Changes in Fibrinolytic Activity and Coagulation Factors after Left Atrial Appendage Closure in Patients With Atrial Fibrillation (HEART-CLOT Study)

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Abstract

Background The left atrial appendage (LAA) is known to be the primary source of thrombus formation in atrial fibrillation (AF). Our aim was to investigate if LAA elimination (LAAO) from the cardiovascular system has an effect on the coagulation and prothrombotic status in AF. Methods Twenty two patients with nonvalvular AF not currently on anticoagulation therapy participated in a single-center prospective study. We measured fibrinogen and plasminogen levels along with Ks, clot lysis time (CLT), and endogenous thrombin potential (ETP) before LAAO procedure, at discharge and 1 month follow-up. Results 1 month after the LAAO procedure compared to baseline value, we found Ks improved by 39.3% measured in clots prepared from peripheral blood (p=0.019) and also after adjustment for fibrinogen (p=0.027). Higher Ks was associated with improved clot susceptibility to lysis (r=-0.67, p=0.013). We found shortened CLT by 10.3% (p=0.0020); a 52% lower PAI-1 antigen levels (p=0.023) along with 8.9% increased plasminogen activity (p=0.0077). A tendency to decreased thrombin generation, reflected by decreased ETP and peak thrombin generated, was observed 1 month after the LAAO procedure (p=0.072 and p=0.087). No differences were found in tPA and TAFI plasma levels (both p>0.05). Conclusions We confirm, that LAA plays a key role in thrombogenesis and is the main source of thrombus in AF. LAA elimination from the circulatory improve fibrin clot permeability and susceptibility to fibrinolysis in peripheral blood.

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Short title: Changes in Blood Coagulation before and after LAAO

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Conclusions

We confirm, that LAA plays a key role in thrombogenesis and is the main source of thrombus in AF. LAA elimination from the circulatory improve fibrin clot permeability and susceptibility to fibrinolysis in peripheral blood.

Keywords

atrial fibrillation, stroke, left atrial appendage, thrombus, LAAO

Declarations

Funding

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Conflict of interest

Bartus K and his institutions Jagiellonian University Medical College and John Paul II Hospital in Krakow Poland were recipients of the above research grant from the Polish National Science Centre. Other authors declare no conflicts of interest concerning this study

Ethics approval

The study design was in accordance with the guiding principles of the Declaration of Helsinki and approved by the Jagiellonian University Ethical Committee.

Consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material: Not applicable

Code availability; Not applicable

Authors' contributions

R. Litwinowicz: Conception and design, manuscript writing, collection and assembly of data; J. Natorska: Data analysis and interpretation, collection and assembly of data, Manuscript writing; M. Zabczyk; Data analysis and interpretation, collection and assembly of data, Manuscript writing; B.Kapelak: Collection and assembly of data; D. Lakkireddy : Manuscript writing: R.J. Lee: Manuscript writing; V. Vuddanda: Manuscript writing; K.Bartus: Conception and design, manuscript writing, collection and assembly of data

Introduction

Near 15% of ischemic stroke is caused by atrial fibrillation (AF). AF is associated with a prothrombotic state which increases the risk of stroke fivefold compared to the general population. It is well known, that increase thromboembolism risk in AF is associated with a combination of pathophysiological mechanism, a Virchow's triad[1]: (1) blood stasis; (2) abnormalities of the vessel wall; and (3) regional and systemic inflammation resulting in a prothrombotic and hypercoagulable state[2]. Over 90% of all intracardiac thrombus formation in patients with AF are observed in the left atrial appendage (LAA).

Elimination of the LAA from the circulatory system became an alternative method for stroke prevention in patients with AF. The ESC and ACC/AHA guidelines give the surgical LAA occlusion or exclusion (LAAO) concomitant to cardiac surgery or thoracoscopic AF surgery a Class 2B recommendation[3,4]. Interventional, percutaneous LAA ligation or occlusion procedure had a Class 2B recommendation only for AF patients contraindicated for oral anticoagulation (OAC)[3,4].

Multiple observational studies indicate the feasibility and safety of surgical or percutaneous LAA occlusion/exclusion procedure[3-6]. However, the assessment of the effectiveness of these procedures is always based on clinical observation with stroke or other thromboembolic incidences as an endpoint. Importantly, those observations are not supported by the results of the basic science or translational research study based on biomarkers approach in thromboembolic risk assessment after LAA elimination [3-6]. Also, a large randomized trial such as LAAOS III, that is currently underway, asses only the clinical outcomes of LAA elimination[7]. Therefore, there is a great need to support existing observational studies of LAA elimination through the implementation of basic research on the coagulation system and effect on the prothrombotic status.

