Biomarkers and epigenetic markers associated with pain in patients with symptomatic atrial fibrillation compared with controls

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Abstract

Background

Atrial fibrillation (AF) is the most common sustained arrhythmia and the number of patients with AF is expected to increase substantially in the coming decades. One third of patients with AF report no AF-associated symptoms, but up to one-fourth report severe symptoms. It is unclear why patients' experience of AF-related symptoms varies so much. We have previously shown that patients with symptomatic AF exhibit lower pain tolerance than patients with asymptomatic AF, as well as impaired pain inhibitory control and facilitated summation of pain, indicating that pain sensitisation may be of importance in symptomatic AF. In patients with chronic pain conditions, several biomarkers and epigenetic markers associated with generation and /or maintenance of chronic pain have been identified. Previous research of biomarkers and epigenetic markers associated with pain is sparse in patients with AF.

Objective and methods

To study levels of biomarkers and epigenetic markers in blood in patients with symptomatic paroxysmal AF (n=100), in relation to severity of AF symptoms, and compared to age- and sex-matched controls without AF (n=100). Blood will be obtained before and after AF ablation and levels of biomarkers, epigenetic markers and cardiac and inflammatory markers, analysed. Patients will complete an AF-specific symptom and a generic health-related quality of life questionnaire.

Clinical relevance

In the future, biomarkers and epigenetic markers associated with pain may be used as a tool for evaluation of patients with AF and have an impact on individualized management. Another possibility is a rationale for future studies of novel analgesics that neutralize biomarkers or antagonizes its receptors.

Trial Registration

The study protocol will be registered at ClinicalTrials.gov after approval by the Swedish Ethical Review Authority.

Keywords

Atrial fibrillation, Symptoms, Quality of life, Pain

Abbreviations

AF	Atrial fibrillation
AF6	Atrial fibrillation 6 questionnaire
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
NGF	Nerve growth factor
RNA	Ribonucleic acid
SF-36	Short form 36 health survey

Introduction

Atrial fibrillation

Atrial fibrillation (AF) is the most common sustained arrhythmia affecting approximately 3% of adults aged 20 years or older in Western countries, and is expected to increase further in the coming decades(1). The presence of AF is independently associated with an increased risk of all-cause mortality and morbidity, largely due to stroke and heart failure, dementia, symptoms, and impaired quality of life(2-7). The management of AF aims to prevent AF-related complications using oral anticoagulation therapy in patients with a high risk of stroke and to reduce symptoms and improve health-related quality of life using a rate (slowing of the ventricular rate) or rhythm control (restoration and maintenance of normal sinus rhythm) strategy(8).

About one third of patients with AF have no AF-associated symptoms, but up to one-fourth of patients report severe symptoms such as palpitations, dyspnea, chest discomfort or pain, dizziness and syncope(9-11). In addition, one third of patients with symptomatic AF suffer from psychological distress(11, 12). It is well recognized, but unclear why patients' perception of AF varies so much. Several studies have evaluated the relationship between patient characteristics and the presence of AF symptoms and have found that symptomatic AF is more frequent in women than in men, and in younger age(13, 14). Inconsistent results have been reported for associations between symptomatic AF and comorbidities(15). Furthermore, symptoms are more common in patients with paroxysmal and persistent AF than in patients with permanent AF(16). However, asymptomatic AF episodes are common even in highly symptomatic patients and AF interventions such as AF ablation increase asymptomatic episodes(17). In conclusion, little is known of the pathophysiological

mechanisms underlying AF symptomatology but both somatic and psychological factors are likely involved.

Pain mechanisms

Patients with chronic pain conditions such as osteoarthrosis, chronic pancreatitis and irritable bowel syndrome, show a high degree of central sensitisation, i.e. facilitated pain responses to repeated painful stimulation and impaired conditioned pain modulation (CPM) compared with controls(18-21). Furthermore, these patients often show a discrepancy between pain intensity and objective measures of disease severity and healthcare personnel often underestimate patients' pain intensity(22, 23). It is also well-known that stress influences pain sensitivity and has been correlated to hyperalgesia in patients with ischemic heart disease(24, 25). The importance of pain sensitisation in visceral ailments such as AF is emerging, and the authors have previously shown that patients with symptomatic permanent AF exhibit both impaired pain inhibitory control as well as facilitated summation of pain, and therefore, pain sensitisation(26).

