Soluble ST2 biomarker analysis for fibrosis development in atrial fibrillation. A case control study

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Abstract

Atrial fibrillation (AF) is a common and clinically relevant supra-ventricular arrhythmia which represents an independent risk factor for development of heart failure as well as for ischemic stroke. Clinical management of this pathology can be still challenging in many patients, in particular the older ones and/or those which present comorbidity. The interest in biomarkers for diagnosis and management of the AF becomes more evident in recent years. We studied the possible role of the soluble sST2 as biomarker to assess the fibrosis development of patients with first diagnosed or permanent AF. The serum concentrations of these biomarkers have been measured in a group of 58 patients (mean age 83.6 ± 6.0 years) and in a control set of 40 individuals. The mean serum concentration of sST2 is 26.1 (22.7-30.5) ng/mL in the AF group, while in the control is 17.3 (15.7-18.9) ng/mL (p<0.05). Remarkable differences have been obtained for the two subsets of patients with first diagnosed and permanent AF (sST2: (23 (21.2-24) ng/mL vs 30.5 (28.6-32) ng/mL). The analysis has been completed with a trans thoracic echocardiographic exam to evaluate the left atrium size and the left ventricular ejection fraction. The results have been discussed to enhance the correlation between the instrumental and laboratory results. The present casecontrol study suggests a possible clinical valuable role of the sST2 to evaluate the fibrosis level in the fibrillating patients as the cohort here studied.

Key words: Atrial fibrillation, Biomarkers, Fibrosis, Echocardiography

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia and it is established since long time that it has an important role in the clinical practice, by representing a significant load in terms of health burden, in particular for Western country. Recent estimations of atrial fibrillation prevalence display an increasing trend for North America, Western Europe and Australia, (1-4) so far it is an important cause of hospitalization and is one of the most important causes of cardiovascular morbidity and mortality, in particular ischemic stroke, heart failure and sudden death.

The role of fibrosis is important in the pathophysiology of AF, with particular reference to atrial fibrosis which has been addressed as one of the dominant factors for the development of the arrhythmia (5). Furthermore, its level shown to be an important parameter affecting the therapeutic strategy, in example for the success rates of rhythm control (6-7).

Identification of atrial fibrosis is based, at the moment, on invasive (electro-anatomical mapping (8)) or non-invasive (i.e. delayed-enhancement magnetic resonance imaging DE-MRI (9)) methods. Furthermore, circulating biomarkers are potentially promising tools to evaluate the cardiac fibrosis (6, 10-15).

In the last years, the pertinent scientific literature faced an increasing number of studies concerning biomarkers of myocardial damage (i.e. troponin) (16-18), cardiac stress and impairment (i.e. growth differentiation factor 15 GDF15, natriuretic peptides pro-BNP) (19-20), myocardial fibrosis (soluble suppression tumorigenicity 2, galectin-3, fibroblast growth factor 23 FGF-23, procollagen) (6,10-15,21) renal dysfunction (creatinine, cystatin C) (22-23), inflammation (cytokines, C-reactive protein) (24-25).

Here the attention is given to sST2 (soluble Suppression Tumorigenicity 2) as biomarker related to the inflammation, oxidative stress and fibrosis development in the cardiac tissue, a marker that received noticeable attention in cardiological diseases (26).

ST2 is a member of Interleukin 1 (IL-1) receptors family discovered in 1989. Recently Wienberg at al. showed its relationship with cardiovascular diseases (27-28).

The marker exists in two isoforms: a transmembrane receptor ST2L, with interleukin 33 (IL-33) as natural ligand, that triggers anti-apoptotic, anti-hypertrophic and cardio-protective actions (27). The second isoform sST2 is a soluble protein that behaves as a decoy receptor thus promoting the inhibition of the IL-33/ST2L signaling pathway (29).

The marker has been extensively studied to evaluate the ventricular fibrosis associated to heart failure and many papers proved its significant prognostic role in both acute and chronic heart failure (12,26, 29).
In acute processes the sST2 does not reveal to be highly specificity, showing marked increased valued also in other inflammatory conditions as sepsis, pneumonia, COPD (30). On the contrary, in chronic heart failure condition, it is now well acknowledged the prognostic importance of high sST2 levels (>35 ng/mL) as a predictive marker of mortality and the usefulness of the time pattern in specific medical treatment (12,26,30-39).

