JAFIB
Original Research
Journal of Atrial Fibrillation

www. jafib.com

Comparison of Immature Platelet Fraction and Factors Associated with Inflammation, Thrombosis and Platelet Reactivity Between Left and Right Atria in Patients with Atrial Fibrillation

Olga Perelshtein Brezinov[1,2], Ziv Sevilya[1,2], Ella Yahud[1,2], Michael Rahkovich[1,2], Yonatan Kogan[1,2], Gergana Marincheva[1,2], Yana Kakzanov[1,2], Eli Lev[1,2], Avishag Laish-Farkash[1,2]

[1] *Department of Cardiology, Assuta Ashdod Medical Center, Ashdod*
[2] *The Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel*

Abstract

Background: Recent trials found poor temporal relationship between atrial fibrillation (AF) episodes and strokes. Thus, stroke in AF patients probably involves more mechanisms than cardiac embolism. We compared factors of inflammation, thrombosis and platelet reactivity between left (LA) and right atria (RA) and femoral vein (FV) in patients with AF.

Methods:Blood samples were collected from patients undergoing AF-ablation from the FV, RA and LA for neutrophil to lymphocyte ratio (NLR), immature platelet fraction (IPF) and count (IPC), CD40 ligand, P-selectin and E-Selectin. IPF was measured by an autoanalyzer; CD40 ligand, P-selectin, and E-Selectin were measured by ELISA and NLR was calculated from complete blood counts.

Results: Sixty-seven patients were included (age 65±10y, 63% male, CHA_2DS_2-VASc score 2.8±1.8, LA volume index 40±24 mL/m², 63% paroxysmal AF). There was no difference between FV, RA and LA regarding NLR and CD40 ligand. Factors associated with platelets activity: P-selectin, IPC and IPF% were higher in RA vs LA (60.3 IQR 49.0-76.4 ng/ml vs. 59.3 IQR 49.0-74.7, respectively, p=0.03 for P-selectin, 7.5 IQR 5.2-10 10^3/µL vs. 7.1 IQR 5-9.8, p<0.01 for IPC, and 3.6 IQR 2.7-5.0 % vs. 3.6 IQR 2.6-4.8, p<0.01 for IPF%). Similar trends were for E-selectin (41.2 IQR 31.1-51.2 ng/mL vs. 38.7 IQR 27.9-50.4 p=0.09). Similar significant differences were found in patients with CHA_2DS_2-VASC≥2 but not in patients with low score.

Conclusions:Patients with AF, especially those with CHA_2DS_2-VASc≥2, have higher markers of thrombogenicity in RA compared to LA. There was no difference in inflammatory properties between the atria.

Introduction

Atrial fibrillation (AF) confers a 3-6 fold increase in the risk of ischemic stroke, depending on CHA_2DS_2-VASc score[1]. Emerging evidence shows that temporal relationship between subclinical AF and stroke occurs only in a minority of patientsand that strokes often occur without subclinical AF detected within 30 days before the event[2,3]. Thus, stroke in patients with AF probably involves other mechanisms in addition to cardiac embolism. AF might simply be a marker of stroke risk — possibly indicating myocardial fibrosis or hypertrophy, thrombogenic tendency and platelet hyper-reactivity, as well as a pro-inflammatory state[3]. Although in patients with AF, thrombi often originate in the left atrial appendage, anatomical considerations alone cannot completely explain the higher frequency of thrombi in the left heart chambers.

Immature or Reticulated platelets (RPs) are hyper-reactive platelets that are larger platelets with higher dense granules content and contain more RNA compared with mature platelets [4,5]. They are associated with thrombotic propensity and have a greater predilection for thrombus formation. Increased levels of RPs are associated with arterial thrombotic events including acute coronary syndrome and acute stroke[6–8]. Measuring the level of RPs is technically difficult. Recently, an automated assay - immature platelet fraction (IPF) - was introduced and correlates directly with reticulated platelets level[9].

Other markers associated with plateletshyper-reactivity, thrombogenicity and inflammation are markers expressed by platelets such asthe platelet glycoproteins P-selectin (CD62), CD 40 ligand and E-selectin expressed by endothelial cells[10–13]. P-selectin and CD 40

Key Words:
Atrial Fibrillation, Thrombosis, Inflammation, Platelet Activation, P-Selectin, IPF, Neutrophil To Lymphocyte Ratio, Left Atrium.

