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

Changes in intracardiac haemostasis parameters during catheter ablation of atrial fibrillation in relation to the ablation technology and the periprocedural anticoagulation

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The date of PhD Defense is 21.06.2021. 11:30 a.m. We provide the publicity online. If you wish to participate in the discussion, please send an e-mail to orsolya.hajas@gmail.com until 20.06.2021. 4 p.m.

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List of abbreviations

AAD: antiarrhythmic drug

ACT: activated clotting time

AF: atrial fibrillation

aPTI: aktivated parcial thromboplastin time

CRYO/CB: cryoballon catheter

cc/min: cubic centimeter/perc

CHA_2DS_2-VASC: Congestive heart failure, Hypertension, Age 65-74 years, ≥75 years, Diabetes mellitus, Stoke/transient ischaemic attack/thromboemboli, Vascular disease, Age

COPD: chronic obstructive pulmonary disease

DOAC: direct oral anticoagulation

DW-MR: diffusion-weighted magnetic resonance imaging

EPS: electrophysiology study

FEU: fibrinogen equivalent unit

Fr: French

FVIII: VIII factor

hsCRP: high sensitivity C-reaktív protein

ICE: intracardiac echocardiography

IRF: irrigated radiofrequency catheter

INR: interntional normalized ratio

IQR: interquartile range

IU/kg: international unit/kilogramm

KVA: K-vitamin antagonist

LMWH: low molecular weight heparin

MES: microemboli signal

min: minute

OAC: oral antikoagulation

PAI-1: plazminogen activator inhibitor-1

PAP: plasmin-antiplasmin

PBP: point-by-point ablation

PVI: pulmonary vein isolation

PV: pulmonary vein

PVAC: pulmonary vein ablation catheter

s: second

SD: standard deviation

sVCAM-1: solubilis vascular cell adhesion molecule-1

SVT: supraventricular tachycardia

TCD: transcranial doppler

TIA: transient ischemic attack

VWF: von Willebrand factor

W: Watt

Introduction

1.1. Epidemiology and clinical relevance of atrial fibrillation

Atrial fibrillation (AF) is the most common arrhythmia with clinical relevance, and may be associated with reversible causes, e.g. acute myocardial ischemia, hyperthyroidism, infection, or alcohol abuse, however, it is much more common that such a direct cause of the arrhythmia cannot be explored. In the elderly, especially those over 65 years of age, common comorbidities include hypertension, heart failure, chronic renal failure, obstructive sleep apnea syndrome, and diabetes mellitus. In developed countries, the prevalence of PF is increasing exponentially, due in part to an aging population, a growing proportion of these comorbidities, and other so far unknown factors. In the latest 2020 guideline of the European Society of Cardiology currently, the estimated prevalence of atrial fibrillation in adults is between 2–4%, and a 2.3-fold increase is projected to increase by the middle of the 21st century as the average life expectancy of the population increases. AF alone is not life-threatening, but it significantly affects the quality of life, increases hospitalization, heart failure, may cause so-called tachycardiomyopathy, and because there is no atrial contraction during the arrhythmia, left atrial (appendage) stasis can lead to the formation of an intracardiac thrombus, which can enter the bloodstream and lead to peripheral or central embolization. AF is an increasing burden on the health care system.

1.2. The pathophysiology of atrial fibrillation

The initiation of AF is triggered directly by rapid electrical impulses, often from arrhythmogenic foci in muscle fibers creeping into the pulmonary veins. However, a number of different factors play a role in the persistence of arrhythmia. Such substrates include atrial tension fibrosis, hypocontactility, fatty infiltration, inflammation, vascular remodeling, ischemia, ion channel dysfunction, and calcium instability. All of these increase the development of ectopia and conduction disorders, increase the chances of AF development or persistence, and promote the development of a hypercoagulable state associated with AF. Lack of contractions reduces local endothelial shear, which increases plasminogen activator inhibitor expression, and ischemia-induced inflammation increases the expression of endothelial adhesion molecules or promotes

endothelial cell detachment, thereby making tissue factor available to the bloodstream. During persistent AF, electrical remodeling occurs, which further favors the persistence of arrhythmia. These lesions are initially reversible when the sinus rhythm is restored, however, further structural transformations occur upon AF persistence, which may explain the progressive nature of the disease.

1.3. Treatment of atrial fibrillation

There are three pillars for treating atrial fibrillation, one is stroke prevention, which means preventing of intracardiac thrombus formation, another is frequency control, which means optimizing ventricular rate, and the third is rhythm control, which aims to restore and maintain the normal sinus rhythm.

1.3.1. Anticoagulation

The essence of anticoagulation is stroke prevention, the necessity is determined by the CHA_2DS_2-VASc score system based on common stroke risk factors. The score system takes into account heart failure, diabetes mellitus, age over 65 and 75 years, previous ischemic stroke and transient ischemic attack (TIA), peripheral vascular disease, and female gender. Inhibition of coagulation is recommended for men with 1 or more points and for women with 2 or more points, which may be by LMWH or by oral anticoagulant administration. Oral anticoagulants include traditional vitamin K antagonists (VKAs) (acenocoumarol, warfarin), which have an indirect effect by inhibiting the synthesis of vitamin K-dependent coagulation factors (II, IX, X) and protein C and S inhibitors in the liver, and also the most commonly used direct oral anticoagulants (DOACs), the direct factor Xa inhibitor apixaban, edoxaban and rivaroxaban, and the direct thrombin inhibitor dabigatran. If anticoagulant therapy is contraindicated, implantation of special left atrial appendage closure device also should be considered.

1.3.2. Pharmacological rhythm and frequency control

For acut and long-term pharmacological frequency control frequency decelerators can be used, including beta-blockers, non-dihydropyridine-type

calcium channel blockers, and digitalis. For acut rate control pharmacologycal and electrical cardioversion can be performe, and for long-term rhythm control antiarrhythmic drugs (AAD) (amidoarone, propafenone, sotalol) can be used.
However, the chance of maintaining the sinus rhythm for 1 year with the available drugs does not exceed 50%. This limited efficacy explains the growing interest in non-drug methods, primarily catheter ablation treatment.