The possible role in atrial fibrillation has been considered more recently. Some studies (40-43) found the sST2 levels higher in fibrillating subjects with respect to those with sinus rhythm and the relationship of the pathophysiology of sST2 with myocardia fibrosis level suggests a prognostic function of its serum concentration (6,44) but the available data are still scarce and not conclusive.

We evaluated the serum levels of sST2 in two groups of fibrillating subjects. The first group consists of patients where the AF condition was not diagnosed before. The second group is represented by permanent AF subjects where the condition can be considered as long standing.

Finally, the study is completed by an echocardiography analysis aimed at the investigation of the left atrial size. In fact, recent contributions (45,46) pointed out the important role of the left atrial (LA) size to predict heart failure development both in patient with sinus and atrial fibrillation rhythm. In particular, Taniguchi et al. (45,46), recently highlighted the indexed LA volume as an independent risk factor to the heart failure progress.

Materials and methods

Study populations

The authors performed a study on a cohort of 120 patients with atrial fibrillation admitted at Emergency Department of the Policlinico Umberto I of Rome, from 1 July 2019 to 31 December 2019.

A control set of 40 assumed healthy people, without atrial fibrillation was also considered.

Exclusion criteria are selected in cardiac diseases (history of myocardial infarction, ACS, significant heart failure (NYHA3), dilated cardiomyopathy, hypertrophic cardiomyopathy, congenital pathologies), acute inflammatory states (sepsis, COPD in acute phase), cancer, autoimmune pathologies (25). As a consequence, among the 120 patients, 32 were excluded for significant heart failure and 30 for the other causes.

Finally, 58 subjects with AF were enrolled to the study, moreover, the patients were split into two subgroups: first diagnosed and permanent AF. Fig. 1 summarizes the admission process.

The present study is carried out in agreement with the Declaration of Helsinki, approved by medical ethics committees and with the written informed consent of both patients and control group enrolled.

Laboratory analysis

The serum concentrations of sST2 has been evaluated in the two groups.

Specifically, the sST2 serum concentration was evaluated by a high-sensitivity, second-generation enzyme-linked immunosorbent assay (Presage™ ST2; Critical Diagnostics) with a detection limit of 2 ng/ml, a within-run coefficient of variation (CV) of 2.5% (47).

The analysis has been carried out on chemical analyzer COBAS 600 provided by Roche®.

2.3 Echocardiographic analysis

A transthoracic echocardiography exam was carried out for all the patients by experienced echocardiographers, to evaluate the left ventricular ejection fraction and the left atrium size (48-49).

Reference limits for the left atrium was established in terms of maximum diameter (3.8 cm for women and 4.0 cm for men) or maximum area (20 cm² for both women and men) as suggested by Lang et al. (50).

Statistical analysis

The analysis has been carried out by IBM software SPSS 24.0.

The continuous data (i.e. age, concentrations, left ventricular ejection fraction) are given as median and percentiles (25th - 75th), while the categorical variables (gender, AF characteristics, left atrium enlargement) are represented as counts and percentage.

The statistical significance of the results has been evaluated by the non-parametric test U of Mann-Whitney and the chi-squared test for the continuous and categorical variables, respectively. The p-value <0.05 has been assumed as limit for a statistically significant result.

Results

The average age of the enrolled 58 patients was 83.6 ± 6.0 (30% were male and 70% female). The 38% of the patients have a first diagnosed atrial fibrillation with respect to 62% of permanent atrial fibrillation. The AF characteristics...
were evaluated on the basis of the previous clinical history and the clinical data recorded during the admission period in the hospital. The permanent atrial fibrillation condition was confirmed by at least 3 12-leads electrocardiograms exploited in different days.

All patients in atrial fibrillation, regardless of new diagnosed or permanent condition, were under anti-coagulant therapy when the marker concentration was measured.

