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EARLY DEVIATIONS IN THE COAGULATION AND FIBRINOLYTIC SYSTEM IN PAROXYSMAL ATRIAL FIBRILLATION

THESIS SUMMARY

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CONTENTS

FREQUENTLY USED ABBREVIATIONS

APPT	-	activated partial thromboplastin time
PT	-	prothrombin time
DIC	-	disseminated intravascular coagulation syndrom
DNA	-	deoxyribonucleic acid
RNA	-	ribonucleic acid
cAMP	-	cyclic adenosine monophosphate
AHA	-	American Heart Association
AT III	-	antithrombin III
Acc	-	accuracy
BMI	-	body mass index
Ca2+	-	calcium ions
EGF	-	epidermal growth factor
ESUS	-	embolic stroke of undetermined source
F1+2	-	prothrombin fragment 1 + 2
FDP	-	fibrin degradation products
FM	-	fibrin monomers
FPA	-	fibrinopeptide A
FPB	-	fibrinopeptide B
GpIIb/IIIa	-	glycoprotein IIb/IIIa
HMWK	-	high-molecular-weight kininogen
IL-6	-	interleukin-6
MPV	-	mean platelet volume
NT-proBNP	-	N-terminal pro-natriuretic peptide type B
PAI-1	-	plasminogen activator inhibitor - 1
PK	-	prekallikrein
SD	-	standard deviation
SE	-	standard error
Se	-	sensitivity
Sp	-	specificity
TAFI	-	thrombin activating fibrinolysis inhibitor
TAT	-	thrombin-antithrombin III complex
TF	-	tissue factor
TFPI	-	tissue factor pathway inhibitor
TNFα	-	tumor-necrotizing factor-alpha
t-PA	-	tissue plasminogen activator
u-PA	-	urokinase plasminogen activator
uPAR	-	urokinase plasminogen activator receptor
TnT	-	high-sensitivity troponin T
TnI	-	high-sensitivity troponin I

I. LITERATURE REVIEW

1. Atrial fibrillation: epidemiological data and thromboembolic risk

Atrial fibrillation is the most commonly diagnosed arrhythmia in clinical practice. Data from epidemiological studies show almost doubled incidence of the disease over a period of ten years, reaching 3% of the total population over 20 years of age (Krijtheetal., 2013; Benjaminetal., 2019). Both current epidemiological data and projected values for the next few decades are disturbing. In Europe, the number of people affected by arrhythmia in 2060 is expected to exceed 17 million, and number of newly diagnosed cases to reach 215 thousand per year (Krijtheetal., 2013). The same unfavorable trend is predicted worldwide (Zulkiflyetal., 2018).

The main clinical problem in AF are thromboembolic accidents: cerebrovascular and peripheral arterial (Paciaronietal., 2019). Stroke risk in atrial fibrillation, although age-adjusted, is five times higher than in the non-arrhythmic population (Friberget al., 2010). Globally, 20% to 30% of all strokes occur in atrial fibrillation (Chaetal., 2014). Extracranial systemic embolic events are less common, but mortality reaches 25% by day 30 (Wolfetal., 1991).

The most commonly used clinical classification of AF is based on episode duration and its temporal characteristics (Hindricks et al., 2021):

First diagnosed, AF not diagnosed before, irrespective of its duration or the presence/severity of AF-related symptoms.

Paroxysmal, AF that terminates spontaneously or with intervention within 7 days of onset.

Persistent, AF that is continuously sustained beyond 7 days, including episodes terminated by cardioversion (drugs or electrical cardioversion) after ≥7 days.

Long-standing persistent, continuous AF of >12 months' duration and decision to adopt a rhythm control strategy.

Permanent, AF with adopted heart control rate therapeutic strategy.

Paroxysmal atrial fibrillation accounts for about 30% of all reported cases (Zoni-Berissoetal., 2014). It is believed that its actual prevalence is much more significant than the established, due to often asymptomatic course. In some studies, its incidence reaches 2.5% of the total study population (Primoetal., 2017). Despite brief episodes and often mild clinical manifestation, incidence of thromboembolic complications is not only significant but also

central for mortality and disability in the disease as a whole (Chenetal., 2015; Christensenetal., 2014; Nieuwlaatetal., 2008). Paroxysmal atrial fibrillation is one of the main causes of acute ischemic stroke. One in five ischemic strokes is associated with the disease (Wohlfahrtetal., 2014). In some studies, it occurs even more often, in on in three cases (Arauzetal., 2019).

Presented epidemiological data on paroxysmal AF not only do not allow for it to be underestimated due to short duration of the episodes, but also emphasize its social and clinical significance. Moreover, they *requireprecise assessment of thromboembolic risk and thromboprophylaxis in paroxysmal atrial fibrillation*, while demonstrating *need for its reliable prediction.*

2. Thromboembolic risk assessment in atrial fibrillation. Anticoagulant prophylaxis

2.1. Long-term clinical approach

Long-term thromboembolic risk and long-term thromboprophylaxis in paroxysmal atrial fibrillation, as in other forms, are defined as a direct function of the patient's risk characteristics and are determined as duration-independent atrial fibrillation. *CHA_2DS_2-VASc score remains the most commonly used stratification scale to assess long-term thromboembolic risk and need for long-term anticoagulant therapy.* (L. Chenetal., 2018; Hindricks etal., 2021) (Table 2). It defines the low-risk patients who do not require long-term anticoagulant treatment: men with CHA_2DS_2-VASc score = 0 and women with CHA_2DS_2-VASc score = 1.

Table 2. *CHA_2DS_2-VASc score components (according to Camm et al., 2010)*

Risk factors	
Congestive heart failure / Left ventricular dysfunction	1
Hypertension	1
Age ≥75 years	2
Diabetes mellitus	1
Stroke / Transient ischemic attack	2
Vascular disease (previous myocardial infarction, peripheral arterial disease)	1
Age: 65-74 years	1
Female	1

Compared to previously created scales, CHA_2DS_2-VASc score significantly improves risk stratification of patients. However, *there are discussions about its accuracy* (Fribergetal., 2015; Foxetal., 2017; Lipetal., 2018). *According to the American Heart Association (AHA), the thromboembolic risk in atrial fibrillation is a dynamic phenomenon and the temporal characteristics of the disease are a key factor for its thromboembolic potential* (L. Chenetal., 2018). In this sense, the binary representation of the disease (absence/presence of atrial fibrillation), on which CHA_2DS_2-VASc score applicability is based, is incorrect (L. Chenetal., 2018). A number of evidence from clinical practice on the role of temporal characteristics for the thromboembolic potential of the disease are presented, including data on the brief clinical form of paroxysmal atrial (Disertori et al., 2013; Ganesan et al., 2016; Go et al., 2018; Inoue et al., 2014; Primo et al., 2017).The European Society of Cardiology also sees duration as an important domain in the clinical presentation of the disease and offers a 4S-AF structured pathophysiological-based approach to it, in which the key component is atrial fibrillation burden (Hindricks et al., 2021; Potparaetal., 2020). Duration undoubtedly contributes to the thromboembolic potential of the disease, including cases of short paroxysmal episodes, where it is appropriate to specify its limits.

2.2. Periprocedural decisions in cardioversion

Patients with atrial fibrillation undergoing cardioversion have an increased risk of periprocedural thromboembolism, especially in the absence of oral anticoagulation (Airaksinen et al., 2013; Hansen et al., 2015; Nuotio et al., 2014). It results directly from the procoagulant disorders that occurred during the atrial fibrillation episode itself, most significant during and immediately after the arrhythmia (Lip, 1995a). According to clinical observational studies, they resolve within four weeks after sinus rhythm retention (Goldman et al., 1999; Kleemann et al., 2009; Oltrona et al., 1997; Rankin & Rankin, 2017; Stoddard et al., 1995).

Acute cardioversion in paroxysmal atrial fibrillation with a duration of <48 hours is a standard clinical approach, presented as early as 2001 in the recommendations of the European Society of Cardiology (Fusteretal., 2001). Periprocedural anticoagulant prophylaxis has remained controversial for years after its introduction (Fuster et al., 2006). In 2010, the Society issued a firm statement that in the absence of risk factors,a four-week anticoagulation is not necessary after an episode of atrial fibrillation lasting up to 48 hours (Cammetal., 2010). However, clinical data has accumulated on periprocedural thromboembolic risks in these cases (Cotter et al., 2013; Glotzer & Ziegler, 2015; Kleemannetal., 2009; Sposatoetal., 2015). The

statement has been revised, and the latest guidelines presented in 2020, defined for the first time the lowest risk window before the 48th hour of the disease, allowing post-procedural anticoagulation to be skipped only after short (≤24 hours) paroxysmal atrial fibrillation episodes in low CHA_2DS_2-VASc score risk (CHA_2DS_2-VAScscore = 0 in men/ 1 in women) (Class IIb recommendation; level C evidence) (Cammetal., 2010; Hindricks et al., 2021). There is a clear tendency to narrow the low-risk time window, which is indirect evidence of the importance of episode duration for the periprocedural thromboembolic potential of the disease. At the same time, large clinical trials have shown increased periprocedural thromboembolic risks in the lowest risk episodes (Airaksinen et al., 2013; Jaakkola et al., 2016; Kaplan et al, 2019). They became a prerequisite for refining the significance of the CHA_2DS_2-VASc score risk characteristic in the decision for periprocedural thromboprophylaxis in conditions of acute cardioversion.

Undoubtedly, the periprocedural thromboembolic potential of brief episodes of paroxysmal atrial fibrillation (≤24 hours) is still debatable. The lack of certainty in the decision for periprocedural thromboprophylaxis in them requires clarification of the role of the risk profile of patients and episode duration.

3. Prothrombotic changes in atrial fibrillation: data from clinical and experimental studies on the coagulation and fibrinolytic system

As in all prothrombotic states, atrial fibrillation thrombosis is subject to the Virchow's triad (Rastegaretal., 2003). Undoubtedly, blood stasis and structural myocardial damage are part of the mechanisms of thrombosis in atrial fibrillation. However, coagulation imbalance remains a key factor for thrombosis. Objective evidence for this is the established abundance of fibrin in the structure of thrombi, formed in the course of atrial fibrillation and exceeding many times platelet content (Wysokinski et al., 2004). This determines the significant experimental and clinical interest in the coagulation and fibrinolytic system in atrial fibrillation. In recent years, studies have multiplied on this topic.They assess the systems in the disease, their role in the process of thrombosis, as well as the relationship with the disease itself.

Summarizing data from studies on the coagulation and fibrinolytic system in atrial fibrillation, we could say that the systems are object of considerable research and clinical interest. The results unequivocally show presence of hypercoagulability in the long-term forms of the disease: persistent and permanent atrial fibrillation (Fu et al., 2011; Gustafsson et al.,

1990; Kahn et al., 1997; Lip et al., 1995c; Lopez-Castaneda et. al., 2018; Toth et al., 2017; Wang et al., 2001). It is associated with significant activation of the coagulation system, thoughthe intimate mechanisms for this remain unclear. However, data on fibrinolytic activity are insufficient and at the same time contradictory, presenting with both extremes: from hypofunction to hyperfunction (Berge et al., 2013; Drabik et al., 2015; Jabati et al., 2018; Marin et al., 1999; Toth et al., 2017; Wang et al., 2001). Research on the paroxysmal form is extremely insufficient. *The coagulation and fibrinolytic systems in paroxysmal atrial fibrillation with a duration of ≤24 hours remain unstudied. We believe that the lack of knowledge about the pathophysiological substrate of thrombosis, namely coagulation balance, is the reason why the periprocedural thromboembolic potential of short paroxysmal atrial fibrillation episodes (≤24 hours) is unspecified and still under discussion.*

Coagulation and fibrinolytic parameters are analyzed in terms of their predictive value for the occurrence of thromboembolic complications. This is quite logical and natural, given the indisputable causal relationship between arrhythmia and thromboembolism (Ehrlichetal., 2011; Freynhoferetal., 2013a; Sieg; Sieg; Youetal., 2918). Their importance for prediction of the disease itself is increasingly being studied, the justification for which is their key role in various pathological processes such as oxidative stress, inflammation, atherosclerosis and others. (Alonso et al., 2012; Ellis et al., 2018; Wu et al., 2015; Zoluetal., 2012). Research in both directions is undoubtedly of scientific interest, but data remain insufficient.

4. Structure and normal functioning of the coagulation and fibrinolytic system

In order to properly analyze the identified abnormalities in the coagulation and fibrinolytic system, it is necessary to know the structure and functions of the systems in physiological conditions.

4.1. Coagulation system

Although the concept of coagulation cascade has been a successful model for several decades, advances in laboratory and clinical studies have shown that the cascade hypothesis does not adequately reflect *in vivo* hemostasis (Becker, 2005; Robertsetal., 2006). The weaknesses of the cascade model are clearly outlined by a number of facts (Cimmino & Cirillo, 2018; Riddel et al., 2007). The idea that the two pathways that activate coagulation do not act

independently as two alternative options is becoming more and more pronounced. A cell-based coagulation model has been developed, displacing the coagulation cascade theory. In the light of modern hemostasiology, coagulation is a complex interaction between different physical, cellular and biochemical processes in a series of stages, rather than two independent initiating pathways merging into a final common pathway. The cell-based model presents coagulation as a process with overlapping phases of initiation, amplification, and propagation, and a final phase of termination occurring primarily on the platelet surface. Plasma factors of the coagulation cascade remain central in it. It is necessary to emphasize several facts about this model:

- TF is considered to be absolutely necessary for *in vivo* initiation of the coagulation process. Sufficient data already exist that TF is also present in the bloodstream (blood-born TF) in at least several pools (Chiva-Blanchetal., 2017). Some authors even believe that TF circulating in blood is a key participant in the process of thrombosis (Brambillaetal., 2015; Cugnoetal., 2014). Once bound to FVII, TF quickly activates it;
- FXII and other contact factors are not always necessary for the coagulation process, contrary to the cascade model;
- There are two sources of FIXa: direct activation from the TF/FVIIa complex and platelet-bound XIa. This allows the formation of a functional tenase and prothrombinase complex, and respectively, functional thrombin, even in FXI deficiency.
- Early coagulation indicators remain highly sensitive markers that register abnormalities in the coagulation profile at a very early stage, called the subclinical stage. Increased levels of these indicators are a reliable criterion for hypercoagulability, and decreased levels, for hypocoagulability or a tendency for such (Borrisetal., 2011; Dasgupta et al., 2007).

In summary, we can say that the cell-based model allows for a better understanding of both *in vivo* coagulation process, as well as some clinical symptoms in the course of various coagulation disorders.*The model convincingly shows that FXII and FXI, considered to be indispensable for coagulationby the cascade model, are not essential for thrombin synthesis.* These data *necessitate refinement of the intimate mechanisms of coagulation in the specific prothrombotic state and study of the coagulation cascade in its entirety.*

4.2. Fibrinolytic system

According to the classical notion, the main function of fibrinolysis is to localize and limit blood clot formation, lyse fibrin clots and restore vascular patency. Recent studies show that this system is involved in other physiological and pathological processes, namely in the breakdown of extracellular matrix, embryogenesis, cell migration, angiogenesis, activation of growth factors of myelopoiesis, apoptosis, etc. (Mahmoodetal., 2018).

The precise balance between fibrinolytic and coagulation systems is of multifaceted importance. First of all, it provides local (in the area of the thrombus) significant increase in fibrinolytic activity at a specific time. This moment coincides with the recovery of the damaged vessel and is associated with increased t-PA and decreased PAI-1 secretion (Khalafallah et al., 2014). Plasmin is the major proteolytic protease that mediates the lysis of fibin coagulum and fibrinogen to fibrin/fibrinogen degradation products (FDPs), among which D-dimer occupies a special place (Kolodziejczyk et al., 2013). D-dimer is the smallest and most specific FDP found in circulation (Favresseetal., 2018; Olson, 2015). It is known as a "unique biological marker for haemostatic abnormalities" due to the following facts:

- it is product of only plasmin-mediated degradation of stabilized fibrin, which is known to mark the moment of conversion of the coagulum into stable;
- due to its high sensitivity, D-dimer is a valuable marker in suspected activation of coagulation and fibrinolysis (for diagnosing and monitoring a number of clinical conditions in which the patient is at high risk of bleeding or thrombosis);
- increase in D-dimer levels after anticoagulation in a thrombotic event show increased risk of recurrent thrombosis (Tripodi, 2011; Weitzetal., 2017).

The coagulation and fibrinolytic systems are multicomponent, closely related and precisely interregulated systems. The essence of disturbed balance between them in state of thrombosis can be established only in their detailed study.

5. Main conclusions of the literature review

1. *Frequent thromboembolic events*, leading to significant disability and mortality, are a major clinical problem in paroxysmal atrial fibrillation, as well as in non-paroxysmal forms. *Proper assessment of the thromboembolic potential of the disease and optimal anticoagulation are crucial for their effective prophylaxis.*

Thrombosis in atrial fibrillation is a structural *manifestation of hypercoagulability* due to imbalance between the multicomponent and precisely interregulated systems of coagulation and fibrinolysis. This necessitates *detailed, simultaneous study of the coagulation status of patients with atrial fibrillation.*

Numerous studies have been conducted on the coagulation and fibrinolytic systems in atrial fibrillation, and they present the following facts:

- The systems remain *incompletely* characterized due to the small number of studied indicators. Most often they are analyzed unilaterally (either coagulation or fibrinolysis), usually in comorbid populations, and the essential mechanisms of coagulation imbalance *are unclear*;
- Mostly the *persistent and permanent forms* of atrial fibrillation have been studied, where *hypercoagulability* with increased coagulation system activity, often accompanied by similar deviations in fibrinolysis, has been indisputably established;
- Data on *paroxysmal atrial fibrillation are insufficient*, especially in short episodes (≤24 hours) of the disease. Deviations in coagulation balance in them remain unspecified.

There is no clinical study that analyzes comprehensively and in detail the systems of coagulation and fibrinolysis in the course of short episodes (≤24 hours) of paroxysmal atrial fibrillation.

The peripheral thromboembolic potential of short episodes (≤24 hours) of paroxysmal atrial fibrillation and need for postprocedural anticoagulation in very low-risk patients (CHA_2DS_2-VASc score = 0 for men/1 for women) *remain unspecified*.

The significance of CHA_2DS_2-VASc score and episode duration for the periprocedural thromboembolic potential of short episodes (≤24 hours) of paroxysmal atrial fibrillation are debatable.

The predictive value of coagulation and fibrinolytic parameters for the manifestation of paroxysmal atrial fibrillation, as well as related thromboembolic events is *unclear*.

Given the clinical significance of thromboembolic events in atrial fibrillation and indisputable need for precise anticoagulation in them, the conclusions of the literature review are in favor of a need for a comprehensive study of the coagulation and fibrinolytic system in the early (first 24 hours) hours of the clinical manifestation of atrial fibrillation.

II. AIM AND TASKS OF THE STUDY

1. AIM

To study coagulation status of patients in paroxysmal atrial fibrillation and episode duration ≤24 hours, examining the coagulation and fibrinolytic systems.

2. TASKS

To achieve the aim, the following tasks were set:

1. To study the extrinsic pathway of hemocoagulation by examining plasma TF levels and FVII coagulation activity.
2. To study the intrinsic pathway of hemocoagulation by examining XII, XI, IX, VIII coagulation factors and vWF plasma glycoprotein.
3. To study the common pathway of the coagulation cascade by determining:
   3.1. activity of the main elements of the prothrombinase complex: FX and FV, as well as thrombin itself (FIIa).
   3.2. F1+2 and FPA levels as early markers of haemocoagulation.
4. To study main regulators and indicators of fibrinolysis: plasminogen, t-PA, PAI-1, α2-antiplasmin and vitronectin, as well as plasma levels of the specific end product of fibrinolysis: D-dimer.
5. To analyze the power of t-test for testing the hypothesis for equality of mean values of coagulation and fibrinolytic indicators for the studied sample size of patient and control groups.
6. To study the influence of thromboembolic risk characteristics of patients, defined by CHA_2DS_2-VASc score, on coagulation and fibrinolytic indicators.
7. To search and estimate possible functional dependences of the values of studied hemostatic indicators on duration of the episode of paroxysmal atrial fibrillation.
8. To assess the prognostic value of coagulation and fibrinolytic indicators using the probability of manifestation of paroxysmal atrial fibrillation.
9. To analyze the predictive value of studied coagulation and fibrinolytic indicators for manifestation of arterial thromboembolic events.

III. MATERIALS AND METHODS

1. Study design

The study was conducted at the First Clinic of Cardiology with Intensive Care Unit at the University Hospital St. Marina- Varna with approval by the Research Ethics Committee at the same hospital (№35/October 29, 2010) and Medical University of Varna (№9/October 14, 2010), and in accordance with the requirements of the Declaration of Helsinki (The World Medical Association Declaration of Helsinki, 2008).

Two groups were formed, patients and controls, for the period October 2010 - May 2012, on the basis of clearly formulated *inclusion and exclusion criteria* (see below). The patient group was formed by selecting consecutive patients admitted to the ward with a first episode of paroxysmal atrial fibrillation. Controls were volunteers, referred by a GP for screeningto participate in the study after their annual prophylactic examination.

A key point in the study design was the alignment of demographic and clinical characteristics of the two groups (patient and control) in order to ensure maximum objective comparison and minimize the possibility of selection error (Kangetal., 2008; Suresh, 2011).

For the purpose of the study, a total of twenty indicators of the coagulation and fibrinolytic system in peripheral venous blood from a cubital vein were examined once in each participant.

Patients were monitored for manifestation of ischemic stroke through annual medical examinations until the end of December 2020 or earlier in case of discontinuation of monitoring for reasons other than stroke, but before it had occurred (refusal to participate, death or detection of cancer).

2. Study participants

The study included 51 patients (26 men and 25 women) with a first episode of paroxysmal atrial fibrillation and a mean age of 59.84 ± 1.60 years (31-77 years). They were sequentially selected from a total of 338 screened patients, admitted to the ward for paroxysmal atrial fibrillation during the study period.

The study also included 52 controls, 26 men and 26 women, with mean age 59.50 ± 1.46 years (30-76 years) with no history or ECG evidence of atrial fibrillation to date. Out of 378 volunteers referred by a GP to researchers, 169 controls were selected after consulting the *inclusion and exclusion* criteria. From these 52 participants remained, in order to balance the two study groups (patient and control).

Inclusion criteria for the patient group participants

1. Atrial fibrillation with a duration less than twenty-four hours;
2. Clearly defined onset of arrhythmia, persistent at the time of blood sampling;
3. No history or ECG data for previous episodes of atrial fibrillation;
4. Lack of exclusion criteria.

Inclusion criteria for the control group participants

1. No history or ECG data for previous episodes of atrial fibrillation;
2. Lack of exclusion criteria.

Exclusion criteria

1. Cardiovascular diseases: ischemic heart disease, severe/resistant hypertension; chronic and acute heart failure, implanted device for treatment of arrhythmias, inflammatory heart disease (myocarditis, pericarditis, infectious endocarditis), congenital heart defects, moderate or severe acquired valve defects, cardiomyopathies;
2. Other diseases: acute or chronic kidney disease, acute or chronic liver disease, ischemic/haemorrhagic stroke, inflammatory and/or infectious diseases in the last three months, neoplastic or autoimmune diseases, chronic lung disease, diseases of the endocrine system (with exception of type 2 diabetes mellitus, non-insulin dependent, well controlled), thromboembolic event (stroke, peripheral arterial embolism, deep vein thrombosis, pulmonary thromboembolism), haemorrhagic diathesis;
3. Drug intake: hormone replacement therapy, contraceptives, anticoagulants, antiplatelets and systemic analgesics and NSAIDs; alcohol intake>2 drinks per week, BMI> 35;
4. Pregnancy and spontaneous abortion (for women);
5. Inability to determine the onset of arrhythmia (in patients);
6. Unrecovered sinus rhythm after administration of propafenone according to the established scheme (criteria only for patients).

3. Sample collection and storage. Laboratory methods. Studied indicators

The amount of blood taken for each blood sample was 14 ml in 3.2% sodium citrate coagulation vacutainers (VacuetteTube, GreinerBio-One North America, Inc.) and heparin vacutainers (VacuetteTube 4.0 ml/LiHep, GreinerBio-One North America, Inc.). The blood was immediately centrifuged and resulting plasma was frozen and stored according to the requirements of tests used.

A total of twenty coagulation and fibrinolytic parameters were studied in each participant, namely: activity of FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, plasminogen, α2-antiplasmin, PAI-1; vWF activity and levels; plasma levels of TF, FVIII, FPA, F1+2, t-PA, vitronectin, D-dimer. We used reagents from Technoclone (Austria), DiagnosticaStago (France), Siemens Diagnostics (Germany), SekisuiDiagnostics (USA), BioMedicaDiagnostics (USA) and USCN Life Science (China), based on kinetic, photometric or ELISA methods.

4. Echocardiographic methods

One-dimensional (M-mode), two-dimensional (B-mode) and Doppler echocardiography of all study participants was performed using AlokaProSound SSD-4000, Aloka, Japan. All echocardiographic methods and measurements were in accordance with the recommendations for assessment of cardiac cavities proposed and published by the European Society of Cardiology and the American Heart Association (Evangelistaetal., 2008; Galiutoetal., 2011; Langetal., 2006).

5. Statistical analysis methods

Statistical analysis of the data was performed using the specialized software STATISTICA 13.3.0 by StatSoft and R free software for statistical computing and graphics.

We used descriptive statistics to calculate means, standard deviations, standard errors of the mean, relative shares and central tendency (Mo = mode). Testing of hypotheses for equality of mean values and indicators for relative share in the dissertation was done using Student's t-test at a significance level of p = 0.05 (Jobson, 1991). Normal distribution of the samples of studied coagulation and fibrinolytic system indicatorswas confirmed by the Kolmogorov-

Smirnov-Lillefors test (Lilliefors, 1965). An analysis of the power of the t-test for deviations in coagulation and fibrinolytic indicators in the studied groups was performed in order to establish the adequacy of the sample size (Suresh & Chandrashekara, 2012). ANOVA assay was used to compare values of plasma indicators of the coagulation and fibrinolytic system depending on the qualitative variables sex (female/male) and thromboembolic risk profile of the patient, defined by the CHA_2DS_2-VASc score. Linear regression was used to model each of the studied hemostatic indicators using the following predictors: age, BMI and duration of atrial fibrillation. One-dimensional logistic regression was applied to model the presence/absence of paroxysmal atrial fibrillation complications, depending on variable coagulation and fibrinolytic plasma parameters. In the dissertation, we constructed ROC-curves with their respective optimal threshold points, determined by Youden's criterion, estimating the predictive value of the indicators for manifestation of paroxysmal atrial fibrillation. Survival function was assessed by the Kaplan-Meyer estimator. Cox's model was used in the dissertation to assess the importance of the studied hemostatic indicators as predictors of ischemic stroke.