1.3.3. Pulmonary vein isolation

The cornerstone of transcatheter treatment of AF is the electrical isolation of the pulmonary veins and the atrial muscle fibers that grew into them and the left atrium. According to the latest 2020. recommendation of the European Society of Cardiology, the risk of intervention and risk factors for the recurrence of arrhythmias should be considered before the ablation procedure, as well as the patient's preference. For rhythm control of patients with symptomatic proxysmal and persistent AF with a low risk of arrhythmia recurrence, catheter ablation could be the first-line therapy, and also PVI is recommended for rhythm control after one failed AAD, to improve symptoms of AF recurrences in symptomatic patients with paroxysmal and persistent AF without structural heart disease. For therapy refractory patients with heart failure with reduced left ventricular function, regardless of symptoms, PVI should be considered to improve quality of life and ejection fraction, and to reduce hospitalizations and mortality.
During the procedure, after penetration through the femoral vein, catheters are inserted into the right atrium, and after the puncture of the atrial septum, we reach the cavity of the left atrium, where the antrum of the pulmonary veins can be found. Pulmonary vein isolation (PVI) is traditionally performed with a focal irrigated radiofrequency (IRF) catheter, and also „single shot" ablation procedures are widely used, which include a circular multipolar phase radiofrequency ablation catheter (PVAC) and cryoballoon (CB) isolation.

1.3.3.1. *Pulmonary vein isolation with focal irrigated fadiofrequency catheter*

The "gold standard" or traditional technique of PVI is focal radiofrequency (RF), which is the "point by point" technique of enclosing pulmonary veins. To

ensure that these 3-4 mm diameter ablation lesions form a continuous line, the location of each ablation lesion is visualized with a 3-dimensional navigation or mapping system, making the anatomy of the left atrium and pulmonary veins visible, while the real time position and movement of the tip electrode of the catheter movement is also visible [1].

The creation of connected and transmural ablation circles is a complex mission that requires a lot of practice, which is time consuming, and there can be significant differences between centers in the incidence of success and complication rate [2].

1.3.3.2. *Single-shot ablation techniques*

These technical challenges have given rise to single-shot PVI techniques that are simpler, faster to master, and widely reproducible in terms of results [3-6]. A common feature of these is that by placing the ablation catheter in the antrum of veins, without constant movement, it is possible to form the ablation line around the antrum with either a single or a few energy releases. The following methods have become more widespread.

Phased radiofrequency ablation

On the Pulmonary Vein Ablation Catheter (PVAC: Pulmonary Vein Ablation Catheter, Medtronic Inc. Minneapolis, MN, USA) originally 10 (5 pairs) electrodes were placed and it was modified to 9 electrodes in the improved version. The electrodes form an almost complete circle through which phased RF energy can be delivered to all or only certain poles at the same time. In the case of phased-RF, the generator connected to the catheter (GENiusTM; Medtronic Inc. Minneapolis, MN, USA) delivers energy not continously, but active phase s alternate with pause phases, which ensures that the electrodes are cooled by the blood before overheating.

The catheter is positioned in each PV orifice using a guidewire through a transseptal sheath, which is monitored by contrast injection. Energy transmission to all poles simultaneously can result in complete isolation.

In addition to intermittent energy transfer, a further difference from conventional RF ablation is that the GENiusTM ablator has a bipolar: unipolar ratio that can be set: in unipolar mode, RF energy flows between the electrode and the indifferent placed ont he back, in bipolar mode the RF energy flows between adjacent electrodes. In unipolar mode, the tissue lesion will be deeper, bipolarly, the lesion will be more continuous. The target temperature is 60° C, which can be measured separately per pole.

Cryoballon ablation

The freezing-based cryoballon ablation (CB) is also a single-shot technique, which is a "tissue-friendly" method of creating tissue lesions. Compared to "burning" RF ablation, it is less thrombogenic, reducing tissue rupture, and thus the risk of even the most severe complications. The components of the system are the balloon catheter, a guidewire that allows the positioning of the catheter and the display of the electrograms in the PV orificle, and a freezing console. The balloon catheter has a double lumen and liquid nitrous oxide flows from the console into the inner circuit, which can cool it to -70 ^{o}C. The balloon catheter has a variable curvature to aid positioning in the PV's orificle, after the balloon is inflated, angiography follows to check the occlusion of the vein. Circumferential electrodes of a special guidewire (Achieve, Medtronic Inc, Minneapolis, MN, USA) continously allows to monitor the PV potentials during freezing.

Results with single shot techniques

The results of several, mostly single-center, limited-sample studies suggest that single-shot ablation methods are similar to those obtained with focal RF ablation in terms of short-term efficacy and risk of complication, but with a shorter intervention time.

With phase RF ablation, a research reported 68% 1-year arrhythmia free [7]. Nardi reported a similar success rate of 68.5% in 22 ± 5 months [8], Wieczorek reported a success rate of 79% at 1 year follow-up [9], while Boersma reported a success rate of 55% at 1 year and 49% at 2 years [10].

In a multicenter European registry after 2.3 ± 1 years follow up, a success rate of 82% was reported in paroximal AF and 70% in persistent AF [11], and these results were also reproduced in another metaanalysis [12]. Similar results have been reported with CB ablation. Van Belle found SR maintaining at 49% with one intervention and a mean follow-up of 225 ± 137 days without antiarrhythmic drug, which increased to 59% with repeated intervention [13]. With long-term follow-up (5 years), Neumann reported a success rate of 53% [14] and a Polish researcher group 77% [15]. Brugada [16] and Chun [17] found 57.5% of success rate with single intervention and 48% with repeated. In a retrospective observational study, based on 605 patients nearly 3 years of follow-up, had a single procedure success rate 61.6%, which increased to 76.9% after up to 3 repeated interventions performed as needed [18]. Studies directly comparing single-shot and conventional ablation techniques [19–24] conclude that with a similar success rate, single-shot ablation methods can be performed with a shorter intervention time. Our own initial results [25] are similar. At a mean follow-up of 2.5 years without antiarrhytmic terapy, with phased RF ablation 55% and with CB ablation 41% succes rate was documented, which increased to 65% (PVAC) and 47% (CB) in case of using antiarrhythmic agent.