Corresponding Author:
Olga Perleshtein Brezinov
Cardiology Department
Assuta Ashdod University MC, Ashdod, Israel

ligand are glycoproteins translocatedto the surface of platelets upon activation, and then cleaved and released into the circulation. They promote platelets activation, aggregation and thrombus formation[14,15]. E-selectin is an endothelial surface molecule that acts as an adhesion molecule. E-selectinis enhanced as a result of endothelial activation.High E-selectin levels are found in patients with AF[6,13]. Another marker that was recently correlated with thromboembolic events and stroke in non-valvular AF is the neutrophil-to-lymphocyte ratio (NLR)[16,17]. NLR is an inflammatory marker that has been correlated with atherosclerosis and coronary cardiac events[18] and was shown to be an independent risk factor for spontaneous echo contrast of LA appendage and LA thrombus formation[17,19].

There is limited data to suggest that there is chamber specific platelet activation that could explain, in part, the propensity for LA thrombus formation in patients with AF[10,12,20]. It is still unclear whether there is any difference between right and left atria regarding platelets activation.

The aim of this study is to examine whether inflammatory markers, such as NLR, C-reactive protein (CRP) and CD40 ligand, and/or pro-thrombogenic markers (such as P-selectin,E-selectin and CD-40) differ between systemic circulation and both atria or between LA and RA, thereby potentially explain the higher proportion of stroke in AF patients.

Methods

We enrolled consecutivepatients withAF who underwent ablation as per European guidelines indications- pulmonary vein isolation and substrate ablation as needed[1]. All patients were enrolled during the index hospitalization for the ablation. The study was approved by the ethical review board of our institution (Institutional Helsinki Board) and all subjects provided written informed consent. The study was registered in the Ministry of Health website of clinical trials.

We excluded patients with chronic hemato-oncologic diseases, anemia (hemoglobin < 10 g/dL), or thrombocytopenia (platelet level < 100 K/μL) or any other condition that could affect blood count. Any patient with acute coronary syndrome or an acute infectious or inflammatory disease was excluded as well.

Three blood samples of 5 cc were drawn from each patient during the ablation procedure: one sample from peripheral vein was taken from the femoral vein (FV); one sample was taken from the RA; and a third sample was taken from the LA immediately after trans-septal puncture and prior to the administration of intravenous Heparin. From each sample (three per patient), we measured NLR, IPF, CRP, CD40 ligand, P-selectin and E-selectin.IPF assessment was performed by automated analyzer (Sysmex XN-3000, Sysmex America Inc. Mundelein, Illinois) that uses fluorescent dyes containing polymethine and xazine. This system discriminates between mature and immature platelets and reports the immature platelet fraction[9]. Plasma level of soluble P selectin, E selectin and CD40 ligand were analyzed using enzyme-linked immuno-adsorbent assay (Human P selectin Quantkine ELISA DPSE00, Human E selectin Quantkine ELISA DSLE00 and Human CD 40 Ligand Quantkine ELISA DCDL 40, R&D systems, Minneapolis).

Statistical analysis of continuous variables between the groups was conducted using student paired t-test for normal distributed variables or Wilcoxon rank test for non-normal distribution as appropriate. Categorical variables were compared by a chi-square ($x2$) test. Data are presented as mean ± SD for normally distributed continuous variables, as median with interquartile range (IQR) for continuous variables that are not normally distributed, and as frequency (%) for categorical variables. Statistical significance was accepted at $p < 0.05$. All analyses were performed with the SPSS version 22 statistical software (IBM Inc. Chicago, Illinois).

Results

Baseline patients' characteristics

Sixty-seven patients who underwent AF ablation wereenrolled in this study.Table 1 describes baseline clinical and echocardiographic characteristics. Mean age was 65.1 ± 10.3 years and 42 (62.7%) were men. Forty-twopatients (62,7 %) had paroxysmal AF and 25 (37.3%) had persistent AF. MeanCHA_2DS_2-VAScscorewas 2.8± 1.8, with 19 patients (28%) having low score and inherent differences in baseline co-morbidities accordingly (Table 1).