IV. RESULTS AND DISCUSSION

1. Demographic and clinical characteristics of study participants

As we can see from the results presented in Table 5 and Table 6, there were no statistically significant differences between the patient and control group in terms of demographic and clinical characteristics. The thromboembolic clinical risk profile of patients was defined according to the established in clinical practice CHA_2DS_2-VASc score in both categories, namely: low-risk patients without anticoagulant (CHA_2DS_2-VASc score = 0 in men/1 in women) and all others with indications for anticoagulant prevention (CHA2DS2-VASc score ≥1 in men/ ≥2 in women) (Hindricks etal., 2021).

Table 5. *Demographic characteristics of patients and controls*

	Patients	**Controls**	**P value**
Number of participants	51	52	0.89
Mean age (years)	59.84±1.60	59.50±1.46	0.87
Men/Women	26/25	26/26	1/0.93
BMI (kg/m^2)	23.85±0.46	24.95±0.45	0.09

Table 6. *Clinical characteristics of patients and controls*

	Patients (%)	**Controls (%)**	**P value**
Comorbidities			
Hypertension	37 (72.54)	34 (65.38)	0.44
Type 2 diabetes mellitus	3 (5.88)	2 (3.84)	0.62
Dyslipidemia	4 (7.84)	3 (5.77)	0.69
Drug treatment			
Beta blockers	19 (37.25)	17 (32.69)	0.62
ACE inhibitors /sartans	26(50.98)	23 (44.23)	0.68
Other antihypertensive drugs (calcium antagonists, diuretics)	11(21.56)	15 (28.84)	0.43
Statins	4 (7.84)	3 (5.77)	0.69
Metformin	3 (5.88)	2 (3.84)	0.62
CHA_2DS_2-VASc score			
CHA_2DS_2-VASc score 0*	13	not applicable	
CHA_2DS_2-VASc score ≥1**	38	not applicable	

**low-risk patients without anticoagulant (CHA_2DS_2-VASc score = 0 in men/ 1 inwomen)*

***with indications for anticoagulant prevention (CHA_2DS_2-VASc score ≥1 in men/ ≥2 in women)*

Alignment of the groups according to demographic and clinical characteristics presented above was embedded in the study design (see*Materials and Methods*). This provided balance between the groups and objective comparison of the data obtained.

The performed transthoracic echocardiography did not show a statistically significant difference between the two groups in terms of the values of studied indicators (Table 8).

Table 8. *Transthoracic echocardiography data*

Echocardiographic indicators	Patients	Controls	P value
LVEDD (mm)	52.57±0.58	52.29±0.57	0.73
LVESD (mm)	34.43±0.56	34.73±0.48	0.69
EF (%)	62.98±0.70	61.54±0.58	0.12
IVS (mm)	10.37±0.23	9.92±0.26	0.20
PW (mm)	10.24±0.21	9.73±0.28	0.16
LA volume (ml/m²)	22.81±0.45	23.82±0.48	0.13
RVEDD (mm)	30.54±1.58	29.17±1.52	0.18

The duration of the episode of paroxysmal atrial fibrillation was analyzed in detail in connection to the aims and tasks of the dissertation. The mean value was 8.14 ± 0.76 hours, with a minimum of 2, and maximum of 24 hours. Hemostatic indicators were most commonly studied in episodes lasting 5 hours (Mo = 5; 10 of all 51 patients).

2. Deviations in indicators of the coagulation system

2.1. TF plasma levels and FVII coagulation activity

TF values shown in Figure 14 were significantly higher in the patient group compared to the control group (268.63 ± 12.69pg/mL vs 170.21 ± 9.18pg/mL, p <0.001). FVII plasma activity in paroxysmal atrial fibrillation was significantly higher (170.82 ± 8.32% vs 95.17 ± 5.26% vs, p <0.001; Figure 15).

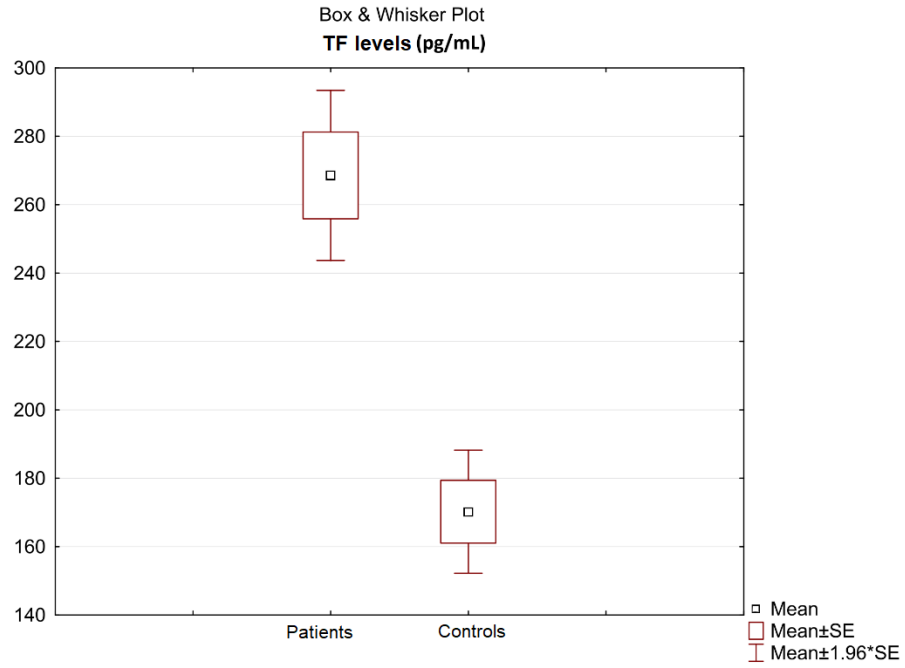
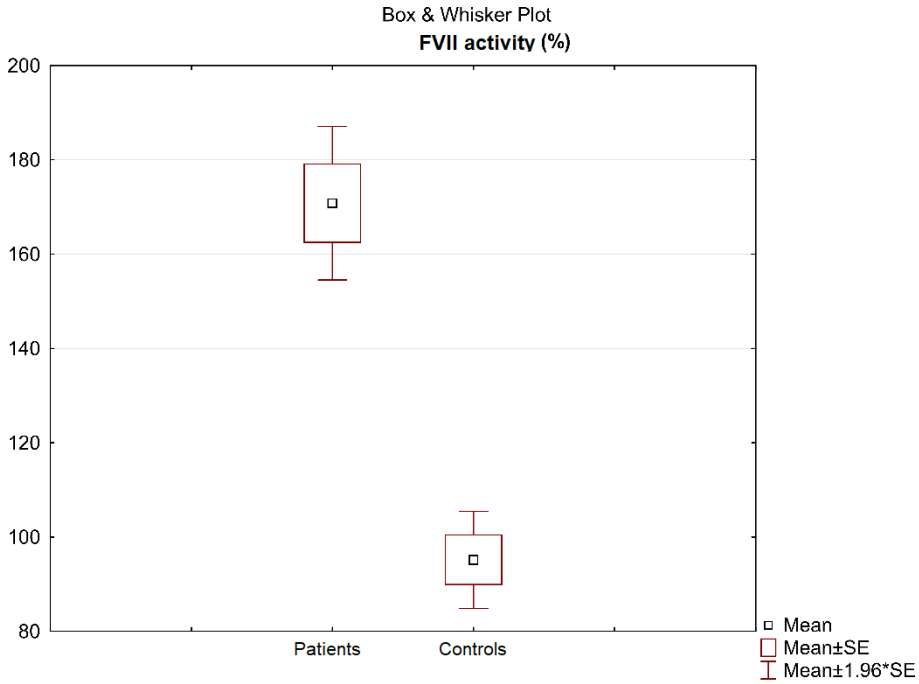


Figure 14. *Comparison of TF plasma levels in patients with paroxysmal atrial fibrillation and controls.*



Фигура15. *Comparison of FVII coagulation activity in patients with paroxysmal atrial fibrillation and controls.*

The established deviations show significant activation of the extinsic pathway of the coagulation cascade in the studied patient population. In the first twenty-four hours of the clinical manifestation of the arrhythmia there was indisputable initiation of coagulation due to increased TF expression. This is the absolutely necessary prerequisite for amplification and propagation phases of the coagulation signal, but not a sufficient component for fibrin formation. Elevated TF andFVII plasma activity levels suggest increased FX and FIX

activation, but not of the coagulation process as a whole. This is a consequence of the synchronous and complementary action of the intrinsic and common coagulation pathway factors. The multicomponent nature of the coagulation cascade and complex mechanisms of inhibition of each stage require separate monitoring of coagulation phases.

A number of studies have shown an activated coagulation process in atrial fibrillation. As we can see from the literature review presented above, despite the great scientific and clinical interest, only single indicators have been studied and the nature of identified changes has not been clarified. There are very few data on TF and FVII / FVIIa in paroxysmal atrial fibrillation. Their study,as initiating coagulation molecules, allows to specify the triggers of the process and its beginning. The presented results give us ground to draw some important conclusions, namely:*early initiation of the coagulation process in paroxysmal atrial fibrillation, manifested even in short episodes (duration ≤24 hours) of the disease*. It is due to an activated extrinsic coagulation pathway *inincreased TF expression*.

2.2. Coagulation factors XII, XI, IX, VIII and vWF plasma glycoprotein

We found increased FXII coagulation activity in patients with paroxysmal atrial fibrillation (218.31 ± 11.77% vs 148.41 ± 7.48%, p <0.001; Figure 16). FXI (178.41 ± 7.99% vs 111.75 ± 5.50%, p <0.001; Figure 17) and FIX (170.43 ± 6.62% vs 117.72 ± 5.95%, p <0.001; Figure 18) activity was also higher, compared to the control group.

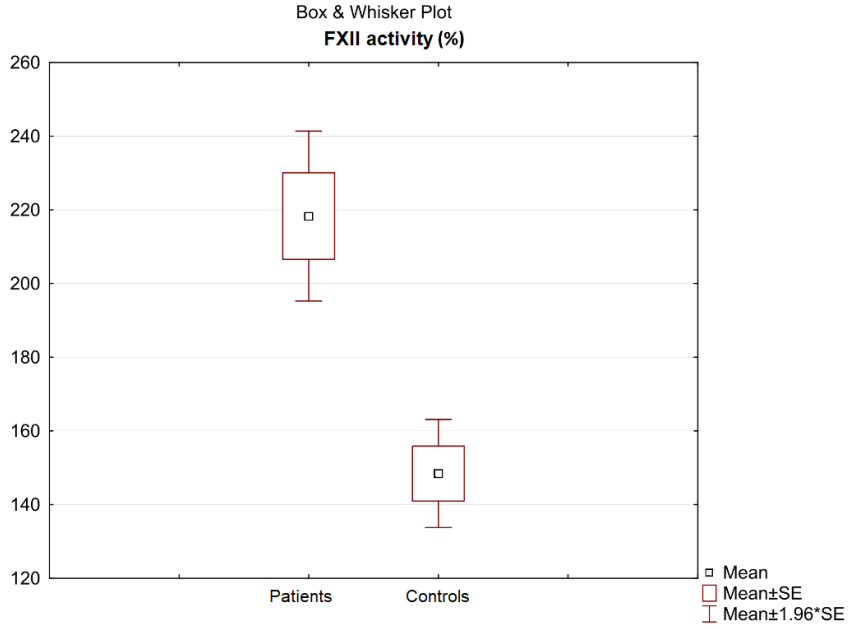


Figure 16. *Comparison of coagulation activity of FXII in patients with paroxysmal atrial fibrillation and controls*

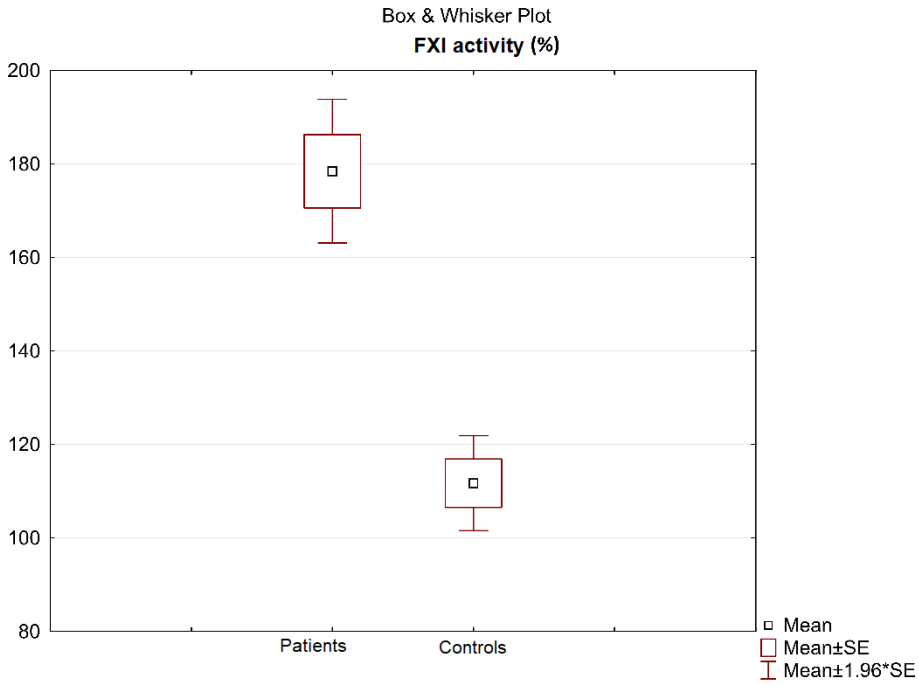


Figure 17. *Comparison of coagulation activity of FXI in patients with paroxysmal atrial fibrillation and controls*

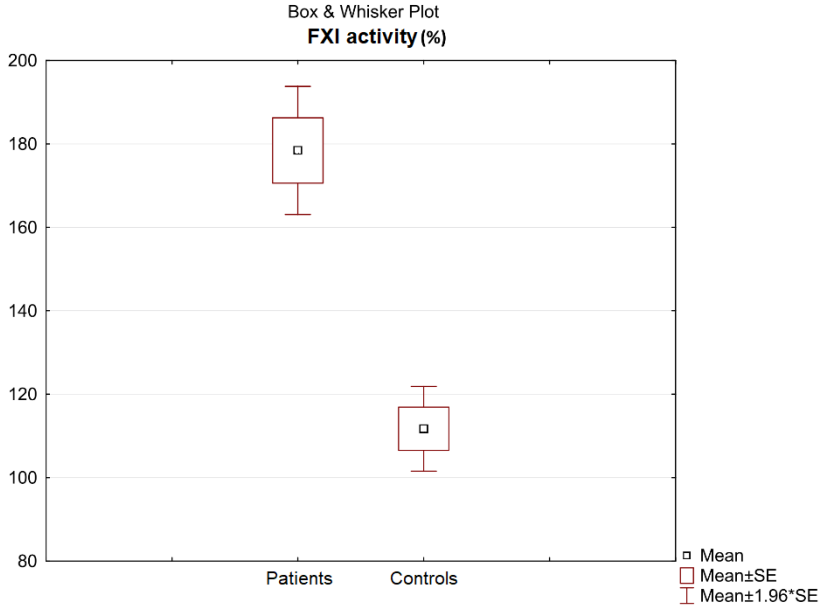


Figure 18. *Comparison of coagulation activity of FIX in patients with paroxysmal atrial fibrillation and controls*

Plasma levels and FVIII activity (107.52 ± 4.36% vs 93.85 ± 2.93%, p <0.05; 200.03 ± 11.11% vs 109.73 ± 4.90%, p <0.001) were significantly higher in the patient group. (Figure 19, 20). Plasma levels of vWF (178.40 ± 12.95% vs 119.53 ± 6.12%, p <0.001; Figure 21) and its collagen-binding activity (200.92 ± 12.45% vs 110.80 ± 5.14%, p <0.001; Figure 22) showed the same deviations.

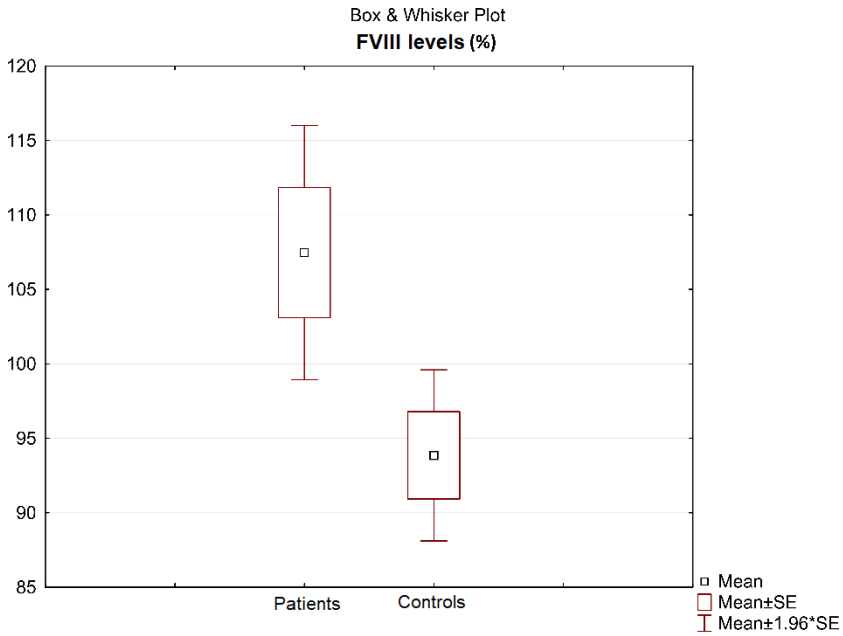


Figure 19. *Comparison of FVIII plasma levels in patients with paroxysmal atrial fibrillation and controls*

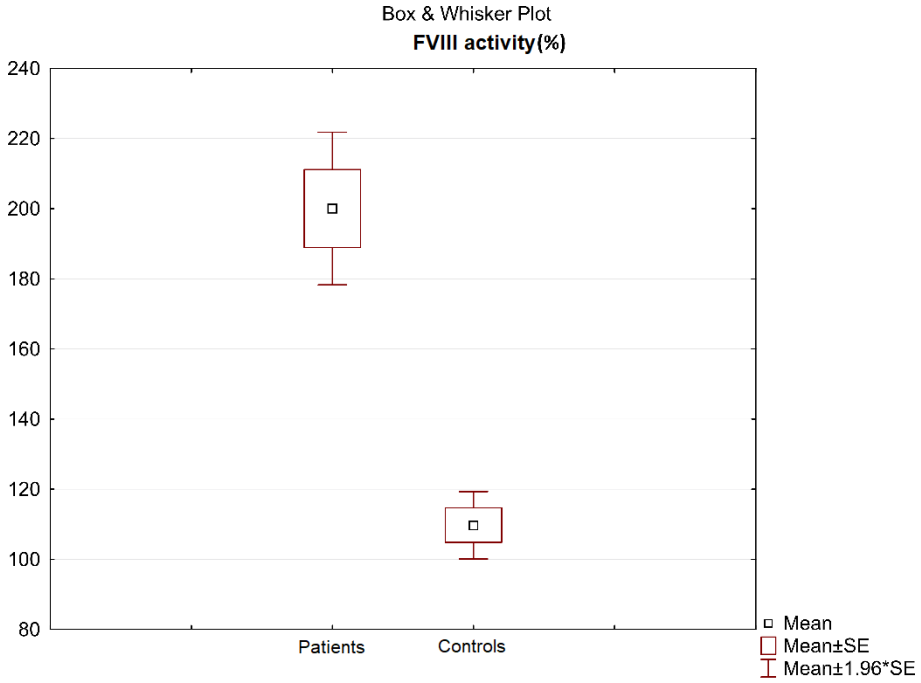


Figure 20. *Comparison of coagulation activity of FVIII in patients with paroxysmal atrial fibrillation and controls*

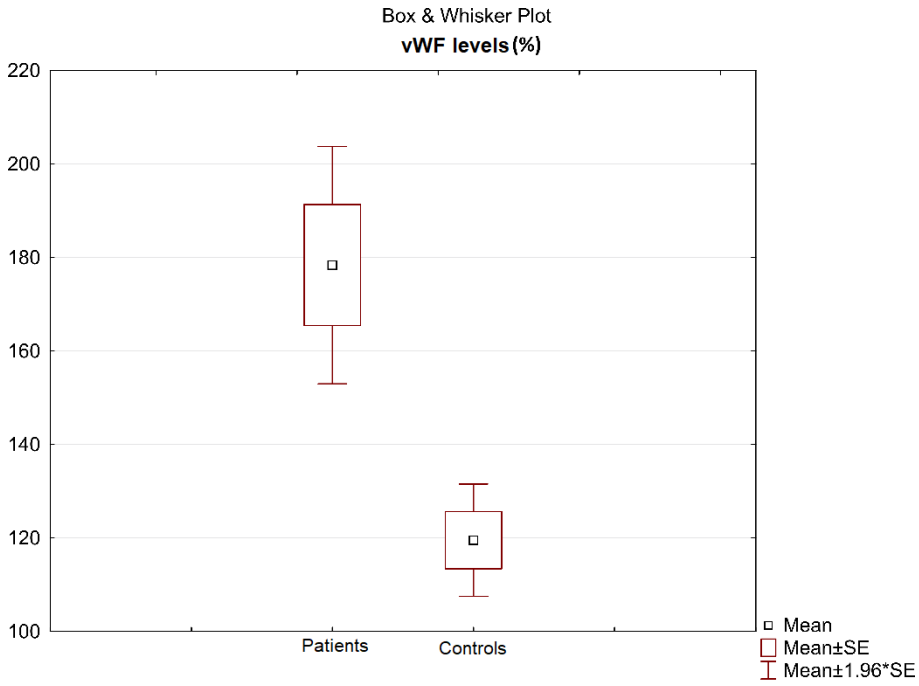


Figure 21. *Comparison of vWF plasma levels in patients with paroxysmal atrial fibrillation and controls*

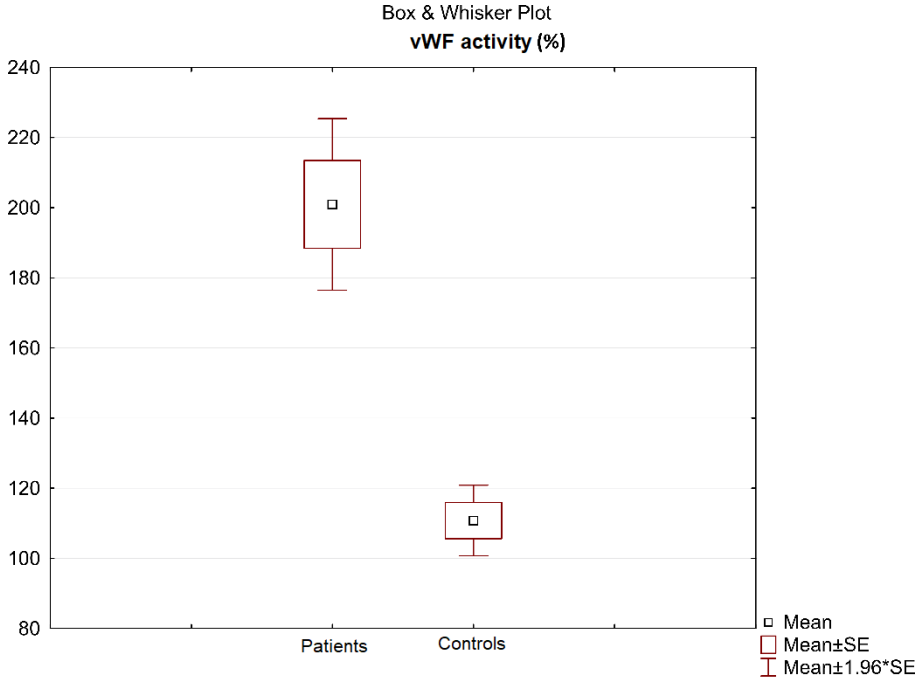


Figure 22. *Comparison of vWF collagen-binding activity in patients with paroxysmal atrial fibrillation and controls*

FXII involvement in *in vivo* coagulation is debatable, since normal hemostasis capacity and lack of abnormal bleeding in congenital FXII deficiency have been established (Geddings & Mackman, 2014). FXI role also remains a subject of discussion. Increasingly, it is defined as "supportive" rather than primary, given clinical observations that have shown mild bleeding in factor FXI deficiency (Vojacek, 2017). The cell-based theory provides a scientific explanation

for this by showing direct FXI activation by thrombin in FXII absence. The TF/VIIa complex, in its turn, can directly activate FIX, even in FXI deficiency. Theoretically, FXI and FXII are not mandatory and absolutely necessary participants in the coagulation process. At the same time, sufficient clinical evidence has been accumulated on the importance of the intrinsic pathway in prothrombotic states. For example, the RE-ALIGN study showed an unacceptably high incidence of thromboembolic events in dabigatrane treatment in patients with mechanical valve prostheses, due to thrombin generation by internal coagulation involving FXII in amounts higher than clinically applicable concentrations of dabigatrane (Jaff al., 2015; van de Werf et al., 2012). This is an example and reason for FXII and FXI not be neglected in prothrombotic states, their study being appropriate for any prothrombotic state. In our study, they showed significantly higher activity in atrial fibrillation (Figure 16 and Figure 17; $p < 0.001$). These results are extremely important from both scientific and clinical points of view. They show *FXII and FXI involvement in the coagulation process in the disease*. It is well known that once activated, FXII and FXI have a greater contribution to thrombosis than to physiological coagulation. In this sense, their inhibition is expected to lead to effective inhibition of coagulation activity. At the same time, this is thought to be associated with a lower risk of bleeding, compared to FXa- and FIIa-inhibition. *The significant FXII and FXI activation found in our study is a serious prerequisite for further experimental studies and search for a new and safer pharmacological approach in anticoagulant therapy in atrial fibrillation by inhibiting FXII and FXI activity.*

Modern notions about hemostasis define FIX as the first link that directly connects the extrinsic and intrinsic pathways of prothrombin activation, being directly responsible for the propagation phase of any procoagulant stimulation, regardless of its nature and origin (Smith, 2009; Tormoenetal., 2013). The non-enzymatic cofactor FVIIIa is a key factor for its functionality and adequacy. The FIXa/FVIIIa intrinsic tenase complex is responsible for the significant activation of FX, which is critical for coagulation, as well as for the explosion in the formation of thrombin, a molecule that directly stimulates fibrin synthesis (Girolami et al., 2004; Goodeve et al., 2010). Very often FVIII is tested simultaneously with the glycoprotein vWF. A prerequisite for this is the well-known fact, that when combined with vWF in a 1:1 ratio in the non-covalent FVIII/vWF complex, FVIII is protected from early proteolytic degradation. This prolongs its half-life in circulation and determines a more effective procoagulant action (Kosteretal., 1995).

