



The role of pro-fibrotic biomarkers in paroxysmal and persistent atrial fibrillation

Adina Elena Stanciu^{a,1,*}, Radu Gabriel Vatasescu^{b,1}, Marcel Marian Stanciu^c, Nafija Serdarevic^d, Maria Dorobantu^b

^a Institute of Oncology Bucharest, Department of Carcinogenesis and Molecular Biology, 252 Fundeni, 022338 Bucharest, Romania

^b Clinic Emergency Hospital Bucharest, Department of Cardiology, 8 Calea Floreasca, 014461 Bucharest, Romania

^c University Politehnica of Bucharest, Electrical Engineering Faculty, 313 Splaiul Independentei, 060042 Bucharest, Romania

^d Institute for Clinical Chemistry and Biochemistry, University of Sarajevo Clinics Center, Bolnicka 25, 71000 Sarajevo, Bosnia and Herzegovina

ARTICLE INFO

Keywords:

Atrial fibrillation

TGF- β 1

Matrix metalloproteinase-9: tissue inhibitor of metalloproteinase-1 ratio

NT-proBNP

Left atrial diameter

Atrial remodeling

ABSTRACT

Purpose: Signaling pathways involved in electrical, structural and contractile remodeling processes behind development and progression of atrial fibrillation (AF) have not been completely elucidated, but it seems to be related to complex interactions among neurohormonal and cellular mediators. We aimed to investigate interleukin-6 (IL-6), transforming growth factor-beta1 (TGF- β 1), matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1), as biomarkers of atrial remodeling, in patients with paroxysmal and persistent AF, and their correlation with N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and left atrial (LA) diameter.

Methods: Thirty-seven patients (22M/15F) with paroxysmal AF, 32 patients (22M/10F) with persistent AF and 30 healthy control subjects (18M/12F) were enrolled in the study. Serum levels of biomarkers were measured by ELISA. Cardiac function was assessed echocardiographically.

Results: IL-6 levels and MMP-9/TIMP-1 ratio were significantly higher in AF patients than in non-AF controls ($P < .001$), and in persistent than in paroxysmal AF ($P < .001$), in line with NT-proBNP and LA diameter. In contrast, TGF- β 1 levels declined with increasing AF duration (from 51.2 pg/mL, IQR: 38.9–87.9 pg/mL in paroxysmal to 23.9 pg/mL, IQR: 16.9–43.6 pg/mL in persistent AF). TGF- β 1 was negatively correlated with NT-proBNP ($r = -0.53$, $P = .001$ in paroxysmal AF and $r = -0.71$, $P < .001$ in persistent AF) and LA diameter ($r = -0.44$, $P = .006$ in paroxysmal AF and $r = -0.51$, $P = .003$ in persistent AF).

Conclusions: Our results demonstrate that AF development and progression (from paroxysmal to persistent) is associated with a gradual increase in serum levels of NT-proBNP, IL-6 and MMP-9/TIMP-1 ratio. Moreover, this study suggests that the relationship between TGF- β 1, NT-proBNP and LA diameter allows for the progression of atrial remodeling during AF, despite compensatory changes in the TGF- β 1 signaling pathway.

1. Introduction

Atrial fibrillation (AF), the most common cardiac rhythm abnormality, is a major risk factor for cardiovascular morbidity, including heart failure and stroke. AF represents an increasing public health challenge with profound social and economic implications [1]. A recent analysis of medical costs associated with AF, conducted on 38 million individuals in the United States, has shown that people with AF had higher medical costs by 73% than those of appropriate controls [2]. The real global burden of AF is unknown, however the estimated number of

individuals with AF globally in 2010 was 33.5 million [3], future projections predicting at least a doubling of AF cases by 2050 [1]. AF was defined as “paroxysmal” when the AF episode terminates spontaneously or with intervention within 7 days of onset (at least 1 day of AF but < 7 consecutive days with > 23 h of AF) and “persistent” when the AF episode persists for at least 7 consecutive days or pharmacological or electrical cardioversion are required to terminate the arrhythmia (at least 7 consecutive days with > 23 h of AF) [4–6].

