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Coagulation factors and fibrinolytic activity in the left atrial appendage and other heart chambers in patients with atrial fibrillation: is there a local intracardiac prothrombotic state? (HEART-CLOT study)☆

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ABSTRACT

Introduction: Atrial fibrillation (AF), a risk factor for stroke and systemic thromboembolism, is associated with unfavorable fibrin clot properties and increased thrombus formation in peripheral blood. The left atrial appendage (LAA) is known to be the primary site of thrombus formation.
Aim: We investigated the relative differences in plasma fibrin clot features including plasma fibrin clot permeability (K_s) and clot lysis time (CLT) between the right atrium (RA), right ventricle (RV), left atrium (LA), left ventricle (LV), LAA, and peripheral blood.
Methods: Sixteen patients with nonvalvular AF who stopped oral anticoagulant therapy at least 2 days before a LARIAT procedure participated in a single-center prospective study. We measured fibrinogen and plasminogen levels along with K_s, CLT, and endogenous thrombin potential (ETP) during the LARIAT procedure in blood obtained from the right femoral vein, RA, RV, LA, LV and LAA.
Results: LAA clot porosity was reduced by 16.2% compared to peripheral blood ($p = 0.026$), also after adjustment for fibrinogen levels ($p = 0.038$). K_s was similar for the RA, RV, LA, LV, and LAA (all $p > 0.05$). We found 14.7% prolonged CLT for clots prepared from blood samples obtained from the LAA compared to those prepared from peripheral blood, but no differences between the RA, RV, LA and LV (all $p > 0.05$) were found. There were no significant differences in other parameters, including ETP, between heart chambers.
Conclusions: Patients with AF are characterised by a local prothrombotic state as reflected by formation of compact fibrin clots in the LAA compared to peripheral blood, which may contribute to LAA thrombus formation and device-related thrombi.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia resulting in a prothrombotic state [1,2]. Significant hemodynamic abnormalities in AF are caused by irregular contractions of the ventricles and a lack of effective atrial contraction with resulting turbulent blood flow [3,4]. The structural and functional changes in the left atrium (LA) and left atrial appendage (LAA) impact their flow dynamics and coagulation status by significantly increasing the risk of thrombus formation and subsequent thromboembolization.

AF patients are characterised by a significantly higher prothrombotic state as reflected by elevated thrombin generation markers (F1 + 2 prothrombin fragments and D-dimer [5–7]), and a prolonged clot lysis time (CLT) [5]. Increased levels of plasminogen activator inhibitor-1 (PAI-1) [8] and thrombin activatable fibrinolysis inhibitor (TAFI) [9] have also been described in AF patients [10]. Moreover, AF is associated with increased levels of plasma fibrinogen, von Willebrand Factor (vWF), soluble P-selectin, and α_2-antiplasmin (α_2AP) [11]. Vascular endothelial cell damage is also increased as measured by elevated soluble thrombomodulin (sTM) [9,12,13]. More compact plasma fibrin clot structure, reflected by reduced clot permeability (K_s), along with decreased susceptibility to lysis as measured by prolonged CLT were found in patients

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with sinus rhythm experiencing a short-term AF episode [14] and in patients with permanent AF [15].

However, these altered parameters have mostly been studied in peripheral blood samples [9,12,13]. Although a systemic increase in prothrombotic state exists in AF patients, thrombosis is mostly observed in cardiac chambers, and evidence that the blood in certain cardiac chambers in AF patients is more thrombogenic than the peripheral blood [16] supports clinical observations that thrombi develop mostly in the LA and LAA. In conclusion, the regional differences in coagulation and fibrinolytic factors levels are poorly understood.

In the current study we evaluated the differences in plasma fibrin clot features between all heart chambers, including K_s and CLT, known to be associated with increased risk of thromboembolism in AF patients.

2. Methods

2.1. Patient characteristics

A prospective study was performed on 16 consecutive patients enrolled between September 2016 and November 2018. The study design was in accordance with the guiding principles of the Declaration of Helsinki and approved by the Jagiellonian University Ethical Committee. All patients signed a written informed consent form prior to inclusion. This study was funded by the Polish National Science Center (research grant UMO-2014/13/D/NZ5/01351).