For each of the different ablation techniques performed for PVI, it can be declared that after an acutely successful intervention, later repeated intervention may be necessary [26 - 32], due to the electrical reconnection of PV [33 - 35]. In doing so, SR is expected to be maintained from transcatheter re-isolation of PV. Repeated PVI can be performed using the ablation method used at the first intervention or a different one, in this perspective the practice of the centers is heterogeneous. In our institute, we achieved 79% of success rate after nearly 2 years of follow-up by phased RF re-isolation after CB ablation [36].

1.4. Periprocedural anticoagulation

Transcatheter treatment of AF is a complex intervention performed in patients with an inherently higher risk of thromboembolic complications, including stroke, than in healthy population. Therefore, it is not surprising that cerebrovascular complications are among the most dreaded complications since the beginning of PF ablation [37].

Morady et al. [38] studied 755 patients who underwent AF ablation with continuous VKA therapy, and the thromboembolic complication after intervention was 1.1%. The results of the study clearly demonstrated that early postprocedural thromboembolic events occurred within the first 2 weeks after intervention and developed as a result of left atrial ablation regardless of postprocedural rhythm and the patient's risk of stroke. The most likely cause was considered to be hypercoaguability due to left atrial endocardial tissue injury. Postoperative manifest cerebral embolization may result in asymptomatic, clinically silent cerebral ischemia (SCI) as evidenced by Diffusion-Weighted Magnetic Resonance Imaging (DW-MRI) within 48 hours after AF ablation [39], as a small-scale white matter lesion that was not yet detectable before ablation. The occurrence of SCI after AF ablation has been confirmed in several studies, and its frequency has been confirmed to be between 5-40% [40 - 45]. The importance of SCI, although disputed, cannot be ruled out as a possible role in the long-term deterioration of patients' cognitive function. For a more accurate understanding of the phenomenon, in addition to comparative DW-MR results, several working groups reported their results in the analysis of microembolic signals (MES: Microembolic Signal) detectable in the cerebral artery by transcranial Doppler (TCD) [46,47].

Kilicaslan used TCD monitoring already during AF ablations to measure microembolisms generated by RF energy [48] and found a correlation between MES numbers and between the amount of bubbles that can be displayed in the left atrium by intracardiac echocardiography (ICE), based on a semiquantitative scale. A comparative study by another group found that CB ablations generate less microembolus formation than RF (focal or phase) techniques [49]. Our group also investigated the phenomenon of microembolization with transcranial Doppler and intracardiac echocardiography during both cryoballoon and multipolar phase RF ablation [50 - 53]. We demonstrated that CB ablations generate microembolization to a significantly lower extent than the phase RF technique with intraprocedural hepatinization with either a lower or higher ACT target. Our studies also demonstrated that while catheter manipulations in the left atrium during CB ablations are associated with uniform MES formation, the formation of microembolus during phase RF interventions is concentrated during energy transfer. We also considered it important to demonstrate that,

using any technique, 80% of the microemboluses were gas. We have also observed that the rate of microembolus formation can show extreme values within an intervention, depending on the location of ablation and the degree of tissue contact. These results also support the importance of aggressive intra- and periprocedural anticoagulation. The usual practice during the procedure is to administer intravenous fractionated heparin until an activated clotting time (ACT) of at least 300 seconds is reached during left atrial manipulation, however, there is no consensus on the preprocedural anticoagulation strategy.

Transeptal puncture and catheter manipulations in the thin-walled atrium, as well as the release of ablation energy, may pose a risk of a life-threatening bleeding complication that precludes continuous administration of any preoperative anticoagulant. Despite these concerns, randomized clinical trials have demonstrated the safety or even superiority of VKA-type anticoagulants administered continuously at therapeutic doses during the perioperative period [54 - 55]. Recently, a numerous of different studies have been conducted that have examined DOACs from a similar perspective, and factor Xa inhibitors have demonstrated the same or greater safety with VKA with similar efficacy. However, conflicting results have been published for the thrombin inhibitor dabigatran. In a multicenter, non-randomized observational study, the rates of thromboembolic and bleeding complications were also statistically significantly higher with dabigatran administered continuously than with VKA administered similarly [56]. In other randomized trials with continuous administration of dabigatran [57-58], there were significantly fewer bleeding complications than with continuous VKA therapy. Recent studies suggest that the extent of local hemostasis activation and endothelial damage in patients with atrial fibrillation may be more accurately reflected by an intracardiac blood sample than by a systemic circulation sample, however, due to the difficulty of sampling, the direct effects of various anticoagulants during PVI from intracardiac samples have not been previously studied.

2. Aims

In our research, we examined 2 issues.

1, What are the changes in hemostasis in left atrial samples when different perioperative anticoagulation strategies are used during cryoballoon abation, which is currently considered to be the most tissue-sparing and safe.

2, We were also looking for an answer to how different ablation techniques effect hemostasis changes associated with PVI in addition to the currently widely used periprocedural anticoagulation method, uninerrupted vitamin K antagonist treatment.

3. Patients and methods

3.1. Patients

Consecutive patients undergoing radiofrequency ablation for symptomatic paroxysmal or persistent AF were enrolled in the study based on the following: age 18–75 years, documented, symptomatic paroxysmal or persistent AF, failure of at least one antiarrhythmic drug, and willingness to sign a written informed consent. Exclusion criteria included long-standing persistent AF, reversible cause of AF (e.g. hyperthyroidism), presence of a left atrial thrombus, previous heart surgery, valvular heart disease, left ventricular ejection fraction (LVEF) ≤30%, heart failure of New York Heart Association functional classification (NYHA) class III or IV, documented carotid stenosis, history of ischemic stroke or TIA, prior cardiac surgery, unstable angina or myocardial infarction within the last 3 months, severe chronic obstructive pulmonary disease, known bleeding or thrombotic disorders, acute inflammation, contraindication to oral anticoagulation, and pregnancy.

The study design was in accordance with the guiding principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the University of Debrecen and the Ethics Committee of the National Medical Research Council. All patients signed a written informed consent form prior to inclusion.