Factors IX and VIII key role in the phases of amplification and propagation of the coagulation signal determines the great scientific and clinical interest in them in prothrombotic states (diseases) (Banketal., 2005; Bosmaetal., 2007; Heikal et al., 2013; Kuoetal ., 2015; Kyrleetal., 2000; Vechtetal., 2018). These results are a prerequisite for the scientific interest in FIX, FVIII and vWF in atrial fibrillation. (Hobbeltetal., 2016; Kusak et al., 2016; Wuetal., 2015). However, the populations studied are generally significantly comorbid and often heterogeneous in terms of arrhythmia duration. There are currently no data on FIX, FVIII and vWF in the early hours (first 24 hours) of the disease to allow an assessment of procoagulant activity at this stage. Our study is the first in this direction. The results seen in Figures 18 and 20 showed significantly higher coagulation activity of both FIX and its cofactor FVIIIa ($p<0.001$), which is a prerequisite for generating a significant amount of thrombin, which determines fibrin formation.

FVIII functional adequacy along with its procoagulant effect depend not only on molecule's activity but also on its levels (Kamikubo et al., 2017; Siegler et al., 2015; Wang et al., 2003). Although, there is usually a direct causal link between them, each of these indicators has its own value and provides specific clinical information (O'Donnell et al., 1997). This gave us reason to study them simultaneously. Similar to plasma activity, FVIII levels were also found to be significantly higher in patients ($p<0.05$, Figure 19). The unidirectional changes in both indicators and known causal relationship between them lead us to believe that there was increased synthesis and secretion of FVIII under conditions of procoagulant stimulation in the studied patient population. Probably the high FVIII concentration is associated with prolonged plasma half-life and stabilization of the molecule, given the high vWF plasma levels of ($p <0.001$, Figure 21).

Von Willebrand factor is released into plasma mainly by endothelial cells and to a lesser extent by activated platelets (De Meyeretal., 2009). Well-known data on physiological and functional characteristics of vWF suggest that high vWF levels in the study group were indicative of *endothelial damage in the course of short (≤24 hours) episodes of arrhythmia* (Figure 21), and *its increased activity*, a marker of platelet adhesion (Figure 22). It is well known that atrial fibrillation itself contributes to structural remodeling of the myocardium. As a result of dyssynchronous atrial contraction, electron microscopy revealed endocardial "coarsening", edema, and denudation, followed by myocyte hypertrophy and monocyte cell infiltration (Khan, 2003). High-frequency uncoordinated atrial contraction leads to local endothelial burden and injury (Guazzi & Arena, 2009). The high vWF plasma levels in

patientswere most likely due to their expression and an integral part of the procoagulant state, formed during the short (≤24 hours) episodes of paroxysmal atrial fibrillation.

As can be seen from figures 16-22 presented above, all studied indicators of the internal coagulation pathway showed significant unidirectional changes, which is indisputable evidence of its activation. *In the course of short (≤24 hours) episodes of paroxysmal atrial fibrillation, a procoagulant state occurred.* It hadthe following specific features:

- *it was associated with significantly activated FXII and FXI*. According to modern cell-based theory, their participation in *in vivo* coagulation is optional and debatable. In this sense, their established activation provides opportunities to seek new pharmacological approaches for adequate and safe anticoagulation in the disease;
- vWF plasma levels and activity were significantly high, suggesting *early endothelial damage and enhanced platelet adhesion*.

2.3. Activity of the main elements of the prothrombinase complex: FX and FV and of thrombin itself (FIIa). F1+2 and FPA levels

Activity of the main elements of the prothrombinase complex: FX and FV and of thrombin itself (FIIa).

We established higher coagulation activity of FX (193.20 ± 11.85% vs 116.20 ± 5.86%, p <0.001; Figure 23) and FV (198.47 ± 10.88% vs 121.53 ± 4.79%, p <0.001; Figure 24)in the patient group compared to controls. FII coagulation activity was also higher (167.81 ± 9.12% vs 100.43 ± 5.77%, p <0.001; Figure 25).

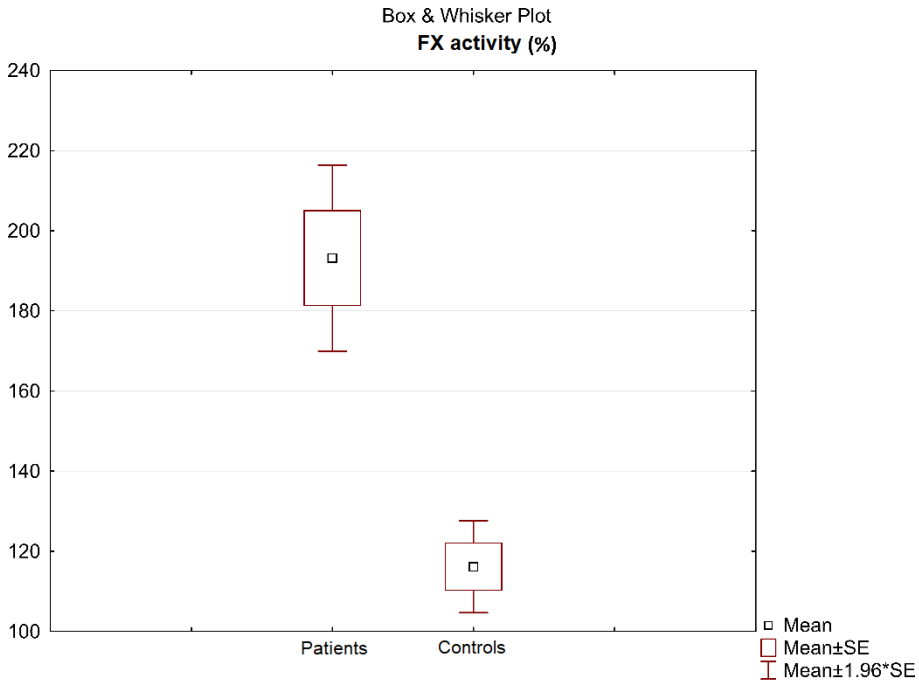


Figure 23. *Comparison of coagulation activity of FX in patients with paroxysmal atrial fibrillation and controls*

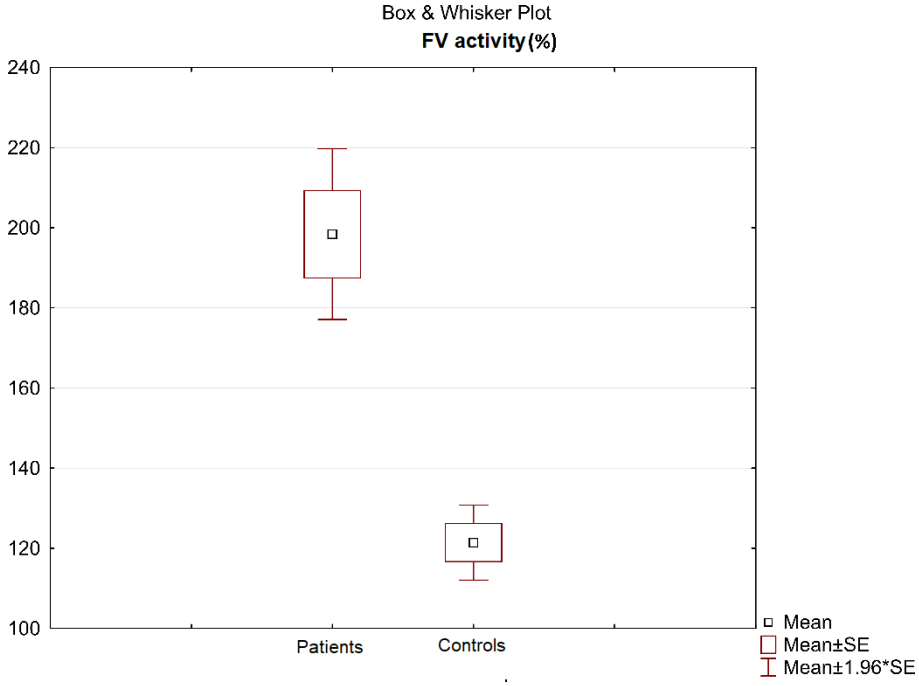


Figure 24. *Comparison of FV coagulation activity in patients with paroxysmal atrial fibrillation and controls*

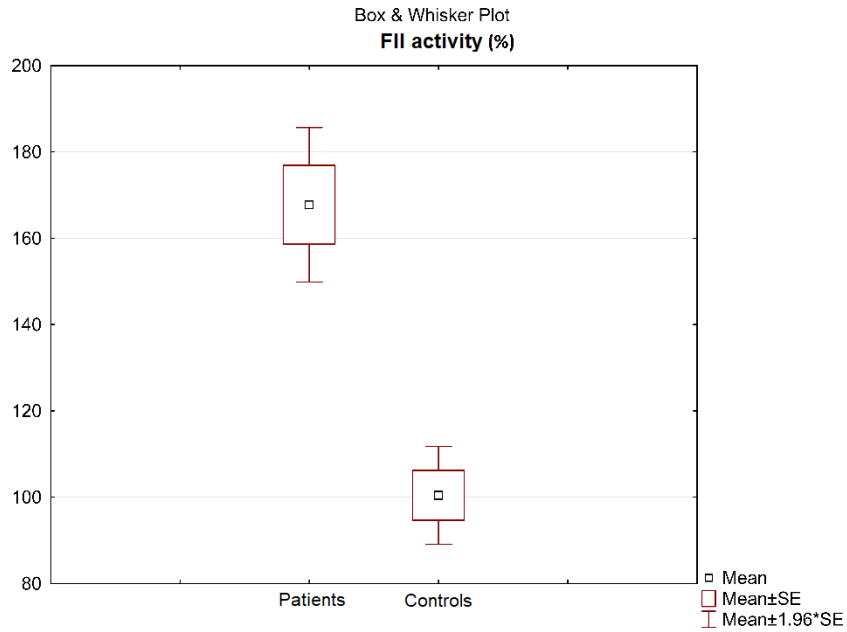


Figure 25. *Comparison of coagulation activity of FII in patients with paroxysmal atrial fibrillation and controls*

Study of FX, FV and FII coagulation activity in our study was an absolutely necessary addition to the coagulation factors presented in 2.1 and 2.2, which stemmed from their association with the final common coagulation pathway.

Our results showed significantly higher values of both proteases,which make up the prothrombin complex, namely FXa and FVa ($p < 0.001$, Figure 23 and Figure 24), as well as FIIa among patients ($p < 0.001$, Figure 25). These abnormalities are evidence of activation of the common coagulation cascade pathway in the first twenty-four hours of the clinical manifestation of paroxysmal atrial fibrillation. They are a good prerequisite for the propagation of the coagulation process and increased fibrin formation.

F1+2 and FPA levels

F1+2 plasma levels were significantly higher in patients compared to controls (292.61 ± 14.03 pmol/L vs 183.40 ± 8.38 pmol/L, $p<0.001$; Figure 26). Fibrinopeptide A also showed higher values (4.47 ± 0.25 ng/mL vs 3.09 ± 0.15 ng/mL, $p<0.001$; Figure 27). These changes wereunidirectional, following the previously presented trend established in FX, FV and FII levels.

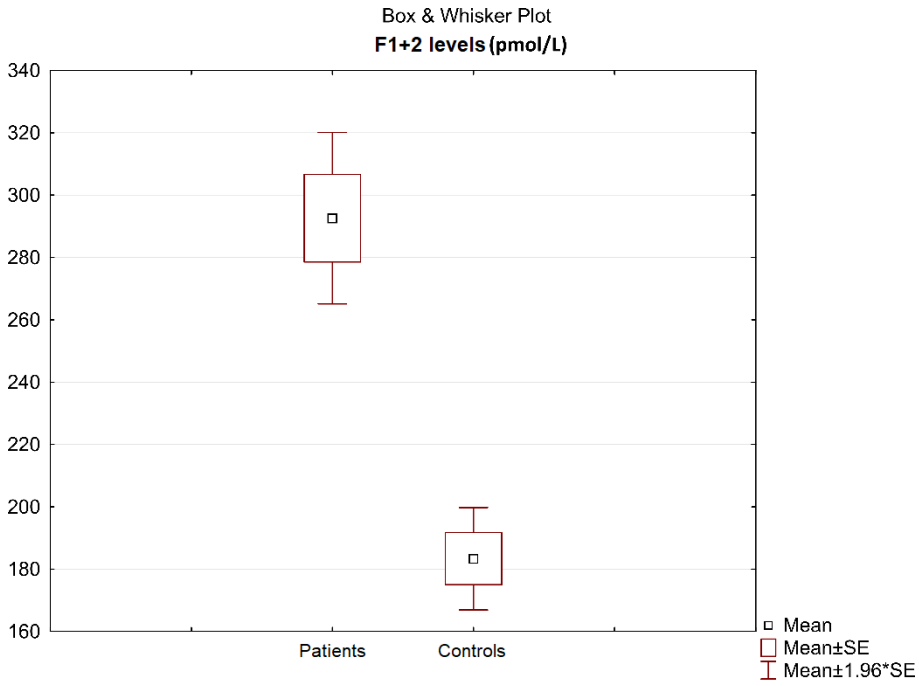


Figure 26. *Comparison of F1+2plasma levels in patients with paroxysmal atrial fibrillation and controls*

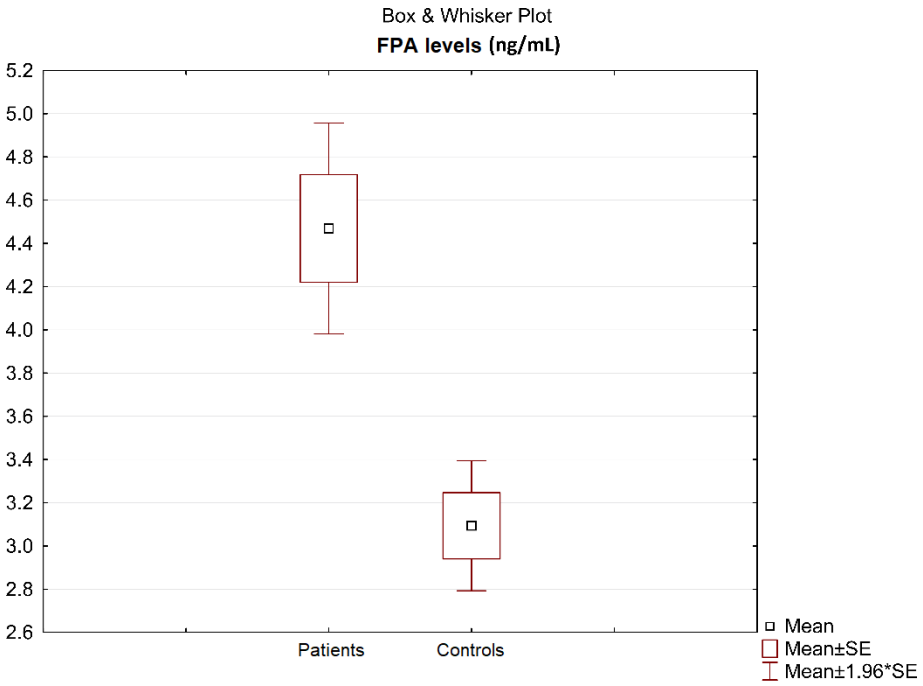


Figure 27. *Comparison of FPAplasma levels in patients with paroxysmal atrial fibrillation and controls*

It is well known that F1+2 is released when prothrombin is converted to its active form, thrombin, a key step in the final common pathway of the coagulation cascade. The prothrombin fragment F1+2 is of great clinical importance, since direct measurement of thrombin is difficult (Haeberli, 1999). Quantitative measurement of the indicator is a reliable method for measuring thrombin synthesis, and its values are a *specific and accurate marker of thrombin formation in vivo* (Bauer et al., 1988; van der Poll et al., 1990). Therefore, F1+2 presents us with good

monitoring of thrombin synthesis. In its turn, FPA plasma levels are considered a good marker of thrombin activity. Obtained by proteolytic degradation of the fibrinogen molecule by thrombin activity, they allow for an indirect assessment of the last step of the coagulation cascade, namely,fibrinogen to fibrin conversion (Haeberli, 1999).

Specific formation characteristics identify F1+2 and FPA as early markers of activated coagulation. They are considered a good diagnostic indicator in the search for thrombotic states. The precise selection of participants in our study gave us a serious precondition to consider F1+2 and FPA high levels to be related to the disease itself and represent procoagulant changes resulting from it. There was undoubtedly increased thrombin generation, as evidenced by high F1+2values, an indirect indicator of thrombin synthesis ($p < 0.001$; Figure 26). Increased F1+2activity was also registered, leading to increased fibrin formation, during which a significant amount of FPA was generated ($p < 0.001$; Figure 27).

The unidirectional deviations in FXa, FVa, FIIa, F1 + 2 and FPA gave us reason to believe that brief manifestations of paroxysmal atrial fibrillation (duration of the episode ≤24 hours) have a procoagulant effect. They show a *tendency to hypercoagulability extremely early* in the course of the disease, which is an indisputable prerequisite for thrombosis.

The generalized analysis of the results presented in 2.1, 2.2 and 2.3 gave us an objective reason to believe that short (≤24 hours) episodes of paroxysmal atrial fibrillation are associated with significantly increased coagulation system activity, which has its own systemic specifics. We observed hypercoagulability, which is an indisputable prerequisite for thrombosis in short (≤24 hours) episodes of paroxysmal atrial fibrillation.

3. Deviations in the main regulators and indicators of fibrinolysis: plasminogen, t-PA, PAI-1, α2-antiplasmin, and vitronectin and D-dimer plasma levels

As shown in Figure 28 and 29, plasminogen activity (159.40 ± 4.81% vs 100.20 ± 2.88%, $p<0.001$; Figure 28) and t-PA levels (11.25 ± 0.35 ng/mL vs 6.05 ± 0.31 ng/mL, $p < 0.001$; Figure 29) were significantly higher in patients compared to controls. PAI-1 (7.33 ± 0.37 AU/mL vs 15.15 ± 0.52 AU/mL, $p < 0.001$; Figure 30) and α2-antiplasmin activity (112.92 ± 2.80% vs 125.60 ± 3.74%, $p<0.05$; Figure 31), as well as vitronectin plasma levels of (134.73 ± 5.83 mcg/mL vs 287.31 ± 10.44 mcg/mL, $p<0.001$; Figure 32) were lower in atrial fibrillation.

In contrast, D-dimer levels showed significantly higher values among patients (0.53 ± 0.07 mg/L vs 0.33 ± 0.02 mg/L, p <0.05, Figure 33).

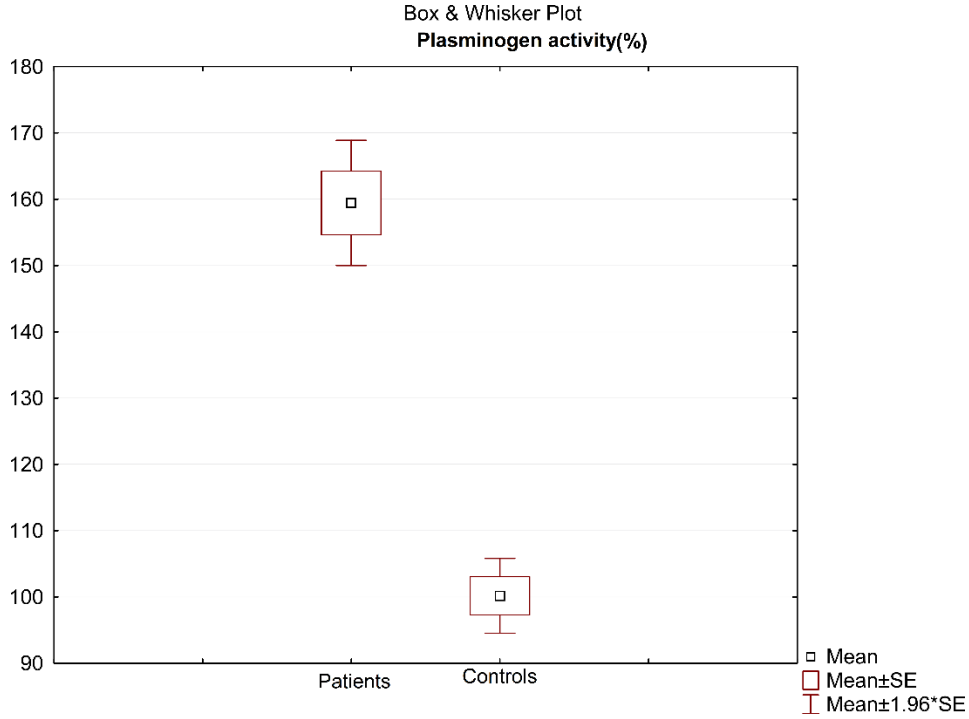


Figure 28. *Comparison of plasminogen activity in patients with paroxysmal atrial fibrillation and controls*

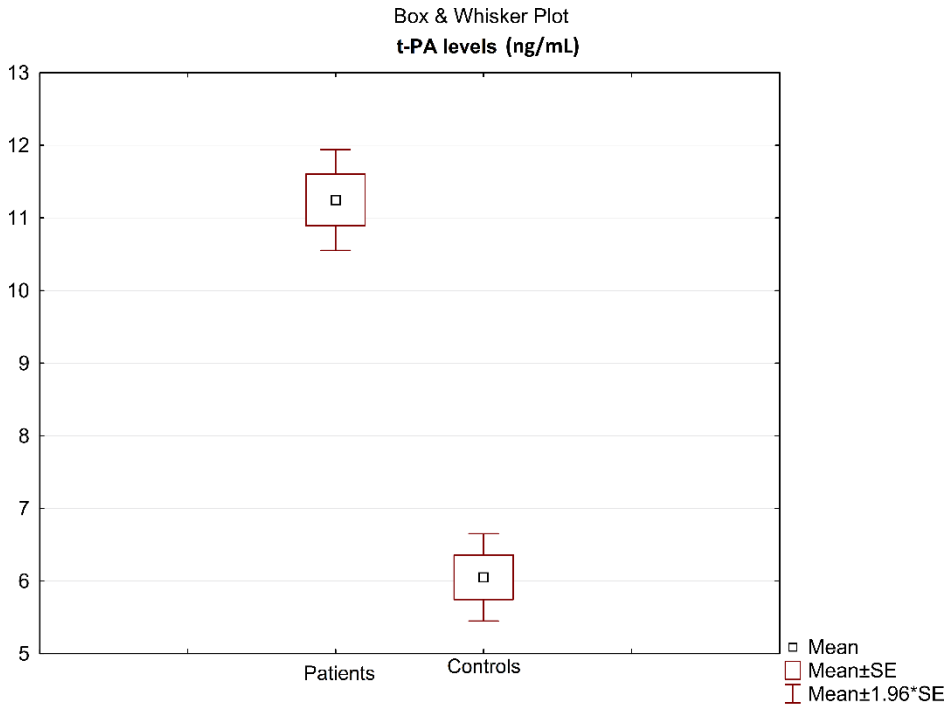


Figure 29. *Comparison of t-PA plasma levels in patients with paroxysmal atrial fibrillation and controls*

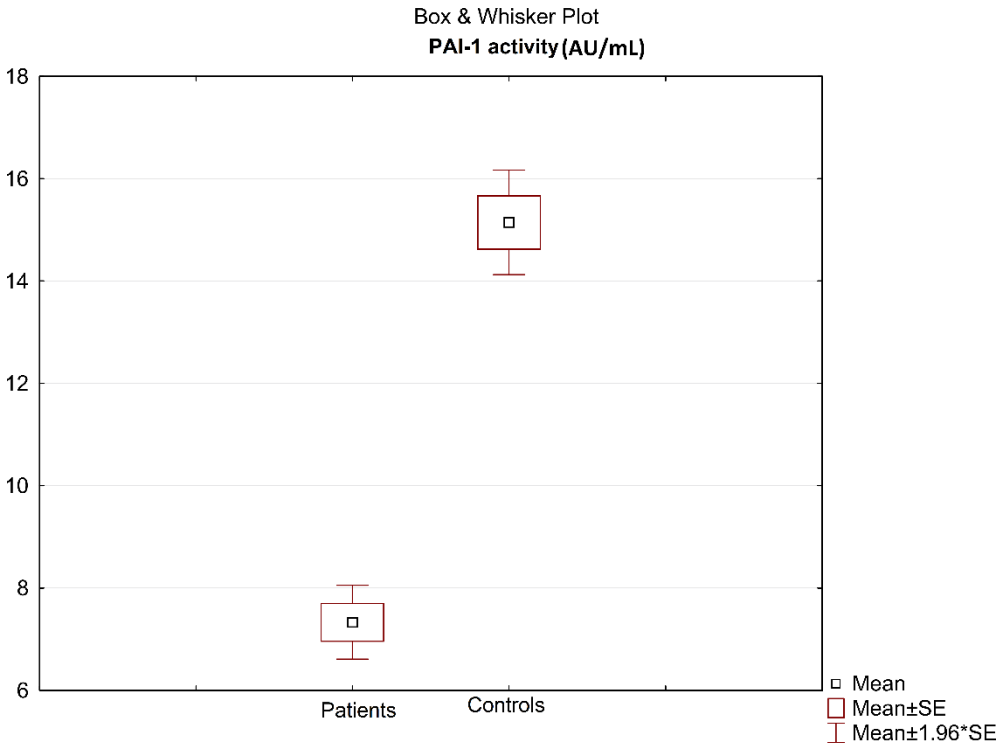


Figure 30. *Comparison of PAI-1 plasma activity in patients with paroxysmal atrial fibrillation and controls*