All patients met the following inclusion criteria: 1) age 18 years or older; 2) nonvalvular AF of >6 months; 3) at least 1 risk factor of embolic stroke ($CHADS_2$ 1); 4) a poor or ineligible candidate for long term oral anticoagulation therapy. The exclusion criteria for Lariat procedure were: 1) previous cardiac surgery; 2) acute illness; 3) known cancer; 4) hepatic or renal dysfunction; 5) heart failure (New York Heart Association III or IV); 6) Left Ventricle Ejection Fraction (LVEF) < 35%; 7) myocardial infarction <3 months; 8) autoimmune disease; 9) steroid administration; or 10) any other cardiac disease required cardiac surgery procedure. Patients meeting enrollment criteria underwent a screening contrast cardiac computed tomography (CT) scan. Exclusion criteria based on LAA anatomy included: 1) LAA width 45 mm; 2) superiorly oriented LAA with the LAA apex directed behind the pulmonary trunk; 3) bilobed LAA or multilobed LAA in which lobes were oriented in different planes exceeding 45 mm; and 4) a posteriorly rotated heart.

2.2. Left atrial appendage occlusion procedure and blood draw

Patients were hospitalized 1 or 2 days before the procedure. Patients receiving vitamin K antagonist (VKA) or direct oral anticoagulant (DOAC) on a long-term-basis were eligible if their anticoagulation was stable within the previous 3 months. Before the procedure, bridging therapy with low molecular weight heparin (LMWH) was performed. VKA was ceased five days and DOAC was ceased a minimum of two days before the LAAO procedure. In patients receiving aspirin or without oral anticoagulants, LMWH was started at least 2 days before the procedure. The last dose of LMWH was administered >12 h before the procedure.

All epicardial LAAO procedures were performed with the Lariat device (SentreHEART Inc, Redwood, CA). The LAAO procedure was described in detail in our previous studies [17–19]. Pericardial access was obtained using a telescoping-micropuncture technique at the beginning of the procedure followed by femoral venous access. Blood samples were quickly taken: (1) peripheral femoral venous (FV) sheath; (2) right atrium (RA); (3) right ventricle (RV); (4) left atrium (LA); (5) left atrial appendage (LAA); (6) left ventricle (LV) after gaining access to the right and left chambers of the heart. All blood samples were collected before the administration of unfractionated heparin.

Briefly, punctures of the right femoral vein were performed using the Seldinger technique and a femoral cannula sheath was placed in the vein. Twenty ml of blood was drawn through the side arm of a short introducer immediately after access to the vein via 5F pigtail, from which the first 5 ml of blood was discarded in order to exclude intra-sheath hemostasis activation (FV sample). After femoral access, the 5F pigtail catheter (Medtronic, Kirkland, QC, Canada) was used again to draw all blood samples from all chambers with the first 5 ml discarded in order to exclude hemostasis activation. The blood was collected from the right atrium (RA sample). The 5F pigtail was then placed inside the right ventricle and the blood sample collected (RV sample). Next, transseptal catheterization was performed. A 5F pigtail catheter was inserted through the transseptal sheath to obtain blood samples from the left atrium, left ventricle and LAA. The order of obtaining LA, LAA and LV blood samples was rotated between patients to eliminate temporal effects of collecting left-sided blood samples. Immediately after the collection of left heart blood samples, 150 IU/kg body weight intravenous heparin was administered and an epicardial LAAO procedure with suture-based LARIAT device was performed according to standard protocols [17,20,21].

2.3. Laboratory investigations

Blood samples were collected in vacutainer tubes (tubes anticoagulated with K_3-EDTA for complete blood count, tubes containing 0.109 M sodium citrate and CTAD (buffered citrate, theophylline, adenosine, and dipyridamole) for hemostasis and fibrinolysis tests. In all cases, before the procedure, anti-Xa activity (IU/mL) was measured.