Biomarkers

Biomarkers associated with chronic pain, such as nerve growth factor (NGF), are proteins fundamental during embryonal development for the growth, differentiation, and survival of sensory and sympathetic afferent neurons. During adulthood, they have been found to play a key role in the modulation of nociception through short- (direct sensitisation of peripheral nociceptors) and long-term (changes of gene expression in the dorsal root ganglion) effects. These modifications lead to a wider input from peripheral nociceptors resulting in increased signaling to the central nervous system(27), (28). AF ablation has been shown to increase the plasma level of NGF in humans in the days following the procedure(29), and chronic high

levels of NGF and noradrenaline increased the incidence of inducible AF and its duration in animal studies(30). The use of monoclonal NGF antibodies has been administered to patients with osteoarthritis, providing a significantly greater pain relief compared to NSAIDs and opioids(31).

Epigenetic markers

Epigenetic mechanisms such as DNA methylation, covalent histone modifications, and non-coding RNAs, have been observed to play an important role as mediators of long-term changes in central and peripheral nervous systems in chronic pain. Epigenetic markers can be measured in blood or tissue(36-37). To our knowledge, pain-related epigenetic markers have not been analysed in patients with AF.

Purpose

To study levels of biomarkers and epigenetic markers associated with pain as well as cardiac and inflammatory biomarkers in patients with symptomatic paroxysmal AF, in relation to severity of AF symptoms, and compared to age- and sex-matched controls without AF.

Hypothesis

We hypothesize that

1. Biomarkers and epigenetic markers increase with AF severity
2. Patients with symptomatic AF display increased levels of biomarkers compared with controls, thereby further proving the involvement of central sensitisation mechanisms in patients with symptomatic AF

Clinical relevance

In the future, biomarkers and epigenetic markers associated with pain could be used as tools for evaluation of patients with AF, and have an impact on individualized patient management. Treatment may include medication that neutralizes the biomarker or antagonizes its receptors.

Material and methods

Study design

Single-center cohort study, Örebro University Hospital.

Subject inclusion criteria

Consecutive study participants will be recruited from the waiting list of all patients planned for AF ablation (pulmonary vein isolation) in Örebro University Hospital. Only patients with symptomatic AF are planned for AF ablation.

Further inclusion criteria:

- Male or female subject ≥ 20 years and ≤ 75 years
- Paroxysmal AF (AF that terminates spontaneously or with intervention within 7 days (8))
- Written informed consent

Subject exclusion criteria

- Persistent AF (AF that is continuously sustained beyond 7 days, including episodes terminated by cardioversion after ≥7 days(8))

- Previous pulmonary vein isolation
- Cognitive or psychiatric condition
- Diabetes mellitus
- Ischemic heart disease
- Heart failure
- Asthma
- Pregnancy
- Previous/current drug or alcohol abuse
- Previous neurological or concomitant musculoskeletal disorders
- Continuous analgesic medication

Patient consent and inclusion

All potential patients will receive written information about the study and at the standard physician visit the day before the AF ablation, be asked to participate in the study. After obtaining written informed consent, patients will be asked to complete the Atrial fibrillation 6 questionnaire (AF6) and the Short form 36 health survey (SF-36) questionnaires before a 12-lead ECG is obtained. Comorbidities, medications and duration of AF will be documented and a routine clinical examination will be performed, and the modified European Heart Rhythm Association symptoms scale assessed.

Blood samples

Blood samples for biomarkers and epigenetic markers associated with pain, cardiac biomarkers, and inflammatory markers, will be obtained when standard blood samples are taken the day before the ablation, and the day after ablation. In total 30 ml of blood per time will be obtained. In controls, the same blood samples will be obtained once only. Blood

samples will be stored in a biobank for possible later analysis. Blood samples may be analysed in another laboratory in Sweden or in the EU/ESS.