Regarding the biomarkers for AF, the Guidelines for the Management of Patients with AF [4,5] have shown that serum N-

Abbreviations: AF, atrial fibrillation; ECM, extracellular matrix; IL-6, interleukin-6; IQR, interquartile range; LA diameter, left atrial diameter; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; MMP-9, matrix metalloproteinase-9; SD, standard deviation; TIMP-1, tissue inhibitor of metalloproteinase-1; TGF- β 1, transforming growth factor-beta 1

* Corresponding author.

E-mail address: adinaelenastanciu@yahoo.com (A.E. Stanciu).

¹ The first two authors equally contributed to this article.

terminal prohormone of brain natriuretic peptide (NT-proBNP) is a more powerful biomarker than any other clinical covariate, as well as echocardiographic assessment (left atrial and ventricular diameters).

Unfortunately, the precise mechanisms and signaling pathways involved in electrical, structural and contractile remodeling processes behind development and perpetuation of AF have not been completely elucidated, but it seems to be related to complex interactions among neurohormonal and cellular mediators. There are data indicating the association between biomarkers of inflammation (C reactive protein and proinflammatory cytokines) and the presence and persistence of AF [7], and accumulation of risk factors in AF [8]. Transforming growth factor-beta 1 (TGF- β 1) is a key factor in the signal cascade reaction during the process of tissue fibrosis [9]. TGF- β 1 promotes and controls the production of extracellular matrix (ECM) which leads to atrial fibrosis and to the development of AF [10]. Another important factor that contributes together with TGF- β 1 to ECM remodeling in the atria is the abnormal balance between matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) [11].

We hypothesized that altered expression and distribution of several biomarkers of inflammation (interleukin-6) and fibrosis (TGF- β 1, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1) will be associated with changes in atrial fibrosis in different types of AF patients. Identification of potential new biomarkers encompassing several subclinical states and measurements of these biomarkers might enhance the understanding of the pathophysiology, early disease detection and optimize treatment in individual patients with AF. According to Guidelines for the Management of Patients with AF [4,5], elevated serum concentrations of NT-proBNP and left atrial (LA) enlargement are associated with an increased risk of AF.

Consequently, the aim of the present study was to investigate interleukin-6 (IL-6), TGF- β 1, matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1), as biomarkers of atrial remodeling, in patients with paroxysmal and persistent AF, and their correlation with serum levels of NT-proBNP and LA diameter.

2. Materials and methods

2.1. Patients and study protocol

Seventy-seven patients with AF scheduled for electrophysiological study or radiofrequency catheter ablation procedures at the Clinic Emergency Hospital Bucharest, between March 2011 and December 2012, were included in the study. The exclusion criteria were as follows: structural heart disease, severe peripheral atherosclerotic disease, antecedents of neoplasia (e.g. patients with antecedents of differentiated thyroid cancer that have thyroid hormone suppressive therapy [12], etc.), hyperthyroidism [13], acute or chronic infection, inflammatory disease, burning, post-surgery status, pulmonary, hepatic, or renal impairment, ongoing treatment with anti-arrhythmics, lipid lowering medication and steroidal or non-steroidal anti-inflammatory drugs. After data pre-processing, 69 samples remained for analysis. Additional samples were excluded for the following reasons: (i) serum samples were strongly lipemic or hemolyzed ($N = 4$); (ii) patients that were classified as outliers, using Interquartile Range (IQR) rule, for some of the investigated variables ($N = 4$). The remaining 69 patients were divided into two groups: 37 paroxysmal AF patients (22M/15F, mean age 52.10 ± 9.08 years, at least 1 episode per month) and 32 persistent AF patients (22M/10F, mean age 50.60 ± 9.25 years, continuous AF for at least 2 months). For each enrolled patient, a detailed medical and drug history was obtained. All patients underwent physical examination and routine laboratory tests. A number of 30 healthy volunteers with no clinical and ECG evidence of AF and no structural heart disease (18M/12F, mean age 50.76 ± 10.32 years) were randomly selected. All exclusion criteria with regards co-morbidities that were applied to the AF groups were also applied to the controls.

The study was done respecting ethical standards in the Helsinki Declaration and was approved by the Clinic Emergency Hospital ethics committee. Enrolled patients and volunteers signed an informed consent.

