

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

**Long-term changes in serum biomarker concentrations in patients
with atrial fibrillation following transcatheter pulmonary vein
isolation.**

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1 INTRODUCTION

1.1. Epidemiology of atrial fibrillation

Atrial fibrillation (AF) is currently the most prevalent sustained cardiac arrhythmia, the significance of which extends beyond routine cardiological practice to represent a major global public health and economic burden. While it affects approximately 2–3% of the adult population, its prevalence increases significantly with age, reaching nearly 10% in individuals over the age of 75. Due to an aging society, the rising frequency of comorbidities, and the continuous advancement of diagnostic tools (e.g., wearable smart devices), the prevalence of the disease is projected to double in the coming decades. AF is not limited to the elderly; its occurrence in younger populations highlights the critical importance of early detection and the implementation of effective therapeutic strategies. In the absence of such interventions, patients face a substantial decline in quality of life, alongside significantly elevated rates of hospitalization and mortality.

1.2 Clinical classification of atrial fibrillation

To determine the appropriate therapeutic strategy, it is essential to categorize AF based on its temporal patterns. Five distinct forms are identified: (1) newly diagnosed AF; (2) paroxysmal AF, which terminates spontaneously within one week of onset; (3) persistent AF, which lasts longer than seven days but terminates spontaneously or via intervention (pharmacological or electrical cardioversion) within one year; (4) long-standing persistent AF, which remains continuous for more than one year; and (5) permanent AF, where the restoration of sinus rhythm (SR) is no longer pursued, and the therapeutic goal is limited to rate control.

1.3 Pathophysiology of atrial fibrillation

Atrial cardiomyocytes that extend like "finger-like" projections into the pulmonary veins (PVs) play a pivotal role in initiating the arrhythmia. Rapid electrical discharges originating from these cells serve as focal triggers that initiate—and in a smaller subset of cases, maintain—the arrhythmia. This landmark discovery by Michel Haïssaguerre and his colleagues in Bordeaux nearly 30 years ago paved the way for the development of effective transcatheter treatment strategies for AF.

For AF to become sustained, the presence of an atrial substrate is generally required. This refers to structural alterations within the left atrium (LA) that allow the arrhythmia to become self-

sustaining. The substrate encompasses structural changes detectable by imaging modalities—such as echocardiography and magnetic resonance imaging (MRI)—as well as electrical remodelling, the nuances of which are currently less understood.

Structural remodelling primarily involves atrial dilatation and fibrotic transformation. These processes lead to the formation of small connective tissue "islands" that provide the anatomical basis for microreentry circuits. The pathological impulses originating from the PVs can circulate around these circuits, thereby perpetuating the arrhythmia. While this fibrotic transformation can result from aging and various comorbidities, the persistently high atrial rate itself can induce further electrical and structural changes—a phenomenon often summarized as "AF begets AF." Chronic wall stress, inflammatory processes, and oxidative stress also contribute to myofibroblast proliferation and interstitial fibrosis, leading to the chronicity of the arrhythmia.

The relative contribution of the atrial trigger (mostly PV-derived) and the atrial substrate to the maintenance of AF varies among individuals. In young patients without comorbidities and with structurally normal atria (as confirmed by imaging), the role of triggers is assumed to be dominant. Conversely, in older patients with multiple comorbidities, the significance of the substrate increases.

1.4 The role of comorbidities and risk factors

The development and progression of AF are closely linked to numerous cardiac and extracardiac conditions, which play an active role in the development of atrial myopathy and remodelling. Among the comorbidities, hypertension, diabetes mellitus, coronary artery disease, heart failure, obesity, and obstructive sleep apnea (OSA) are attributed a prominent role.

Hypertension is the most common risk factor; in inadequately managed cases, elevated systemic blood pressure can lead to left ventricular hypertrophy and diastolic dysfunction. These processes increase left ventricular end-diastolic pressure, which, by affecting the LA, increases atrial pressure and wall stress. Wall stress activates the local renin-angiotensin-aldosterone system (RAAS), leading to the release of inflammatory cytokines and the development of fibrosis.

Diabetes mellitus is an independent risk factor for AF. Hyperglycemia promotes the accumulation of advanced glycation end-products in the myocardium, which create cross-links between collagen fibers, thereby reducing atrial compliance. In the context of diabetes, it is essential to mention the role of obesity, the prevalence of which has increased drastically in recent years. Both obesity and insulin resistance are associated with increased accumulation of epicardial adipose tissue. This epicardial fat is a metabolically active tissue that secretes inflammatory cytokines (e.g., interleukin-6, tumor necrosis factor-alpha (TNF- α)), thereby promoting apoptosis and fibrosis.

AF and HF often coexist, mutually exacerbating patient symptoms and prognosis while increasing mortality. The hemodynamic overload occurring in HF can lead to atrial stretching, dilatation, and consequently fibrosis, providing an ideal substrate for the formation of microentry circuits. During AF, the loss of atrial contraction—and thus active ventricular filling—along with irregular, tachycardic cardiac activity, can lead to impaired pump function and HF, or further aggravate existing HF symptoms.

Research over the past decade has highlighted a strong association between OSA and AF. The high negative intrathoracic pressure, hypoxia, and sudden increase in sympathetic tone during nocturnal apnoeic episodes cause acute electrical instability in the atria. To this day, this condition remains underdiagnosed.

Inadequately managed comorbidities significantly impair the success rate of catheter ablation for AF, thus the importance of treating these conditions must be emphasized.

Age is also a significant risk factor for AF; although it is a non-modifiable factor, it plays a crucial role in AF pathophysiology. With advancing age, physiological connective tissue transformation occurs in the heart. In addition to increased interstitial fibrosis, impulse conduction also slows down. Furthermore, cellular aging is associated with oxidative stress and mitochondrial dysfunction, which increases the sensitivity of the atria to triggers.

1.5. Definition of atrial myopathy

Atrial myopathy is defined as any structural or electrical alteration within the atrial myocardium. The term was first introduced by Zipes in 1997, suggesting a close correlation between AF and atrial remodelling. Today, the relationship between AF and atrial myopathy is considered bidirectional: atrial myopathy may develop due to or in the absence of AF or

independently through other mechanisms, and the myopathy itself is a critical factor in the onset of the arrhythmia.