The left ventricular ejection fraction (LVEF) and the left atrium (LA) size were evaluated for the entire group of fibrillating patients. LVEF presents a median value of 48%, whereas for the two subgroups of first diagnosed and first diagnosed fibrillating patients, LVEF is 50% and 46%, respectively.

The analysis of the left atrium size gives the 65% of the patients with an enlarged left atrium (35% has a size in the limit).

The first diagnosed fibrillating patients are equally divided (50%-50%) in normal and enlarged left atrium size with a median LVEF equal to 55% and 45% for normal and enlarged size, respectively.

On the other side, the permanent fibrillating group displays a marked increased percentage of enlarged left atrium (76%) with respect to the normal size (24%). The corresponding LVEF are 44% and 50%, respectively. The echocardiographic data are summarized in Table 1.

The median serum concentrations (with interquartile range at 25th and 75th) for the sST2 is 26.1 (22.7-30.5) ng/mL in the AF group, while in the control is 17.3 (15.7-18.9) ng/mL (p<0.05).

The two AF groups present significant concentrations differences: 23 (21.2-24) ng/mL for patients with first diagnosed AF vs 30.5 (28.6-32) ng/mL for the permanent subjects, p<0.05.

All the results are summarized in the Tables 2,3.

Figures 2,3 show the concentrations of the marker related to the AF class (Fig.2) and to the echocardiographic characteristics (Fig.3).

Discussion

The first comment deals with the patients age. We note that the median age is significantly high, but quite homogenous. This result is well representative of a continuously increasing age of the hospitalized subjects nowadays.

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Controls n=40</th>
<th>AF (Total) n=58</th>
<th>p value</th>
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<tbody>
<tr>
<td>58 (49-70)</td>
<td>84 (80-86)</td>
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<tr>
<td>55%</td>
<td>70%</td>
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</thead>
<tbody>
<tr>
<td>30%</td>
<td>45%</td>
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<tbody>
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<td>25%</td>
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<thead>
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<tr>
<td>0%</td>
<td>7%</td>
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<tr>
<td>48%</td>
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<th>AF (Total) n=58</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>65%</td>
<td>65%</td>
<td>0.02</td>
<td></td>
</tr>
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<table>
<thead>
<tr>
<th>sST2 (ng/dL)</th>
<th>Controls n=40</th>
<th>AF (Total) n=58</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.3 (15.7-18.9)</td>
<td>26.1 (22.7-30.5)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

AF=Atrial Fibrillation, LA=Left Atrium, LVEF=Left Ventricular Ejection Fraction

<table>
<thead>
<tr>
<th>AF First Diagnosed n=22 (38%)</th>
<th>AF Permanent n=36 (62%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>82 (78-86)</td>
<td>85 (82-87)</td>
</tr>
<tr>
<td>Gender Female</td>
<td>72%</td>
<td>67%</td>
</tr>
<tr>
<td>Arterial hypertension</td>
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<td>44%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
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<td>22%</td>
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<tr>
<td>Cognitive Impairment</td>
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<td>8.3%</td>
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<tr>
<td>LVEF (%)</td>
<td>50%</td>
<td>46%</td>
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<tr>
<td>LA-Enlarged</td>
<td>50%</td>
<td>76%</td>
</tr>
<tr>
<td>sST2 (ng/dL)</td>
<td>23 (21.2-24)</td>
<td>30.5 (28.6-32)</td>
</tr>
</tbody>
</table>

AF=Atrial Fibrillation, LA=Left Atrium, LVEF=Left Ventricular Ejection Fraction

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<tr>
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<th>AF First Diagnosed n=22 (38%)</th>
<th>AF Permanent n=36 (62%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enlarged LA</td>
<td>Normal LA</td>
<td>Enlarged LA</td>
</tr>
<tr>
<td>65%</td>
<td>35%</td>
<td>50%</td>
</tr>
<tr>
<td>LVEF=45%</td>
<td>LVEF=55%</td>
<td>LVEF=44%</td>
</tr>
</tbody>
</table>

AF=Atrial Fibrillation, LA=Left Atrium, LVEF=Left Ventricular Ejection Fraction
It is important to stress that the clinical management of the old patient with AF will be more and more frequent and important in the proximal next future. The adoption of additional information, as those obtained by biomarkers, can be useful to tailor the therapy and to discriminate better the differences between each single patient.