International Normalized Ratio (INR), activated partial thromboplastin time (APTT), high sensitivity C-reactive protein (CRP), glycated hemoglobin (HbA1c), blood cell count, comprehensive lipid profile including total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured from antecubital vein blood samples of all patients upon hospital admission by routine methods. Tissue plasminogen activator (tPA) antigen, PAI-1 antigen, TAFI antigen, and plasminogen activity were determined by ELISA (Hyphen BioMed, Neuville-Sur-Oise, France).

2.4. Fibrin clot analysis

Plasma fibrin clot permeability was determined as described previously [22]. Briefly, 20 mM calcium chloride and 1 U/mL human thrombin (Merck, Darmstadt, Germany) were added to citrated plasma. Tubes containing the clots were connected to a reservoir of Tris-buffered saline. Its volume flowing through the gels was measured within 60 min. A permeation coefficient reflected by K_s indicating the average size of pores formed in the fibrin network with low values indicating tightly packed fibrin structure was calculated using the equation $K_s = Q \times L \times \eta / t \times A \times \Delta p$, where Q is the flow rate in time t, L is the length of a fibrin gel, η is the viscosity of liquid (in poise), t is the percolating time, A is the cross-sectional area (in cm^2), and Δp is the differential pressure (in $dyne/cm^2$).

Clot lysis time (CLT) was measured using the method described by Pieters et al. [23]. Briefly, citrated plasma was mixed with 20 mM calcium chloride, 0.5 U/mL thrombin (Merck), 15 μM phospholipid vesicles (Rossix AB, Mölndal, Sweden) and 18 ng/ml recombinant tissue plasminogen activator (Actilyse 20 mg, Boerhinger Ingelheim, Germany). The mixture was transferred to a microtiter plate and turbidity measured at 405 nm at 37 °C. CLT was defined as the time from the midpoint of the clear-to-maximum-turbidity transition, which represents clot formation, to the midpoint of the maximum-turbidity-to-clear transition. The interassay coefficients of variation for lysis variables were <8%.

2.5. Calibrated automated thrombogram

Thrombin generation kinetics was measured with the Calibrated Automated Thrombogram (CAT) (Thrombinoscope BV, Maastricht, Netherlands) according to the manufacturer's instructions in the 96-well plate fluorometer (Ascent Reader, Thermo LabSystems OY, Helsinki, Finland) equipped with the 390/460 filter set at a temperature of 37 °C [24]. Briefly, 80 μl of platelet-poor plasma was diluted with 20 μl of the reagent containing 5 pmol/l recombinant tissue factor (TF), 4 μmol/l phosphatidylserine/phosphatidylcholine/phosphatidylethanolamine vesicles, and 20 μl of FluCa solution (HEPES, pH 7.35, 100 mmoL/l $CaCl_2$, 60 mg/ml bovine albumin, and 2.5 mmoL/L Z-Gly-Gly-Arg-amino-methylcoumarin). Each plasma sample was analyzed in duplicate, and the intraassay variability was 7%. The maximum concentration of thrombin formed during the recording time is described as the thrombin peak and the area under the curve represents endogenous thrombin potential (ETP).

2.6. Statistical analysis

Categorical variables were presented as numbers and percentages and analyzed by Pearson's χ^2 or Fisher's exact test. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range. The normality of the data was assessed using the Shapiro-Wilk test. Differences between groups were compared using the Student's t-test for normally distributed continuous variables, and for non-normally distributed continuous variables the Mann-Whitney U test was used. Analysis of variance (ANOVA) was used to compare continuous variables across >2 groups. Associations between nonparametric and parametric variables were assessed by Spearman's and Pearson's tests, respectively. *p*-values of <0.05 were considered statistically significant. All statistical analyses were performed using JMP® PRO 13 version 13.1.0 and SAS 9.4 (SAS Institute Inc).

