABG AF occurrence [32, 33].

IL-6 and IL-8 are often placed into a single group of "inflammatory mediators" and are usually tested simultaneously to simplify the process. We cannot do this if we are looking for predictive significance. Clinical interest in plasma concentrations of IL-8 is significantly less compared to IL-6. The conducted studies are single. It has been established that the high serum levels of cytokines measured preoperatively could be used to identify patients prone to develop post-CABG AF [34]. In acute respiratory distress syndrome elevated levels of IL-8 are associated with the manifestation of new-onset AF [35].

Studies on TNF-α in AF showed that the values of cytokines were statistically significant for the predictionof the clinical manifestation of the disease. A study with forty-four patients who underwent an initial pulmonary vein isolation show that the ablation responders had lower values of TNF-α [36]. The study byVasiletz et al. also established a predictive value of the indicator in hypertensive patients [37]. TNF-α, studied before and after the first episode of persistent AF, proved to be a reliable early predictor of maintenance of sinus rhythm [38].

Our study confirms the results of previous studies in this field. The regression analysis we performed showed undoubtedly that the increased levels of proinflammatory cytokines IL-6, IL-8 and TNF-α are associated with increased probability of PAF. The equations of the fitted models allow us to determine with greater accuracy the probability of developing the disease in clinical practice.

Although the presented idea is not entirely new, the design of our study differs significantly. In this sense, it is right to emphasize that the presented by us patient population differs from the previously tested AF populations. It is characterized by "low burden of concomitant diseases". By eliminating a number of cardiovascular and heart diseases we can avoid their potential effects on the inflammatory status of the organism. The creation of pure groups allows us to present objectively the narrow relationship between the levels of IL-6, IL-8 and TNF-α and the rhythm disorder. Therefore, we believe that the results of the regression analysis are not an accidental finding, but reflect adequately the possibility for predicting the manifestation of PAF. The validation of laboratory indicators parameters with predictive values in clinical practice would influence the approach in the treatment of the rhythm disorder as well as its prognosis.

CONCLUSION

Plasma concentrations of IL-6, IL-8 and TNF-α provide prognostic information on the clinical manifestation of PAF. They could be used in the overall clinical risk assessment, which in turn would affect the prophylactic and therapeutic approach to the disease.

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