Although atrial myopathy is now regarded as a cornerstone in the pathophysiology of AF, assessing its severity remains a challenge in clinical practice. Beyond sustaining the arrhythmia, fibrosis, endothelial dysfunction, and systemic inflammation (measurable via biomarkers) create a prothrombotic status. This environment can increase the risk of cardiogenic stroke or systemic embolization even in the presence of sinus rhythm (SR).

1.6. The cornerstone of atrial fibrillation management

The management of AF is built upon three primary pillars: the prevention of thromboembolism, the restoration and maintenance of SR (rhythm control), and the control of ventricular rate (rate control).

1.6.1. Thromboembolic risk assessment: the CHA₂DS₂-VASc score

Emboic risk factors are traditionally categorized by the CHA₂DS₂-VASc scoring system, which accounts for congestive heart failure (1 point), hypertension (1 point), age (≥ 65 years: 1 point; ≥ 75 years: 2 points), diabetes mellitus (1 point), a history of stroke/transient ischemic attack/thromboembolism (2 points), vascular disease (1 point), and female sex (1 point). Based on this scale, a score of 2 or higher necessitates long-term anticoagulant therapy. This scoring system has provided guidance for anticoagulation for decades and has served as the basis for numerous studies, including our present investigation, where it was used to classify patients.

However, the new 2024 ESC Guidelines—reflecting the latest clinical trial data—mark a paradigm shift: female sex alone, in the absence of other risk factors, does not significantly increase the risk of stroke. Consequently, clinical practice has transitioned toward the CHA₂DS₂-VA score, which no longer includes sex as an independent point-yielding factor. Based on this updated system:

- 0 points: Anticoagulation is not required.
- 1 point: Oral anticoagulant (OAC) therapy should be considered (Class IIa).
- ≥ 2 points: OAC therapy is recommended/indicated (Class I).

1.6.2 Oral anticoagulation therapy

Alongside Vitamin K antagonists (VKAs), direct oral anticoagulants (DOACs)—including dabigatran, rivaroxaban, apixaban, and edoxaban—now constitute the first-line choice for therapy. These agents have proven superior to VKAs both in the prevention of ischemic stroke and in terms of reducing the risk of intracranial and other major bleeding complications. Furthermore, they have improved patient adherence, as they do not require frequent laboratory monitoring.

1.6.3. Rythm control in clinical practice

Both pharmacological and interventional approaches are available for the restoration and maintenance of SR. The efficacy of antiarrhythmic drugs, including amiodarone, remains inferior to that of catheter ablation; the former can maintain SR beyond one year in approximately 50% of cases. In contrast, the success rate of catheter ablation can range between 70% and 80%, since highly effective techniques for the elimination of triggers originating from the PVs have become available.

1.6.4. Ablation techniques

During technological development, various energy sources and catheter designs have emerged, aimed at increasing procedural safety, reducing intervention time, and ensuring the transmural continuity of lesions. The cornerstone of these interventions is the permanent electrical isolation of all pulmonary veins.

- **Point by point radiofrequency (RF) ablation:** During the procedure, thermal (coagulation) necrosis is induced within the tissue using high-frequency alternating current. During ablation, an isolation line is created point-by-point around the antrum of the pulmonary veins (PVs) using an irrigated-tip catheter. The advent of modern 3D electroanatomical mapping systems and contact force-sensing catheters has significantly improved lesion quality. The primary advantage of this technique lies in its high degree of flexibility; however, its disadvantages include a steep learning curve requiring substantial operator experience and longer procedural times compared to "single-shot" devices.
- The essence of **Pulmonary Vein Ablation Catheter (PVAC)** technology involves positioning a decapolar, circular catheter into the PV antrum, followed by the delivery of RF energy through the electrodes to create a circumferential lesion, potentially in a single step

(**single-shot isolation**). The development of PVAC aimed for more rapid PV selection, specifically targeting the reduction of procedural time and technical simplification.

- In contrast to the previous techniques, **Cryoballoon Ablation (CRYO)** utilizes freezing technology rather than high temperatures to induce lesion formation. By employing an inflatable balloon, the PV ostium is occluded, allowing for circumferential isolation to be achieved with a single freezing cycle (also a single-shot technique). The primary advantage of this method is, once again, the shorter procedural duration. Clinical trials (e.g., FIRE AND ICE) have confirmed that its efficacy and safety are non-inferior to those of RF ablation.

1.6.5. Relationships between pulmonary vein isolation and left atrial substrate

PVI is associated with high success rates primarily in young patients with structurally normal hearts; however, during long-term follow-up, the recurrence of arrhythmia is not uncommon, usually driven by the electrical reconnection of one or more PVs. Conversely, recurrence can occur even with persistently isolated PVs in young, structurally healthy patients with paroxysmal AF, and particularly in older patients presenting with significant cardiac and extracardiac comorbidities and persistent or long-standing persistent forms of the arrhythmia. In such cases, other mechanisms related to atrial myocardial fibrosis are presumed to play a role, and limited clinical benefit can be expected from electrical isolation of the PVs alone.

Based on these observations, patients can be didactically categorized into "trigger-based fibrillation," "substrate-based fibrillation," or cases involving mixed mechanisms. Consequently, the identification of atrial myopathy and a more accurate assessment of its staging could facilitate appropriate patient selection for AF ablation strategies utilizing PVI exclusively.

1.6.6 Diagnostics and follow-up of the left atrial substrate

Diagnostic methods for identifying the LA substrate include the echocardiographic measurement of left atrial volume and the assessment of fibrosis severity via Cardiac Magnetic Resonance Imaging (CMRI) utilizing the Late Gadolinium Enhancement technique. Furthermore, preclinical and human studies have compared the plasma or serum levels of biomarkers associated with myocardial fibrosis and inflammation in patients with various forms of AF versus those maintaining SR. These studies evaluated levels of Cancer Antigen 125 (CA-125), Caspase-3, and Cathepsin L across patient cohorts primarily affected by persistent and

long-standing persistent forms of AF, frequently associated with significant comorbidities such as chronic HF.