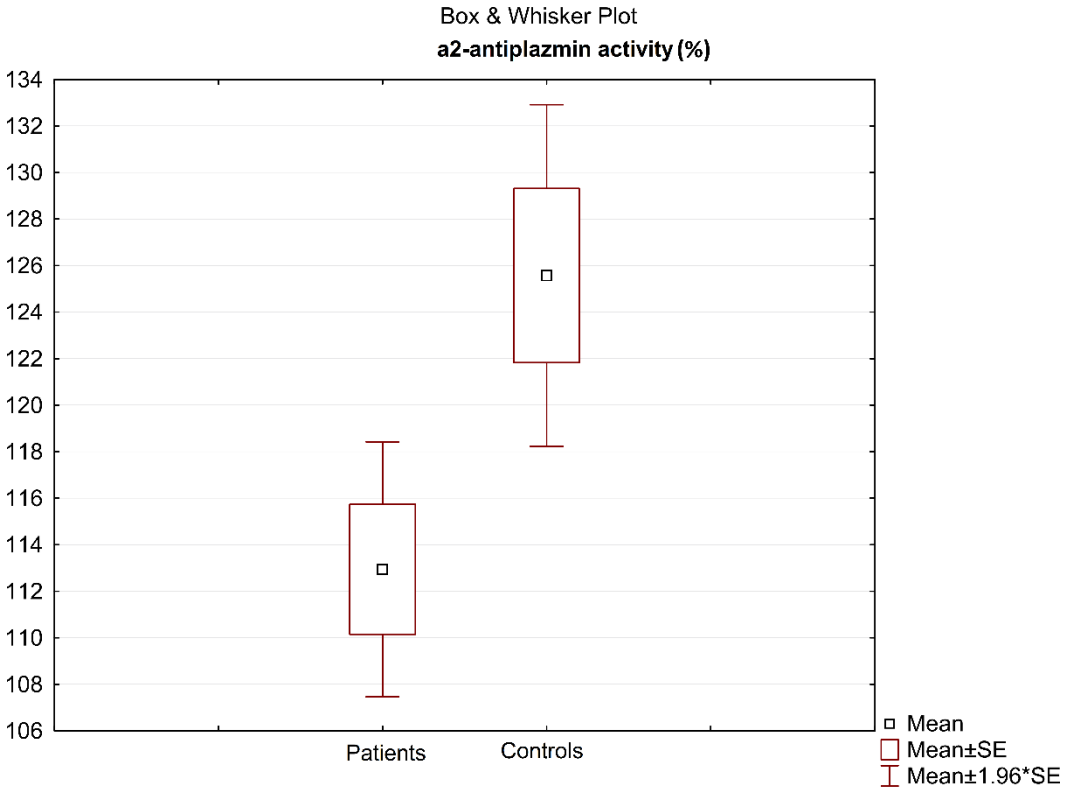


Figure 31. *Comparison of α2-antiplasmin plasma activity in patients with paroxysmal atrial fibrillation and controls*

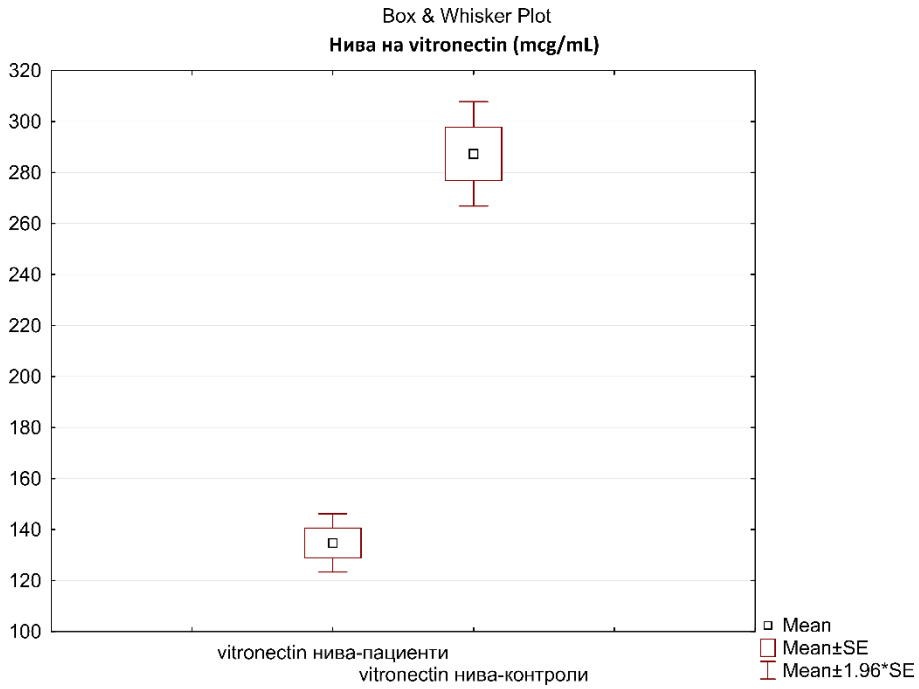


Figure 32. *Comparison of vitronectin plasma activity in patients with paroxysmal atrial fibrillation and controls*

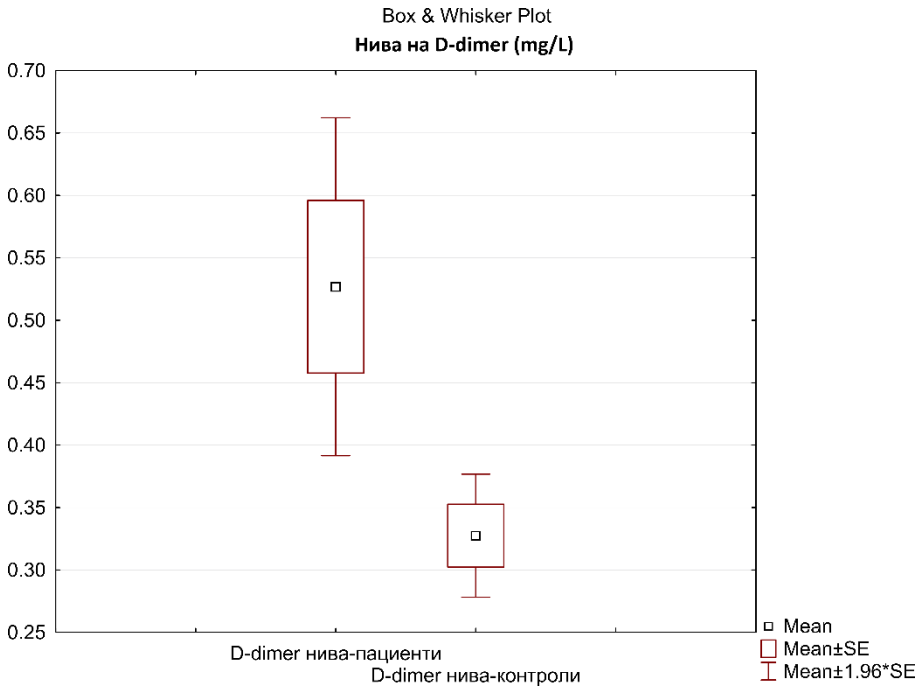


Figure 33. *Comparison of D-dimer plasma levels in patients with paroxysmal atrial fibrillation and controls*

As a major source of plasma proteins and major site of their clearance, the liver plays a significant role in regulating the functions of the fibrinolytic system (Raum et al., 1980). It is responsible for plasminogen synthesis and has the ability to affect the fibrinolytic cascade from the very beginning. Elevated plasminogenplasma levels found in patients with paroxysmal atrial fibrillation suggest increased hepatic synthesis of the proenzyme in the early hours of the clinical manifestation of the disease ($p < 0.001$; Figure 28). At the same time, it is well known

that freely circulating plasminogen in plasma is a key molecule for fibrinolysis. It is a zymogenic form of the active serine protease plasmin, initiating fibrin cleavage (Rijken & Lijnen, 2009; Al-Horani & Desai, 2014). In this sense, elevated plasminogen levels are in themselves a prerequisite for increased plasmin formation and initiation of the fibrinolytic process.

The conversion of plasminogen to plasmin occurs under the influence of two immunologically different physiological activators, t-PA and u-PA, the latter acting mainly extravascularly (Al-Horani, 2014). This establishes t-PA as a major initiator of fibrinolysis, in which the fibrin network cleaves to a variety of soluble FDPs, including D-dimer (Dobrovolsky & Titaeva, 2002; Mosnier & Bouma, 2006). This very fact was a prerequisite for the selection of t-PA for the purposes of the study, therefore t-PA total levels were examined. It is well known that it includes two forms, namely active free t-PA and inactive t-PA, associated mainly in complex with PAI-1 (Chandler et al., 1990). Therefore, the high plasma levels of this tissue activator that we found ($p < 0.001$, Figure 29) may be due to changes in each of the two fractions. It is worth noting that t-PA metabolism in the human body has some characteristic features. A significant portion of it circulates as an inactive form within the t-PA/PAI-1 complex, due to high plasma concentrations of the t-PA-specific inhibitor PAI-1 (Chandler et al., 1997). Changes in the activity and/or PAI-1 levels lead to changes in t-PA/PAI-1 complex levels. In this sense, the measured low PAI-1 activity in patients determined a significantly reduced inhibition of t-PA and correspondingly reduced t-PA/PAI-1 complex levels ($p < 0.001$, Figure 30). Therefore, we believe that high total t-PA values in paroxysmal atrial fibrillation were primarily due to increased free t-PA levels. It exhibits enzymatic activity and is a major regulator in initiating fibrinolysis (Nordenhem & Wiman, 1998). High plasma total t-PA levels, together with already discussed low PAI-1 activity and high plasminogen levels, suggest enhanced t-PA activation of the proenzyme form of plasmin in patients.

The fibrinolytic process depends largely on the delicate balance between proteases and inhibitors. It can be inhibited by two main mechanisms: inhibition of plasminogen activation by plasmogenic activator inhibitors (PAIs) or inhibition of plasmin by α2-antiplasmin (Dubis & Witkiewicz, 2010). Physiologically, the most important t-PA inhibitor is PAI-1, as its active form rapidly and irreversibly inhibits t-PA (Mehta & Shapiro, 2008). Alpha 2-antiplasmin, responsible for about 90% of plasmin inhibition, has the ability to rapidly inactivate plasmin, forming a stable inactive complex (Carpenter & Mathew, 2008; Mutchetal., 2007). PAI-1 and α2-antiplasmin activity determines the degree of inhibition of t-PA and plasmin proteases,

which are essential for the cleavage of the fibrin network in thrombi (Arroyo De Pradaetal., 2002).

In this sense, simultaneous study of t-PA and α2-antiplasmin is an integral part of the evaluation of the fibrinolytic system. Similar to PAI-1, α2-antiplasmin showed lower activity in patients compared to controls ($p < 0.001$, Figure 30; $p < 0.05$, Figure 31, respectively). These data are of critical clinical importance. They complemented already presented results and provided accurate information about the intimate regulatory mechanisms of the fibrinolytic cascade in patients. *The activity of the two main pathways for inhibition of fibrinolysis, PAI-1 and α2-antiplasmin, was reduced.*

In this regard we should alsoconsider the decreased vitronectin levels in the patient group ($p < 0.001$, Figure 32). As we know, it is an indirect, non-catalytic inhibitor of plasminogen proteolysis to plasmin (Zhou et al., 2004). Its interaction with active PAI-1 leads to stabilization of the structure of the latter, prolongation and enhancement of its inhibitory effect on t-PA (Zhou, 2007). Low values of this indicator could explain the reduced PAI-1 activity. Moreover, they complemented already considered fibrinolytic parameters and gave us grounds to assume that there was an increased conversion of plasminogen to plasmin, and therefore *increased fibrinolytic activity in short (≤24 hours) episodes of arrhythmia.*

This assumption was directly confirmed by the results obtained in the study of D-dimer ($p < 0.05$; Figure 33). The molecule is a direct product of the formation and subsequent lysis of only FXIII-associated cross-fibrin in thrombi, unlike other FDPs, produced by degradation of other coagulation proteins such as fibrinogen. In this sense, D-dimer is a specific laboratory marker that accurately represents intravascular formation of fibrin and its subsequent cleavage (Adam et al., 2009). Its plasma levels reflect both effectively activated coagulation (complete fibrin formation) and the process of fibrinolysis (Sadanaga et al., 2010). *In this sense, the high plasma D-dimer levels are an absolute complement and confirmation of the results presented in 2. and 3. of the Results and Discussion section, namely that short (≤24 hours) episodes of paroxysmal atrial fibrillation are a prothrombotic state. They are associated with hypercoagulability in increased activity of the coagulation and fibrinolytic system. Early changes in fibrinolytic activity, due to increased plasmin synthesis and decreased activity of the fibrinolytic inhibitors PAI-1 and α2-antiplasmin, are most likely a pathophysiological response to hypercoagulation.*

4. Adequacy of group sizes: analysis of the t-test power for coagulation and fibrinolytic deviations

A critical point for any study is the sample size. It determines the reliability of the conclusions made (Bland, 2000; Munro, 2005). Analysis of an incorrect sample size can completely compromise a study.

Table 13 below shows the power of the t-test for testing the hypothesis of equality of means (in patients and controls) of each hemostatic indicator studied at a significance level of 0.05. Results show that there was a very good statistical power of the t -test (> 0.9) for the sample size we used (patient and control group size), except for FVIII plasma levels, where the power was borderline (0.79).

Table 13. *Power of the t-test for two independent samples in the presented mean values and SD of studied hemostatic parameters*

Hemostatic indicator	***Mean±SD in patients***	***Mean±SD in controls***	***t-test power***
FII activity (%)	167.81±65.12	100.43±41.61	0.99
FV activity (%)	198.47±77.68	121.53±34.45	0.99
FVII activity (%)	170.82±59.39	95.17±37.90	0.99
FVIII activity (%)	200.03±89.18	109.73±35.36	0.99
vWF activity (%)	200.92±46.33	110.80±37.10	0.99
FIX activity (%)	170.43±84.61	117.72±42.53	0.99
FX activity (%)	193.20±84.61	116.20±42.27	0.99
FXI activity (%)	178.41±55.94	111.75±37.33	0.99
FXII activity (%)	218.31±84.04	148.41±53.94	0.98
TF levels (pg/mL)	268.63±90.62	170.21±66.19	0.99
FVIII levels (%)	107.52±31.13	93.85±21.15	0.79
vWF levels (%)	178.40±92.50	119.53±44.15	0.89
F1+2 levels (pmol/L)	292.61±100.18	183.40±60.41	0.99
FPA levels (ng/mL)	4.47±1.78	3.09±1.11	0.97
Plasminogen activity (%)	159.40±34.36	100.20±20.75	1.00
t-PA levels (ng/mL)	11.25±2.53	6.05±2.21	1.00
PAI-1 activity (AU/mL)	7.33±2.63	15.15±3.76	1.00
α2-antiplasmin activity (%)	112.95±19.96	125.57±26.74	0.85
Vitronectin levels (mcg/mL)	134.70±41.63	287.30±75.27	1.00
D-dimer levels (mg/L)	0.53±0.49	0.33±0.18	0.80

In summary, the performed t-test power analysis shows that the number of selected participants for each group was sufficient and adequate for the obtained means and SD of studied coagulation and fibrinolyticindicators. Presented results and conclusions were reliable

and correctly described hemostatic changes in the studied populations, and were not an accidental finding or result of experimental error.

5. Thromboembolic risk characterization defined by the CHA2DS2-VASc score: influence on coagulation and fibrinolytic indicators

In paroxysmal atrial fibrillation, the clinical and demographic characteristics of the patient presented in the CHA2DS2-VASc score have an indisputable role in the formation of long-term thromboembolic risk and in the long-term forms of the disease (L. Chen et al., 2018; Hindricks et al., 2021). However, its significance for periprocedural thromboembolic potential of paroxysmal atrial fibrillation episodes and corresponding periprocedural anticoagulant approach is unclear. Clarification of this issue is of great clinical importance for patients without indications for long-term anticoagulation. Periprocedural thromboembolic risk is mainly a function of prothrombotic abnormalities that occurred during the episode itself, and in this regard we decided to investigate the significance of the thromboembolic risk characteristic presented by the CHA_2DS_2-VASc score for periprocedural thromboembolic potential by examining its influence on the coagulation status of patients. For this purpose, we divided the group into two subgroups, namely a subgroup of low-risk patients not indicated for long-term anticoagulation (CHA_2DS_2-VASc score = 0 in men/1 in women) and a subgroup of all other patients with increased thromboembolic risk and indications for long-term anticoagulation (≥1 regardless of sex). We compared the values of the indicators between the two subgroups as well as with the controls.

The results obtained by comparing the values of the indicators in the two subgroups with the control group are presented in Table 14 and Table 15. In the low-risk patient subgroup the values of all twenty indicators differed significantly from those of the controls ($p<0.05$, Table 14), the deviations were unidirectional with those found between the entire patient group and controls ($p<0.05$, Figures 14-33). This gave us reason to believe that prothrombotic abnormalities in coagulation status occur in low-risk patients during the short (≤24 hours) episodes of paroxysmal atrial fibrillation, similar to the entire patient group. Almost identical abnormalities were found in the patient subgroup with increased thromboembolic risk. All studied parameters, except for plasma activity of α2-antiplasmin ($p> 0.05$, Table 15) differed significantly from those in controls ($p<0.05$, Table 15), and the deviations were again unidirectional with those in the entire patient population. ($p<0.05$, Figures 14-33). There were also prothrombotic abnormalities in coagulation in the patient subgroup with CHA_2DS_2-VASc score ≥1 (for both sexes). It appears that brief episodes (≤24 hours) of the disease are associated

with hypercoagulability in both low-risk and high-risk patients with increased thromboembolic risk. Hypercoagulability determines the thromboembolic potential of the disease during and immediately after the episode and gives us reason to assume that short episodes of paroxysmal atrial fibrillation pose an increased periprocedural thromboembolic risk even in patients with CHA_2DS_2-VASc score = 0 in men/1 in women.

Table 14. *Coagulation and fibrinolytic indicators in patients at low risk and controls*

Indicator	**Patients with CHA_2DS_2-VASc score = 0 in men/ 1 in women**	**Controls**	**P value**
FII activity (%)	151.57 ± 17.42	100.43 ± 5.77	<0.05
TF levels (pg/mL)	263.01 ± 25.55	170.21 ± 9.18	<0.05
FV activity (%)	175.82 ± 19.72	121.53 ± 4.79	<0.05
FVII activity (%)	184.33 ± 15.87	95.17 ± 5.26	<0.05
FVIII levels (%)	114.82 ± 8.62	93.85 ± 2.93	<0.05
FVIII activity (%)	177.63 ± 18.88	109.73 ± 4.90	<0.05
vWF levels (%)	163.00 ± 22.96	119.53 ± 6.12	<0.05
vWF activity (%)	184.61 ± 25.25	110.80 ± 5.14	<0.05
FIX activity (%)	166.41 ± 14.12	117.72 ± 5.95	<0.05
FX activity (%)	169.10 ± 23.37	116.20 ± 5.86	<0.05
FXI activity (%)	184.52 ± 15.69	111.75 ± 5.50	<0.05
FXII activity (%)	222.53 ± 24.12	148.41 ± 7.48	<0.05
F1+2 levels (pmol/L)	289.30 ± 29.18	183.40 ± 8.38	<0.05
FPA levels (ng/mL)	4.24 ± 0.47	3.09 ± 0.15	<0.05
Plasminogen activity (%)	158.72 ± 5.33	100.20 ± 2.88	<0.05
t-PA levels (ng/mL)	11.11 ± 0.45	6.05 ± 0.31	<0.05
PAI-1 activity (AU/mL)	7.30 ± 0.43	15.15 ± 0.52	<0.05
α2-antiplasmin activity (%)	108.80 ± 3.08	125.6 ± 3.74	<0.05
Vitronectin levels (mcg/mL)	135.07 ± 6.73	287.31 ± 10.44	<0.05
D-dimer levels (mg/L)	0.47 ± 0.09	0.33 ± 0.02	<0.05

Table 15. *Coagulation and fibrinolytic parameters in patients at increased thromboembolic risk and controls*

Indicator	Patients CHA_2DS_2-VASc score ≥1 (both sexes)	Controls	P value
FII activity (%)	173.32 ± 10.67	100.43 ± 5.77	<0.05
TF levels (pg/mL)	270.53 ± 14.81	170.21 ± 9.18	<0.05
FV activity (%)	206.11 ± 12.85	121.53 ± 4.79	<0.05
FVII activity (%)	166.21 ± 9.76	95.17 ± 5.26	<0.05
FVIII levels (%)	105.00 ± 5.06	93.85 ± 2.93	<0.05
FVIII activity (%)	207.70 ± 13.34	109.73 ± 4.90	<0.05
vWF levels (%)	183.62 ± 15.58	119.53 ± 6.12	<0.05
vWF activity (%)	206.53 ± 14.45	110.80 ± 5.14	<0.05
FIX activity (%)	171.76 ± 7.58	117.72 ± 5.95	<0.05
FX activity (%)	201.44 ± 13.67	116.20 ± 5.86	<0.05
FXI activity (%)	176.34 ± 9.39	111.75 ± 5.50	<0.05
FXII activity (%)	216.90 ± 13.65	148.41 ± 7.48	<0.05
F1+2 levels (pmol/L)	293.70 ± 16.20	183.40 ± 8.38	<0.05
FPA levels (ng/mL)	4.55 ± 0.29	3.09 ± 0.15	<0.05
Plasminogen activity (%)	161.65 ± 11.03	100.20 ± 2.88	<0.05
t-PA levels (ng/mL)	11.67 ± 0.46	6.05 ± 0.31	<0.05
PAI-1 activity (AU/mL)	7.46 ± 0.74	15.15 ± 0.52	<0.05
α2-antiplasmin activity (%)	125.17 ± 5.07	125.6 ± 3.74	**>0.05**
Vitronectin levels (mcg/mL)	133.90 ± 12.12	287.31 ± 10.44	<0.05
D-dimer levels (mg/L)	0.55 ± 0.09	0.33 ± 0.02	<0.05

Comparison of the values of the studied hemostatic indicators between the two subgroups of patients with paroxysmal atrial fibrillation revealed a significant difference only in α2-antiplasminplasma activity, which was significantly lower in the low-risk subgroup without indications for long-term anticoagulation, $p<0.05$. Other indicators did not show significant differences in the two subgroups ($p>0.05$, Table 16). These results give us reason to assume that there were no significant differences in coagulation status, determining hypercoagulability in both subgroups, in the course of early (≤24 hours) clinical manifestation of the disease between low-risk patients (CHA_2DS_2-VASc score = 0 in men and 1 in women) and those with increased thromboembolic risk (CHA_2DS_2-VASc score ≥1 for both sexes).

Table 16. *Mean values ± SE of coagulation and fibrinolytic parameters in patients with low and increased thromboembolic risk**

Indicator	Patients with CHA_2DS_2-VASc score = 0 in men/1 in women*	Patients with CHA_2DS_2-VASc score ≥1 (both sexes)*	P value
FII activity (%)	151.57 ± 17.42	173.32 ± 10.67	>0.05
TF levels (pg/mL)	263.01 ± 25.55	270.53 ± 14.81	>0.05
FV activity (%)	175.82 ± 19.72	206.11 ± 12.85	>0.05
FVII activity (%)	184.33 ± 15.87	166.21 ± 9.76	>0.05
FVIII levels (%)	114.82 ± 8.62	105.00 ± 5.06	>0.05
FVIII activity (%)	177.63 ± 18.88	207.70 ± 13.34	>0.05
vWF levels (%)	163.00 ± 22.96	183.62 ± 15.58	>0.05
vWF activity (%)	184.61 ± 25.25	206.53 ± 14.45	>0.05
FIX activity (%)	166.41 ± 14.12	171.76 ± 7.58	>0.05
FX activity (%)	169.10 ± 23.37	201.44 ± 13.67	>0.05
FXI activity (%)	184.52 ± 15.69	176.34 ± 9.39	>0.05
FXII activity (%)	222.53 ± 24.12	216.90 ± 13.65	>0.05
F1+2 levels (pmol/L)	289.30 ± 29.18	293.70 ± 16.20	>0.05
FPA levels (ng/mL)	4.24 ± 0.47	4.55 ± 0.29	>0.05
Plasminogen activity (%)	158.72 ± 5.33	161.65 ± 11.03	>0.05
t-PA levels (ng/mL)	11.11 ± 0.45	11.67 ± 0.46	>0.05
PAI-1 activity (AU/mL)	7.30 ± 0.43	7.46 ± 0.74	>0.05
α2-antiplasmin activity (%)	108.80 ± 3.08	125.17 ± 5.07	<0.05
Vitronectin levels (mcg/mL)	135.07 ± 6.73	133.90 ± 12.12	>0.05
D-dimer levels (mg/L)	0.47 ± 0.09	0.55 ± 0.09	>0.05

** Definition of the thromboembolic risk profile of patients was performed in accordance with the recommendations of the European Society of Cardiology from 2020, where low-risk patients had CHA_2DS_2-VASc score = 0 in men and 1 in women, high-risk patients ≥1 for both sexes (*Hindricks etal., 2021).

Summarizing the data presented in Tables 14-16, we consider it reasonable to conclude that clinical manifestation of short (≤24 hours) episodes of paroxysmal atrial fibrillation is associated with significant prothrombotic abnormalities in coagulation status, which are not predetermined by deteriorated thromboembolic risk characteristic in patients (CHA2DS2-VASc score ≥1 for both sexes), being a major determinant of long-term thromboembolic risk. The thromboembolic risk characteristic did not determine early hypercoagulability that occurred during the short (≤24 hours) episodes of paroxysmal atrial fibrillation, which gave us a reason to believe that it had no significant influence on the resulting increased periprocedural thromboembolic risk. In this sense, we believe that it is appropriate to conduct periprocedural anticoagulation until restoration of coagulation balance in all patients with a short episode of atrial fibrillation (≤24 hours), including low-risk patients with no indication for long-term anticoagulation. Regarding its duration, it is permissible to assume a 4-week period after recovery of sinus rhythm, which will be in accordance with established time intervals for lysis of each hypercoagulant state, currently accepted in good clinical practice (Goldman et al.,

Hindricks et al., 2021; 1999; Kleemann et al., 2009; Oltrona et al., 1997; Rankin & Rankin, 2017; Stoddard et al., 1995). Its application should, of course, be fully in line with good clinical practice and in this regard discussed only in acceptable HAS-BLED score risk of bleeding (Hindricks et al., 2021).

Regarding the observed exceptions from the general trend in α2-antiplasmin activity (Table 15, Table 16), we believe that they do not contradict the generalized analysis, but suggest additional longer-term studies to clarify their scientific and clinical value.

Current recommendations of the European Society of Cardiology for prevention of periprocedural thromboembolic events after short (≤24 hours) episodes of paroxysmal atrial fibrillation in patients with low thromboembolic risk (CHA_2DS_2-VASc score = 0 in men/1 in women) are not explicit and allow for it to be omitted (Class IIb recommendation; level C evidence) (Hindricks et al., 2021). This is due to conflicting results on the incidence of thromboembolic events after such episodes (Airaksinen et al., 2013; Garg et al., 2016; Hansen et al., 2015; Kleeman et al., 2009; Palomäki et al., 2016; Själanderetal., 2016). One possible reason for this is clinical heterogeneity of studied patient populations and usually significantly higher CHA_2DS_2-VASc score risk in patients receiving anticoagulant therapy. It is also noteworthy that studies in this area compare directly the incidence of thromboembolic events in different categories of thromboembolic risk according to CHA_2DS_2-VASc score, most often defined according to class IA recommendations of the European Society of Cardiology for long-term oral anticoagulation. However, the relationship between the pathophysiological substrate that determines their manifestation, namely coagulation status, and the clinical thromboembolic risk profile of patients, usually defined by the CHA_2DS_2-VASc score model, has not been directly studied. Our study is the first to present results in this direction, and studied patients were selected according to strict criteria in order to avoid the effect of the usual comorbidity of studied populations. This gives us objective and reliable results.