3.2. Pre-procedural anticoaculation

For all included patients, antiplatelet medications were dicontinues before the procedure for a period at least 7 days. Transoesophageal echocardiography was performed within 24 h prior to the procedure to rule out the possibility of a cardiac thrombus.

3.2.1. Anticoagulation in the cryoballoon ablation group (1. study)

In the part of our research in which we examined the effect of different perioperative anticoagulation methods, we used one of the following 3 therapeutic options:

1, No anticoagulation (OAC free) group. In this group patients didn't take any anticoagulant medicine.

2, Patients in VKA group were an uninterrupted VKA therapy for at least 30 days pre-ablation to maintain a therapeutic international normalised ratio (INR) between 2-3, which was confirmed ont he morning of the procedure.

3, Dabigatran 150 mg BID for at least 30 days with the last dose given 2 h prior to the procedure (Dabigatran group). Dabigatran was administered to all patients exactly 2 h before the scheduled start of the ablation, by the form of controlled pill intake as inspected by a nurse.

Pre-procedural anticoagulation was kept unchanged as compared to the treatment patients had been receiving at the time the ablation was scheduled, this was our study wasn't randomized.

3.2.2. Antikoagulálás a fázisos rádiófrekvenciás, cryoballon és fokális irrigált rádiófrekvenciás csoportban (2. kutatás)

A különböző ablációs eljárások (PVAC, CRYO vagy IRF) során létrejövő hemosztázis változásokra irányuló vizsgálatban, a betegek egységesen KVA-t szedtek, INR értékük a beavatkozás reggelén a terápiás tartományban volt.

3.3. Catheter ablation

Procedures were performed under conscious sedation, using midazolam and fentanyl according to standard practice at our institution. Puncturing the femoral vein was performed using the Seldinger technique and introducers with side arms were placed in the vein. A multipolar electrode catheter was placed in the coronary sinus and an intracardiac echocardiography (ICE) catheter in the right atrium to guide transseptal puncture and positioning the catheter. A single transseptal puncture was performed with a Brockenbrough needle (Medtronic, Minneapolis, MN, USA) and a Mullins sheath (Medtronic Inc., Minneapolis, MN, USA) was advanced in the left atrium. Immediately after transseptal puncture and pre-ablation blood sample collection, a 150 IU/kg body weight intravenous heparin bolus was given, followed by a continuous infusion to maintain a minimum target activated clotting time level of >300 s.

PVI was then performed using one of the 3 ablation techniques detailed below. If necessary, cardioversion was performed at the end of the procedure.

The following ablation techniques were used: cryoballon (CB), phased radiofrequency (PVAC), or focal irrigated radiofrequency (IRF) ablation. Among these, the choice was determined by the preference of the patient and the operator.

3.3.1. Cryoablation

Cryoablations were carried out with the 28 mm second-generation CB (Arctic Front Advance; Medtronic Inc., Minneapolis, MN, USA). The Mullins sheath (Medtronic Inc., Minneapolis, MN, USA) was replaced for the deflectable 12-Fr long FlexCath sheath (FlexCath, Medtronic CryoCath LP, Kirkland, Quebec, Canada) and introduced into the LA over a stiff long guidewire. An 8-pole, circular electrode catheter was advanced through the lumen of the cryoballoon and used as a guidewire to cannulate specific side branches of the PVs to allow the continuous monitoring of PV electrograms during each freezing cycle. The balloon was manipulated to obtain the potentially most antral position while maintaining a sufficient seal of the vein as assessed by contrast injection and ICE Doppler. Two freezing cycles of 3–4 min duration were usually applied in each PV based on the achieved temperature and the time to PVI. Temperatures between −40 °C and −55 °C were considered suitable for the procedure.

Cryoapplication was terminated in case of lower values in order to minimize the risk of collateral damage. Procedural end-point was PVI, defined as PV-LA entrance block verified with pacing maneuvers according to standard practice.

3.3.2. Phased radiofrequency ablation

Phased RF ablation protocol at our center and technical details of the 2nd-generation PVAC have been described earlier. Briefly, the Mullins sheath (Medtronic Inc., Minneapolis, MN, USA) was exchanged for a deflectable 12-Fr long FlexCath sheath (FlexCath, Medtronic CryoCath LP, Kirkland, Quebec, Canada) and advanced into the LA over a 220 cm guidewire. Submerged loading of the circular PVAC- Gold containing 9 electrodes of gold alloy into the introducer as well as continuous flushing of the sheath with heparinized saline was performed to minimize air ingress. Duty-cycled bipolar and unipolar phased RF energy to all or selected electrode pairs was delivered in a temperature-controlled and power-limited fashion (60°C, maximum 10 W) with typical ablation duration of 60 s. Pulmonary veins (PVs) were electrically isolated by targeted ablation of each PV-LA antrum.

3.3.3. A point-by-point abláció

After 2 separate transseptal punctures, a Mullins transseptal and a 9F steerable Agilis sheath were placed in the LA. A circular decapolar Lasso catheter (LASSO™, Biosense Webster Inc., Diamond Bar, CA, USA) and a contact force ablation catheter were advanced through the Mullins and the Agilis sheath, respectively. A 3D anatomical map of the LA was obtained using the Carto Merge System (Biosense Webster Inc., Diamond Bar, CA, USA). Predefined lines for the ablation circles were tagged in the antrum of the PVs. Point-by-point ablation was performed with focal irrigated ablation catheter (Smarttouch, Biosense Webster Inc., Diamond Bar, CA, USA) isolating the left and the right PVs in separate circles. RF energy was delivered in power-controlled mode without ramping using 30–35W on anterior segments and 20–25W on posterior wall with an irrigation flow of 17 cc/min. Energy was delivered for 30 s at each

site after obtaining a target contact force >6 g. Ablation tags were displayed using Visitag (Biosense-Webster Inc, Diamond Bar, CA, USA). PVI in each PV was assessed based on signals recorded through electrodes of the Lasso catheter.