Limited data are available regarding the correlation between pre-ablation Galectin-3 levels and AF recurrence during the one-year post-procedural follow-up period. Although the indications for AF ablation have recently expanded to include patients with significant comorbidities—primarily congestive HF—and persistent forms of the arrhythmia, the procedure remains predominantly recommended for patients with shorter AF duration, structurally intact hearts (including near-normal LA size), and few comorbidities. In these specific patient populations, there is insufficient data concerning both the correlation of these biomarkers with the long-term success of PVI and the longitudinal changes occurring in these biomarker levels.

1.7. The Role of Biomarkers in Atrial Fibrillation

The most significant challenge in the treatment of AF is the inherent heterogeneity of the patient population. While clinical parameters (e.g., age, comorbidities, echocardiographic indices) often fail to accurately reflect the true extent of atrial myopathy, the determination of circulating biomarkers may be particularly useful for monitoring the degree of left atrial remodelling, inflammation, fibrosis, and apoptosis.

1.7.1. General Characteristics of the Studied Biomarkers

Based on literature data, the biomarkers considered for monitoring the complex pathophysiology of AF represent various aspects of atrial remodelling, including inflammation, fibrosis, apoptosis, and extracellular matrix restructuring.

- **CA-125:** A large transmembrane glycoprotein, it is the largest of the membrane-associated mucins. Its production increases in the mesothelial cells of serous membranes (pleura, peritoneum, and pericardium) in response to mechanical irritation or inflammation. Although not a conventional cardiovascular marker, its levels are elevated in malignancies—such as ovarian, lung, and breast cancers—as well as in nephrotic syndrome, liver cirrhosis, and heart failure. Functioning as a secondary cytokine, its concentration rises in response to primary cytokines (e.g., TNF- α or IL-1), thereby exerting a pro-inflammatory effect. Plasma CA-125 levels are independent of age, sex, body weight, and renal function. Numerous studies have correlated elevated CA-125 levels with an increased risk of AF. The close

anatomical and paracrine relationship between the left atrium and the pericardium may explain why atrial dilation and inflammation manifest as an elevation in CA-125 levels.

- **Galectin-3:** A beta-galactoside-binding lectin. Upon activation, it promotes macrophage chemotaxis and the differentiation of resting fibroblasts into active myofibroblasts, leading to increased collagen deposition. Consequently, it may play a significant role in fibrosis, tissue regeneration, and cardiac remodelling. Plasma levels are elevated in AF, heart failure, renal insufficiency, liver cirrhosis, chronic obstructive pulmonary disease, breast and gastric malignancies, and obesity. While higher Galectin-3 levels may predict the recurrence of arrhythmia—potentially indicating more severe damage to the atrial substrate—these studies frequently involved patient populations with significant comorbidities. In contrast, no correlation was found between elevated biomarker levels and the occurrence of AF in idiopathic cases; thus, the relationship between Galectin-3 levels and the incidence of AF remains to be fully elucidated.
- **Caspase-3:** A key enzyme in the apoptosis pathway, which can be activated by Caspase-8. In a study by Trappe et al., the Caspase-3 gene was inactivated in the left atrium of knock-out pigs using an adenovirus vector; as a result, the frequency and duration of AF occurrences were significantly reduced compared to the control group. Caspase-3 levels have not been previously investigated in the context of AF recurrence following PVI during long-term follow-up. Some studies suggest that tissue-level elevations of Caspase-3 can be detected within 12 hours following a stressor (e.g., acute atrial stretch or ischemia), indicating that Caspase-3 may be a suitable biomarker for the dynamic monitoring of atrial myopathy.
- **Cathepsin L:** A lysosomal cysteine protease responsible for the degradation of extracellular matrix (ECM) proteins, thereby playing a role in normal tissue turnover and regeneration. However, elevated levels may serve as a predictor of atrial myopathy. In cases of idiopathic AF, higher plasma levels of Cathepsin L have been associated with an increased risk of developing the arrhythmia, potentially indicating early ECM remodelling prior to the complete transformation of structural fibrosis. To date, Cathepsin L levels have not been investigated in relation to AF ablation and arrhythmia recurrence.

Existing results regarding these biomarkers are based on single-point measurements; the findings of longitudinal studies have not yet been published. Nevertheless, it can be

hypothesized that long-term changes in peripheral blood biomarker levels may correlate with the progression or regression of atrial myopathy. Long-term freedom from atrial arrhythmia, or a significant reduction in arrhythmia burden, may facilitate the regeneration of the atrial myocardium, for which the biomarkers could serve as suitable monitoring tools. These processes may also be influenced by other factors, including the potential presence of comorbidities.

2 OBJECTIVES

In our prospective observational study, we primarily sought to determine whether the pre-procedural serum concentrations of CA-125, Galectin-3, Caspase-3, and Cathepsin L are predictive of long-term (3-year) arrhythmia recurrence in patients with structurally intact hearts undergoing PVI for paroxysmal or persistent AF. Furthermore, we aimed to analyse the longitudinal changes in the serum concentrations of these biomarkers by comparing samples collected at baseline and at the end of the 3-year follow-up period. Additionally, we investigated the correlations between these biomarker dynamics and arrhythmia recurrence, changes in LA dimensions, and baseline comorbidities.

3 PATIENTS AND METHODS

3.1 Patients

Patients aged 18–75 years treated at the Department of Cardiology and Cardiac Surgery of the University of Debrecen between March 2018 and December 2022 were enrolled if they underwent PVI for paroxysmal or persistent AF. Further inclusion criteria included consent to participate in the clinical trial—including biomarker measurements prior to ablation and after a 3-year period—as well as compliance with outpatient follow-up visits.

The exclusion criteria were as follows:

1. Long-standing persistent or permanent AF.
2. Reversible causes of AF (e.g., hyperthyroidism).
3. Presence of a LA thrombus.
4. History of cardiac surgery.
5. Valvular heart disease.
6. Reduced left ventricular ejection fraction (LVEF <50%) associated with symptoms of heart failure.
7. Unstable angina or myocardial infarction within the preceding 3 months.
8. Acute inflammatory disease.
9. Pregnancy.
10. Active or previous history of malignant disease.
11. Prior AF ablation.