6. Dependence of coagulation and fibrinolytic indicators on the duration of the paroxysmal atrial fibrillation episode

Using linear regression analysis, we presented the values of all studied indicators as a function of time. We used simple linear regression with a single predictor (see Material and Methods) to create models for predicting changes in studied indicators in connection to the duration of the paroxysmal atrial fibrillation episode.

Table 17 shows the calculated coefficients b_0 and b_1 from the obtained simple linear regression equations, as well as values of r and r^2. Only the models of the equations with statistically significant correlation are graphically presented, where episode duration is a significant predictor of deviations in indicators' values (Figures 37-43). Strong correlation was found in coagulation activity of FII (r=0.83, p(b_1) <0.001; Figure 37, Table 17). This was also the case for FVIII plasma levels and coagulation activity (r=0.85, p(b_1) <0.001; r= 0.83, p(b_1)<0.001; Figures 38 and 39, Table 17). FIXa showed a weaker dependence (r=0.39, p(b_1)=0.04; Figure 40, Table 17). Linear regression models for FXII coagulation activity (r=0.78, p(b_1) <0.001; Figure 41, Table 17), F1+2 plasma levels (r=0.83, p(b_1) <0.001 (Figure 42, Table 16)) and FPA (r=0.84, p(b_1) <0.001; Figure 43, Table 17) also presented a strong correlation between the indicators and episode duration. The coefficient of determination r^2 calculated for each indicator showed that 68%, 69% and 61% of the changes in FII, FVIII and FXII plasma activity, respectively, were due to change in the duration of the arrhythmia. For FVIII, F1+2 and FPA plasma levels this percentage was 72%, 68% and 70%, while for FIX it was only 15%.

The dynamic nature of detected deviations logically raises a specific interest in their course, namely: is the dependence present for the entire observed period or there is a time threshold determining the change in it. In the obtained models, it can be seen that the scattering of observations in patients with arrhythmia>6 hours affected accurate modeling of data from patients with paroxysmal atrial fibrillation≤ 6 hours (in 28 patients the episode was ≤6 hours). Therefore, we divided the patients into two subgroups according to the temporal characteristics of the disease: patients with a duration of arrhythmia ≤6 hours and patients with a duration of arrhythmia> 6 hours. Thus, subgroups of 28 and 23 patients were formed, respectively.

Table 17. *Regression model parameters for linear relationship between studied hemostatic indicators and duration of paroxysmal atrial fibrillation episode*

Indicator	b_O*	b_1*	r *	r^2 *
FII activity (%)	86.63, p<0.001	9.95, p<0.001	0.83	0.68
TF levels (pg/mL)	245.53, p<0.001	2.83, p=0.24	0.16	0.03
FV activity (%)	211.23, p<0.001	1.57, p=0.44	0.10	0.01
FVII activity (%)	189.01, p<0.001	2.23, p=0.15	0.20	0.04
FVIII levels (%)	67.46, p<0.001	4.91, p<0.001	0.85	0.72
FVIII activity (%)	100.29, p<0.001	12.23, p<0.001	0.83	0.69
vWF levels (%)	198.97, p>0.05	-2.53, p=0.30	-0.14	0.02
vWF activity (%)	217.79, p<0.001	-2.07, p=0.38	-0.12	0.01
FIX activity (%)	150.48, p<0.001	2.45, p=0.04	0.39	0.15
FX activity (%)	207.26, p<0.001	-1.73, p=0.44	-0.11	0.01
FXI activity (%)	195.63, p<0.001	2.11, p=0.15	0.20	0.04
FXII activity (%)	118.84, p<0.001	12.20, p<0.001	0.78	0.61
F1+2 levels (pmol/L)	167.68, p<0.001	15.31, p<0.001	0.83	0.68
FPA levels (ng/mL)	2.22, p<0.001	0.28, p<0.001	0.84	0.70
Plasminogen activity (%)	158.34, p=0.09	0.14, p=0.88	0.02	0.01
t-PA levels (ng/mL)	11.11, p=0.10	0.02, p=0.80	0.04	0.01
PAI-1 activity (AU/mL)	7.61, p=0.20	-0.03, p=0.63	-0.07	0.01
α2-antiplasmin activity (%)	110.27, p=0.06	0.33, p=0.54	0.09	0.01
Vitronectin levels (mcg/mL)	125.24, p=0.08	1.16, p=0.29	0.15	0.02
D-dimer levels (mg/L)	0.65, p=0.33	-0.01, p=0.69	-0.16	0.03

**b_O– constant (intercept parameter); b_1 – regression coefficient for the independent variable; r – correlation coefficient; r^2 coefficient of determination)*

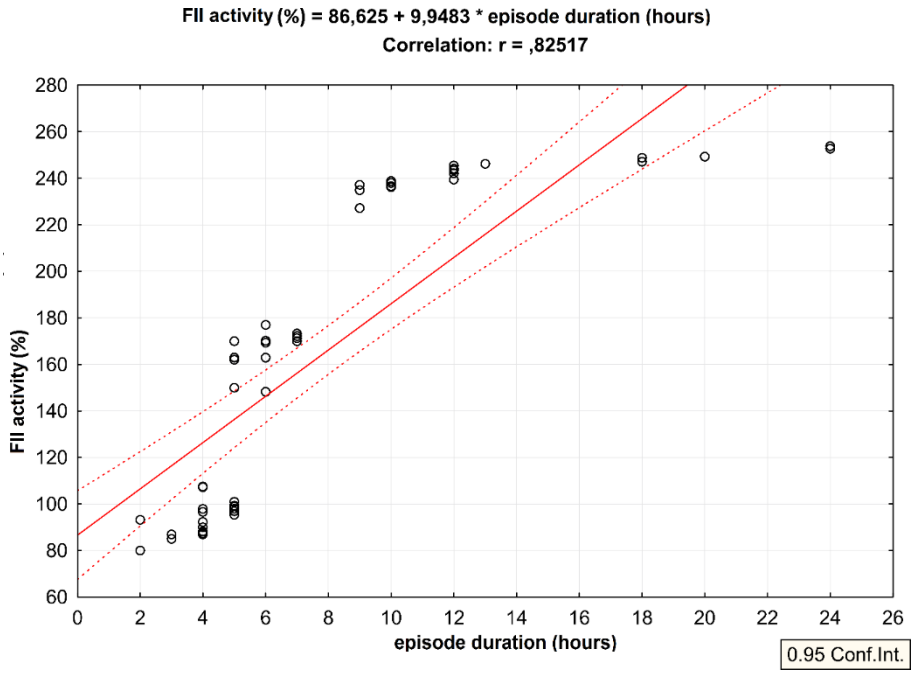


Figure 37. *Correlation between FII coagulation activity and paroxysmal atrial fibrillation episode duration*

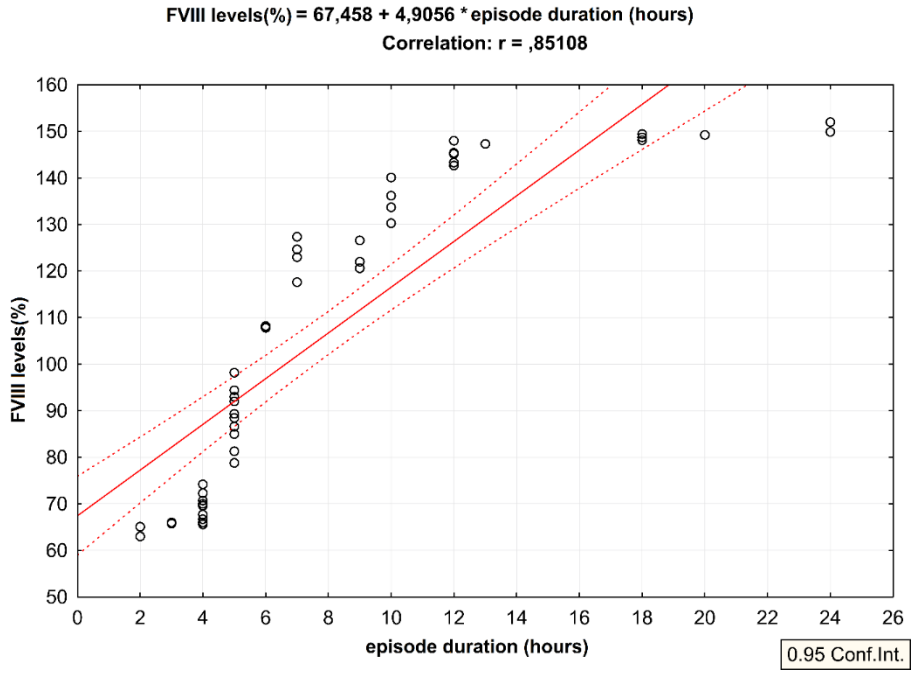


Figure 38. *Correlation between FVIII plasma levels and paroxysmal atrial fibrillation episode duration*

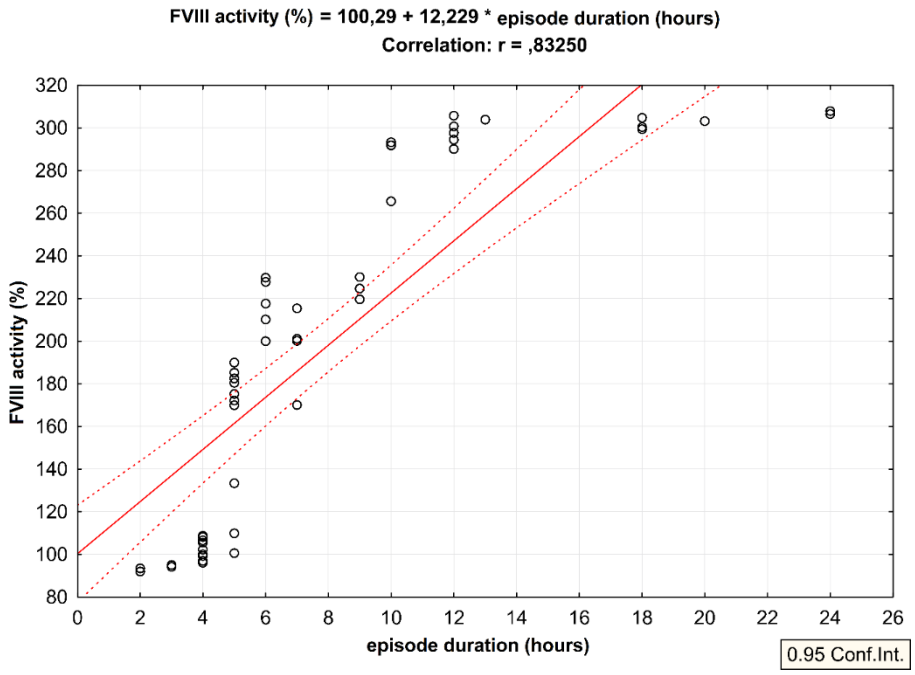


Figure 39. *Correlation between FVIII coagulation activity and paroxysmal atrial fibrillation episode duration*

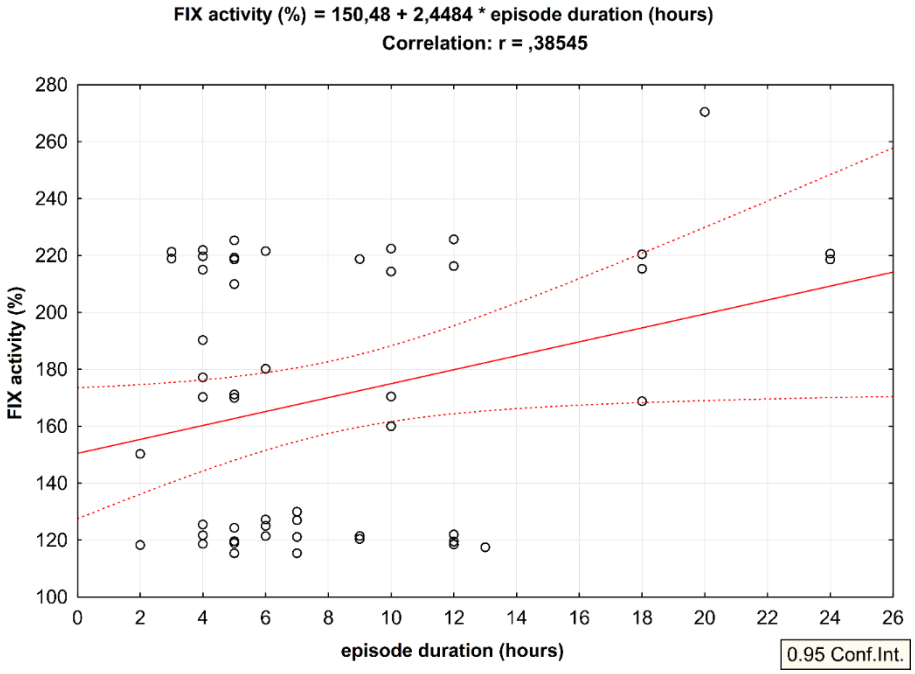


Figure. 40. *Correlation between FIX coagulation activity and paroxysmal atrial fibrillation episode duration*

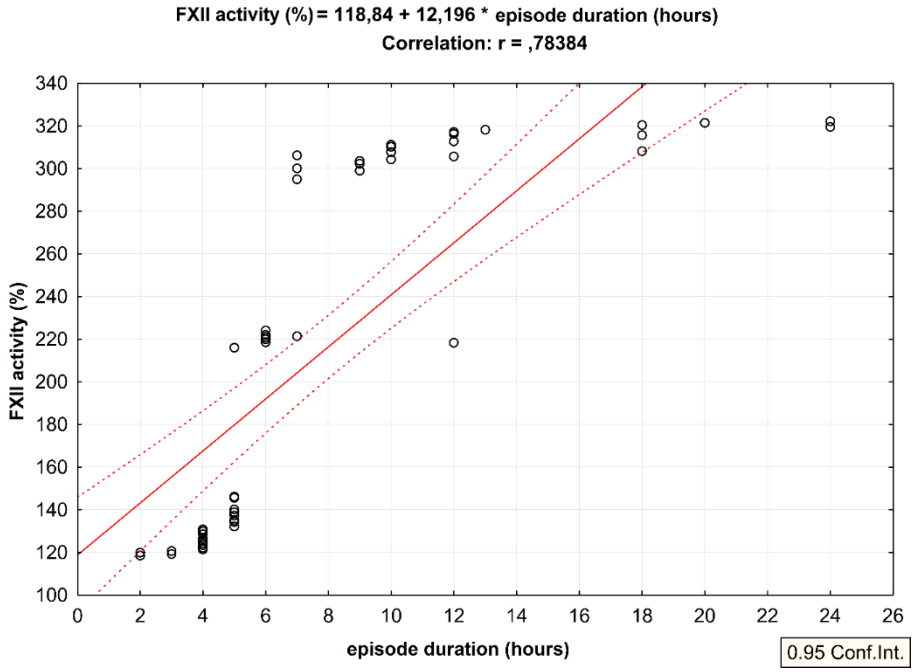


Figure 41. *Correlation betweenXI coagulation activity and duration of paroxysmal atrial fibrillation episode*

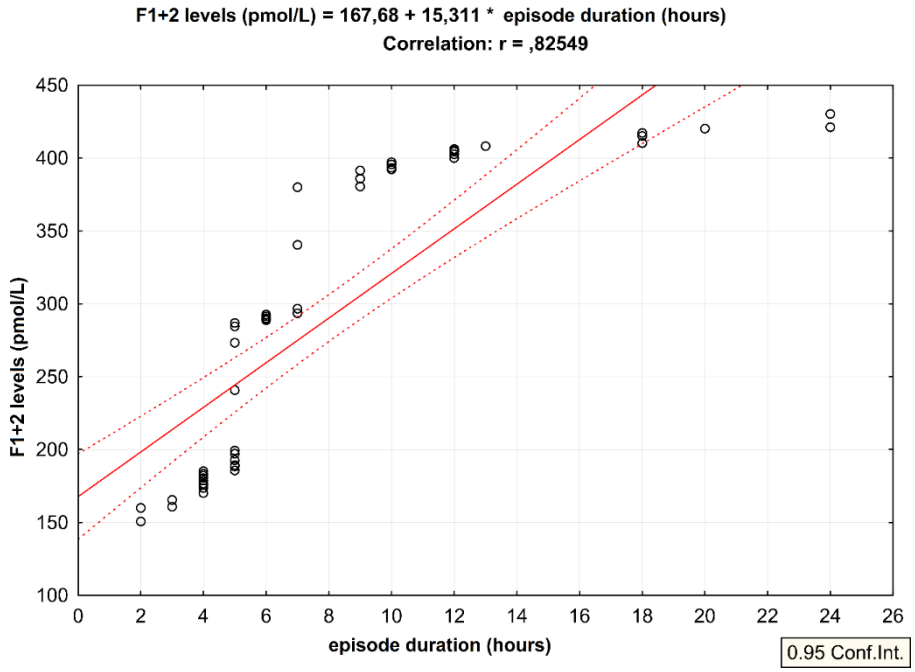


Figure 42. *Correlation between F1+2 plasma levels and paroxysmal atrial fibrillation episode duration*

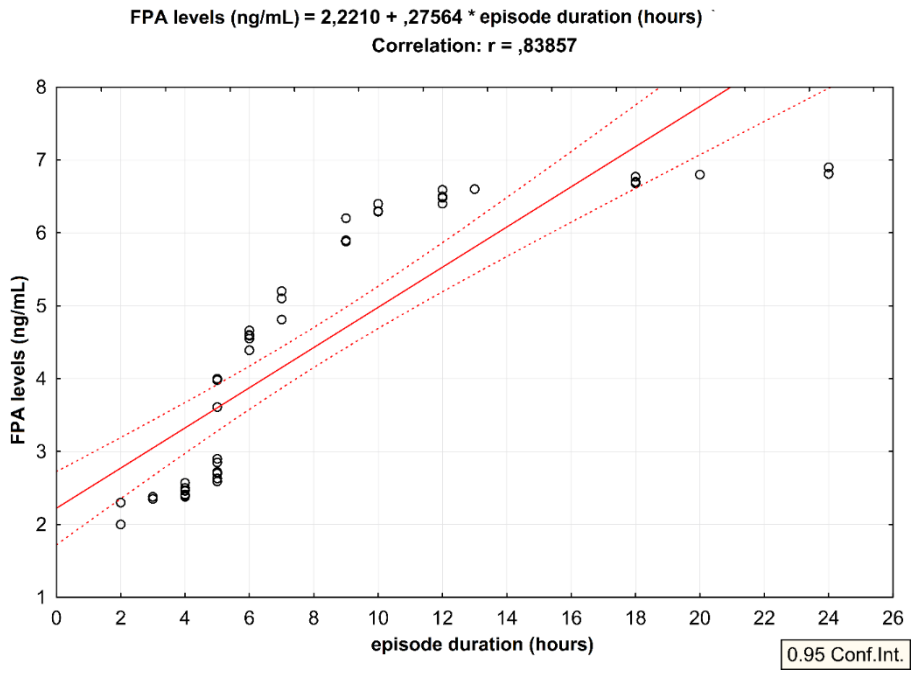


Figure 43. *Correlation between FPA plasma levels and paroxysmal atrial fibrillation episode duration*

Data from the regression analysis concerning episode duration ≤6 hours are summarized below in Table 18. In the first six hours of the clinical manifestation of the arrhythmia there was a strong correlation in six of the studied indicators, namely: FII coagulation activity ($r=0.73$, $p(b_1)<0.001$; Figure 44, Table 18), FVIII levels and activity ($r=0.89$, $p(b_1)<0.001$, Figure 45, Table 18; $r=0.83$, $p(b_1)<0.001$, Figure 46, Table 18, respectively), FXI activity ($r=0.75$, $p(b_1)<0.001$, Figure 47, Table 18), and F1+2 plasma levels ($r=0.81$, $p(b_1)<0.001$, Figure 48, Table 18) and FPA ($r=0.81$, $p(b_1)<0.001$, Figure 49, Table 18). Weaker correlations were found for the following three of the indicators: TF levels ($r=0.25$, $p(b_1)<0.05$, Table 18), FVII activity ($r=0.25$, $p(b_1)<0.05$, Table 18) and FXII activity ($r=0.25$, $p(b_1)<0.05$, Table 18). For all these nine indicators, the regression models presented a positive linear dependence (with a positive regression coefficient b_1), where their values increased with increase in episode duration. (Table 18).

In the second group of patients with an episode duration >6 hours, no statistically significant dependence was observed in any haemostatic indicator values ($p>0.05$, Table 19). These results gave us reason to believe that in the studied patient population after six hours from the onset of the disease, *the duration of atrial fibrillation was not a significant predictor of plasma levels and activity of analyzed coagulation and fibrinolytic parameters.*

Table 18. *Regression model parameters for linear relationship between studied hemostatic parameters and episode duration of paroxysmal atrial fibrillation within the first 6 hours of the disease*

Indicator	b_0*	b_1*	r*	r^2*
FII activity (%)	15.46, p<0.05	22.45, p<0.001	0.73	0.53
TF levels (pg/mL)	165.05, p<0.05	19.66, p=0.04	0.25	0.06
FV activity (%)	204.82, p0.11	2.10, p=0.88	0.03	0.01
FVII activity (%)	116.71, p<0.05	14.01, p=0.04	0.25	0.06
FVIII levels (%)	24.66, p<0.001	12.85, p<0.001	0.89	0.79
FVIII activity (%)	-23.89, p<0.05	36.92, p<0.001	0.83	0.69
vWF levels (%)	255.41, p=0.08	-14.52, p=0.21	-0.17	0.03
vWF activity (%)	315.64, p=0.11	-22.29, p=0.11	-0.31	0.10
FIX activity (%)	183.17, p=0.07	3.11, p=0.69	0.08	0.01
FX activity (%)	207.87, p=0.09	-3.79, p=0.81	-0.05	0.01
FXI activity (%)	31.04, p<0.05	26.26, p<0.001	0.75	0.57
FXII activity (%)	27.78, p<0.05	26.95, p=0.04	0.25	0.06
F1+2 levels (pmol/L)	44.29, p<0.05	37.25, p<0.001	0.81	0.65
FPA levels (ng/mL)	0.20, p<0.05	0.63, p<0.001	0.81	0.65
Plasminogen activity (%)	132.73, p=0.09	5.80, p=0.30	0.21	0.04
t-PA levels (ng/mL)	13.22, p=0.08	-0.47, p=0.24	0.23	0.05
PAI-1 activity (AU/mL)	8.72, p=0.06	-0.23, p=0.62	-0.10	0.01
α2-antiplasmin activity (%)	85.02, p=0.10	5.69, p=0.15	0.28	0.08
Vitronectin levels (mcg/mL)	77.44, p=0.07	12.72, p=0.32	0.13	0.02
D-dimer levels (mg/L)	0.76, p=0.06	-0.05, p=0.49	-0.13	0.02

**b_0– constant (intercept parameter); b_1 – regression coefficient for the independent variable; r – correlation coefficient; r^2 coefficient of determination)*

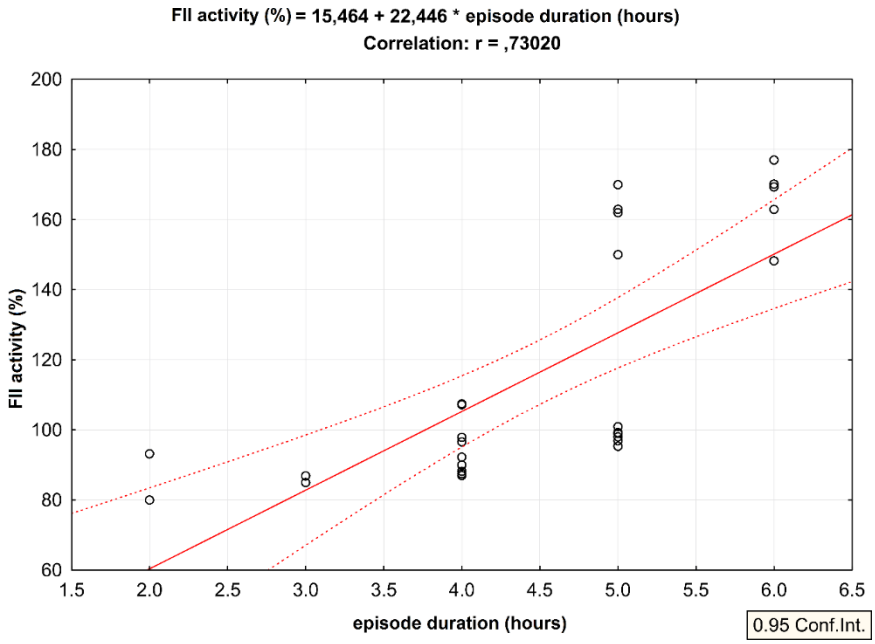


Figure 44. *Correlation between FII coagulation activity and duration of paroxysmal atrial fibrillation episode within the first 6 hours of disease*

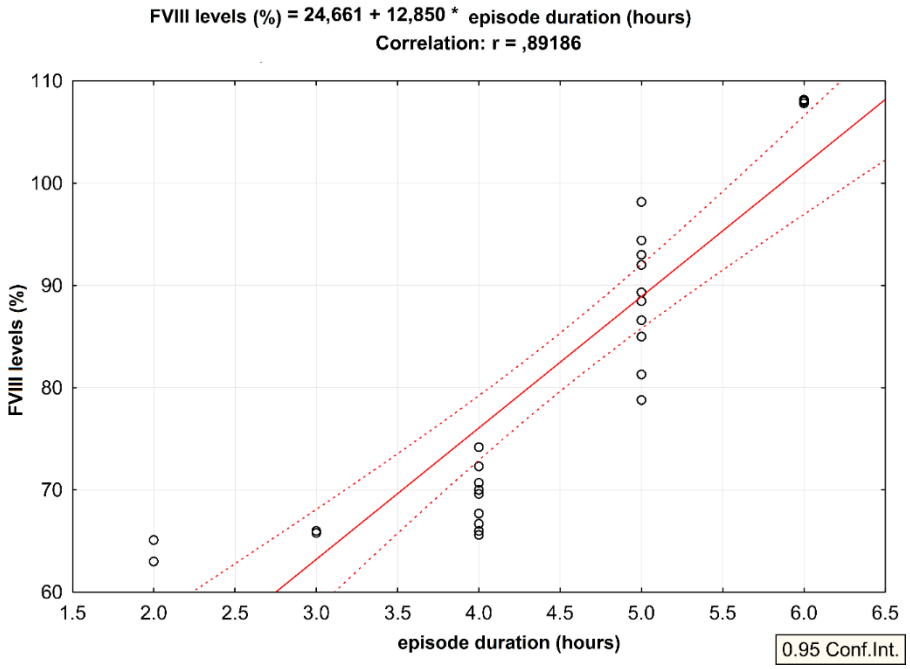


Figure 45. *Correlation between FVIII plasma levels and duration of paroxysmal atrial fibrillation episode within the first 6 hours of disease*