3.4. Blood sampling and laboratory investigations

Sampling was performed in the same manner in both studies. Preablation blood samples were collected through the Mullins sheath from the LA immediately after transseptal puncture and the removal of the dilator, before the intravenous administration of unfractionated heparin. Post-ablation blood samples were drawn through the LA sheath after removal of the CB catheter, immediately after the last ablation was completed. Forty-five ml blood samples were collected, of which the first 15 mL was discarded to exclude intra-sheath activation of hemostasis. Blood was drawn into vacutainer tubes (tubes containing 0.109 M sodium citrate, and tubes containing no anticoagulant with polymer gel separator: SST tubes, Becton Dickinson, Franklin Lakes, NJ, USA). Citrated blood samples were centrifuged twice at 1500 *g*, room temperature for 15 min, while SST tubes were centrifuged once at 2000× *g*, at room temperature, for 20 min. Plasma and serum samples were aliquoted and stored at −80 °C until further analysis. Screening tests of hemostasis (prothrombin time, activated partial thromboplastin time, thrombin time) and fibrinogen levels (based on Clauss method) were assessed from freshly separated plasma samples using standard methods (Siemens Healthcare Diagnostic Products, Marburg, Germany). Stored plasma samples were used to perform specific hemostasis and fibrinolysis assays, all measurements were carried out by investigators blinded to clinical data. Quantitative D-dimer levels were measured by a particle-enhanced, immuno-turbidimetric assay (Innovance D-dimer) on a BCS coagulometer following the manufacturer's instructions (Siemens Healthcare Diagnostic Products, Marburg, Germany). The levels of von Willebrand factor (VWF) antigen, chromogenic factor VIII (FVIII) activity, and α2-plasmin inhibitor (α2-PI) activity were measured by commercially available methods on a BCS coagulometer (Siemens Healthcare Diagnostic Products, Marburg, Germany). The levels of plasmin-α2-antiplasmin (PAP) complex were measured using an ELISA test (Technozym PAP complex ELISA kit, Technoclone, Vienna, Austria). Soluble fibrin monomer (FM) levels were determined using the Liatest FM assay (Diagnostica Stago, Asnieres, France). High sensitivity C-reactive protein (CRP) was

measured from stored serum samples by routine methods (Roche Diagnostics, Mannheim, Germany). A direct thrombin inhibitor assay was used to assess dabigatran peak levels, as measured from antecubital vein blood samples drawn at the beginning of the ablation procedure (INNOVANCE DTI, Siemens Healthcare Diagnostic Products, Marburg, Germany; reference range in local laboratory: 64–443 μg/mL) [59].

3.5 Statistical Analysis

Statistical analysis was carried out using GraphPad Prism Software version 6.0 (La Jolla, CA, USA) and the Statistical Package for Social Sciences (SPSS, Release 26.0, Chicago, IL, USA). Normality of the data was evaluated by the Shapiro-Wilk and the D'Agostino and Pearson omnibus tests. A paired t-test or Wilcoxon matched-pairs rank-sum test was performed when comparing results obtained from pre-ablation and post-ablation intracardiac samples. ANOVA with Bonferroni post-hoc test or Kruskal-Wallis test using Dunn's-Bonferroni post-hoc test was applied for multiple comparisons of unpaired data. In order to adjust for covariates associated with selection, analysis of covariance (ANCOVA) was performed (after logarithmic transformation of data when necessary). Differences between categorical variables were studied using the χ^2 or Fisher's exact test. $p<0.05$ was considered statistically significant.

4. Results

4.1. Haemostasis activation and fibrinolysis during cryoballoon ablation with different anticoagulation strategies

4.1.1. Baseline patient and procedure characteristics

A total of 52 patients were enrolled in the study. No pre-ablation anticoagulation was applied in 24, uninterrupted VKA in 11, and uninterrupted dabigatran in 17 patients. No difference in baseline patient characteristics (age, sex, smoking body-mass index), or in relevant comobidity (hypertension, hypercholesterolaemia, diabetes mellitus), or in LA dwelling times were found. No thromboembolic or major bleeding complications

occurred in any of the patient groups. No patients experienced cerebral events during or after the procedure.

4.1.2. Haemostasis and fibrinolysis parameters

D-dimer levels increased significantly in LA blood samples in all three groups after ablation. Measured values (median; IQR = interquartile range) before vs. after ablation were (0.48; IQR 0.81 vs. 1.09; IQR 1.30 mg/L, p <0.001) in the OAC free group, (0.33; IQR 0.21 vs. 0.74; IQR 0.26 mg/L, p <0.01) in the VKA group and (0.10; IQR 0.20 vs. 0.27; IQR 0.36 mg/L, p <0.001) in the dabigatran group. Both pre- and post-ablation values were significantly lower in patients on dabigatran as compared to what was obtained in the OAC free and in the VKA groups (p <0.01). Of note, post-ablation levels of D-dimer did not exceed the 0.5 mg/L cut-off in patients treated with dabigatran.

Similarly to D-dimer, PAP complex levels increased significantly after the ablation in both the OAC free (317.57; IQR 140.43 vs. 373.07; IQR 144.93 ng/mL, p <0.05) and the VKA groups (237.56; IQR 102.77 vs. 253.88; IQR 99.51 ng/mL, p <0.05), but only a non-significant trend was observed in the dabigatran group (216.82; IQR 104.33 vs. 235.67; IQR 142.89 ng/mL, p > 0.05). Of note, significantly increased post-ablation values were found in patients not treated with any OAC as compared to those on VKA (p <0.05) or on dabigatran (p <0.01).

A significant decrease in α2-PI activity, indicating enhanced fibrinolysis post-ablation, was demonstrated in the OAC free group (110; IQR 18 vs. 104; IQR 16%; p <0.01) but not in patients on either VKA or dabigatran therapy. In line with these results, a significant consumption of fibrinogen after the ablation was only observed in the OAC free group (3.11; IQR 0.65 vs. 2.99; IQR 0.81 g/L; p <0.01), but not in patients treated with any pre-procedural OAC.