The aim of the current study was to investigate if epicardial LAA elimination from the cardiovascular system has an effect on the coagulation system and prothrombotic status in AF patients. We also analyzed the relationship between the level of hypercoagulability, fibrinolytic markers and clot lysis time depending on the presence of LAA.

Material and Methods

The study design was in accordance with the guiding principles of the Declaration of Helsinki and was approved by the Jagiellonian University Ethical Committee (27 September 2012, number KBET/282/B/2012). All patients signed a written informed consent form prior to inclusion.

Patients characteristic

A prospective study was performed in 22 consecutive patients with permanent nonvalvular AF of 6-months duration of longer who were qualified for epicardial LAAO procedure with Lariat or AtriClip device. All eligible patients had electrocardiographically confirmed long-term AF.

These patients were high stroke and bleeding risk for continued long term systemic anticoagulation. Patients were enrolled between 2016 September and 2018 November.

Data on demographics, cardiovascular risk factors, comorbidities and current treatment were collected from all patients using a standardized questionnaire. The diagnosis of stroke was based on the World Health Orgnization criteria. Diabetes was defined as a history of diabetes regardless of duration of the disease, a need for hypoglycemic agents, or fasting glycemia greater than 7 mmol/l or 126 mg/dl. CAD was confirmed angiographically (>50% stenosis in at least 1 major epicardial artery). Patients were enrolled between 2016 September and 2018 November.

Left atrial appendage occlusion procedure and blood drawing

Patients receiving vitamin K antagonist (VKA) or novel oral anticoagulation (NOAC) on a long term-basis were eligible if their anticoagulation was stable within the previous 3 months. Before procedure, bridge therapy was performed. VKAs was interrupted 5 days and NOAC was interrupted minimum 2 days before LAAO procedure and switched to low molecular heparin (LMWH).

All left atrial appendage occlusion were performed using epicardial devices: 72.7% cases were performed with Lariat device (SentreHEART Inc, Redwood, CA, USA) and 27.3% cases were performed using AtriClip (AtriCure, Inc., West Chester, PA, USA).

LAAO procedure with Lariat device use pericardial access obtained via a telescoping-micropuncture technique at the beginning of the procedure followed by femoral venous access and was described in detail in our previous study [8-10]. LAAO procedure with AtriClip device was performed by a stand-alone totally thoracoscopic left atrial appendage exclusion using minimally invasive approach was also described elsewhere[11]

Day after procedure in all patients LMWH was continued till patient hospital discharge. All patients were discharged on aspirin monotherapy (150mg/day).

Echocardiography

To evaluate presence of postprocedure leak, transthoracic echocardiography was performed at discharge and 1 month follow-up. Leaks were categorized into 3 categories: complete or < 1mm leak; < 2mm leak; < 3mm leak.

Laboratory investigations

At the date of procedure, before LAAO, baseline blood samples were collected. Control blood samples were collected at the discharge and 1 month follow-up.

Blood samples were collected into vacutainer tubes (tubes anticoagulated with K3-EDTA for complete blood count, tubes containing 0.109M 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 from peripheral blood.

International Normalized Ratio (INR), activated partial thromboplastin time (APTT), high sensitivity C-reactive protein (CRP), glycated hemoglobin (HBA1C), morphology including platelets 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, plasminogen activator inhibitor type 1 (PAI-1) antigen, thrombin activatable fibrinolysis inhibitor (TAFI) antigen, and plasminogen activity levels were determined by ELISA (Hyphen BioMed, Neuville-Sur-Oise, France).

Fibrin clot analysis

Plasma fibrin clot permeability was determined as described previously[12]. 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 a Tris-buffered saline. Its volume flowing through the gels was measured within 60 min. A permeation coefficient reflected by K_s, which indicates the average size of pores formed in the fibrin network with low values indicating tightly packed fibrin structure, was calculated from 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 percolating time, A is the cross-sectional area (in cm^2), and Δp is a differential pressure (in dyne/cm^2).

CLT was measured using the method of Pieters M, et al[13] . Briefly, citrated plasma was mixed with 20 mM calcium chloride, 0.5 U/mL thrombin (Merck), 15 μM phospholipid vesicles (Rossix) and 18 ng/mL recombinant tissue plasminogen activator (Actilyse 20 mg, Boerhinger Ingelheim, Germany). The mixture was transferred to a microtiter plate and its turbidity was measured at 405 nm at 37°C. CLT was defined as the time from the midpoint of the clear-to-maximum-turbid transition, which represents clot formation, to the midpoint of the maximum-turbid-to-clear transition. The interassay coefficients of variation for lysis variables were <8%.