In the second phase of the study, patients were selected from this cohort who remained in SR during follow-up—documented by electrocardiogram (ECG)—and reported no symptoms suggestive of AF recurrence throughout the follow-up period.

3.2 Baseline cardiac evaluation

At the commencement of the study, all patients underwent routine cardiovascular evaluation, which included a detailed medical history, physical examination, a 12-lead ECG, and transthoracic echocardiography (TTE) to measure LVEF and left atrial (LA) parameters prior to the intervention. To exclude intracardiac thrombi, transoesophageal echocardiography (TEE) was performed within 24 hours of the procedure. During the collection of medical histories, particular attention was paid to comorbidities such as hypertension (blood pressure >140/90 mmHg), heart failure, diabetes mellitus (HbA1c $\geq$ 6.5%), coronary artery disease, and prior stroke or transient ischemic attack (TIA). These conditions were validated based on medical records and clinical symptoms, and treatments were optimized in accordance with current clinical guidelines. Blood samples for biomarker measurements were collected from peripheral veins within 24 hours preceding the intervention.

3.3 Ablation procedure

Procedures were performed under local anaesthesia, with intravenous midazolam administered as required. The femoral vein was cannulated using the Seldinger technique. Multipolar electrode catheters were positioned in the coronary sinus and the right ventricle. Transseptal puncture was executed using a Brockenbrough needle under intracardiac echocardiography (ICE) guidance. Following the transseptal puncture, an intravenous heparin bolus of 150 IU/kg was administered, followed by a continuous infusion to maintain an activated clotting time (ACT) between 300–400 ms. All PVI procedures were performed using one of the following three ablation techniques based on the operator's discretion:

(1) Point-by-point irrigated radiofrequency ablation with contact force monitoring, utilizing a Thermocool Smarttouch catheter (D133602, Biosense Webster Inc., Johnson & Johnson Medtech, Irvine, CA, USA).

(2) Phased RF ablation with the second-generation Pulmonary Vein Ablation Catheter (PVAC) Gold (Medtronic Inc., 990078, Minneapolis, MN, USA).

(3) Cryoballoon ablation using the Arctic Front Advance catheter (Medtronic Inc., 2AF283, Minneapolis, MN, USA).

The procedural endpoint for all three techniques was the complete isolation of all pulmonary veins, verified by standard local PV electrograms using differential pacing from the coronary

sinus (CS) or near the vein ostium (entry block), as well as from within the vein ostium (exit block), as necessary. If required, sinus rhythm was restored via electrical cardioversion. All three ablation protocols employed at our institution have been previously described in detail.

3.3.1 Point by point pulmonary vein isolation

A circular decapolar Lasso catheter was advanced through a Mullins transseptal sheath and positioned sequentially at each PV ostium. Following a second transseptal puncture, a 9F steerable Agilis sheath was used to advance a contact force ablation catheter into the LA. Point-by-point RFA delivery was performed in a temperature- and power-controlled manner, guided by a three-dimensional (3D) electroanatomical mapping system. The average duration of individual RF applications was 30 seconds.

3.3.2 Ablation with Pulmonary Vein Ablation Catheter (PVAC)

The Mullins sheath was exchanged for a 12 Fr FlexCath sheath, through which a second-generation, circular PVAC-Gold catheter (Medtronic Inc., 990078, Minneapolis, MN, USA), featuring 9 gold-alloy electrodes, was loaded into the introducer. The circular portion of the catheter was positioned within the PV antrum to ensure maximal electrode-to-tissue contact. Phased RF energy was delivered in a temperature-controlled and power-limited manner (60°C, maximum 10 W). The average duration of each ablation cycle was 60 seconds.

3.3.3 Cryoballoon ablation

The Mullins sheath was exchanged for a 12 Fr FlexCath sheath, through which a second-generation Arctic Front Advance balloon catheter was positioned. PV potentials were continuously monitored using a circular electrode catheter (Achieve Mapping Catheter, 990063-020, Medtronic Inc., Minneapolis, MN, USA) advanced into the PV through the balloon catheter. Prior to ablation, the balloon position and the degree of occlusion were assessed with the administration of contrast injection. The target temperature was set between -40°C and -55°C; the duration of individual freezing cycles was typically determined to be between 180–240 seconds, based on the achieved temperature and the time to isolation.

3.4 Laboratory measurements of biomarkers

Baseline blood samples were collected from the cubital vein within the 24 hours preceding the intervention. Follow-up samples were similarly collected from the cubital vein at the end of

the 3-year follow-up period. Blood samples were obtained using Vacutainer tubes. Ethylenediamine-tetraacetic acid (EDTA) anticoagulant tubes (3 mL Vacuette tube with K3EDTA, 454086, Greiner Bio-One GmbH, Bad Haller Str., Kremsmünster, Austria) and serum separator tubes (5 mL BD Vacutainer SST II Advance Plus blood collection tubes, 367955, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were utilized. Within two hours, the samples were centrifuged at 1500 g for 20 minutes at room temperature. The plasma phase from the EDTA tubes and the serum phase from the serum separator tubes were pipetted, divided into aliquots, and stored at -70°C until analysis.

Serum CA-125 levels were determined using an electrochemiluminescence microparticle immunoassay (Cobas® e602, Roche Diagnostics, Mannheim, Germany). The concentrations of the remaining biomarkers were measured using enzyme-linked immunosorbent assay (ELISA) methods. Plasma Caspase-3 concentrations were measured with the Human Caspase-3 ELISA kit (Thermo Fisher Scientific Inc., Carlsbad, CA, USA, Cat. No.: BMS2012INST), while plasma Galectin-3 levels were determined using the Human Galectin-3 DuoSet ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA, Cat. No.: DY1154), and plasma Cathepsin L levels were measured using the Human Cathepsin L DuoSet ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA, Cat. No.: DY952), in accordance with the manufacturers' instructions.