Similarly to earlier published studies [60], pre-ablation median and IQR levels of fibrin monomers were above the upper limit of reference in all groups, due to hemostasis activation related to the catheterization procedure and left atrial sampling. Due to the short (t (1/2) =2.3 h) half-life of this protein [61], a significant decrease was observed after the ablation procedure in all patient groups (OAC free group: 64.35; IQR 52.83 vs. 26.34; IQR 30.04 mg/L; p <0.001; VKA group: 36.14; IQR 92.56 vs. 10.12; IQR 16.01 mg/L; p <0.01; dabigatran group: 38.37; IQR 153.06 vs. 3.98; IQR 2.0 mg/L; p <0.001). Of note, in case of the dabigatran group, remarkably low levels of fibrin monomers were

found post-ablation. In all patients of the dabigatran group, fibrin monomer levels returned to below the limit of reference of coagulation activation, indicating significantly lower additional hemostasis activation during the ablation procedure in this patient group as compared to the VKA group and the OAC free group. In the OAC free group, post-ablation fibrin monomer levels were mostly above the reference range, suggesting a lasting activation of coagulation during the ablation procedure.

4.1.3. Local endothelium activation in the left atrium during cryballoon ablation with different preprocedural anticoagulation strategies

VWF antigen levels showed a similar increase in all groups post-ablation as compared to pre-ablation levels, suggesting comparable procedure-related endothelial damage (OAC free: 138.6; IQR 38.83 vs. 214.1; IQR 58.55%; p <0.001; VKA: 138.9; IQR 133.8 vs. 196.1; IQR 123.6%; p <0.01; dabigatran: 148; IQR 87.5 vs. 192.0; IQR 112.0%; p <0.01).

As expected, FVIII activity showed a similar pattern to what was observed in case of VWF antigen levels, with no difference amongst groups of various pre-procedural anticoagulation strategies (OAC free: 107; IQR 72 vs. 164; IQR 62.25%; p <0.001; VKA: 154; IQR 135 vs. 226; IQR 149%; p <0.01; dabigatran: 110; IQR 32 vs. 144; IQR 88.5%; p <0.01).

4.2. Hemostasis changes during pulmonary vein isolation with different ablation techniques in case of patients treated with vitamin K antagonist therapy

4.2.1. Baseline of patients and procedure characteristics

Total of 31 patients were included in this study. PVI was performed with phased RF in 7, with cryoballoon in 10, and with point by point IRF ablation in14 patients. Acute PVI was achieved in all PVs in all patients. No difference in baseline characteristics was found between the 3 groups regarding demographics (age, male sex), body-mass index, relevant comorbidities (hypertension, hypecholesterolaemia, diabetes mellitus), echocardiographic parameters (LA diameter, ejection fraction), and thromboembolic risk (CHA_2DS_2VASc score) (p value >0,05). Left atrial access times were

significantly longer with IRF ablation (p value <0,001). No procedural complication occurred in any of the patients.

4.2.2. Changes of fibrinolysis parameters during pulmonary vein isolation with different ablation techniques

Levels of D-dimer in left atrial blood samples increased significantly after ablation with the PVAC (pre-ablation median: 0,34, IQR: 0,24-0,50 mgFEU/L, post-ablation median: 0,70, IQR: 0,61-1,31 mgFEU/L; p=0.0313), cryo (pre-ablation median: 0,33, IQR: 0,28-0,49 mgFEU / L; post-ablation median: 0,79, IQR: 0,65-0,93 mgFEU/L; p=0.0078), and IRF (pre-ablation median: 0,33, IQR: 0,21-0,44 mgFEU/L; post-ablation median: 0,83, IQR: 0,56-1,21 mgFEU/L; p=0.0001). Postablation values exceeded the cutoff value of D-dimer (0.5 mg FEU/L) with all 3 ablation modalities. Levels of PAP complex demonstrated no significant change with phased RF ablation (pre-ablation median: 344,9, IQR: 273,6-577,5 ng/ml; post-ablation median: 361,5, IQR: 313,5-548,9 ng/ml; (p=0.2969), but significantly increased after cryo (pre-ablation median: 247,3, IQR: 199,9-331,6 ng/ml, post-ablation median: 270,9, IQR: 227,9-346,7 ng/ml; p=0.0020) and irrigated RF (pre-ablation median: 265,3, IQR: 202,0-800,1 ng/ml, postablation median: 325,6, IQR: 250,2-701,9 ng / ml; p=0.0166). However, preablation and post-ablation median PAP values did not exceed the cutoff value proposed by the manufacturer. PAI-1 activity decreased significantly during ablations with the PVAC (pre-ablation median: 1,931, IQR: 0,508-3,859%; post-ablation median: 0,735, IQR: 0,240-2,707%; p=0.0313) and cryo (pre-ablation median: 0,361, IQR: 0,080-1,575%; post-ablation median: 0,378, IQR: 0,111-0,915%; p=0.0313). A nonsignificant trend was observed with IRF (pre-ablation median: 0,548, IQR: 0,303-1,710%; post-ablation median: 0,500, IQR: 0,122-1,328%; p=0.0676).

4.2.3. Local endothelial damage during pulmonary vein isolation with different ablation techniques

VWF antigen levels increased significantly during ablations with all 3 types of catheters. Significant increase was observed after PVAC ablation (pre-ablation median: 175,4, IQR: 146,3-212,8%; post-ablation median: 200,1; IQR: 174,4-248,9%; p=0.0313), after CB ablation (pre-ablation median: 138,9, IQR: 91,6-186,7%; postablation median: 189,9, IQR: 124,1-254,1%; p=0.0039), and a highly significant increase occurred after IRF

ablations (preablation median: 136,0, IQR: 113,7-203,5%; postablation median: 203,0, IQR: 143,3-255,7%; p=0.0002). The increase in FVIII activity levels during the ablation procedures was concordant with the increase in VWF antigen levels. A significant elevation in FVIII activity was observed after phased RF (pre-ablation median: 158,5, IQR: 129,0-174,0%; post-ablation median: 198,9; IQR: 180,0-302,1%; p=0.0355), after CB (pre-ablation median: 153,0, IQR: 94,25-185,8%; post-ablation median: 229,0; IQR: 155,7-333,1%; p=0.0078), and a highly significant elevation was found after irrigated RF ablations (pre-ablation median: 152,0; IQR: 99,0-186,5 %; post-ablation median: 194,6, IQR: 139,8-316,0%; p=0.0002). The levels of soluble VCAM-1 (ng/ml) were not significantly different in the left atrium before and after the PVAC procedure (pre-ablation median: 644,6; IQR: 544,1-798,2 ng/ml; post-ablation median: 698,1; IQR: 586,5 -801,9 ng/ml; p=0.0963); however, significant increase was detected after cryo (pre-ablation median: 542,6, IQR: 428,5-753,1 ng/ml; post-ablation median: 619,2, IQR: 499,8-799,0 ng/ml; p=0.0005) and also after IRF (pre-ablation median: 679,3, IQR: 505,0-744,7 ng/ml; post-ablation median: 770,9, IQR: 631,9-894,0 ng/ml; p <0.0001).