AF severity assessment

The patient-reported AF6 questionnaire includes six items (breathing difficulties at rest and upon exertion, limitations in day-to-day life, feeling of discomfort, tiredness and worry/anxiety due to AF) and a score of 0 (no symptoms) to 10 (severe symptoms) is reported for each item, and all scores are added into a single sum score. Sum scores range from 0 to 60, with higher values reflecting more severe AF-related symptoms. The AF6 includes a recall period of the last 7 days(32, 33).

The physician-assessed modified European Heart Rhythm Association symptoms scale is used for symptom severity assessment in patients with AF, relating specifically to the time when patients feel symptoms of AF. Patients in class I are considered to have no symptoms, in class IIa mild, class IIb moderate, class III severe and in class IV disabling symptoms of AF(8, 34).

Health-related quality of life

The patient-reported SF-36 has a recall period of four weeks and consists of 36 items assessing eight domains ranging from 0 to 100, with higher values indicating better health-related quality of life. The eight domains generate two summary measures: the physical component summary and the mental component summary scores, which will be used to assess health-related quality of life(35).

Control group

Sex and age-matched controls will be recruited by advertisement. The same blood samples will be obtained from controls as on patients with AF but only once, and controls will be asked to complete the SF-36 questionnaire and a questionnaire about previous and current diseases and current medication.

Follow-up

No follow-up visits are scheduled.

Primary endpoint

The primary objectives are (1) to study the association of biomarkers and epigenetic markers and the severity of AF symptoms, and (2) to assess differences in the levels of biomarkers and epigenetic markers in patients with symptomatic AF compared with participants without AF.

Secondary endpoint

Secondary objectives are to study the association of age, sex, AF duration, health-related quality of life, and biomarkers and epigenetic markers.

Ethics approval

The study protocol is submitted to the Swedish Ethical Review Authority for approval.

Statistics and data management

Data collected during the study will be coded.

Statistical analysis

Data will be presented as mean and standard deviation. Independent t-test will be used to compare biomarkers and epigenetic markers in AF patients and control subjects. Pearson correlations will be used for linear estimates and Spearman´s correlations for non-linear parameters. Multiple linear regression analysis will be performed to show associations between explanatory variables (age, sex, symptoms) and biomarkers and epigenetic markers associated with pain. Analyses will be performed using IBM SPSS Statistics 22 (Armonk, NY, USA) or STATA release 14 (College Station, TX, USA).

Database and Case report form

A case report form will be filled out and coded for each patient. The investigators are responsible for ensuring the accuracy, completeness, legibility and timeliness of the data recorded in the case report forms.

Documentation and data collection

Criteria for inclusion, informed consent, the decision to include the subject and the subject number will be documented in the subject's hospital record.

Insurance

The subjects in the study are covered by the Swedish patient insurances.

Economy

The trial is an academic study conceived and conducted by healthcare personnel. The study is independent of commercial interests. Study logistics, handling of data and statistical assessment will be financed by the Department of Cardiology, Örebro University Hospital, Sweden. The investigators will apply for grants from public funds. Neither patients nor controls will receive economic compensation.

Ethical considerations

The study will be conducted in accordance with the Declaration of Helsinki. The protocol is submitted to the Swedish Ethical Review Authority and the study will be initiated when the protocol has been approved. There are no known risks or complications associated with additional blood samples in association with standard blood samples being obtained, but it may cause some temporary discomfort.

Risks, side effects, advantages and disadvantages in participation

Subjects will be treated according to standard clinical practice. We do not expect that subjects will have any advantages or disadvantages from participating in the study.

Guidelines for obtaining informed consent

Subjects will enter the study after signing the informed consent form. Candidate participants will receive written information of the study, and oral information by a physician participating in the study. The subjects will be given time to think through participation in the study and to ask questions. Informed consent will be obtained by a Good Clinical Practice qualified physician participating in the study.

Withdrawal

A subject can withdraw from the study at any time. Data collected up to the end of follow-up will be used in the final analysis of the study.

Publication

Results, positive as well as negative or inconclusive, will be published in an international medical journal.

End of trial and archiving

The study will end when the last subject has signed informed consent, completed the questionnaires and has undergone clinical examination and had blood samples taken. The steering committee reserves the right to terminate the study prematurely e.g. if the study participant recruitment is too slow, if study participant retention in the study is insufficient. Data collected during the study will be archived for at least 10 years after the study has been completed.

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