Moreover, the geriatric patients usually present a multi-pathology condition and is typically under a multidrug therapy, a clinical frame that makes more difficult the AF treatment.

The analysis shows that most of the patients (62%) fall in the permanent subgroup, while the remaining 38% are classified as first diagnosed where often the triggering cause of the AF is related to COPD exacerbation, pneumonia or a worsening of a heart failure state. The adoption of the exclusion criteria gives a cohort of few patients (58), but with an acceptable median LVEF=48%. As reported in Table 1, the first diagnosed AF subgroup shows a better LVEF and a lower percentage of LA enlargement.

The results compare favorably with data present in literature (49) and coherently display the typical behavior of the structural remodeling and fibrosis development associated with age and AF that leads to irreversible enlargement of the LA (50).

Finally, the lower LVEF obtained for the permanent AF subgroup is consistent with the chronic pathological condition.

The serum concentration of the sST2 for the complete cohort has a median value 26.1 ng/mL, that is comparable to the value 21.69 ng/mL reported by Ma et al. (41). The difference between the two values can be related to the higher mean age of our group with respect to the patients studied in (41).

The difference regarding the control group is apparent, statistically significant (p<0.05), and consistent with the pertinent literature. The difference between the mean ages of the two groups is an additional factor that enhance the gap, in fact is known (51) that sST2 is positively associated with older ages (regression coefficient 0.027 for every 10 years (51)). In any case, also by adjusting the sST2 values, the difference between the AF and the control groups is still remarkable.

The comparison between the two subgroups, first diagnosed and permanent AF, shows a marked and meaningful difference (23 ng/mL vs 30 ng/mL, p<0.05), see Table 3 and Fig. 2.

By considering that acute conditions (as sepsis, pneumonia, COPD acute exacerbations) and significant heart failure are excluded, the difference observed between the two subgroups can be thought related to the fibrosis level. Permanent AF patients develop higher fibrosis due to the more longstanding irreversible structural remodeling processes.

This last comment is also confirmed by the weak correlation observed between the LVEF and the sST2 concentration values. In fact, the Pearson index, -0.26 with p<0.05, shows a lower sST2 value as the LVEF increases.

Conclusions

In this study the serum concentrations of sST2 have been measured in a population of atrial fibrillating patients.

The values of the marker are found to be higher in the chronic condition of permanent AF with respect to the cases where the AF is of new onset or follow a paroxysmal pattern. This outcome is coherent with the expected higher degree of fibrosis in the long-standing condition where the irreversible processes that lead to the fibrosis are much represented.

Furthermore, the association with echocardiographic characteristics was positively proved, with larger concentrations of the marker in subjects with enlarger left atrium where again the fibrosis is expected more important.
We conclude that the sST2 could be a useful marker to evaluate the degree of fibrosis related to the left atrium of fibrillating patients.

An open issue concerns the specificity of the marker. It is known that other inflammatory processes can activate the expression of the sST2 and the real cardiac fibrosis can be masked by fibrosis of different origin. This point needs further investigation to prove the possible application in a clinical scenario.

Finally, we observe that the present study has the limit of a small sample size, which could affect the statistical analysis of some results and refined exclusion criteria should be applied to remove other possible causes of increase of the biomarkers concentration not related to the AF.

Compliance with Ethical Statement

Conflicts of interest

The authors declare that they have no conflict of interest.

Statement of human and animal rights

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional or national research committee and with the Helsinki Declaration of 1975, as revised in 2000. This article does not contain any studies with animals performed by any of the authors.

Informed consent

Informed written consent was obtained from the patients and control group individuals included in this study.

References

38. O’Meara E, Prescott MF, Claggett B, et al. Independent Prognostic Value of Serum Soluble ST2 Measure- ments in Patients with Heart Failure and a Reduced Ejection Fraction in the PARADIGM-HF Trial (Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure), Circ Heart Fail, 2018; 11: 5
39. Maisel AS, Di Somma S. Do we need another heart failure biomarker: focus on soluble suppression of tumorigenicity 2 (sST2), Eur Heart J, 2017; 38, N.30:2325–33