3. Results

A total of 16 AF patients were evaluated, with patient characteristics presented in Supplemental file 1. The cohort constituted mostly males (60%) with a $CHADS_2$ score of 2.6 ± 1.2 and a CHA_2DS_2-VASc score of 3.8 ± 1.8. Most patients (87.5%) presented with hypertension as an additional risk factor of thromboembolism. More than 60% of patients had a history of previous stroke or transient ischemic attack (TIA). Patients were also at high risk of bleeding based on the clinical scale HAS-BLED (3.2 ± 1.5). Baseline hematological characteristics are presented in Supplemental file 2.

The median baseline plasma fibrinogen level was 2.19 [range 1.71–2.66] g/L. Fibrinogen levels did not differ between particular heart chambers (all $p > 0.05$). There were no differences between peripheral blood and intracardiac fibrinogen levels (all $p > 0.05$). K_s was similar for the RA, RV, LA, LV, and LAA (all $p > 0.05$), but a tendency of reduced K_s was observed in the LAA compared to other intracardiac locations (Table 1, Fig. 1A). Clot porosity, reflected by K_s, was found to be 16.3% reduced in the LAA compared to peripheral blood (4.58 [4.14–5.16] × $10^{-9}cm^2$ vs. 5.47 [4.74–7.59] × $10^{-9}cm^2$, $p = 0.026$). After adjustment for fibrinogen this difference remained significant ($p = 0.038$). Similarly, there was 14.7% prolonged CLT for clots prepared from blood samples obtained from the LAA (Table 1), compared to peripheral blood clots (133 [122–140] min vs. 116 [108–125]

Table 1
Plasma fibrin clot characteristics, thrombin generation parameters, and fibrinolysis activators and inhibitors assessed in particular heart chambers and peripheral blood in AF patients undergoing the LAAO procedure.

Variable	RA	RV	LA	LV	LAA	Peripheral blood
Fibrinogen, g/L	2.32 [1.57-2.59]	2.03 [1.70-2.37]	2.48 [1.87-2.75]	2.31 [1.93-2.76]	2.35 [2.21-2.58]	2.19[1.76-2.44]
K_s, $\times 10^{-9}cm^2$	4.95 [4.24-8.11]	4.89 [4.02-8.02]	4.74 [4.37-7.56]	4.81 [4.29-7.21]	4.58 [4.14-5.16]	5.47 [4.74-7.59]
CLT, min	122 [103-127]	125 [105-125]	123 [110-129]	125 [113-135]	133 [122-140]	116 [108-125]
ETP, nM×min	1289 [1178-1486]	1337 [1217-1526]	1412 [1306-1645]	1435 [1326-1692]	1542 [1214-1942]	1380 [1263-1450]
Peak thrombin, nM	181 [141-249]	192 [149-239]	190 [151-257]	194 [143-262]	202 [148-268]	198 [151-242]
tPA antigen, ng/mL	9.83 [5.94-13.78]	9.18 [4.93-12.72]	9.30 [3.82-9.96]	9.42 [3.83-14.87]	9.71 [4.02-11.66]	9.48 [5.06-11.54]
PAI-1 antigen, ng/mL	3.72 [2.14-5.07]	4.11 [2.12-6.03]	3.31 [1.90-4.90]	3.32 [1.92-4.30]	3.78 [1.86-4.95]	4.29 [1.76-6.44]
TAFI antigen, %	88.8 [78.6-99.4]	90.6 [78.9-103.13]	89.8 [77.7-96.1]	88.3 [71.6-102.3]	84.1 [66.7-94.0]	81.0 [63.8-91.2]
Plasminogen, %	87.6 [81.2-95.6]	89.4 [80.2-93.7]	85.8 [76.6-91.7]	86.2 [74.9-92.1]	81.3 [70.4-88.8]	90.0 [85.1-96.9]

min, $p = 0.015$). Such a difference was not observed for other cardiac chambers, but a trend toward a prolonged CLT (Fig. 1B, $p = 0.074$) was observed.