Regarding medical treatment, patients with CHA_2DS_2-VASc≥2 were treated more with statins and angiotensin converting enzyme inhibitor (ACE-I) or angiotensin II receptor blocker (ARB) than patients with CHA_2DS_2-VASc<2: 28(58.2%) vs. 5 (26.3%) and 36 (75.0%) vs. 6 (31.6%) respectively (p=0.02 and p=0.001). There was no difference regarding antithrombotic therapy, including aspirin, clopidogrel, ticagrelor or prasugrel. Almost all patients in our cohort -65 (97.0%) - were treated with anticoagulation according to their CHA_2DS_2-VAScscore. (Table 1)

Regarding echocardiographic characteristics of the cohort: mean measurements of ejection fraction (EF) were55.3 ± 9.1 %, LA diameter 43.1± 5.6 mm, LA volume 79.5±26.4 mL and indexed to body surface 40.1± 23.9 mL/m2. Patients with CHA_2DS_2-VASc≥2 had higher estimated pulmonary artery wedge pressure (36.3 ±9.4 mmHg vs. 29.7 ±8.8, respectively; p<0.05). There was no difference in LA diameter, volume and indexed volume to body surface area (Table 1).

Platelets reactivity and inflammatory markers

Platelet activation was evaluated by soluble P-selectins,IPF and CD40 ligand levelsand activated endothelium was evaluated by soluble E-selectinlevel (Table 2). Soluble P-selectin mean levels were significantly higher in the RA compared to LA (60.3 IQR 49.0-76.4 ng/ml vs. 59.3 IQR 49.0-74.7 ng/ml, respectively, p=0.03). CD40 ligand levels did not differ between RA and LA. There was also a trend towards higher soluble E-selectin levels in the RA vs LA (41.2 IQR 31.1-51.2 ng/ml vs. 38.7 IQR 27.9-50.4 ng/ml, respectively, p=0.09) (Table 2). No difference was found in soluble P-selectin, E-selectin and CD40 levels between peripheral blood and both atria.

The level of immature platelet counts (IPC) was higher in the RA compared to LA, 7.5 IQR 5.2-10.0103/μL vs. 7.1 IQR 5.0-9.8 103/μL (p <0.01) and 8.2±4.2 103/μL in RA vs. 7.9±4.1 103/μL in LA