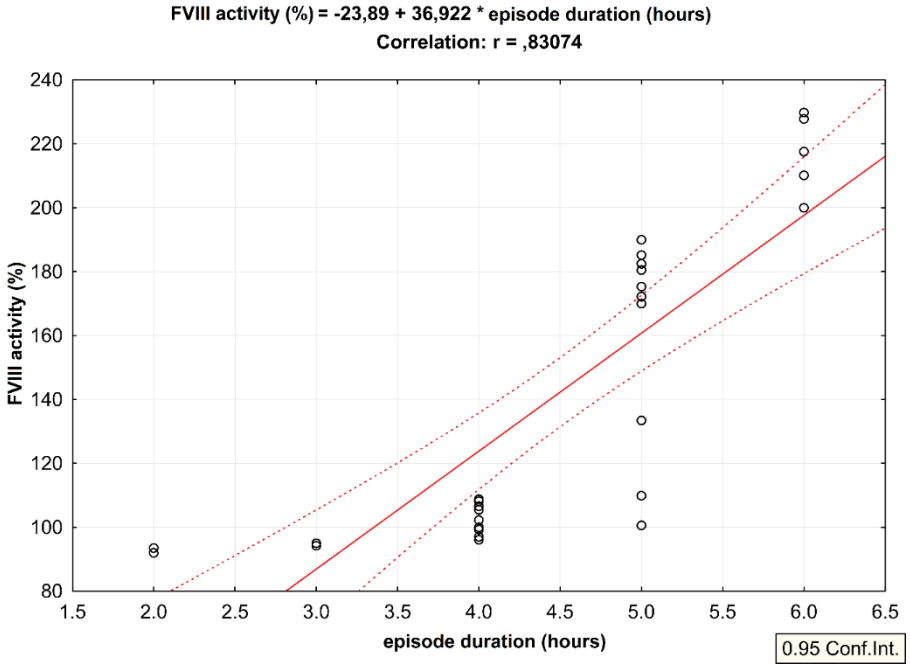


Figure 46. *Correlation between FVIII coagulation activity of and duration of paroxysmal atrial fibrillation episode within the first 6 hours of disease*

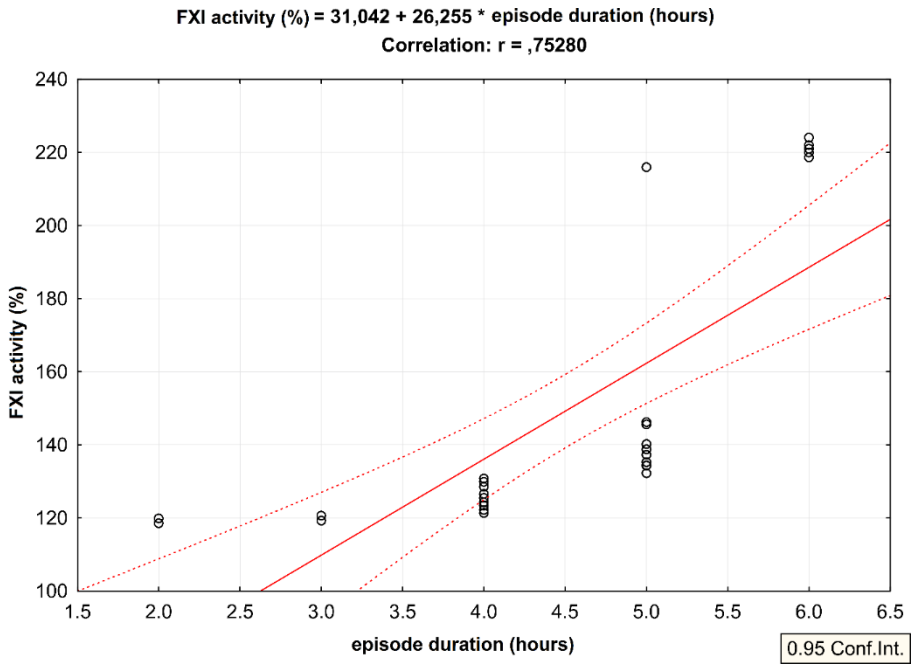


Figure 47 *Correlation between FXI coagulation activity and duration of paroxysmal atrial fibrillation episode duringthe first 6 hours of disease*

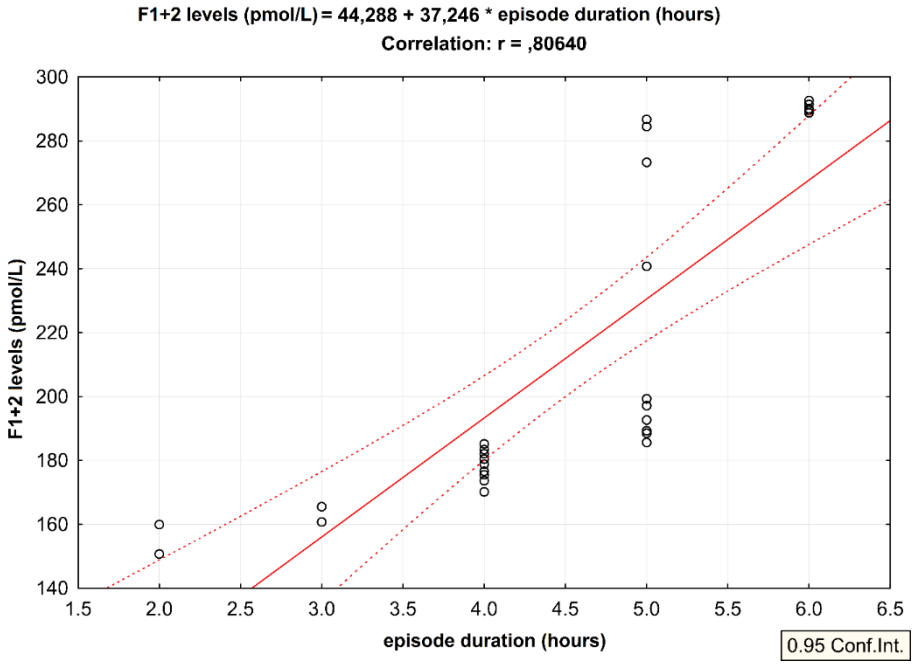


Figure 48. *Correlation between F1+2 plasma levels and duration of paroxysmal atrial fibrillation episode during the first 6 hours of the disease*

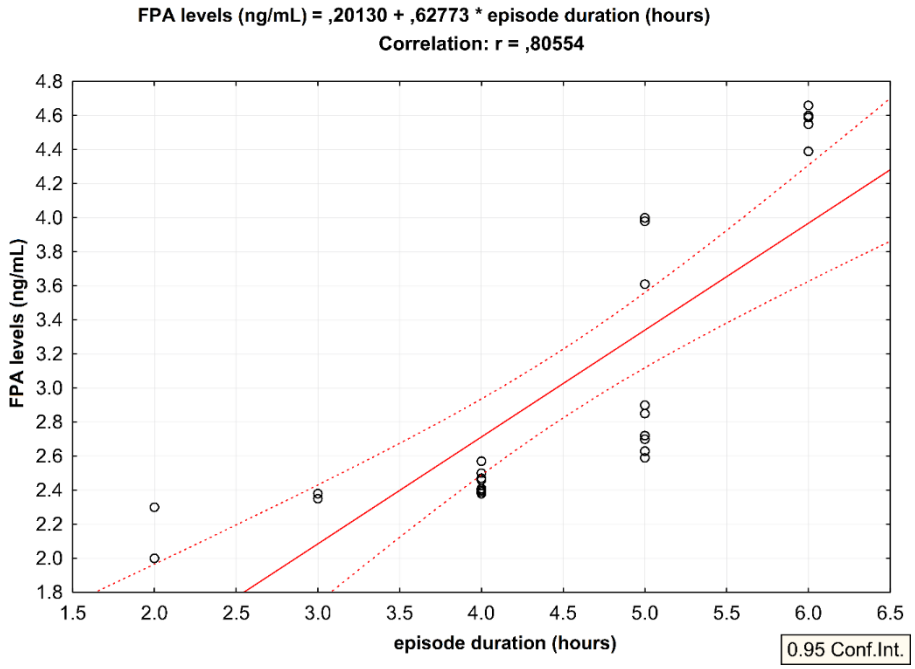


Figure 49. *Correlation between FPA plasma levels and duration of paroxysmal atrial fibrillation episode during the first 6 hours of the disease*

Таблица 19. *Linear regression model for relationship between studied hemostaticindicators and episode duration > 6 hours*

Indicator	b_0*	b_1*	*r* *	r^2 *
FII activity (%)	142.23, p=0.21	3.05, p=0.15	0.21	0.04
TF levels (pg/mL)	285.62, p=0.47	0.10, p=0.98	0.01	0.01
FV activity (%)	205.74, p=0.11	-0.73, p=0.78	-0.04	0.01
FVII activity (%)	195.63, p=0.32	-2.83, p=0.22	-0.25	0.06
FVIII levels (%)	9.70, p=0.25	1.70, p=0.10	0.24	0.06
FVIII activity (%)	161.68, p=0.07	4.75, p=0.07	0.26	0.07
vWF levels (%)	223.62, p=0.19	-6.02, p=0.06	-0.29	0.09
vWF activity (%)	217.08, p=0.27	-2.14, p=0.46	-0.11	0.01
FIX activity (%)	146.00, p=0.07	3.26, p=0.21	0.312	0.10
FX activity (%)	149.57, p=0.11	5.36, p=0.05	0.28	0.08
FXI activity (%)	175.27, p=0.62	0.82, p=0.72	0.08	0.01
FXII activity (%)	276.49, p=0.08	2.06, p=0.06	0.39	0.16
F1+2 (pmol/L)	195.33, p=0.08	1.76, p=0.38	0.13	0.02
FPA (ng/mL)	3.70, p=0.06	0.09, p=0.11	0.23	0.05
Plasminogen activity (%)	163.15, p=0.32	0.23, p=0.88	0.03	0.01
t-PA levels (ng/mL)	11.12, p=0.42	0.02, p=0.86	0.04	0.01
PAI-1 activity (AU/mL)	6.18, p=0.67	0.06, p=0.60	0.12	0.01
α2-antiplasmin activity (%)	120.82, p=0.09	-0.40, p=0.57	-0.12	0.01
Vitronectin levels (mcg/mL)	97.16, p=0.19	2.91, p=0.06	0.41	0.17
D-dimer levels (mg/L)	2.72, p=0.23	-0.10, p=0.54	-0.14	0.018

**b_0– constant (intercept parameter); b_1 – regression coefficient for the independent variable; r – correlation coefficient; r^2 coefficient of determination)*

Summarizing the results of the functional relationship between the duration of the paroxysmal atrial fibrillation episode and values of studied coagulation and fibrinolytic parameters, we found that longer episodes of arrhythmia were associated with significantly more pronounced deviations in key coagulation indicators (Table 17, 18).

Seven of the thirteen studied markers of coagulation activity showed significant sensitivity to the duration of the paroxysmal atrial fibrillation episode for entire time interval studied in the dissertation (first 24 hours of clinical manifestation of the disease) (Figures 37-43, Table 17). Increase in duration was associated with increased FII, FVIII, FIX and FXII activity and FVIII, F1+2 and FPA plasma levels. These observations show that time-dependent coagulation factors are key factors for the activity of the intrinsic and final common pathway of the coagulation cascade. It is well known that the intrinsic pathway is primarily responsible for the phases of amplification and propagation of the coagulation signal, in contrast to the extrinsic pathway, which determines the initiation of coagulation (Becker, 2005; Riddel et al., 2007). The common pathway determines the processes of thrombin and fibrin formation. In this sense, longer episodes of arrhythmia are associated with increased propagation of the coagulation signal and formation of thrombin and fibrin. Procoagulant deviations in short (≤24 hours) episodes of paroxysmal atrial fibrillation are dynamic and duration is their significant predictor. This suggests that duration plays a role for the thromboembolic potential. It could provide a logical clinical-laboratory explanation of the opposite results presented regarding thromboembolic potential of paroxysmal atrial fibrillation, which is defined from "significant and almost identical to non-paroxysmal forms" to significantly lower (Y. Chenetal., 2015; Christensen et al., 2014; Ganesan et al., 2016; Lip et al., 2008; Nieuwlaatetal., 2008). The large differences in temporal characteristics of the disease in individual studies are a possible and logical explanation for the different thromboembolic potential.

Logically, dividing the patient group into two subgroups according to episode duration (≤6 hours and > 6 hours) made it possible to refine the time characteristic values of established hypercoagulability in the short (≤24 hours) paroxysmal atrial paroxysmal episodes. In the first six hours, increase in episode duration showed significant increase in the deviations of nine of the studied thirteen coagulation parameters, namely: FII, FVII, FVIII, FXI and FXII coagulation activity and TF, FVIII, F1+2 and FPA plasma levels (Table 17). The established dynamic early changes (up to 6 hours) in coagulation status with procoagulant character show the need for early initiation of anticoagulant therapy, immediately after establishment of the arrhythmia. The data we obtained confirm the empirical rule introduced in clinical practice for immediate

initiation of anticoagulant treatment in patients with atrial fibrillation. This was also confirmed in the current recommendations of the European Society of Cardiology (Hindricks et al., 2021). It will help clinicians to make decisions based on indisputable laboratory data, which to this moment are not known.

It is noteworthy that dynamic changes in TF plasma levels and FVII coagulation activity were observed only in the first six hours of the clinical manifestation of the disease (Figure 50, Figure 51). These results have their own logical explanation. The modern cell-based coagulation model defines TF and FVII molecules as directly related and responsible for the initiating coagulation phase. In this sense, it turns out that time is a limiting and predictive factor for the initiation of the coagulation process in paroxysmal atrial fibrillation. This dependence is clearly expressed in the first hours (≤6 hours) of the disease. After the sixth hour of the disease, none of the studied hemostatic indicators presented a statistically significant dependence on the duration of the arrhythmia (Table 19). It turns out that episode duration in the later hours (>6 hours) did not have this key and determining role in the activity of the coagulation and fibrinolytic system.

Undoubtedly, the results presented in Table 17-19 show that procoagulant deviations in short episodes (≤24 hours) of paroxysmal atrial fibrillation are not static but dynamic in nature, with the most pronounced changes in the first six hours of the disease. Episode duration proved to be a critical predictor of the dynamics of procoagulant deviations during the first six hours of the clinical manifestation of the arrhythmia. As it increased, so did they. This fact makes early restoration of sinus rhythm important. The first six hours of paroxysmal atrial fibrillation were the defining threshold, before which shortening the episode of atrial fibrillation in itself effectively and significantly reduced procoagulant deviations. In the later hours of the disease, the influence of duration was greatly reduced, as can be seen from regression analysis data (Table 19). These findings are reflected in clinical practice. As is well known, the modern approach to hemodynamically stable patients with paroxysmal atrial fibrillation allows delayed cardioversion, within the first twenty-four hours of illness. This is the so-called “wait-and-watch” strategy, adopted as a reasonable alternative to immediate cardioversion in patients with a recent onset of the disease and part of the latest recommendations of the European Society of Cardiology (Hindricks et al., 2021). At the heart of this decision is the tendency of paroxysmal atrial fibrillation to self-restraint, according to some studies in almost 70% of cases (Dan et al., 2018; Danias et al., 1998). However, our data show the need for the earliest possible cardioversion, its benefits far outweighing the immediate benefits from shortening episode

duration and related symptoms. It would limit procoagulant deviations and resulting periprocedural thromboembolic risk.

The role of episode duration regarding periprocedural thromboembolic potential, decision for acute cardioversion, and need for subsequent periprocedural anticoagulation has been increasingly discussed in recent years. The imposed rule for "low-risk" first 48 hours of the disease in the last few years has not received absolute support from studies conducted in this direction, which is naturally a prerequisite for defining a new low-risk limit and adjusting the clinical approach for these short episodes. A series of articles by Airaksinen et al., present arguments on the topic (Airaksinen et al., 2013; Nuotio et al., 2014; Gronberg et al., 2016; Bah et al., 2017; Hellman et al., 2018; Hellman et al., 2017). They analyzed data from the Finnish FinCV study in a total of 6 reports and generally found expected low thromboembolic risk of 0.7% in cardioversion performed up to 48 hours after the onset of paroxysmal atrial fibrillation. However, the risk varied significantly according to arrhythmia duration. The time to cardioversion proved to be a very strong predictor for 30-day risk of thromboembolism, which was significantly higher in patients with episodes lasting 12-48 hours than those <12 hours (0.3% vs 1.1%). This difference persisted in both patients with CHA_2DS_2-VASc score 0-1 and >1. Rankin & Rankin complemented these considerations, believing that the decision for acute cardioversion is appropriate to be based on estimated by CHA_2DS_2-VASc score thromboembolic risk and duration of the episode (Rankin & Rankin, 2017). This view has been taken into account in the latest recommendations of the European Society of Cardiology for atrial fibrillation behavior (Hindricks et al., 2021). Here, for the first time, episode duration was presented as a factor relevant to the decision for cardioversion and episodes with a duration of <12 hours were presented as lower risk. Our results support this reasoning. Although the incidence of periprocedural thromboembolic events has not been directly examined, their pathophysiological substrate has been objectively studied. The results allow us to define the first 6 hours of arrhythmia as the hours with the lowest procoagulant activity. Considering periprocedural thromboembolic events in atrial fibrillation as a clinical manifestation and function of procoagulant changes, we have reason to assume that the first 6 hours of the clinical manifestation of paroxysmal atrial fibrillation have a lower periprocedural potential and define the time window for safer hours for acute cardioversion and postprocedural thromboembolic events. The critical importance of the first 6 hours of paroxysmal atrial fibrillation has been observed in other studies (Turakhia et al., 2015). A follow-up of 9,850 patients with paroxysmal atrial fibrillation revealed that a 5.5 hour burden of atrial fibrillation was the limit for stroke. Our results provide an objective clinical and laboratory basis for the need to distinguish the

periprocedural thromboembolic potential of short (≤24 hours) episodes of paroxysmal atrial fibrillation, depending on their duration, despite the narrow time interval (≤24 hours).

7. Logistic regression models of studied hemostatic indicators. Evaluation of obtained models by ROC analysis

Based on constructed logistic models and their calculated correctness, seven of the studied twenty coagulation and fibrinolytic parameters represented a good opportunity (over 70% probability) for correct classification of observed cases (absence/presence of paroxysmal atrial fibrillation), namely: FVIII, vWF, plasminogen and PAI-1 plasma activity, as well as vWF, t-PA and vitronectin plasma levels (Table 20-21).

Table 20. *Estimated coefficients of the logistics models and p-value from the Wald test*

Indicator	B_0	B_1	P value
FII activity (%)	-2.854	0.022	<0.001
TF levels (pg/mL)	-3.243	0.015	<0.001
FV activity (%)	-3.379	0.022	<0.001
FVII activity (%)	-3.893	0.030	<0.001
FVIII levels (%)	-2.118	0.021	<0.05
FVIII activity (%)	-3.626	0.025	<0.001
vWF levels (%)	-1.641	0.011	<0.001
vWF activity (%)	-2.928	0.020	<0.001
FIX activity (%)	-3.585	0.025	<0.001
FX activity (%)	-2.529	0.017	<0.001
FXI activity (%)	-4.119	0.029	<0.001
FXII activity (%)	-2.435	0.013	<0.001
F1+2 (pmol/L)	-0.011	0.002	**>0.05**
FPA (ng/mL)	-2.303	0.611	<0.001
Plasminogenactivity (%)	-10.354	0.083	<0.001
t-PA levels (ng/mL)	-8.835	1.034	<0.001
PAI-1 activity (AU/mL)	9.450	-0.885	<0.001
α2-antiplasminactivity (%)	2.772	-0.023	<0.05
Vitronectinlevels (mcg/mL)	11.035	-0.057	<0.001
D-dimerlevels (mg/L)	-0.706	1.747	<0.05

Table 21. *Correctness of the statistical model for classification of observations*

Indicator	OR	Correctly classified cases
FII activity (%)	2.49	61.17%
TF levels (pg/mL)	2.70	62.14%
FV activity (%)	4.52	67.96%
FVII activity (%)	3.52	65.05%
FVIII levels (%)	2.12	59.22%
FVIII activity (%)	5.94	70.87%
vWF levels (%)	8.53	72.82%
vWF activity (%)	5.94	70.87%
FIX activity (%)	2.41	60.78%
FX activity (%)	2.30	60.19%
FXI activity (%)	2.11	59.22%
FXII activity (%)	2.11	59.22%
F1+2 (pmol/L)	n.a.	n.a.
FPA (ng/mL)	1.43	54.37%
Plasminogenactivity (%)	17.88	80.58%
t-PA levels (ng/mL)	12.11	77.67%
PAI-1 activity (AU/mL)	34.55	85.44%
α2-antiplasminactivity (%)	4.04	66.67%
Vitronectinlevels (mcg/mL)	76.44	88.35%
D-dimerlevels (mg/L)	1.98	56.86%

Figure 56 shows an increase in the probability of manifestation of the disease with increasing plasminogen activity. At 95% activity, the probability is only 10% and reaches 90% at 150% plasminogen activity.

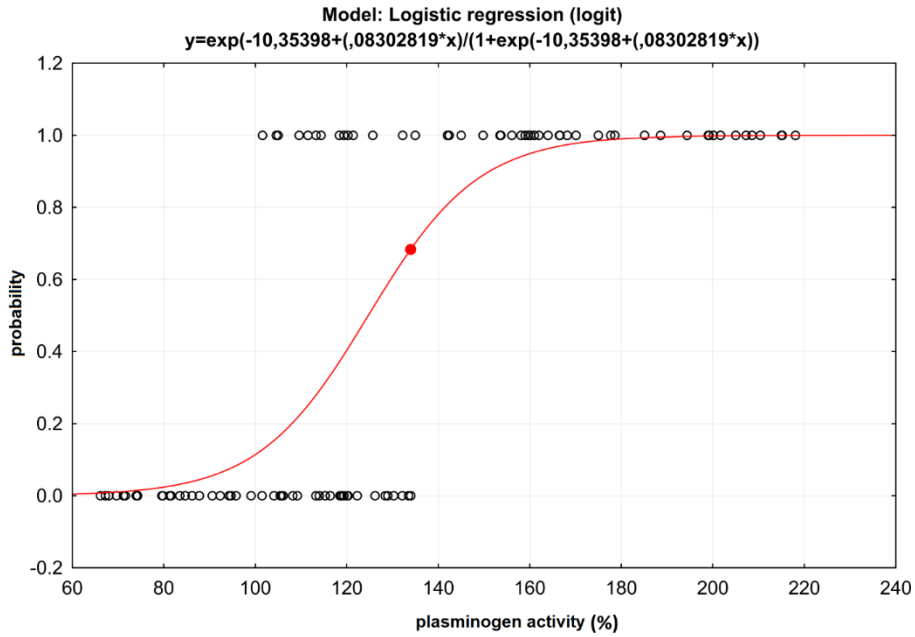


Figure 56. *Paroxysmal atrial fibrillation manifestation probability in relationship to plasminogen plasma activity (red dot - optimal manifestation threshold)*

As can be seen from Figure 57, at t-PA plasma levels of 6.5 ng/mL, a probability of 10% is reported, and when they rise to 10.5 ng/mL, it increases to 90%.

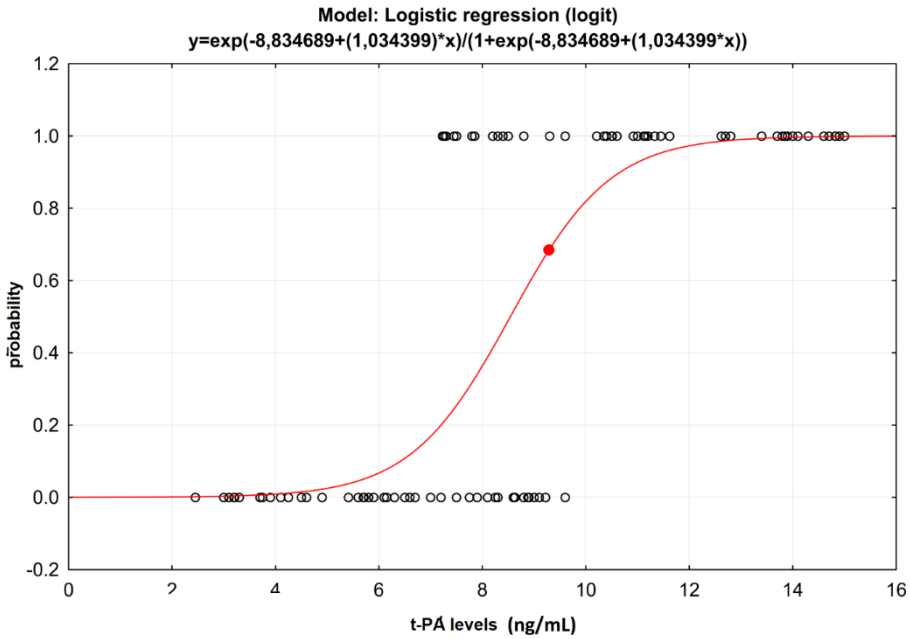


Figure 57. *Paroxysmal atrial fibrillation manifestation probability in relationship to t-PA plasma levels (red dot - optimal manifestation threshold)*

Figure 58 presents the relationship between arrhythmia probability and the values PAI-1 plasma activity index. The dependence is the opposite of that found in the above hemostatic parameters, when PAI-1 activity increases, probability decreases. At an activity of 8.5 AU/mL, the probability is 90% and decreases to 10% at an activity of 13.2 AU/mL.

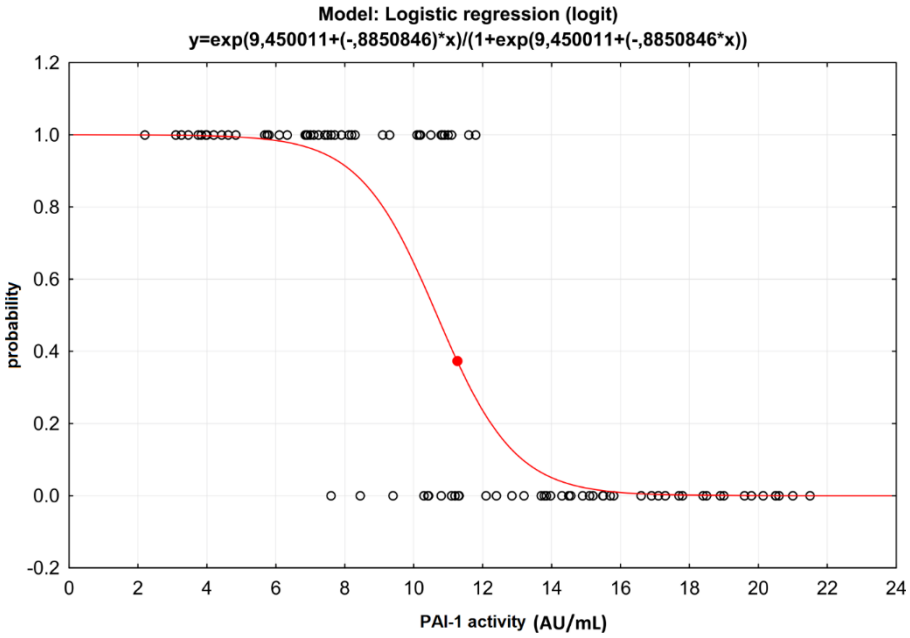


Figure 58. *Paroxysmal atrial fibrillation manifestation probability in relationship to PAI-1 plasma levels (red dot - optimal manifestation threshold)*

An inverse relationship was also found in plasma vitronectin levels (Figure 59). At a value of 157 mcg/mL, probability is 90%. The curve shows a 20% probability at 230 mcg/mL. It does not allow direct reading of levels at 10% probability.