There were no significant differences in the thrombin generation parameters (ETP and peak thrombin levels) between peripheral blood and the intracardiac chambers. However, a tendency of increased ETP was observed in the LAA when compared to other intracardiac samples (borderline significance $p = 0.053$ for ANOVA, Fig. 1C). No differences were found for PAI-1, tPA, and TAFI levels between any cardiac chamber and the peripheral blood (Table 1) as well as between intracardiac blood samples (all $p > 0.05$, data not shown). We observed a significantly reduced plasminogen activity in the LAA compared to peripheral blood (81.3 [70.4–88.8]% vs. 90 [85–97]%, $p = 0.043$).

In the LAA samples, K_s was inversely associated with CLT ($r = -0.73$, $p < 0.001$, Fig. 2A), while CLT correlated with PAI-1 ($r = 0.71$, $p < 0.002$, Fig. 2B) and tended to correlate inversely with plasminogen activity ($r = -0.43$, $p = 0.062$).

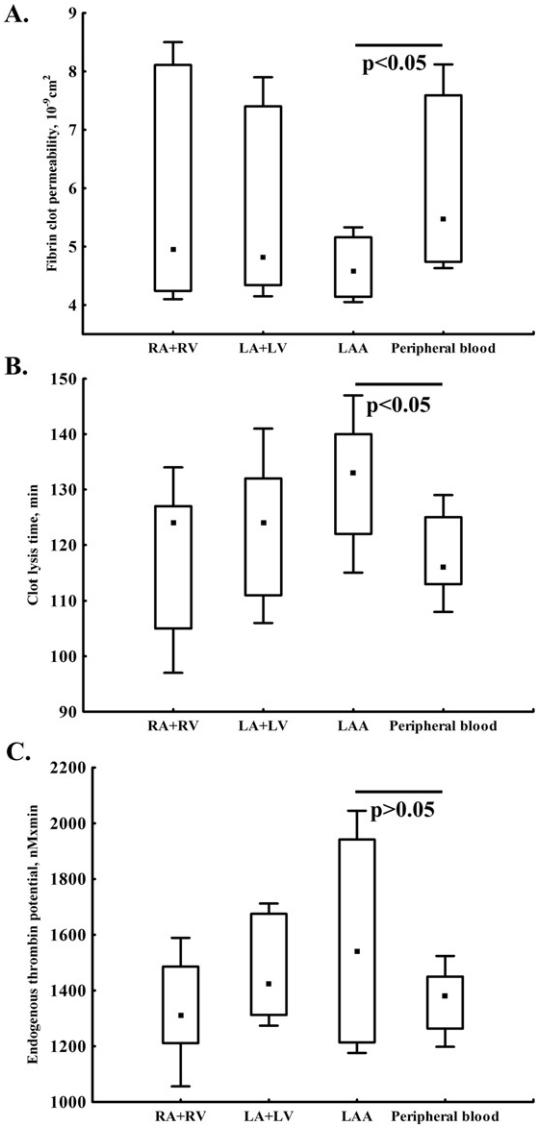

Fig. 1. Fibrin clot permeability (panel A), clot lysis time (panel B), and endogenous thrombin potential (ETP, panel C) measured in plasma obtained from the right atrium (RA) and ventricle (RV), left atrium (LA) and ventricle (LV), and left atrial appendage (LAA) compared to peripheral blood.

4. Discussion

Our study is the first to show an unfavorably altered fibrin clot phenotype in the LAA, with reduced clot permeability and decreased susceptibility to clot lysis in patients with permanent AF. The inverse correlation between CLT and K_s and the direct correlation between CLT and reduced plasminogen activity in the LAA presents a unique prothrombotic environment for the formation of compact fibrin clots with impaired lytic susceptibility (Fig. 3).

Unfavorably modified fibrin properties could at least be partially related to the tendency of increased thrombus generation and decreased plasminogen activity in the LAA compared to the other cardiac chambers. Thrombin generation could be an important modulator of fibrin features in AF. It has been postulated that some mechanisms, including a lack of effective atrial contraction (blood stagnation), endothelial dysfunction, atrial tissue fibrosis and increased platelet activity, could be related to enhanced thrombin generation [25–27]. Drabik et al. [14] showed that vWF, a key to effective hemostasis (independent predictor of both K_s and CLT), is elevated in AF patients [28]. Moreover, vWF along with FVIII were elevated in both peripheral and intracardiac blood samples from AF patients [16].