3.5 Post-ablation follow-up

The previously prescribed antiarrhythmic drugs were continued after the procedure and stopped three months later. All patients received anticoagulation therapy for at least three months, then the decision on long-term treatment was based on the CHA₂DS₂-VASc score. Follow-up visits were conducted every six months post-ablation, including a 12-lead ECG and 24-hour Holter monitoring. Patients were also provided with transtelephonic ECG devices for up to six weeks, allowing them to transmit ECG recordings in case of any symptom suggestive of an AF episode. Recurrence was defined as any ECG-documented AF episode lasting longer than 30 seconds. During the 3-year follow-up visit, LVEF and LA diameter were measured using TTE.

3.6 Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0.0 (GraphPad Software, San Diego, CA, USA). Normality of data distribution was assessed using the Kolmogorov–Smirnov test. Normally distributed continuous variables were expressed as mean $\pm$ standard deviation,

while not normally distributed variables were presented as median and interquartile range. Categorical variables were presented as counts and percentages within brackets. For patients with missing values at the end of the follow-up period, the Last Observation Carried Forward (LOCF) method was applied for data imputation. Comparisons between the two groups were performed using Student's t-test for normally distributed data and the Mann–Whitney test for not normally distributed data. Comparisons between baseline and follow-up values were conducted using paired t-tests or Wilcoxon signed-rank tests. Differences between categorical variables were evaluated using the Pearson's chi-square (χ^2 -) or Fisher's exact test. Correlations were examined using the Spearman's correlation coefficient. A $p < 0.05$ was considered statistically significant.

Univariable regression analysis was applied to investigate independent associations between fibrosis biomarkers (dependent variables) and comorbidities (independent variables). The standardized β linear beta coefficients representing linear correlations between the two parameters were determined. The B-regression coefficient (with 95% confidence intervals) indicated the independent associations between dependent and independent variables during changes. General linear model repeated measures analysis of variance (RM-ANOVA) was performed to determine the additional effects of comorbidities with the 3-year biomarker changes. Adjusted analyses were performed using linear mixed-effects models on log-transformed biomarker levels, with comorbidity group, time, and their interaction entered as fixed effects, and the patient as a random intercept. The models were adjusted for age, sex, AF type, and the ablation technique.

4 RESULTS

4.1 Relationship between biomarker levels and arrhythmia recurrence

Out of a total of 89 consecutive patients undergoing their first PVI procedure, 77 patients provided written informed consent and had blood samples collected prior to ablation. A total of 63 patients (23 females; mean age: 61.4 ± 8.8 years) completed the follow-up, including the repeated blood sampling after 3 years. Among these 63 patients, arrhythmia recurrence was confirmed in 26 cases, while 37 patients remained arrhythmia-free. Thirty-six patients (57.1%) were receiving antiarrhythmic medication prior to the intervention. PVI was performed using point-by-point irrigated RFA in 24 cases, PVAC ablation in 11 cases, and cryoablation in 28 cases. Acute PV isolation was successfully achieved in all patients, and no complications occurred during the procedures.

4.2 Changes in biomarker levels during follow-up

During the follow-up period, which averaged 36.3 ± 6.3 months, AF recurrence occurred in 26 patients (41.3%). In the arrhythmia recurrence group, 23% of patients had persistent AF, whereas this proportion was only 8% in the arrhythmia-free group. The only statistically significant difference between the two groups was a higher proportion of females in the recurrence group (53.8% vs. 24.3%; $p=0.017$). Among all patients with recurrence, the arrhythmia returned in paroxysmal form. No significant differences were observed between the two groups in baseline biomarker levels (CA-125, Galectin-3, Caspase-3, Cathepsin L) prior to ablation. Similarly, biomarker levels measured 36.3 ± 6.3 months post-ablation showed no significant differences between the two groups. The comparison between baseline and follow-up biomarker levels yielded the following results:

- CA-125 levels did not change significantly in either the arrhythmia-free group (9.7 (7.6–13.0) kU/L vs. 8.7 (6.7–12.8) kU/L; $p=0.104$) or the recurrence group (11.3 (7.8–15.1) kU/L vs. 11.0 (7.2–14.0) kU/L; $p=0.681$).
- Galectin-3 levels decreased significantly in both groups (arrhythmia-free group: 4209.9 (3226.2–5662.4) pg/mL vs. 3391.4 (2856.2–4182.5) pg/mL; $p=0.014$; recurrence group: $p=0.037$).
- Caspase-3 levels decreased significantly during the follow-up period in both groups (arrhythmia-free group: 564.1 (463.0–731.6) pg/mL vs. 376.9 (310.9–467.6) pg/mL;

$p=0.001$; recurrence group: 579.0 (523.9–735.6) pg/mL vs. 413.5 (341.8–563.5) pg/mL; $p=0.032$).

- Cathepsin L levels also decreased significantly in both groups (arrhythmia-free group: 406.9 (200.4–763.3) pg/mL vs. 309.2 (131.7–460.4) pg/mL; $p=0.025$; and recurrence group: 422.4 (248.3–600.4) pg/mL vs. 294.4 (158.2–470.7) pg/mL; $p=0.036$).

4.3 Echocardiographic parameters in the arrhythmia-free and AF recurrence groups

Left atrial diameter decreased slightly in the arrhythmia-free group (41.8 ± 4.1 mm vs 40.5 ± 4.6 mm; $p = 0.231$), while a slight increase was observed in the recurrence group (41.3 ± 5.2 mm vs 42.7 ± 4.9 mm; $p= 0.087$); however, neither change reached the level of statistical significance. LVEF also showed no significant change in either group (arrhythmia-free group: $58.5 \pm 5.3\%$ vs $57.2 \pm 4.3\%$; $p=0.248$; recurrence group: $57.0 \pm 7.7\%$ vs $56.5 \pm 5.5\%$; $p=0.786$). A positive correlation was observed between Caspase-3 levels and left atrial diameters in the recurrence group both pre-ablation ($r=0.477$; $p=0.018$) and post-ablation ($r=0.533$; $p=0.019$). No significant correlations were found between the other biomarkers (CA-125, Galectin-3, Cathepsin L) and LA dimensions.