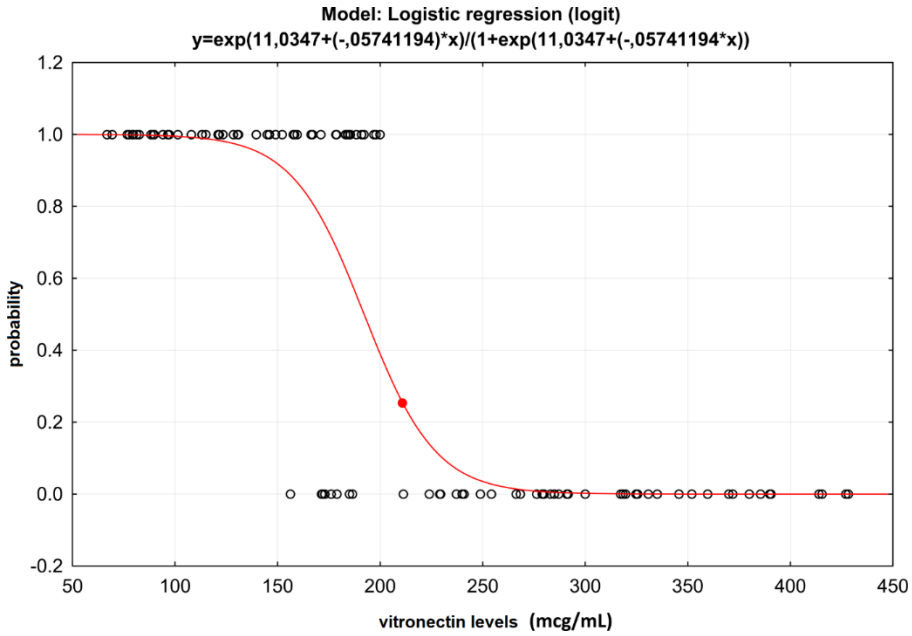


Figure 59. *Paroxysmal atrial fibrillation manifestation probability in relationship to vitronectin plasma levels (red dot - optimal manifestation threshold)*

To assess the quality of obtained logistics models, and respectively, the accuracy of studied hemostatic indicators as possible laboratory diagnostic markers for paroxysmal atrial fibrillation, we conducted a ROC analysis of those who presented statistical significance in logistic regression. These were all studied coagulation and fibrinolytic markers without F1+2 plasma levels (Table 20). The calculated AUC, optimal threshold according to Youden's index and its corresponding accuracy, sensitivity and specificity for each indicator are presented in Table 22.

Comparison of ROC curves by estimated AUC showed as the most effective models for prediction of paroxysmal atrial fibrillation. These were the models of: vitronectin plasma levels (AUC=0.96, Figure 60), PAI-1 activity (AUC=0.96, Figure 61), t-PA levels (AUC=0.93, Figure 62) and plasminogen activity (AUC=0.92, Figure 63), in descending order, respectively (Table 22). Their model quality was determined to be excellent (AUC>0.9).

Table 22. *Data from ROC analysis of studied coagulation and fibrinolytic indicators.*

Indicator	AUC	Optimal threshold point	Youden's index	Accuracy	Sensitivity	Specificity
FII activity (%)	0.79	160.10	0.59	0.80	0.99	0.59
TF levels (pg/mL)	0.81	264.00	0.57	0.79	0.94	0.63
FV activity (%)	0.80	172.77	0.63	0.82	0.99	0.63
FVII activity (%)	0.81	148.20	0.59	0.79	0.99	0.59
FVIII levels (%)	0.63	124.10	0.35	0.68	0.98	0.37
FVIII activity (%)	0.85	158.89	0.69	0.85	0.99	0.69
vWF levels (%)	0.64	140.60	0.45	0.73	0.85	0.61
vWF activity (%)	0.79	165.00	0.61	0.81	0.98	0.63
FIX activity (%)	0.80	117.0	0.53	0.77	0.57	0.96
FX activity (%)	0.75	175.00	0.55	0.78	0.99	0.55
FXI activity (%)	0.81	156.90	0.57	0.57	0.99	0.58
FXII activity (%)	0.74	215.48	0.43	0.72	0.87	0.57
F1+2 (pmol/L)	n.a.	n.a	n.a	n.a	n.a	n.a
FPA (ng/mL)	0.72	4.30	0.53	0.77	0.98	0.55
Plasminogenactivity (%)	0.92	134.00	0.73	0.86	0.99	0.73
t-PA levels (ng/mL)	0.93	9.22	0.73	0.86	0.98	0.75
PAI-1 activity (AU/mL)	0.96	11.20	0.79	0.89	0.83	0.96
α2-antiplasminactivity (%)	0.72	131.6	0.47	0.74	0.65	0.82
Vitronectinlevels (mcg/mL)	0.96	211.40	0.83	0.91	0.83	0.99
D-dimerlevels (mg/L)	0.57	0.6	0.20	0.60	0.92	0.27

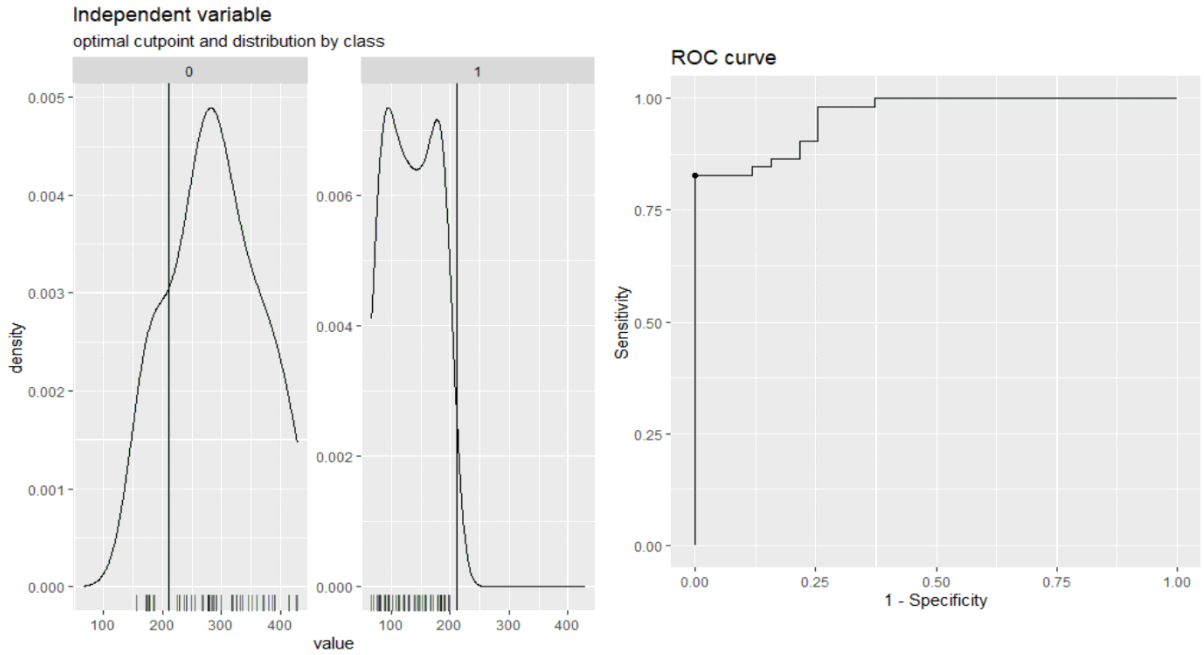


Figure 60. Left: *Distribution of vitronectin values in the absence (0) and presence (1) of paroxysmal atrial fibrillation relative to the optimal threshold vertical line.* ***Right:*** *ROC curve of the indicator with optimal threshold point.*

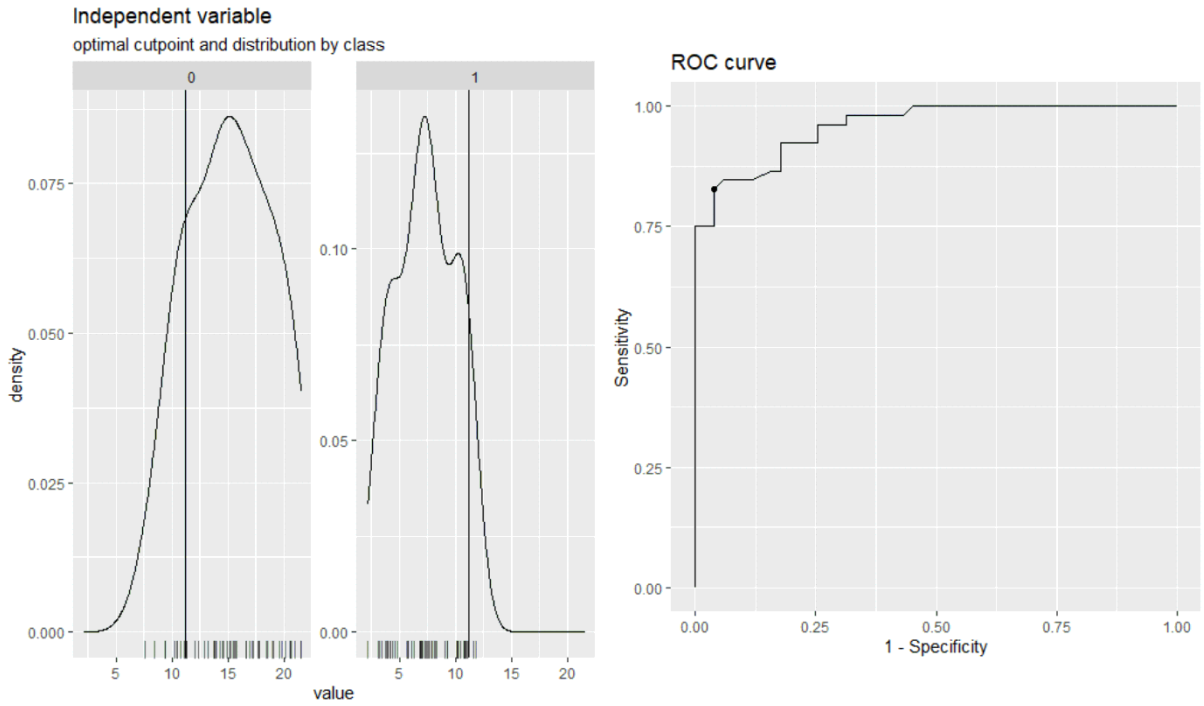


Figure 61. Left: *Distribution of PAI-1 values in the absence (0) and presence (1) of paroxysmal atrial fibrillation relative to the optimal threshold vertical line.* ***Right:*** *ROC curve of the indicator with optimal threshold point.*

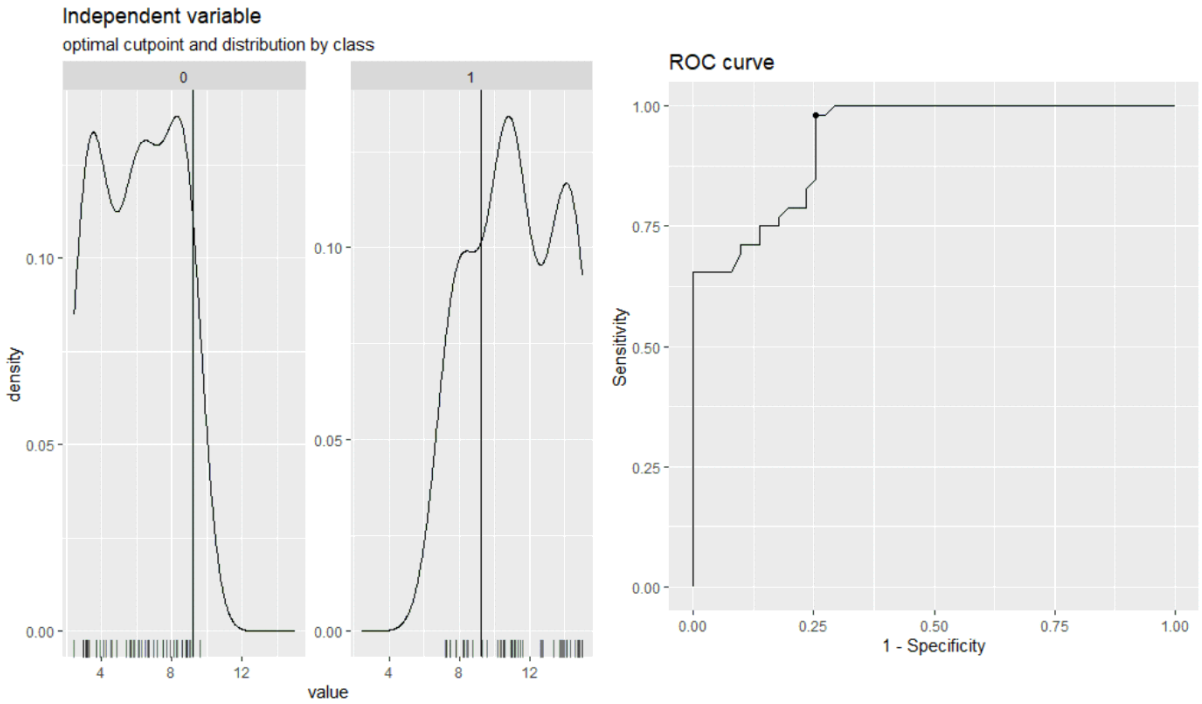


Figure 62. Left: *Distribution of t-PA values in the absence (0) and presence (1) of paroxysmal atrial fibrillation relative to the optimal threshold vertical line.* ***Right:*** *ROC curve of the indicator with optimal threshold point.*

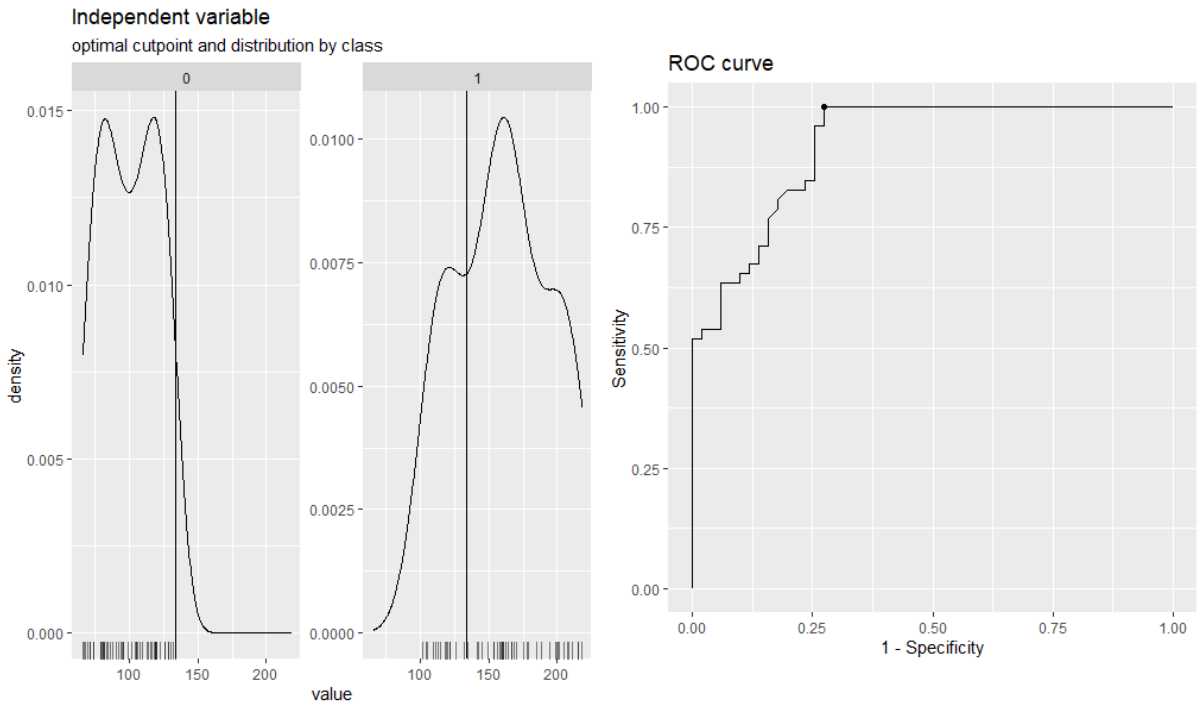


Figure 63. Left: *Distribution of plasminogen activity values in the absence (0) and presence (1) of paroxysmal atrial fibrillation relative to the optimal threshold vertical line.* ***Right:*** *ROC curve of the indicator with optimal threshold point.*

Based on sensitivity and specificity, as well as probability of paroxysmal atrial fibrillation (obtained on the basis of previously built logistic models) at calculated threshold values, we found that plasminogen and t-PA had the best predictive value for manifestation of the disease.

Plasma activity of plasminogen ≥134% determined a probability of ≥69% for manifestation of the disease, the indicator having high sensitivity and specificity in diagnosing the condition (Se = 0.99, Sp = 0.73). Plasma t-PA levels ≥9.22 ng/mL determined ≥69% probability, again with good diagnostic accuracy (Se = 0.98, Sp = 0.75). It is worth noting that both indicators are fibrinolytic. It is well known that in addition to thrombolytic function, the fibrinolytic system plays a key role in a number of other physiological and pathological processes, one of which is tissue remodeling (Nissinen et al., 2014; J. Chen et al., 2017; Cui et al., 2017). The plasminogen/plasmin system has two essential functions: a well-known one, related to the degradation of fibrin to FDP, and another, no less important, responsible for the degradation of cell membranes and extracellular matrix, promoting tissue remodeling and cell migration. Plasmin is a direct activator of important functional enzymes, including more than seven matrix metalloproteinases. Structural remodeling is an indisputable morphological substrate for manifestation of atrial fibrillation, and in this sense, the effects of plasminogen/plasmin system beyond fibrinolytic function can logically explain the relationship between plasminogen and t-PA values with manifestation of the disease.

8. Arterial thromboembolism predictors: modeling data from studied hemostatic parameters using the Cox model

Using the Cox model, we examined the extent to which identified abnormalities in studied hemostatic indicators may be predictors of ischemic stroke. Clinical indicators with already established prognostic values for ischemic stroke, namely: age, BMI (kg/m2) and GFR (ml/min/1.73 m^2) were tested, as well as thromboembolic risk characteristic, defined as low-risk (CHA2DS2-VASc score = 0 in men/1 in women) and high risk (CHA2DS2-VASc score ≥1 for both sexes) (Hindricks et al., 2021).

Assessment of survival function by the Kaplan-Meier estimator showed a mean follow-up period of 8.44 years (1.16-10.08), during which 9 arterial thromboembolic events were reported. The mean annual incidence of thromboembolic events was 2%. Univariate Cox regression analysis revealed the following haemostatic parameters as statistically significant ($p < 0.05$) for manifestation of ischemic stroke: TF plasma levels (HR 1.032 [95% CI, 1.003 - 1.061)]), FVIII plasma levels (HR 1.054 (95% CI, (1.018-1.091)]) and vitronectin plasma levels (HR 0.973 (95% CI, (0.948-0.998)]) (Table 23). As TF and FVIII increase, probability of a cerebrovascular accidents also increase (Beta> 0). From the constructed dichotomous logistic regression models for these two predictors with an indicating variable presence/absence of

stroke in studied patients, we can see that at TF=418.79 pg/mL, probability is 50% (0.5) and increases to 75% (0.75) at a value of 440 pg/mL (the value of the indicator at a probability of 90% cannot be read directly, as can be seen in Figure 67, and extrapolation would lead to some inaccuracy). Regarding FVIII, probability of 50% (0.5) at 46.95% value of the indicator increases to 90% (0.9) at a value of 150% (Figure 68). For the statistically significant predictor vitronectin, Beta factor is <0 and stroke probability is 50% (0.5) at 46 (mcg / mL), increasing to 90% (0.9) at 155 (mcg / mL) (Figure 69).

Table 23. *Univariate Cox regression analysis for stroke manifestation*

Risk factor	Evaluation ofBeta parameter	P-value	HazardRatio (HR) (95% CI for HR)
Age (years)	0.108	0.037	1.113 (1.007-1.231)
BMI (kg/m^2)	-0.065	0.525	0.937 (0.767-1.145)
eGFR (mL/min/1.73 m^2)	0.014	0.338	1.014 (0.986-1.043)
CHA_2DS_2-VASc score *	0.452	0.048	1.157 (1.075-2.534)
FII activity (%)	0.004	0.498	1.004 (0.993-1.014)
TF levels (pg/mL)	0.031	0.029	1.032 (1.003-1.061)
FV activity (%)	0.002	0.722	1.002 (0.993-1.010)
FVII activity (%)	-0.006	0.339	0.995 (0.983-1.006)
FVIII levels (%)	0.054	0.003	1.054 (1.018-1.091)
FVIII activity (%)	-0.003	0.547	0.997 (0.989-1.006)
vWF levels (%)	-0.003	0.502	0.998 (0.990-1.006)
vWF activity (%)	-0.001	0.920	0.999 (0.992-1.007)
FIX activity (%)	0.003	0.671	1.003 (0.989-1.017)
FX activity (%)	-0.009	0.063	0.991 (0.981-1.001)
FXI activity (%)	-0.007	0.260	0.993 (0.980-1.005)
FXII activity (%)	-0.003	0.543	0.998 (0.989-1.006)
F1+2 (pmol/L)	0.003	0.439	1.003 (0.996-1.010)
FPA (ng/mL)	-0.150	0.440	0.861 (0.588-1.260)
Plasminogenactivity (%)	-0.002	0.804	0.998 (0.979-1.017)
t-PA levels (ng/mL)	-0.213	0.137	0.808 (0.610-1.070)
PAI-1 activity (AU/mL)	-0.110	0.397	0.896 (0.694-1.156)
α2-antiplasminactivity (%)	0.013	0.489	1.013 (0.977-1.049)
Vitronectinlevels (mcg/mL)	-0.028	0.036	0.973 (0.948-0.998)
D-dimerlevels (mg/L)	-1.25	0.264	0.287 (0.032-2.573)

**defined in both cathegories CHA_2DS_2-VASc score = 0 in men/1 in women andCHA2DS2-VASc score≥1 for both sexes (*Hindricks etal., 2021)

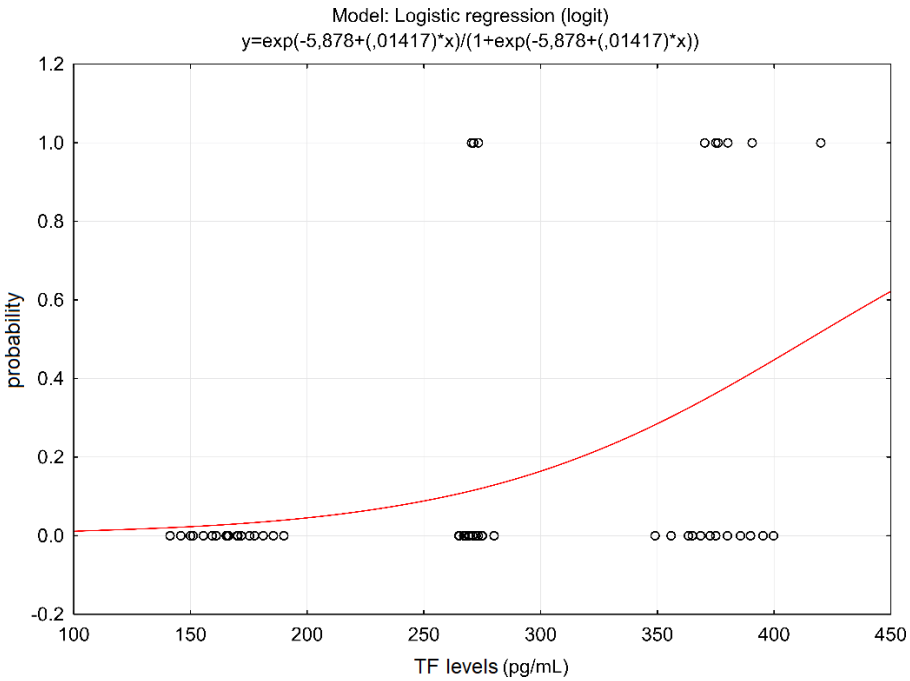


Figure 67. *Probability of ischemic stroke depending on TF plasma levels*

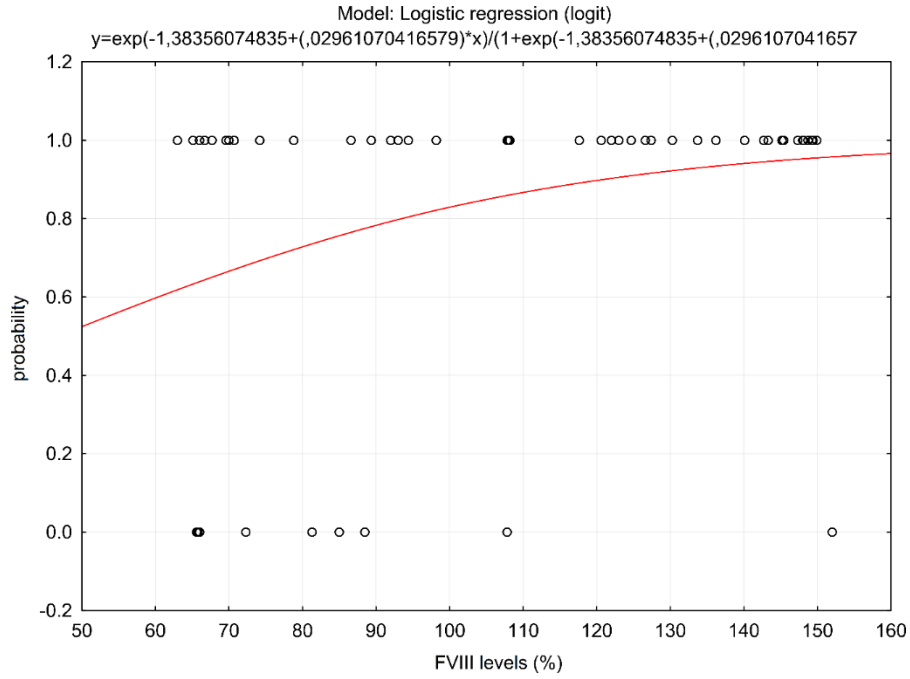


Figure 68. *Probability of ischemic stroke depending on FVIII plasma levels*

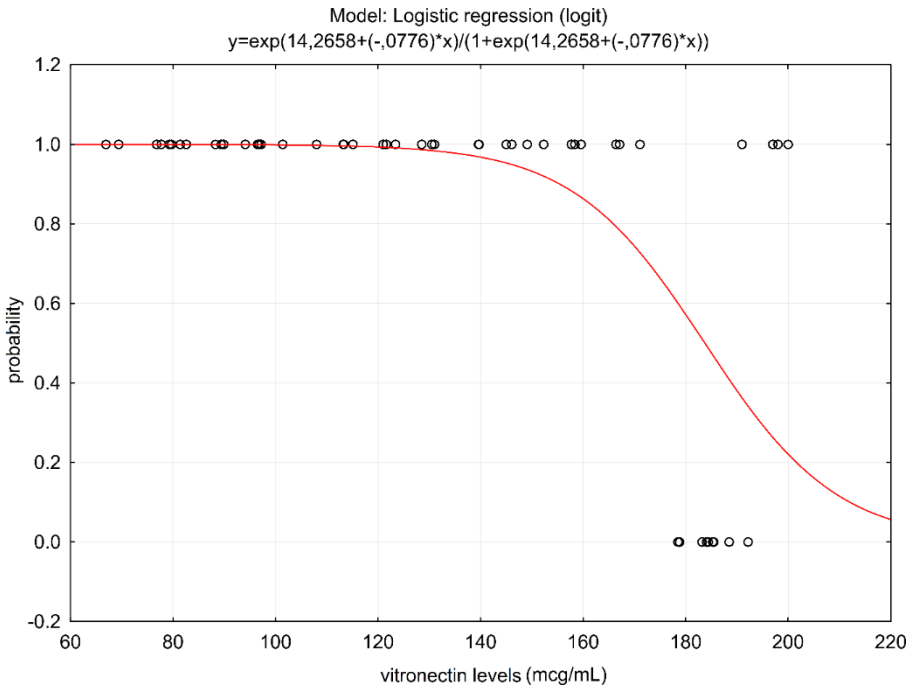


Figure 69. *Probability of ischemic stroke depending on vitronectin plasma levels*

From the studied clinical indicators, only age and CHA_2DS_2-VASC score showed a predictive value for manifestation of ischemic stroke (HR 1,113 [95% CI, (1,007-1,231)], p <0.05; HR 1,157 [95% CI, (1,075-2,534)], p<0.05, respectively.) The Beta coefficient was >0, so with increasing age, probability of stroke increased and CHA2DS2-VASc score≥1 (both sexes) gave a poor prognosis for stroke.