Table 1: Patients'baseline characteristics

	All Patients (N=67)	$CHA_2Ds_2VASC<2$ (N= 19)	$CHA_2DS_2VASC≥2$ (N= 48)	P value
Clinical Characteristics				
Age (years)	65.1 ± 10.3	55.1± 9.2	69.1±7.8	<0.01
Male Gender (%)	42 (62.7)	16 (84.2)	26 (61.9)	0.02
Diabetes Mellitus (%)	22 (32.8)	1 (5.3)	21 (43.8)	<0.01
Hypertension (%)	47 (70.1)	5 (26.3)	42 (62.7)	<0.01
Dyslipidemia (%)	40 (59.7)	7 (36.8)	33 (68.8)	0.02
Smoker (%) *	6 (9.0)	2 (10.50	4 (8.3)	1.00
Ischemic heart disease (%) *	13 (19.4)	0 (0)	4 (27.1)	0.01
Cerebrovascular disease (%) *	6 (9.0)	0 (0)	6 (12.5)	0.17
Peripheral arterial disease (%) *	5 (7.5)	0 (0)	5 (10.4)	0.31
Congestive Heart Failure (%) *	12 (17.9)	1 (5.3)	11 (22.9)	0.16
Chronic renal failure (%)	4 (6.0)	0 (0)	4 (8.3)	0.57
$CHAD_2$-$VASC_2$ Score	2.8 ± 1.8	0.6 ± 0.5	3.6±1.3	<0.001
Arrhythmia Type - Paroxysmal AF (%)	42 (62.7)	14 (73.7)	28 (58.3)	0.24
Persistent AF (%)	25 (37.3)	5 (26.3)	20 (41.7)	
Medical Treatment				
Aspirin (%) *	7 (10.4)	2 (10.5)	5 (10.4)	1.0
Other anti-aggregation (Clopidogrel, Ticagrelor, Prasugrel) (%) *	2 (3.0)	0 (0)	2 (4.2)	1.0
Statins (%)	33 (49.3)	5 (26.3)	28 (58.3)	0.02
ACE-I/ARB (%)	42 (62.7)	6 (31.6)	36 (75.0)	0.001
Beta Blockers (%)	42 (62.7)	12 (63.2)	30 (62.5)	0.96
Anticoagulation (Apixaban, Rivaroxaban, Dabigatran) (%)	65 (97.0)	17 (89.4)	48 (100)	0.16
Antiarrhythmic (%)				
Ic (Propafenone, Fecanide)	11 (16.4)	5 (26.3)	6 (12.5)	
III (Sotalol)	2 (3.0)	0 (0)	2 (4.2)	0.49
III (Amiodarone, Dronaderone)	32 (47.8)	7 (36.8)	25 (52.1)	
Echocardiographic Characteristics				
Ejection Fraction (%)	55.3 ± 9.1	55.8 ± 5.8	55.1±10.1	0.73
LVEDd (mm)	47.4 ± 5.3	48.4 ± 5.3	47.0 ± 5.3	0.33
LVESd (mm)	32.1 ± 5.6	32.7 ± 5.0	31.9 ± 5.9	0.56
Diastolic Dysfunction grade – 0 (%)	19 (28.4)	9 (47.4)	10 (20.8)	0.118
1 (%)	7 (10.4)	1 (5.3)	6 (12.5)	
2 (%)	16 (23.90	2(10.5)	14 (29.2)	
3 (%)	3 (4.5)	0 (0)	3 (6.3)	
Left Atrial Diameter (mm)	43.1 ± 5.9	42.9 ± 6.6	43.1 ± 5.8	0.90
Left Atrial Area (cm^2)	24.4 ± 5.3	22.7 ± 4.8	25.0 ± 5.4	0.14
Left Atrial Volume (mL)	79.5 ± 26.4	73.6 ± 28.8	81.2 ± 25.8	0.46
Left Arial Volume Index (mL/m^2)	40.1 ± 23. 9	35.7 ± 12.5	41.3 ± 14.2	0.26
Right Atrial Area (cm^2)	19.2 ± 5.5	18.9 ± 4.1	19.3 ± 6.0	0.77
Right Atrial Volume (mL)	48.8 ± 23.4	49.7 ± 16.6	48.6 ± 25.1	0.88
Right Arial Volume Index (mL/m^2)	24.4 ± 10.9	25.0 ± 6.7	24.2 ± 11.8	0.82
Estimated Pulmonary Arterial Pressure (mmHg)	34.7 ± 9.6	29.7 ± 8.8	36.3 ± 9.4	0.03

* By Fisher Exact Test

Abbriviations: LVEDd= left ventricular end diastolic diameter, LVESd=left ventricular end systolic diameter, AF= atrial fibrillation, CHA2DS2-VASc score =score for atrial fibrillation stroke risk that includes congestive heart failure, hypertension, age above 65 or 75, diabetes, previous stroke, vascular disease history and female sex, ACE-I= angiotensin converting enzyme inhibitor, ARB=angiotensin II receptor blocker.

(p=0.05). IPF% (immature platelets fraction) was higher in the RA compared to LA as well, 3.6 IQR 2.7-5.0 % vs. 3.6 IQR 2.6-4.8 % (p<0.01) and 4.4±2.9 % in RA vs. 4.1±2.5 % in LA(p=0.04).

Inflammatory markers were evaluated by NLR and CRP (Table 2). There was no difference between RA and LA regarding NLR. However, CRP was lower in the LA vs. RA (2.6 IQR 1.3-5.0 mg/dL, 5.2 mg/dL, p<0.05).

Platelets and inflammatory markers according to CHA_2DS_2-VASc score

We examined separately patients with CHA_2DS_2-VASc≥2 and CHA_2DS_2-VASc<2 (Table 3); patients with low CHA_2DS_2-VASc score had no differences in platelet activation markerssuch as soluble P-selectin, IPF% or soluble E-selectin (Table 3). However, patients with CHA2DS2-VASc≥2 had higher levels of IPF% and soluble P-selectinin the RA compared with the LA (3.6 IQR 2.7-9.6 % vs. 3.5 IQR 2.7-5.2 % (p<0.01) and 69.3 IQR 48.5-79.1 ng/mL vs. 59.3. IQR 49.0-75.6 ng/mL (p<0.05), respectively). There was also a trend towards higher soluble E-selectin levels in the RA vs LA in this group of patients. No difference was found between RA and LA regarding NLR in both groups with either CHA_2DS_2-VASc≥2 and CHA_2DS_2-VASc<2(Table 3).