4.4 Biomarker levels in arrhythmia-free patients based on baseline comorbidities

During the follow-up period, no arrhythmia recurrence was detected in 37 patients. Among them, 12 patients had no recorded comorbidities at the time of PVI, while at least one comorbidity was present in 25 patients. Patients with comorbidities were significantly older (48 [42.3–60] years vs. 59 [54.5–65] years; $p=0.032$); there were no significant differences in other parameters. In the groups with and without comorbidities, we compared biomarker levels measured before ablation and at 33.7 ± 7.9 months post-intervention. In cases where the level of a specific biomarker was below or above the measurement range, the data were excluded from statistical analysis and visualization. Before ablation, there were no significant differences in the levels of CA-125, Galectin-3, Caspase-3, and Cathepsin L between the two groups. However, at the 3-year follow-up, Caspase-3 levels were significantly higher in the group with comorbidities (447.7 [327.6–636.9] pg/mL) than in the group without comorbidities (340.5 [255.9–387.3] pg/mL; $p=0.038$). Univariable regression analysis was performed between Caspase-3 levels at follow-up (dependent variable) and comorbidities (independent variables). At the 3-year follow-up, the only significant correlation observed was between hypertension and Caspase-3 levels $\beta=0.387$; $p=0.024$; $B= 181.54$; 95% CI= 25.6 - 337.5). Within both groups,

we also compared the baseline and 3-year levels of the four biomarkers. Caspase-3 levels decreased significantly compared to pre-ablation values by the end of follow-up in both patients with comorbidities (623.8 [476.9–751.4] pg/mL vs. 447.7 [327.6–636.9] pg/mL, $p=0.039$) and those without (500.9 [345.7–719.5] pg/mL vs. 340.5 [255.9–387.3] pg/mL, $p=0.016$). No significant changes were detectable in the levels of CA-125, Galectin-3, or Cathepsin L in either group. Based on RM-ANOVA tests, the presence of comorbidities had no effect on the 3-year changes in biomarkers. In the adjusted mixed-model analysis, there was no significant interaction between comorbidity status and time for CA-125 ($p=0.262$), Galectin-3 ($p=0.273$), Caspase-3 ($p=0.570$), or Cathepsin L ($p=0.462$). This indicates that the longitudinal changes in biomarkers did not differ significantly based on comorbidity status. For Caspase-3, a significant overall time effect was observed ($\beta=-0.437$, $p=0.010$), which is consistent with the lower concentrations measured at the 3-year follow-up, even after adjusting for age, sex, AF type, and ablation technique. The adjusted difference in Caspase-3 levels between groups at the 3-year follow-up was not significant ($p=0.114$).

4.5 Echocardiographic parameters in groups with and without comorbidities

At 3-year post-ablation LA diameter showed no significant change compared to baseline in either the group without comorbidities (39 [35–46] mm vs. 39 [35–41.5] mm; $p=0.973$) or the group with comorbidities (43 [39.5–45.5] mm vs. 42.5 [38–45] mm; $p=0.177$). Similarly, no significant changes were observed in LVEF among patients without comorbidities (60 [56–64]% vs. 58.5 [55–62]%; $p=0.219$) or those with comorbidities (57 [55–59.8]% vs. 56 [54.3–59.5]%; $p=0.930$).

5 DISCUSSION

5.1 Main findings

Out of the 63 enrolled patients, the procedure proved successful in 37 cases (58.7%) by the end of the 3-year follow-up. The only significant difference between the two groups (recurrence vs. arrhythmia-free) was a higher proportion of females in the recurrence group. In our study, none of the baseline biomarker levels showed an association with AF recurrence. CA-125 levels did not change significantly over the 3 years; however, the concentrations of the other biomarkers (Galectin-3, Cathepsin L, and Caspase-3) decreased significantly in all patients, regardless of the success of the ablation. Average LA diameter and LVEF showed no statistically significant changes. A positive correlation was confirmed between Caspase-3 levels and LA diameter in the AF recurrence group both before and after the intervention.

In the comparison between the group with and without comorbidities, the levels of the 4 biomarkers showed no difference prior to PVI. By the end of the 3-year follow-up, Caspase-3 levels had decreased significantly in both patient groups compared to pre-ablation values, although concentrations remained higher in patients with comorbidities. The serum levels of the other three biomarkers did not change in either group during our follow-up. Among the baseline comorbidities, hypertension showed a significant correlation with Caspase-3 levels measured at the 3-year follow-up; however, the presence or absence of comorbidities had no effect on the 3-year longitudinal change of the biomarkers. The mixed-model analysis confirmed that time was the only factor influencing the decrease in Caspase-3 levels; neither comorbidities, type of AF, nor ablation technique influenced the biomarker reduction.

5.2 Clinical significance of biomarkers in atrial fibrillation

Imaging techniques (delayed gadolinium enhancement cardiac MRI, multidetector computed tomography (MDCT), and LA strain echocardiography) have been proposed to characterize the atrial wall and guide clinical decision to select patients for ablation and to assess the risk of later comorbidities, such as stroke and HF. Given the significant correlation between the dynamics of fibrotic changes and the progression of atrial myopathy, serial measurements of fibrosis-related biomarker levels may portend the clinical course of the disease.

In addition to the information provided by imaging techniques, these biomarkers could offer a simple, rapid, and less expensive method for routine clinical practice, including population-

based screening. For instance, in the STROKESTOP II study, low levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP) identified patients with a negligible risk of stroke, suggesting that anticoagulant therapy may not be warranted regardless of long-term ECG monitoring results.

The identification of other markers indicating the extent of fibrotic processes in the atrial wall could open new avenues for determining stroke risk beyond the CHA₂DS₂-VA score. Furthermore, it could facilitate more specific patient selection for various arrhythmia suppression methods and serve as a tool for monitoring the long-term efficacy of these therapies.