In patients with nonvalvular AF, >90% of the clots originating from the LA appear to come from the LAA [29]. In AF, the classic Virchow triad for thrombogenesis is met. This includes (1) blood stasis; (2) abnormalities of the vessel wall; and (3) regional and systemic inflammation resulting in a prothrombotic and hypercoagulable state [30]. The most common site of intracardiac thrombus formation in patients with AF is the LA, specifically the LAA, the source of embolic thrombi in more than 90% [31] which in some cases may be quite rapid [32].

Blood stasis in the LA of patients with AF is promoted by atriopathy due to significant atrial diastolic dysfunction, loss of atrial contractility due to rapid atrial rates, and atrial chamber enlargement. In transesophageal echocardiograms, this phenomenon may be observed as a spontaneous echo contrast (SEC) or "echogenic smoke". SEC is caused by an interaction of erythrocytes and plasma proteins modulated by shearing forces predisposing to a prothrombotic state [30,33]. Compared to other heart chambers, the LAA presents a complex anatomy making it more prone to stasis and thrombosis. Unlike the LA, the LAA is an outpouching with a neck and a very arborized structure in which blood can get trapped and fail to empty due to loss of effective contraction (rapid atrial rates 400–600 range) [34]. Scanning Electron Microscopy has shown endothelial damage in the LAA with large craters that fibrin plaques and platelet clumps can attach [35]. Histopathologic studies in these patients have confirmed increased levels of Collagens I and III and fibronectin [36], impaired matrix degradation [37] and cardiomyocyte necrosis or hypertrophy with mononuclear cell infiltrations [38].

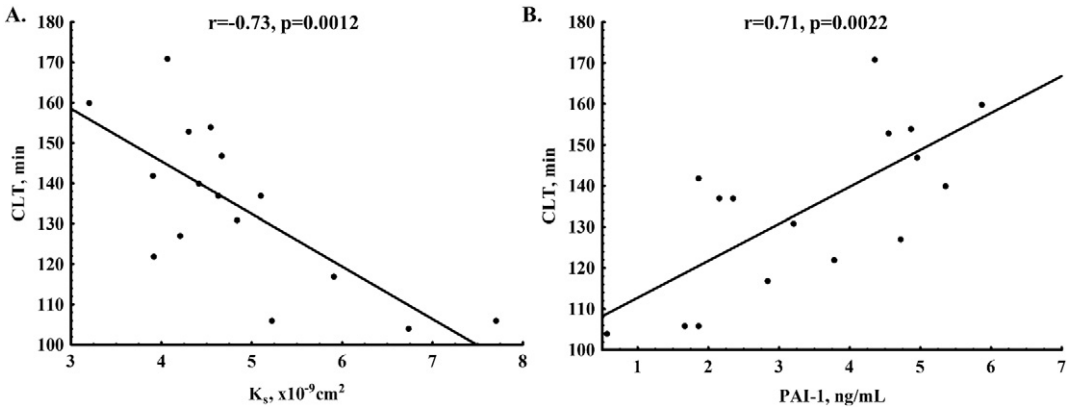

Fig. 2. Associations of clot lysis time (CLT) with fibrin clot permeability (K_s; panel A) and plasminogen activator inhibitor type 1 (PAI-1; panel B) assessed in plasma obtained from the left atrial appendage (LAA).

Therefore, dilatation of the LA and LAA, structural remodeling of chamber walls, endothelial abnormalities, systemic and regional inflammation, and blood stasis with SEC increase the risk of thrombosis in LAA and LA in AF patients [30,33–38] (Supplement file 3).