Calibrated automated thrombogram

Thrombin generation kinetics was measured with the Calibrated Automated Thrombogram (CAT) (Thrombinoscope BV, Maastricht, the Netherlands) according to the manufacturer's instructions in the 96-well plate fluorometer (Ascent Reader, Thermolabsystems OY, Helsinki, Finland) equipped with the 390/460 filter set at a temperature of 37°C[14]. 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 CaCl2, 60 mg/ml bovine albumin, and 2.5 mmol/l Z-Gly-Gly-Arg-amido methyl coumarin). 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 ETP.

Statistical analysis

Categorical variables were presented as numbers and percentages and were 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. Normality of the data was assessed using Shapiro-Wilk test. Differences between groups were compared using the Student's 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® Version 13.1.0 and SAS 9.4 (SAS Institute Inc.).

Results

A total of 22 AF patients were evaluated. Patient characteristics are present in Table 1. The cohort constituted mostly males (54.5%) with $CHADS_2$ score of 2.8±1.1 and CHA_2DS_2-VASc score of 4.3±1.7. Most patients (95%) presented hypertension as an additional risk factor of thromboembolism. More than 72.7% 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 (3.5±1.3).

Plasma fibrinogen level before LAAO in peripheral blood was 2.23 (1.75-2.72) g/L and increased 87% after the procedure (p=0.0051). However, after 1-month follow-up fibrinogen concentrations returned to baseline value (Table 1, p>0.05). At baseline AF patients were characterized by 19.7% reduced K_s of clots prepared from LAA obtained blood compared to peripheral blood. As expected, the LAAO procedure was associated with K_s when compared with the baseline (p=0.011; Fig. 1A). Interestingly, after 1-month follow-up we observed in patients after the LAAO K_s improved by 39.3% compared to baseline value of K_s measured in clots prepared from peripheral blood obtained from AF patients before the surgical procedure (p=0.019), also after adjustment for fibrinogen (p=0.027; Fig. 1A).

Similarly, we found shortened CLT by 10.3% in patients 1-month after the LAAO procedure compared with baseline (p=0.0020; Fig. 1B). Of note, clots prepared from blood obtained from the LAA were characterized by 16.4% prolonged CLT compared with baseline (p=0.016; Fig. 1B). The LAAO procedure was associated with 19.8% prolongation of CLT compared to baseline values (p=0.0007; Fig. 1B). After 1-month follow-up, higher K_s was associated with improved clot susceptibility to lysis (r=-0.67, p=0.013). A tendency to decreased thrombin generation, reflected by decreased ETP and peak thrombin generated, was observed 1 month after the LAAO procedure (Table 2, p=0.072 and p=0.087). Moreover, we found a 52% lower PAI-1 antigen levels (p=0.023) along with 8.9% increased plasminogen activity (p=0.0077) 1 month after the LAAO compared with baseline. No differences were found in tPA and TAFI plasma levels in AF patients before the LAA procedure and after 1-month follow-up (both p>0.05; Table 2). CLT, before the LAAO and 1 month after the procedure was associated with PAI-1 (r=0.68, p=0.0026 and r=0.073, p=0.0018, respectively) and with the plasminogen activity (r=-0.38, p=0.048 and r=-0.36, p=0.044).

Discussion

Our study is the first to show the influence of epicardial LAAO in nonvalvular AF patients on fibrin clot characteristics and thrombin generation assessed 1 month after the procedure. Our novel finding is that the LAAO improves fibrin clot permeability and susceptibility to fibrinolysis. Interestingly, shortened CLT correlated with decreased PAI-1 antigen levels and increased plasminogen activity. Moreover, a tendency to reduced thrombin generation measured 1 month after the LAAO procedure was observed (Figure 2).