5.3 Our results in the context of published data

Based on preclinical studies, all four biomarkers included in our research have been linked to fibrosis and inflammation of the atrial myocardium. Eiras et al. found a positive correlation between CA-125 expression in epicardial cells and markers of inflammation and fibroblasts. Sun et al. reported that Cathepsin L regulates cardiac repair and structural remodelling in mouse models following myocardial infarction. In a knock-out porcine model based on rapid atrial pacing, the deletion of the Caspase-3 gene prevented or delayed the onset of AF compared to the control group. Several preclinical studies have described the role of Caspase-3 in apoptosis, myofibroblast differentiation, and cardiac remodelling. Galectin-3 has been shown to enhance atrial fibroblast proliferation, atrial dilation, and atrial fibrosis.

In human studies, elevated serum levels of these biomarkers have been measured in various forms of AF, most commonly in association with comorbidities such as heart failure (HF) or valvular disease.

While CA-125 is traditionally known as a tumor marker for ovarian and other cancers, it may also be associated with the occurrence of AF. Yilmaz et al. described a link between higher CA-125 levels and an increased incidence of AF episodes. Dudink et al. examined more than 90 circulating cardiovascular biomarkers in 60 patients with idiopathic AF, comparing the results to 120 control patients (without known AF). Elevated CA-125 levels proved to be an independent predictor of AF. In a meta-analysis by Cheung, seven out of nine studies reported that CA-125 levels were higher in patients living with AF compared to a control group in sinus rhythm (SR), with the cutoff for CA-125 levels set at 35 U/L. Elevated CA-125 levels were associated with a 1.39-fold higher risk of AF. However, the patient groups in these studies were

highly heterogeneous, suffering from various comorbidities such as HF or inflammatory conditions.

Notably, neither Dudink's nor Cheung's studies included data on whether there is a correlation between CA-125 levels and the success of AF ablation, or how CA-125 levels change over the long term. In our study—which excluded patients with symptomatic HF or HF confirmed by echocardiography—CA-125 levels were lower (10 U/L before ablation, 9 U/L at the end of follow-up) compared to the 35 U/L threshold suggested in the meta-analysis. Consequently, no difference was found between the recurrence and recurrence-free patient groups. It is important to note that among the four biomarkers we examined, CA-125 was the only one that showed no change during our three-year follow-up. In the context of the published data, our results may suggest that CA-125 concentration is more closely associated with HF or other inflammatory processes than with AF per se, and it does not predict the success of ablation in patients with idiopathic AF.

There is very little data available regarding Cathepsin L as a cardiovascular biomarker. Mehra et al. described that increased expression of Cathepsin L is associated with peripheral blood mononuclear cells and the severity of left ventricular dysfunction in patients with dilated cardiomyopathy. In the study conducted by Dudink et al., Cathepsin L concentrations were higher in patients with AF compared to the control group in sinus rhythm; however, Cathepsin L was not an independent predictor of arrhythmia recurrence. Prior to our study, this was the only publication investigating Cathepsin L levels in the context of AF. In our current study, baseline Cathepsin L levels showed no association with AF recurrence, but its levels significantly decreased by the end of the follow-up period.

The expression of Caspase-3 was investigated by Chen et al. in patients undergoing valve replacement surgery due to rheumatic heart disease. Increased expression of Caspase-3 was observed in permanent AF compared to sinus rhythm. Furthermore, Caspase-3 levels showed a correlation with both LA dimensions and the duration of AF. In our study, Caspase-3 levels prior to PVI showed no association with the recurrence of the arrhythmia; however, its levels decreased significantly by the end of our three-year follow-up. Additionally, Caspase-3 was the only biomarker whose plasma concentration showed a correlation with LA diameter in cases of AF recurrence.

It should be noted that in the AF recurrence group, the proportion of persistent AF at baseline was 23%, while in the recurrence-free group, this proportion was only 8%. Although Caspase-3 levels were associated with AF recurrence, this biomarker showed the most substantial decrease in levels during our follow-up. Based on our observations, it can be hypothesized that Caspase-3 levels may serve as a sensitive marker of fibrosis, particularly in persistent AF or in cases of high AF burden.

For Galectin-3, levels have been consistently higher in AF patients compared to control groups. In these studies, the most of patients had persistent or long-standing persistent AF. Additionally, research has investigated whether Galectin-3 could serve as a predictive marker for AF recurrence following PVI. Clementy et al. studied 160 patients before PVI and 12 months later; 55% of patients had paroxysmal AF, and the average LA diameter was 42 mm. One-year arrhythmia-free survival was 91% among patients with a Galectin-3 level <15,000 pg/mL and an LA diameter <40 mm.

In our study, however, Galectin-3 was not a predictor of AF recurrence, which may be explained by the longer follow-up period and the baseline parameters of our patients. In the cohort studied by Clementy, patients with AF recurrence were older (64 ± 10 years vs. 59.9 ± 8.5 years) and had more comorbidities (e.g., the prevalence of HF was 33%, whereas no HF patients were included in our study). Furthermore, the proportion of persistent AF was also higher (64% vs. 23%) than in our patient population. These differences suggest that Galectin-3 concentration is more likely associated with HF, other comorbidities, and the persistent/permanent forms of AF.

This hypothesis is further supported by another study from the same research group, which included patients with persistent AF and systolic HF (LVEF <40%) undergoing PVI. In that study, the average LA volume was 48 ± 16 mL/m², and serum Galectin-3 levels above 21,000 pg/mL were measured. In this cohort, elevated Galectin-3 levels were predictive of AF recurrence following ablation. In a study by Takemoto et al., which involved patients with long-standing persistent AF, dilated LA (mean LA diameter: 47.8 ± 1.4 mm), and structural heart disease, higher Galectin-3 levels were measured in persistent AF compared to paroxysmal AF in blood samples taken from the LA cavity and CS. Values above 13,050 pg/mL in the CS and 11,900 pg/mL in the LA proved predictive for AF recurrence post-ablation. It is important to note that in our study, we measured significantly lower Galectin-3 levels from peripheral blood

(averaging 4,209 pg/mL in the arrhythmia-free group and 3,810 pg/mL in cases of AF recurrence).