The current study adds additional evidence to support the existing literature on the propensity of the LAA to become the site for thrombus formation and subsequent systemic embolization. The physiologic explanation for these findings is still unclear. Whether it is the slow and delayed interaction of the stagnant blood with the altered endothelium and pathologic myocardial tissue or an increased production of prothrombotic factors from an abnormal fibrillating LAA is yet to be established. These findings clearly support the role that systemic anticoagulation plays to counter the prothrombotic status of the LAA in mitigating against thrombus formation and systemic thromboembolism (STE).

In patients who cannot tolerate long-term oral anticoagulation, mechanical exclusion of a highly prothrombotic LAA has proven to be effective in minimizing the risk of STE and systemic complications [18,19,39–45]. Even though mechanical occlusion reduces the risk of STE to a greater degree, it does not completely eliminate it [40,46]. Device-related thrombus has been reported to occur in 3.5%–7.2% of LAA implants and 1–2% after LAA ligation [39,47]. The observation that LAA manipulation can cause transient changes in clot permeability provides an impetus for further examination. Further understanding of the interaction between these mechanical devices and a prothrombotic state also need further examination.

4.1. Limitations

A major limitation of this study was the relatively small cohort of patients enrolled and the shortcomings of an observational cohort. Despite the small number of patients enrolled in the study, significant differences were still found between the thrombogenicity of the LAA compared to the other heart chambers that would only be enhanced by an increased patient number. It was difficult to control for the introduction of catheters to collect the blood from each sample, which could have influenced the clotting state of the chambers. Moreover, direct thrombin inhibitors such as dabigatran or argatroban have been shown to alter expression of thrombin receptors on human platelets or cardiomyocytes and platelets activation [48,49]. However, investigation of platelet activation influence on coagulation was beyond the scope of our study and the platelet-poor plasma was used in all measurements. This issue need to be elucidated. There was no control group of patients that did not have AF, but in our opinion, the control group has no ethical justification for this type of study and procedure.

5. Conclusion

In patients with AF, the LAA chamber presents procoagulant microenvironment reflected by reduced fibrin clot permeability and prolonged clot lysis time. Additionally, the LAAO can affect fibrin clot permeability leading to potential device related thrombus. Future

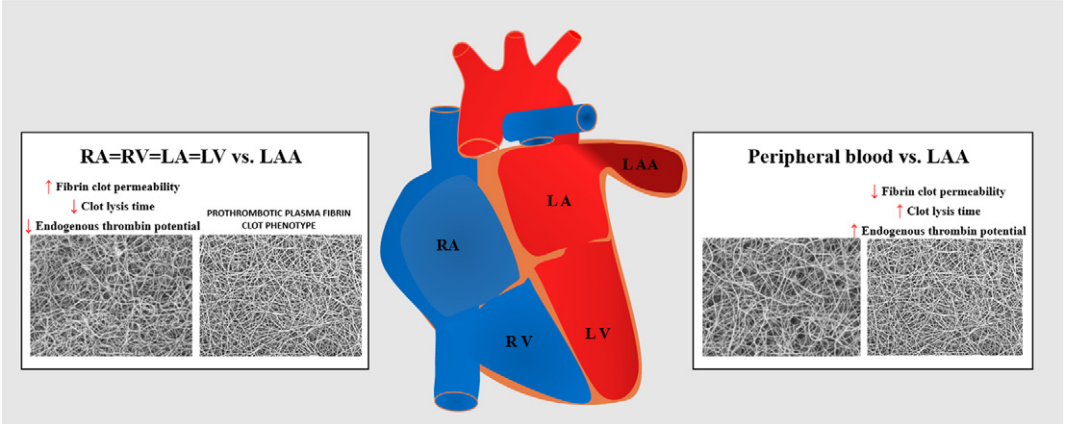

Fig. 3. Comparison of fibrin clot properties assessed in blood from particular heart chambers: right atrium (RA) and ventricle (RV), left atrium (LA) and ventricle (LV), left atrial appendage (LAA) or peripheral blood.

studies are needed to estimate the clinical significance of these findings and identify factors affecting fibrin clot phenotype in patients with AF.

Disclosures

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.09.053.

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