5. Discussion

Limited data and conflicting results are available on hemostasis activation related to invasive electrophysiology procedures. Previous studies evaluated fibrinolysis activation in blood samples obtained from the femoral vein in patients undergoing RF ablation for supraventricular tachycardias (SVTs). Dorbala et al. [62] compared different markers of coagulation and fibrinolytic activation measured in blood samples from the femoral vein obtained immediately after sheath insertion, after a diagnostic electrophysiology study (EPS), and after RF ablation. Significant hemostasis activation was found after EPS as compared to after sheath insertion but no further increase postablation, suggesting that insertion of foreign materials (sheaths, wires, and catheters) in the bloodstream is a significant activator of coagulation. In another report on 37 patients, procedure duration, but not the number of RF ablation, correlated with hemostasis activation. On the contrary, Parizek et al. [63] reported a significant elevation in D-dimer levels after diagnostic EPS and which further increases after ablation. A statistical correlation between *D*-dimer levels and the number of RF applications was also demonstrated. Importantly, well-de- fined, small-size substrates mainly in the right cardiac chambers were targeted by the ablation in patients who were not anticoagulated before the procedure in these studies. Data on hemostasis activation related to AF ablation were

reported by Bulava et al. [64]: D-dimer levels dem- onstrated a rapid elevation after sheaths and intracardiac catheter placement with further increase after RF applica- tions, which still persisted 24 hours after ablation. This study was carried out 15 years ago and thereby represents the AF ablation routine of that time: nonirrigated focal RF lesions were placed around or inside the PVs, vitamin K antagonist was interrupted using low molecular weight heparin bridging before the procedure, and intraprocedural heparin applied according to activated partial thromboplastin time (aPTI). Of note, blood samples in this investigation were also obtained from the femoral vein. As suggested by the results of these studies, insertion of any foreign material into the blood stream initiates hemo- stasis activation. Previously, our group measured hemostasis activation in blood samples obtained sequentially from different sites including the femoral vein, the left atrium, and the left atrial appendage without any ablation therapy. In this earlier work, which was completed in a different patient cohort than the present study, we demonstrated that transseptal puncture itself is a significant signal for further hemostasis activation [65]. In our research, we were the first to analyze complex left atrial hemostasis changes during atrial fibrillation ablation, using different preoperative anticoagulation strategies during cryoballon ablation, and uniformly with a vitamin K antagonist in case of different ablation procedures. In both studies, blood samples were taken from the left atrium before the first application and immediately after the last energy release. Intraprocedurally, after the first sampling, heparin was administered according to the protocol in all cases until the ACT exceeded 300 s.

5.1. Hemostasis activation and fibrinolysis

Our results confirm that pre-ablation OAC treatment provides significant inhibition of hemostasis activation triggered by left atrial ablation with the second generation cryoballoon, a technology widely used for transcatheter treatment of AF today. Moreover, our study is the first to provide a complex hemostasis analysis of the left atrium indicating that during cryoballoon catheter ablation, dabigatran provides greater inhibition against intracardiac activation of hemostasis and consequent fibrinolysis as compared to VKAs. We demonstrated that in patients treated with dabigatran, inhibition of local, intra-atrial coagulation activation related to the ablation procedure is markedly suppressed. Indeed, levels of D-dimer, the most commonly used marker to detect a prothrombotic state, were kept below the generally used cut-off of hypercoagulation (0.5 mg/L) in both pre- and post-ablation intracardiac blood samples by dabigatran only,

but not in patients treated with VKA or those who received no anticoagulation before the procedure. Similar results were obtained when evaluating a complex set of heparin-insensitive markers of hemostasis/fibrinolysis activation. The level of fibrin monomers, a highly sensitive marker of prothrombotic hemostasis balance was strikingly low in dabigatran-treated patients post-ablation. Although less sensitive markers indicating consumption of hemostasis or fibrinolysis factors do not necessarily show the subtle difference between different anticoagulant regimens, those still demonstrated a considerable activation of hemostasis in patients without pre-procedural anticoagulation, despite receiving heparin during the ablation procedure. Overall, the comprehensive evaluation of our results suggests that dabigatran has the strongest potential to prevent the hypercoagulable state related to a left atrial ablation procedure, while patients undergoing AF ablation with effective VKA anticoagulation might still be exposed to significant hemostasis activation. Moreover, marked coagulation activation was observed in patients receiving no pre-ablation OAC therapy, supporting previous clinical observations on the increased hypercoagulable state carrying a potential risk of thromboembolic events in these patients.

In our study, when the effect of different ablation technique on hemostasis was researchedT, blood samples were obtained from the LA before the 1st and immediately after the last energy application. Importantly, our periprocedural anticoagulation scheme represented a common practice of AF ablation centers based on recent guideline recommendations [14]. This included uninterrupted VKA with no bridging, INR level in the therapeutic range before the procedure, and intravenous administration of unfractionated heparin to maintain the ACT level above 300 sec throughout catheter dwelling in the LA. Despite these measures, significant coagulation activa- tion was observed as indicated by the marked change in the levels of different fibrinolysis markers. Post-ablation D-dimer levels exceeded the normal cutoff value and elevated in the range usually observed during clinical events related to thrombus formation, such as deep vein thrombosis or pulmonary embolism. The levels of PAP complex increased significantly after ablations with cryo and IRF but not with the PVAC. As another indication of coagulation activation, a significant decrease in PAI-1 activity was measured with phased RF and cryo, and a trend for lower values was de- tected after IRF ablations. These results suggest that sig- nificant coagulation activation during AF ablation may not be prevented with the anticoagulation scheme representing a common practice of these days.