Atrial fibrillation increase risk of stroke up to 20%[15]. In nonvalvular AF patients, 90% of thrombus is located in LAA what was confirmed by autopsy, transesophageal echocardiography or direct inspection study[16]. In contrast to the anatomy of different hear chambers, LAA present long, tubular structure as well as narrow junction with the atrium[17]. Increased thromboembolism risk in AF is associated with a combination of pathophysiological mechanism, a Virchow's triad[1]. The first factor is left atria dysfunction with stasis observed in spontaneous echocardiography contrast[18]. The second factor, an abnormal change in the vessel are present by vascular endothelial cell damage reflected by elevated soluble thrombomodulin (TM)[19,20]. TM, an integral membrane protein expressed on the surface of endothelial cells, binds thrombin with high affinity inhibits fibrinolysis by cleaving thrombin-activatable fibrinolysis inhibitor (TAFI) into its active form[20,21]. Additionally, unfavourably altered fibrin clot properties have been also described [22]. The third factor, hypercoagulable or prothrombotic state is reflected by increased levels of prothrombin fragments 1 + 2 (F1 + 2), elevated thrombin generation markers, including increased levels of prothrombin fragments, plasm fibrinogen and hypofibrinolysis due to increased levels of plasminogen activator inhibitor type 1 (PAI-1) and plasmin-?$_2$-antiplasmin (PAP)[20,23]. Of note, in our previous study, we have shown, that in patients with AF, the LAA chamber has reduced fibrin clot permeability and prolonged lysis time suggestive increased prothrombotic state (data being published). Additionally, it has been shown that clot lysis time predicts stroke during anticoagulant therapy in patients with atrial fibrillation and that patients with chronic AF and previous stroke are characterized by prolonged CLT and higher TAFI antigen than those free of stroke[20]. Therefore, the mechanisms underlying a thrombus formation in LAA in patients with AF are complex.

However, the CHA_2DS_2-VASc score, clinically applicable stroke risk-stratification model in AF patients, do not incorporate biomarkers[4,15]. Interestingly, CLT, PAI-1, and TAFI activity were positively associated with CHA_2DS_2-VASc scores, reflecting stroke risk in AF [20].

In last decades LAA became a therapeutic target for stroke prevention in patients who are at high stroke risk and have contraindications for long-term OAC (European Society of Cardiology guidelines Class IIb, Level of Evidence B)[24]. Multiple observational studies indicate the feasibility and safety of surgical or percutaneous LAA occlusion/exclusion procedure[3-6], even in high risk patients with increased thromboembolism risk comorbidities[25-27]. Epicardial LAAO complete ligate LAA orifice what cause necrosis and fibrosis permanently eliminating LAA [28].

Our study supports the concept of an LAA elimination from the circulatory system based on biomarkers approach in thromboembolic risk assessment in patients with AF. LAAO procedure improves fibrin clot permeability and susceptibility to fibrinolysis. Importantly, this effect lasts for a long time as evidenced by shortened CLT correlated with decreased PAI-1 antigen levels, increased plasminogen activity and a tendency to reduced thrombin generation measured 1 month after the LAAO procedure. Importantly, this effect was achieved in patients not receiving oral anticoagulation.

Therefore, our study confirms two important practical aspects. First, that LAA plays a key role in thrombogenesis and that LAA may be the main source of all thrombus in AF patients. Secondly, that LAAO procedure decreased the thromboembolic risk not only by elimination the local source of thrombus but also by improves fibrin clot permeability and susceptibility to fibrinolysis in peripheral blood. To our knowledge, the present prospective cohort study is unique in this regard.

Our study that is based on biomarkers concepts of thromboembolic risk in AF, may explain the effectiveness and successful results in the reduction of stroke or other thromboembolic episodes in observational studies that assess percutaneous LAA occlusion/exclusion procedure[29,30].

For cardiac surgery, obtained results are especially important, because they support the idea to perform concomitant surgical LAA occlusion in patients with AF/flutter who are undergoing routine cardiac surgery[7]. However, it should be noted, that in our study all LAAO was performed using Lariat or AtriClip (epicardial devices) as an elective procedure. There were also no leaks or thrombus in 1 months follow-up TTE examination. Is should be noted, that we didn't investigate the effect of amputation of the LAA and closure technique that is allowed in LAAO III trial[7].

Study limitations

The number of patients enrolled in the study was limited. We would like to highlight, however, that the number of patients enrolled in our study is comparable with the average number of patients undergoing this kind of blood sampling as published so far. Based on our findings larger studies are warranted to corroborate our observations

Conclusions

Our findings demonstrate that LAA plays a key role in thrombogenesis and is the main source of thrombus in AF. Our study suggests that LAA elimination from the circulatory system not only eliminate the local source of thrombus but also improve fibrin clot permeability and susceptibility to fibrinolysis in peripheral blood. Result of our study based on biomarkers concept of thromboembolic risk in AF support the results of observational of surgical or percutaneous LAA occlusion/exclusion procedure. Further studies are needed to validate our observations.