2.2. Biomarker measurements

Blood sampling was performed prior to electrophysiological study or catheter ablation procedures. Blood specimens were collected by venipuncture into Vacutainer (Becton Dickinson, Rutherford, NJ, USA) serum separation tubes (6 mL). The serum samples were obtained by clotting (30 min, room temperature) and centrifugation (15 min at 1000g). Strongly lipemic or hemolyzed samples were removed from the study. Serum samples were then immediately aliquoted into labeled cryo-vials and stored at -70°C .

Serum concentrations of NT-proBNP, MMP-9, TIMP-1, TGF- β 1 and IL-6 were measured using commercially available quantitative enzyme-linked immunosorbent assay kits (NT-proBNP from Biomedica Gruppe; Human MMP-9, TIMP-1, TGF- β 1 and IL-6 Quantikine ELISA kits from R & D Systems, Inc., Minneapolis, MN, USA). The precision (intra-assay variation) was tested by twenty measurements of three different samples of known concentrations. The reproducibility (inter-assay variation) for the same three samples was tested, too. The values of the inter-assay imprecision study were similar to those from the intra-assay study with CVs ranging from 2 to 7.4%. The lower limits of detection were 3 pmol/L for NT-proBNP; 0.156 ng/mL for MMP-9; 0.08 ng/mL for TIMP-1; 4 pg/mL for TGF- β 1; 0.70 pg/mL for IL-6.

TGF- β 1 is secreted in a latent form, linked to Latency Associated Protein (LAP). Analysis of TGF- β 1 ELISA requires activation of latent TGF- β 1 to the immunoreactive form by acidification of serum samples. Acidic conditions can denature the LAP and the treatment of the medium with extremes of pH resulted in significant activation of TGF- β 1. Therefore, there were necessary solutions for acid activation (1 N HCl) and neutralization (1.2 N NaOH/0.5M HEPES). The activation protocol was: (a) acidify with 1 N HCl (to 40 μL serum, add 20 μL of 1 N HCl); (b) incubate 10 min at room temperature; (c) neutralize with 20 μL of 1.2 N NaOH/0.5 M HEPES. For each new lot of acidification and neutralization reagents, it was necessary to measure the pH of several representative samples after neutralization to ensure that it was within pH 7.2–7.6. Activated serum samples have been stored for up to 24 h at $2-8^\circ\text{C}$ before use. Polypropylene test tubes for dilution of standards were needed for TGF- β 1, MMP-9 and TIMP-1. The contamination can cause falsely elevating concentrations. Because MMP-9 and TIMP-1 are presented in saliva, protective measures have been taken to prevent contamination of kit reagents while running the assay.

The within CVs for NT-proBNP were 7.7% and 10.6% at a mean concentration of 125 pmol/L and 575 pmol/L, respectively; for MMP-9 were 2.0% and 2.9% at a mean concentration of 83.3 ng/mL and 1100 ng/mL, respectively; for TIMP-1 were 4.2% and 5% at a mean concentration of 48 ng/mL and 710 ng/mL, respectively; for TGF- β 1 were 2.9% and 2% at a mean concentration of 56 pg/mL and 695 pg/mL, respectively; and for IL-6 were 4.4% and 1.7% at a mean concentration of 3.2 pg/mL and 179 pg/mL (for low- and high-concentration patient samples).

As the levels of MMP-9 and TIMP-1 were measured, as indicated above, the MMP-9/TIMP-1 ratio was calculated. All assays were performed in duplicate according to the manufacturers' recommendations and in such a way minimize any effects of repeated freeze-thaw cycles.

2.3. Echocardiographic measurement

An ultrasound system (Vingmed Vivid 7, General Electric-Vingmed, Milwaukee, Wisconsin, USA) was used for the transthoracic 2D and color Doppler echocardiographic examination. Using a 3.5 MHz transducer (16 cm depth), images were obtained in the parasternal (long- and short-axis) and apical (2-, 3-, and 4-chamber) views. Left atrial (LA)

Download English Version:

<https://daneshyari.com/en/article/8629194>

Download Persian Version:

<https://daneshyari.com/article/8629194>

[Daneshyari.com](https://daneshyari.com)