The factors identified as significant in the univariate analysis were included in a multivariate Cox regression model. The aim was to establish their contribution to ischemic stroke manifestation in aggregation and interaction. The obtained results are shown in Table 24. Multivariate regression analysis identified as significant (p<0.05) predictors of ischemic stroke all five factors, found significant in the univariate Cox model.

Table 24. *Multivariate Cox regression analysis for stroke manifestation*

Risk factor	Evaluation of Beta parameter	P-value	HazardRatio (HR) (95% CI for HR)
Age (years)	0.213	0.019	1.237 (1.036-1.478)
CHA_2DS_2-VASc score *	0.281	0.016	1.324 (1.054-1.662)
TF levels (pg/mL)	0.022	0.015	1.022 (1.004-1.040)
FVIII levels (%)	0.081	0.037	1.084 (1.005-1.170)
Vitronectin levels (mcg/mL)	-0.055	0.049	0.946 (0.895-0.999)

**defined in both cathegories CHA_2DS_2-VASc score = 0 in men/1 in women andCHA2DS2-VASc score≥1 for both sexes (*Hindricks etal., 2021)

Undoubtedly, our study is not the first to look for an opportunity to define predictors of ischemic stroke among indicators of coagulation and fibrinolytic system. However, it is the first to study twenty indicators of these two systems simultaneously. The established results have their pathophysiological justification. Tissue factor is a key and irrevocable participant in the process of thrombosis and, more precisely,the initiating phase of coagulation. Circulating TF is even thought to carry TF/FVIIa activity until it meets activated platelets (Brambilla et al., 2015; Cimmino et al., 2015a; Cugno et al., 2014; Ferreira et al., 2010a). In this sense, it is the freely circulating plasma levels of TF that very accurately reflect the body's tendency to thrombosis. The vitronectin molecule is a multifunctional complementary regulator that can be found in almost all cells of the human body (Yu-Ching et al., 2018). It is an important adherent platelet glycoprotein and an indirect inhibitor of fibrinolysis. These facts determine its procoagulant role (Preissner & Seiffert, 1998; Smyth et al., 2009). At the same time, however, it inhibits the final pathway of the complement system and prevents formation of cell-deadly "pore-forming complexes" (Yu-Ching et al., 2018). Of course, the exact mechanisms by which low vitronectin levels predetermine a high probability of stroke in the population we studied are unclear, but we speculatively assume its role in cellular protection by controlling the activity of the complement system. CHA_2DS_2-VASc scores confirm the importance of this indicator as a risk stratifier for long-term ischemic stroke in patients with atrial fibrillation.

The obtained data suggest that TF, FVIII and vitronectin plasma levels should be studied in detail as biomarkers, which, in addition to the well-established CHA2DS2-VASc score, optimize the approach to thromboembolic risk assessment in patients with paroxysmal atrial fibrillation (especially among the low-risk population). However, their application in clinical practice requires a number of preliminary studies. The population studied by us, although sufficient, is small in size and selected under a number of exclusion criteria (imposed by the purpose of the study). In this sense, we believe that *presented results on the predictive value for stroke of TF, FVIII and vitronectin plasma levels are a sufficient prerequisite for future studies in this direction* and their introduction into the therapeutic approach requires further research.

9. Final discussion

The generalized analysis of the obtained results allows to emphasize and highlight the characteristics of coagulation status in short episodes of paroxysmal atrial fibrillation lasting up to twenty-four hours. They are of both fundamental (scientific) and clinical (applied) importance. This conclusion is based on the following facts:

- brief episodes of paroxysmal atrial fibrillation (duration ≤24 hours) are a common clinical finding;
- data on their procoagulant changes are scarce and mixed, despite numerous laboratory and clinical studies on atrial fibrillation in general;
- post-procedural anticoagulant therapy after these episodes is still under discussion (in the absence of indications for long-term anticoagulation);
- the predictive possibilities of coagulation and fibrinolytic indicators for the occurrence of the disease itself and thromboembolic accidents are not clarified.

For us, the following few facts gave special weight to the results obtained:

- a sufficient number of key coagulation and fibrinolytic parameters have been identified at the same time, which allows to adequately to seek and accurately analyze the presented deviations in these two systems;
- a sufficient number of patients and controls were studied, groups were well balanced and duration of the disease was precisely determined, therefore the obtained clinical and laboratory evidence of early hemostasis deviations with their specific features, were reliable and indisputable;
- in order to clarify the need and optimize the decision for post-procedure anticoagulant prophylaxis, we sought a dependence of hemostatic deviations on the thromboembolic risk profile of patients, defined by CHA_2DS_2-VASc score along with episode duration;
- in carefully selected patients we sought the most objective possibility for prediction of manifestation of paroxysmal atrial fibrillation and associated thromboembolic complications.

In the light of modern hemostaseology, *coagulation* is a complex, finely regulated and physiologically well-balanced process, closely related with its counterpoint, the fibrinolytic system. According to Maureane Hoffman, one of the creators of the cell-based theory, the attempt to represent it graphically, resembles a *three-dimensional galactic map* (Hoffman,

2007). *In conditions of pathologically altered coagulation balance, the precise mechanisms of impaired coagulation and fibrinolysis can be understood only in a detailed and simultaneous study of both systems* (Chapin & Hajjar, 2015). We built our study based on these modern ideas, and this gave us the opportunity to draw important conclusions.

Of great importance is the fact that the values of all studied coagulation parameters presented significant deviations (Figures 14-27). In patients, plasma activity of FIIa, FVa, FVIIa, FVIIIa, FIXa, FXa, FXIa, FXIIa, and vWF was significantly higher ($p<0.001$; Figures 15-18, 20, 22, 24, 25). Plasma levels of TF ($p<0.001$; Figure 14), FVIII ($p<0.05$; Figure 19), vWF ($p<0.001$; Figure 21), F1+2 ($p<0.01$; Figure 26) and FPA ($p<0.001$; Figure 27) were also higher compared to controls. The summary analysis of all these unidirectional deviations unequivocally shows presence of *early hypercoagulability in paroxysmal atrial fibrillation, occurring even in short (≤24 hours) episodes.* We consider as particularly important evidence for the procoagulant effect of the arrhythmia, the established activated synthesis and secretion of some of the studied coagulation indicators, namely FVIII and vWF, in addition to their high activity (Figures 19-22). We presented for the *first time convincing laboratory evidence of presence of a prothrombotic state in the course of paroxysmal episodes of atrial fibrillation with a duration of ≤24 hours.* The established systemic hypercoagulability is a natural, objective and sufficient prerequisite to assume that short episodes (≤24 hours) of the disease determine increased periprocedural thromboembolic potential.

The detailed study of the coagulation system in our study not only clearly outlined the hemostatic profile of patients with short episodes of arrhythmia, but also presented characteristic features of activated coagulation, namely significant activation of FXII and FXI ($p<0.001$; Figure 16, Figure 17). For the first time, participation of FXII and FXI in procoagulant changes in atrial fibrillation has been confirmed. It is well known that these coagulation factors are crucial for occlusive thrombus formation, while their absence does not lead to significant, life-threatening bleeding episodes (Geddings & Mackman, 2014; Rankin & Rankin, 2017; Vojacek, 2017). This fact makes FXIa and FXIIa promising targets for effective but safer anticoagulation in atrial fibrillation, compared to currently established direct thrombin (dabigatran) and FXa (rivaroxaban, apixaban, edoxaban) inhibition.

The established deviations in fibrinolytic parameters, namely higher plasminogen activity ($p <0.001$; Figure 28), higher plasma t-PA levels ($p <0.001$; Figure 29), lower PAI-1 activity ($p <0.001$; Figure 30) and α2-antiplasmin ($p <0.05$; Figure 31) and vitronectin levels ($p <0.001$;

Figure 32) showed activated fibrinolysis. D-dimer is a unique molecule that characterizes both the end of coagulation and beginning of fibrinolysis. It is absolutely necessary in assessing the balance and interaction between the two systems. Its high values in our study not only fully confirm activation of fibrinolysis, but are direct evidence of increased intravascular fibrin formation, a major component of thrombi associated with atrial fibrillation ($p < 0.05$; Figure 33). In this sense, we believe that activated fibrinolysis is a response to existing hypercoagulation.

Clinical significance of postprocedural anticoagulation after an episode of recent atrial fibrillation, albeit indirectly, has been presented in the FinCV study, ANTIKoagulationsstudie, results of the Danish cohort, and many others (Hellman et al., 2018; Kleemann et al., 2009; Palomä; et al., 2016). However, it remains controversial after paroxysmal episodes ≤24 hours in patients with no indication for long-term anticoagulant prophylaxis (CHA_2DS_2-VASc score 0 in men/1 woman). Data on postprocedural thromboembolic potential of the disease in the low-risk group of patients are ambiguous (Andrade et al, 2018; Andarde et al., 2019; Gang et al., 2016; Nuotio et al., 2014). The latest recommendations of the European Society of Cardiology remain vague and uncertain about the need for post-procedural prophylaxis in these patients, mainly due to small number of studies providing direct evidence (Hindricks et al., 2021). A 4-week anticoagulant prophylaxis is recommended in all patients after cardioversion in episodes >24 hours, which may be ommitted if the episode lasts ≤24 hours and has a low thromboembolic risk profile (Class IIb recommendation; level C evidence) (Camm et al., 2010; Hindricks et al., 2021).

It was the lack of convincing evidence that allowed us to accept *our results as an objective basis for improving clinical practice in patients with paroxysmal atrial fibrillation ≤24 hours.* Examining the influence of patient's thromboembolic risk characteristics on the coagulation and fibrinolytic system during the short (≤24 hours) episodes of paroxysmal atrial fibrillation, we found a prothrombotic state in low-risk patients (CHA2DS2-VASc score = 0 in men/1 in women) which did not differ significantly from that in patients with increased thromboembolic risk (CHA2DS2-VASc score ≥1 for both sexes) (Tables 14-16). In this sense, we believe that it is appropriate to conduct periprocedural anticoagulation until restoration of coagulation balance in all patients with a short episode of atrial fibrillation (≤24 hours), including low-risk patients with no indication of long-term prophylaxis. Regarding its duration, it is permissible to accept a 4-week period after recovery of sinus rhythm, which will be in accordance with established time intervals for lysis of each hypercoagulant state, currently accepted in good

clinical practice (Goldman et al., Hindricks et al., 2021; 1999; Kleemann et al., 2009; Oltrona et al., 1997; Rankin & Rankin, 2017; Stoddard et al., 1995). Its use, of course, must be fully in line with good clinical practice, requiring a critical assessment of the risk of bleeding using the established HASBLED score (Hindricks et al., 2021).

Episode duration was a significant predictor and limiting factor for the established procoagulant deviations in the study (Tables 17-18). The correlation was emphasized within the first 6 hours of the disease, where coagulation activity of FII, FVII, FVIII, FIX and FXII and plasma levels of TF, FVIII, F1+2 and FPA increased significantly, and in the later hours (after 6 hour) this dependence was greatly reduced and lost (Tables 18-19, Figures 44-52). The identified early changes and their dynamic nature not only support the empirical rule for immediate initiation of anticoagulant treatment in the detection of atrial fibrillation, *but provide the first scientific evidence of the need for this decision*. At the same time, the possibility of limiting the thromboembolic potential of paroxysmal atrial fibrillation by early and rapid recovery of sinus rhythm has been shown (Tables 17-18). *Early cardioversion is limiting and important in reducing periprocedural thromboembolic risk.* Immediate attempts to restore sinus rhythm with immediate initiation of anticoagulant therapy outline clear advantages in reducing procoagulant deviations, and in this regard the *"wait-and-watch" approach during the first 24 hours*, proposed in current therapeutic recommendations, is inappropriate.

The critical threshold of hours with lower thromboembolic potential is defined by the first 6 hours of the disease, according to the results obtained (Table 18). In this sense, we have reason to propose a rethinking of the definition of "ideal candidates for acute cardioversion" proposed by the latest European recommendations, as duration of the atrial fibrillation episode has narrowed down to these early hours. The dynamic nature of the detected prothrombotic changes are inherent laboratory confirmation and direct objective evidence of the conclusion presented in observational clinical trials that the time to cardioversion is a predictor of postprocedural thromboembolism (Nuotio et al., 2014; Hellman et al., 2018).

The performed logistic regression and ROC analysis established promising possibilities for identifying the *occurrence of paroxysmal atrial fibrillation by examining plasminogen plasma activity and t-PA plasma levels* (Tables 20-22). We also showed that *TF, FVIII, and vitronectin plasma levels could be significant predictors of ischemic stroke* (Figure 67, Figure 68, Figure 69; Tables 23-24). Undoubtedly, our study is not the first to look for clinical application of coagulation and fibrinolytic parameters beyond presentation of hemostatic

imbalance in atrial fibrillation. However, we consider the simultaneous study and comparison of such a large number of indicators, as well as strict selection of participants, as factors of particular importance, making our results a serious prerequisite for larger clinical trials to confirm the strength of conclusions and their clinical application. *The obtained results raise a significant question*. Given the role of hemocoagulation in a number of pathological processes, such as oxidative stress, inflammation, tissue remodeling, etc., to what extent established procoagulant abnormalities are caused by atrial fibrillation, and to what extent they precede its clinical manifestation, as part of yet unclear pathophysiological mechanisms, responsible for its initiation.

In summary, we can present for the first time convincing clinical and laboratory data for the development of hypercoagulability in the first 24 hours of paroxysmal atrial fibrillation, caused by significant deviations in the coagulation and fibrinolytic system. Brief (≤24 hours) episodes of the disease are clearly defined as a prothrombotic state, even in low-risk thromboembolic characteristics of patients (CHA_2DS_2-VASc score = 0 in men/1 in women). Episode duration has an effect on the established hemostatic deviations. The presented dissertation has not only original scientific value, but also clearly outlines clinical applications of the results. It shows that FXIa and FXIIa are more promising targets for effective and safer anticoagulation than currently established. It indicates the need for post-procedural anticoagulation even after currently considered lowest risk episodes of non-valvular paroxysmal atrial fibrillation (≤ 24 hours and CHA_2DS_2-VASc score = 0 in men/1 in women). It outlines the first 6 hours of the disease as having lower periprocedural thromboembolic risk. It outlines possibilities for clinical application of some hemostatic indicators beyond hemostatic assessment, namely, possibility of predicting manifestation of paroxysmal atrial fibrillation by plasminogen plasma activity and t-PA plasma levels and resulting ischemic stroke complications by TF, FVIII and vitronectin plasma levels.

V. CONCLUSIONS

1. In the present study, conducted with well-balanced patient and control groups, significant deviations were found in all twenty coagulation and fibrinolytic indicators studied. They unequivocally represent impaired *coagulation status* in the early hours (≤24 hours) of paroxysmal atrial fibrillation. Their specific and dynamic nature is reason to assume that it is closely *related to the disease itself*.

2. *Fibrinolytic activity was also significantly enhanced,* most likely a pathophysiological response to coagulation disorders. It results from increased conversion of plasminogen to plasmin and decreased activity of major inhibitors of PAI-1 and α2-antiplasmin systems.

3. The established high procoagulant *activity lead to hypercoagulability in the early hours (≤24 hours) of paroxysmal atrial fibrillation*, evident from deviations in plasma levels of early coagulation markers F1+2 and FPA, as well as D-dimer.

4. The coagulation process showed specific features in the disease, namely:
- it was associated with significant FXII and FXI activation;
- it was associated with early *endothelial dysfunction and damage*, as evidenced by high vWF plasma levels.

5. The high statistical power of the t-test in testing the hypothesis of equality of means of studied hemostaticindicators *was evidence for adequacy of sample size and correctness of conclusions*.

6. During brief episodes of paroxysmal atrial fibrillation (≤24 hours*), there were no significant differences* in coagulability between *low-risk patients* (CHA_2DS_2-VASc score = 0 for men /1 for women) and those with *high thromboembolic risk* (CHA_2DS_2-VASc score≥1 for both sexes), evident from the comparison of mean values of hemostatic in both categories of thromboembolic risk.

7. *Episode duration* was a critical *predictor of coagulation activity* in the first six hours of clinical manifestation of the arrhythmia, as evidenced by positive correlation of key coagulation indicators with the temporal characteristics of the disease. In later hours (> 6 hours) this strong dependence was lost.

8. The idea of using *coagulation and fibrinolytic indicators as biomarkers in paroxysmal atrial fibrillation* is undoubtedly promising, and our study is a prerequisite for its complementation by establishing:

- plasminogen activity and levels of its major activator t-PA were associated with the highest prognostic probability of paroxysmal atrial fibrillation;
- high plasma levels of TF, FVIII and vitronectin, measured in the early hours (≤24 hours) of the disease, showed a predictive value for occurrence of ischemic stroke.

VI. SCIENTIFIC CONTRIBUTIONS

The obtained results have an original character, on a fundamental and clinical level.

1. We performed a first-of-its-kind clinical study on coagulation balance in brief episodes of paroxysmal atrial fibrillation lasting ≤24 hours. Objective analysis of 20 indicators of the coagulation and fibrinolytic system was performed. Significant facts have been established regarding hemostatic indicators of the disease studied so far.

2. We presented direct convincing evidence for *development of early significant systemic hypercoagulability in the course of brief (≤24 hours) episodes of paroxysmal atrial fibrillation*. It objectively showed an increased *periprocedural thromboembolic risk in these episodes*.

3. We studied directly for the first time the relationship between the periprocedural thromboembolic potential of short (≤24 hours) episodes of paroxysmal atrial fibrillation and patients' thromboembolic risk characteristics and also examined the coagulation status in patients with different thromboembolic risk. It has been shown that there were no significant differences between patients at low (CHA_2DS_2-VASc score = 0 for men/1 for women) and highrisk (CHA_2DS_2-VASc score ≥1for both sexes).

4. We showed that *there was significantly lower procoagulant activity* during *the first six hours* of paroxysmal atrial fibrillation.

5. The presented results not only agree with the latest recommendations of the European Society of Cardiology for treatment of short (≤24 hours) episodes of paroxysmal atrial fibrillation, published in 2020, but significantly build on them:

- there is a clear need for timely periprocedural anticoagulant therapy until complete restoration of hemostatic profile in all patients with a short (≤24 hours) episode of paroxysmal atrial fibrillation, including those at very low risk (CHA_2DS_2-VASc score = 0 for men/1 for women).
- We provided the first scientific evidence to support the empirical rule of immediate anticoagulation in the course of atrial fibrillation;

- we presented arguments that there is need for the *earliest possible attempt to restore sinus rhythm in paroxysmal atrial fibrillation*, which would limit procoagulant deviations and associated periprocedural thromboembolic risk;
- we showed that the "*wait and watch*" approach during the first 24 hours of the disease, proposed in current therapeutic recommendations, *lead to accumulation of prothrombotic masses, and in this sense is inappropriate*;
- we propose to redefine "ideal candidates for acute cardioversion" proposed by the latest European recommendations and narrow down episode duration to the first six hours of the disease.

6. The studied clinical and laboratory indicators provide opportunities for their use beyond the hemostatic assessment. Their introduction in clinical practice would additional studies:

- creation of new effective anticoagulants, based on presented data on FXI and FXII, expecting greatly reduced hemorrhagic risk compared to established anti-FIIa and anti-FXa drugs;
- use of some of studied indicators as markers for prediction of paroxysmal atrial fibrillation;
- prediction of ischemic stroke, the main complication of paroxysmal atrial fibrillation.

Study limitations. Prospects for future research.

We consider main limitation of our study that coagulation and fibrinolytic indicators were studied only once in hospitalization of patients as well as the lack of follow-up after restoration of sinus rhythm. This was predetermined by the set aim and tasks, and embedded in the resulting study design. The need to study patients with CHA_2DS_2-VASc ≥1 (for both sexes), requiring initiation of long-term anticoagulation, precludes the possibility of monitoring indicators over time, as this would require their study against the background of anticoagulant treatment. This would compromise the reliability of the results obtained. At the same time, the presented limitation is a prerequisite for new research with a design that determines an objective possibility for studying the systems in dynamics.

VII. PUBLICATIONS ASSOCIATED WITH THE DISSERTATION

1. Full text publications in *Web of Science/Scopus*:

1. **Negreva MN**, Prodanova KS, Vitlianova KD. Paroxysmal atrial fibrillation is associated with early coagulation activity regardless of risk factors for embolism. Minerva Cardiol Angiol. 2021;69(3):269-276. doi: 10.23736/S2724-5683.20.05209-3. PMID: 32657551

2. **Negreva M**, Zarkova A, Prodanova K, Petrov P. Paroxysmal Atrial Fibrillation: Insight Into the Intimate Mechanisms of Coagulation. Cardiol Res. 2020;11(1):22-32. doi: 10.14740/cr972. Epub 2020 Jan 26. PMID: 32095193; PMCID: PMC7011925

3. **Negreva M**, Prodanova K, Zarkova A. Paroxysmal atrial fibrillation: an independent risk factor for prothrombotic conditions. JAFIB. 2020;13(2):2297. doi: 10.4022/jafib.2297. PMID: 34950291; PMCID: PMC8691306

4. **Negreva MN**, Prodanova K, Vitlianova K, Madjova C. Paroxysmal atrial fibrillation: changes in factor VIII and von Willebrand factor impose early hypercoagulability. Arch Med Sci Atheroscler Dis. 2020;5: e140-e147. doi:10.5114/amsad.2020.97101

5. **Negreva M**, Georgiev S, Vitlianova K. Early effects of paroxysmal atrial fibrillation on plasma markers of fibrinolysis. Medicine (Baltimore). 2016;95(45): e5184. doi: 10.1097/MD.0000000000005184. PMID: 27828845; PMCID: PMC5106051

2. Other full text publications:

1. Prodanova K, **Negreva M**, Vitlianova K. Diagnostic Values of Some Fibrinolytic Indicators for Rejecting the Presence of Paroxysmal Atrial Fibrillation. IJBSAC. 2020;2(12):1-6. doi: 10.35940/ijbsac.L0171.0421220

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3. Abstracts in *Web of Science*:

1. **Negreva M**, A Zarkova, J Ilieva. Paroxysmal atrial fibrillation: insight into the intimate mechanisms of coagulation. J Interv Card Electrophysiol. 2020;57(1):163-205. doi:10.1007/s10840-019-00665-1

2. **Negreva M**, Vitlianova K, Tasheva R. Early changes in FVIII and vWF plasma levels and activity in patients with paroxysmal atrial fibrillation. European Heart Journal. 2018;39(1): P6617. doi: 10.1093/eurheartj/ehy566.P6617

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4. Other abstracts:

1. **Negreva M**, Vitlianova K, Tasheva R. Early Markers of Coagulation Activity Prothrombin Fragments 1+2 and Fibrinopeptide A are Significantly Increased in Patients with Paroxysmal Atrial Fibrillation. JACC: Clinical Electrophysiology. 2017;3(10S): S23. doi:10.1016/j.jacep.2017.09.098

Participations in congresses, symposia, conferences:

1. **Negreva M**, Zarkova A, Ilieva J. Paroxysmal atrial fibrillation: insight into the intimate mechanisms of coagulation. 16th Edition of Venice Arrhythmias, Venice, 3-5.10.2019 (poster presentation)
2. **Negreva M**, Vitlianova K, Georgiev Sv. Paroxysmal atrial fibrillation: early changes in the plasma activity of coagulation factors proaccelerin (F V), antihemophilic globulin B (F IX) and antihemophilic globulin C (F XI). Eurothrombosis: annual meeting of the European Society of Cardiology Working Group on Thrombosis, Barcelona, 4-6.10.2018 (poster presentation)
3. **Negreva M**, Vitlianova K, Tasheva R. Early changes in FVIII and vWF plasma levels and activity in patients with paroxysmal atrial fibrillation. Congress of European Society of Cardiology, Munich, 25-29.08.2018. (poster presentation)
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VIII. CITATIONS ASSOCIATED WITH THE DISSERTATION

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