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Table 1. Patient characteristics

Variable
LAA epicardial device Lariat AtriClip
Age, years (Mean ± SD)
Female
BMI (Mean ± SD) [kg/m^2]
Left Ventricle Ejection Fraction (LVEF) (%)
$CHADS_2$ score (Mean ± SD)
CHA_2DS_2- VASs score (Mean ± SD)
HAS-BLED score (Mean ± SD)
Congestive Heart Failure
Hypertension
Hyperlipidemia

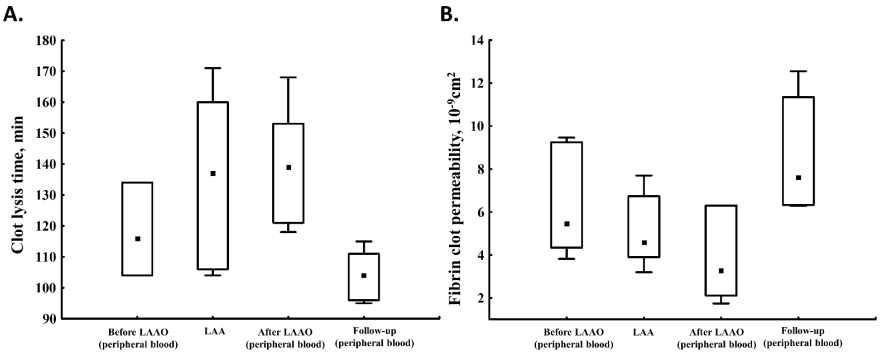
Variable
Diabetes Mellitus 2
Previous ischemic stroke/TIA
Hemorrhagic stroke
Myocardial infraction
Vascular Disease
Alcoholism
Laboratory parameters Platelet count (10^3) PT-INR PT sec PT % APTT (sek) Anti-Xa activity (IU/mL) C-reactive protei

Table 2. Plasma fibrin clot characteristics, thrombin generation parameters, and fibrinolysis activators and inhibitors assessed in peripheral blood before, during, and after the LAAO procedure as well as in the LAA in patients undergoing the LAAO.

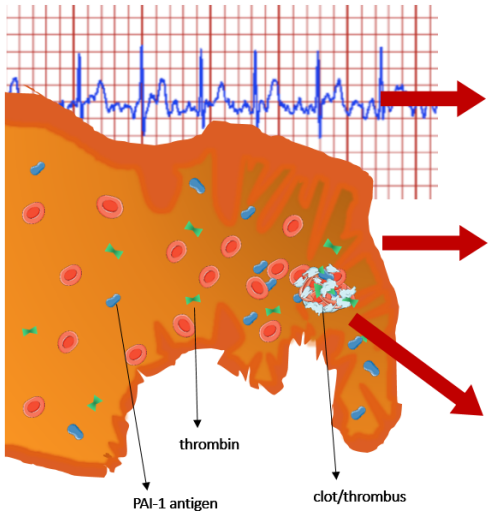
Variable	Peripheral blood before LAAO	LAA	Peripheral blood after LAAO	Peripheral blood after 1-month follow-up
Fibrinogen, g/L	2.23 [1.75-2.72]	2.42 [2.15-2.65]	4.17 [3.69-4.86]	2.31 [2.06-2.63]
ETP, nM×min	1480 [1263-1649]	1572 [1238-2122]	1791 [1547-2214]	1389 [1273-1460]
Peak thrombin, nM	215 [169-262]	212 [152-273]	243 [186-295]	189 [146-256]
tPA antigen, ng/mL	9.48 [5.06-11.54]	9.78 [4.32-12.02]	9.50 [8.12-13.05]	5.42 [3.43-7.46]
PAI-1 antigen, ng/mL	4.29 [1.76-6.44]	3.82 [1.89-5.04]	3.76 [2.83-4.39]	2.06 [0.97-2.74]
TAFI antigen, %	81.0 [63.8-91.2]	84.2 [67.3-95.1]	100.1 [90.4-106.1]	88.3 [75.6-99.5]
Plasminogen, %	90.6 [85.5-98.1]	81.5 [70.6-89.4]	101.3 [84.7-112.4]	98.7 [91.2-113.4]

Figure 1. Characteristics of fibrin clots prepared from blood obtained from the LAA and clots prepared from peripheral blood taken before the LAAO, directly after the procedure, and after 1-month follow-up.

Figure 2. Virchow's triad in left atrial appendage



VIRCHOW'S TRIAD IN LEFT ATRIAL APPENDAGE



I. BLOOD STASIS
- atrial fibrillation
- ineffective contraction of the atria
- turbulent blood flow

II. ENDOTHELIAL CELL DAMAGES
- dilated left atrium
- myocyte necrosis
- myocyte hypertrophy
- extracellular matrix abnormal changes

III. HYPERCOAGULABLE STATE
- prolonged plasma fibrin clot permeability
- prolonged clot lysis time
- decreased plasminogen activity
- increased thrombin genereation
- increased PAI-1 antigen levels