Comparing our results with previously published data, it can be concluded that markers of atrial fibrosis may predict arrhythmia recurrence after PVI in cases of more severely damaged LA myocardium, primarily in patients with significant comorbidities and long-standing AF. However, they are not suitable for this purpose in cases of a structurally intact heart, preserved chamber dimensions, and paroxysmal AF. The lower initial biomarker concentrations measured in our cohort, particularly for Galectin-3, are consistent with this explanation. The discrepancy between our findings and those reported by other researchers may also be explained by differences in follow-up duration and uncertainties arising from the lack of long-term monitoring.

While it may seem reasonable to assume that atrial myopathy is a progressive disease associated with aging, comorbidities, and AF burden—and thus the plasma concentrations of substances related to this pathophysiological process might increase over time—no previous study has provided data on how plasma or serum biomarker concentrations change during long-term follow-up. In our study, we demonstrated a significant decrease in the levels of Galectin-3, Cathepsin L, and Caspase-3 by the end of the 3-year follow-up. Notably, these changes were observed consistently in both patients with and without AF recurrence. As a novel finding, these results suggest that longitudinal changes in biomarker concentrations are not necessarily unidirectional: serum levels may decrease as a result of successful arrhythmia control, whether achieved through catheter-based procedures—as in our patients—or pharmacological treatment. One possible explanation is that the ongoing fibrosis within the atrial wall may have halted or even reversed following the elimination (in the recurrence-free group) or at least a significant reduction (in the recurrence group) of AF episodes post-PVI. This concept is supported by favorable post-ablation changes in LA dimensions demonstrated in several studies using cardiac MRI or 3D echocardiography.

Recent studies have also shown a significant reduction in the frequency of AF episodes even in patients with recurrence, which may explain the significant improvement in outcomes, particularly among patients with HF. This observation has led to a paradigm shift in the assessment of the long-term clinical value of AF ablation. Our results may provide further evidence to support this concept. Prospective studies utilizing continuous monitoring and serial measurements of fibrosis markers could provide definitive evidence to address these questions.

It is important to emphasize that in the first half of our study, we did not separately analyse patients with and without comorbidities, although the effects of age, sex, and cardiac or non-cardiac disease processes on biomarkers are well known. Therefore, a specific question in the second half of our research was whether the presence or absence of baseline comorbidities affects the long-term changes of these biomarkers in patients who remained recurrence-free after ablation, making the potential confounding effect of atrial arrhythmias less likely. We found that Caspase-3 was the only biomarker whose serum levels showed a significant decrease 3 years after ablation, regardless of whether comorbidities were present at the time of the procedure.

The explanation for why only one of the four biomarkers showed such changes may be that Caspase-3 is a more sensitive marker of AF-related atrial myopathy than the others. Thus, in clinical practice, it could serve as an indicator of effective arrhythmia suppression in patients with varying degrees of atrial wall fibrosis, independent of the presence of comorbidities. Instant Caspase-3 ELISA tests are currently available, though likely at a higher cost compared to routine clinical applications. However, we consider our observation to be hypothesis-generating, awaiting confirmation through studies with larger sample sizes.

6 SUMMARY AND CLINICAL IMPLICATIONS OF OUR FINDINGS

Our results demonstrated that the levels of CA-125, Caspase-3, Cathepsin L and Galectin-3 were not associated with AF recurrence after PVI in patients with a structurally normal heart and mainly paroxysmal AF. Except for CA 125, all the other biomarkers demonstrated a significant decrease during a 3-year follow-up post-ablation. Additionally, Caspase-3 levels demonstrated a positive correlation with LA dimensions in patients with AF recurrence. In our study, serum levels of Caspase-3 decreased significantly in patients who demonstrated no recurrence after PVI regardless of the presence or absence of comorbidities, although at 3-year the Caspase-3 levels were higher in patients with comorbidities.

If confirmed by further research on a larger sample, a decrease in Caspase-3 may serve as an indicator to be monitored long-term to detect changes in the atrial myocardium and assess the success of the ablation.

7 NEW SCIENTIFIC FINDINGS

1. This is the first study on the longitudinal changes in the serum levels of biomarkers after PVI.
2. We demonstrated that pre-ablation levels of Ca-125, Caspase-3, Galectin-3, and Cathepsin L were not predictive of long-term arrhythmia recurrence after PVI.
3. The levels of Caspase-3, Galectin-3, and Cathepsin L decreased during the 3-year follow-up regardless of AF recurrence.
4. Caspase-3 was the only biomarker that correlated with LA diameters both before ablation and at the 3-year follow-up in patients with arrhythmia recurrence.
5. In patients with no arrhythmia recurrence either with or without comorbidities, only Caspase-3 levels showed a consistent decrease during follow-up.
6. Despite the decrease from the pre-ablation value, Caspase-3 levels were higher at the end of follow-up in patients with comorbidities; higher levels were significantly associated only with hypertension.
7. The decrease in Caspase-3 levels in patients either with or without comorbidities, was independent from age, sex, type of AF, or the specific ablation technique used.

8 LIST OF PUBLICATIONS



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Doctoral School: Kálmán Laki Doctoral School

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List of publications related to the dissertation

1. **Szuromi, L.**, Hajas, O., Nagy-Baló, E., Forgács, I. N., Barta, J., Nagy, L. T., Fagyas, M., Tóth, A., Nagy, B. J., Kappelmayer, J., Csanádi, Z.: Serum levels of Caspase-3 decrease in patients with long-term arrhythmia suppression after PVI.
Res. Rep. Clin. Cardiol. "Accepted by publisher", 2026.
IF: 0.5 (2024)
2. **Szuromi, L.**, Hajas, O., Nagy-Baló, E., Forgács, I. N., Nagy, L. T., Fagyas, M., Tóth, A., Nagy, B. J., Kappelmayer, J., Csanádi, Z.: Long-Term Changes in the Biomarkers of Left Atrial Fibrosis after Pulmonary Vein Isolation for Paroxysmal and Persistent Atrial Fibrillation.
Rev. Cardiovasc. Med. 24 (6), 171, 2023.
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List of other publications

3. **Szuromi, L.**, Szegedi, A., Csanádi, Z., Homoródi, N.: Posztoperatív pitvarfibrilláció előfordulása és hatása a korai kardiológiai rehabilitációra.
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10 APPENDIX

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