5.2. Endohel activation

Endothelial damage is a known prothrombotic mechanism as a component of Virchow's triad. The association between left atrial appendage thrombus formation and endocardial expression of VWF has been described [66]. Further, previous studies [64, 67] demonstrated that VWF remains elevated for 24–48 hours after PVI with nonirri- gated RF. Prolonged endothelial dysfunction might explain previous observation that thromboembolism mostly occurs within 48–72 hours after AF ablation in the majority of the cases [38]. CB ablation induced significant endothelial activation as indicated by increased VWF antigen levels and FVIII activity in the post-ablation blood samples, regardless of pre-ablation anticoagulation treatment. These results suggest that the ablation technology and the area covered by the ablation might be the dominant driver of endothelial damage and antithrombotic therapy per se has a limited potential to mitigate endothelial activation. Kuhne et al. [68] measured significantly higher levels of Troponin *T* after PVI using IRF as compared to PVI with the 1st-generation cryoballoon. However, other investigations comparing biomarkers of myocardial injury after ablation reported conflicting results. In a multicenter study [69], the highest creatinine kinase MB and troponin I levels were demonstrated after PVI with cryoballoon as compared to IRF with or without contact force and laser ablation, while no difference in high sensitive troponin *T*, microparticles, and high-sensitive CRP was found after PVI with IRF versus with cryoballoon in another work [70]. The effect of various ablation technologies on endothelial activation has not been elucidated. In our further research at our institute, in addition to an unified anticoagulation strategy, we detected significant increases in VWF antigen and FVIII activity levels by all three ablation methods (CB, PVAC, IRF). Postablation increases in sVCAM levels were associated with CB and IRF use, but not with PVAC. Taken together, these results suggest that current ablation techniques cause significant endothelial damage. This finding highlights the importance of rigorous and continuous anticoagulation during the postablation period.

*5.3. **Summary of scientific results, new findings***

In our human research, we were the first to perform a comparative study of various hemostasis markers from blood samples obtained directly from the left atrium before and immediately after catheter ablation due to atrial fibrillation. In some of our studies, we compared the effect of different periprocedural anticoagulation protocols on hemostasis

during cryoballon ablations, which are currently widely considered as tissue-friendly ablation techniques. In the other part of our research, we compared different ablation techniques in addition to the currently routinely used uninterrupted vitamin K antagonist as periprocedural thromboembolism prophylaxis. We consider the following to be new findings of our work:

1, Prolonged and continuous oral anticoagulant therapy before PVI with cryoballoon with both vitamin K antagonist and dabigatran reduces hemostasis activation.

2, Prior to PF ablation, uninterrupted dabigatran therapy is more effective in reducing intracardiac hemostasis activation and consequent fibrinolysis than vitamin K antagonist therapy at therapeutic level. The effect of dabigatran is particularly marked in preventing the increase in D-dimer change due to catheter ablation.

3, Ablation with a 2nd generation cryoballoon catheter induces significant endothelial activation regardless of preoperative anticoagulation strategy.

4, Despite uninterrupted vitamin K antagonist prophylaxis at therapeutic INR values, significant hemostasis activation develops in addition to all three widely used AF ablation methods, cryoballon, focal RF, and multipolar phase RF.

5, The previous ablation techniques cause a similar extend of endothelial activation.

6. Summary

This is the first analysis of complex hemostasis changes in left atrial blood samples in the context of left atrial ablation. In this prospective observational study we demostrated the efficiency of uninterrupted dabigatran (150 mg BID) controlled hemostasis activation before and after PVI with the 2nd generation cryoballoon as evidenced by measuring the levels of different markers in left atrial blood samples. Indeed, dabigatran compared favourably to VKA treatment with a therapeutic INR. All 3 different AF ablation technologies (cryoballoon, focal RF and multipolar phased RF) used in our study provoked a significant hemostasis and endothelial activation despite periprocedural thromboembolic profilaxis with VKA. These results suggest that perioperative anticoagulation needs to be improved for patients' safety when currently available AF ablation technologies are used and uninterrupted administration of DOACs might be better alternatives. Detailed analysis of fibrinolysis and endothelial activation with these novel agents might provide further insights into the mechanism of silent and manifest clinical thromboembolic events with potential implications for risk assessment in the context of AF ablation.

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List of publications related to the dissertation

1. **Hajas, O.**, Bagoly, Z., Tóth, N. K., Urbancsek, R., Kiss, A., Kovács, K. B., Sarkady, F., Nagy, A. C., Oláh, A., Nagy, L., Clemens, M., Csiba, L., Csanádi, Z.: Intracardiac Fibrinolysis and Endothelium Activation Related to Atrial Fibrillation Ablation with Different Techniques.
Cardiol Res Pract. 2020, 1-8, 2020.
DOI: http://dx.doi.org/10.1155/2020/1570483
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2. Bagoly, Z., **Hajas, O.**, Urbancsek, R., Kiss, A., Fiak, E., Sarkady, F., Tóth, N. K., Orbán-Kálmándi, R. A., Kovács, K. B., Nagy, L., Nagy, A. C., Kappelmayer, J., Csiba, L., Csanádi, Z.: Uninterrupted Dabigatran Administration Provides Greater Inhibition against Intracardiac Activation of Hemostasis as Compared to Vitamin K Antagonists during Cryoballoon Catheter Ablation of Atrial Fibrillation.
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List of other publications

3. Szirák, K., Soltész, B., **Hajas, O.**, Urbancsek, R., Nagy-Baló, E., Penyige, A., Csanádi, Z., Nagy, B.: PITX2 and NEURL1 SNP polymorphisms in Hungarian atrial fibrillation patients determined by quantitative real-time PCR and melting curve analysis.
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4. Soltész, B., Urbancsek, R., Pös, O., **Hajas, O.**, Forgács, I. N., Szilágyi, E., Nagy-Baló, E., Szemes, T., Csanádi, Z., Nagy, B.: Quantification of peripheral whole blood, cell-free plasma and exosome encapsulated mitochondrial DNA copy numbers in patients with atrial fibrillation.
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