Discussion

The main findings of our study are that in patients with AF, markers of platelet activation are higher in the RA compared with the LA, while most inflammatory markers do not differ between the atria. These differences are especially pronounced in patients with CHA_2DS_2-VASc≥2.

In this study we examined three microparticles related to platelets reactivity. P-selectin is an important protein in recruitment and aggregation of platelets and is an important marker of activated platelets [21]. Soluble P-selectin is a remnant marker of activated pro-thrombotic platelets.Thus, soluble levels of P-selectin correlate with expressed P-selectin within plateletmembrane[15,21]. Activated platelets also release CD40 ligand. CD40/CD40 ligandactivation results in expression of many pro-inflammatory and pro-thrombotic factors, including IL-1, IL6, and TNF-α and correlatewith pro-thrombotic states such as cerebrovascular ischemia[21]. Another glycoprotein related to thrombogenicity of platelets is E-selectin. E-selectin is expressed on endothelial cells and is related to activated leukocytes; it is also secreted from intracellular granules and is measurable in soluble form. E-selectin has been shown to be an important factor in neutrophil trafficking and platelets recruitment and therefore hasan important key role in thrombus formation. [21,22]

Previous studies showed that platelets of patients with AFare more pro-thrombotic compared to non-AF patients[23]. Patients with AF have higher levels of pro-thrombotic markers such as P-selectin, CD40 and mean platelets volume[11,24–26].

Table 2: A comparison of platelet and inflammatory markers between peripheral vein and both atria

	Right Atria	Left Atria	P value (left vs. right atria)
WBC (103/µL)	6.0 (4.9-7.8)	6.1 (5.0-7.8)	0.70
Neutrophils(103/µL)	3.8 (2.7-5.6)	3.7 (2.8-5.7)	0.70
Lymphocytes (103/µL)	1.6 (1.2-2.1)	1.6 (1.3-2.0)	0.45
Neutrophils (%)	63.6 (55.8-68.9)	63.9 (56.8-68.8)	0.52
Lymphocytes (%)	25.5 (21.6-31.1)	25.5 (21.6-32.3)	0.72
Platelets (103/µL)	195.5 (160.5-229.0)	194.0 (163.3-234.0)	0.99
IPC (103/µL)	7.5 (5.2-10.0)	7.1(5.0-9.8)	<0.01
IPF (%)	3.6 (2.7-5.0)	3.6 (2.6-4.8)	<0.01
NLR (ratio)	2.5 (1.9-3.2)	2.5 (1.7-3.2)	0.37
CRP (mg/dL)	2.7 (1.5-5.2)	2.6 (1.3-5.0)	<0.01
Soluble P-Selectin (ng/mL)	60.3 (49.0-76.4)	59.3 (49.0-74.7)	0.03
Soluble E-Selectin(ng/mL)	41.2 (31.1-51.2)	38.7 (27.9-50.4)	0.09
CD-40 ligand (pg/mL)	512.5 (371.9-746.9)	500.0 (362.5-843.8)	0.72

Abbreviations: WBC= white blood count; NLR= neutrophils to lymphocytes ratio; CRP= C reactive protein; IPF= immature platelets fraction, IPC=immature platelets count.

Thrombogenicity of the LAwas shown to be induced by AF and to return to baseline after return to sinus rhythm[27]; however, the reason why thrombi are more prevalent inthe LA is still an unanswered question. Temporal relationship between subclinical AF and stroke occurs only in a minority of patientsand strokes often occur without subclinical AF detected within 30 days before the event[3,23]. Thus, debate still exists whether thrombus formationis related only to the stunning ofthe atria during and post AF or there are anatomical differences between the atria that cause propensity of LA thrombus (fibrosis, hypertrophy, etc.). Possibly,there are other alternativemechanisms related to cell activation, such as in situ thrombogenic tendency or platelet hyper-reactivity or a pro-inflammatory state that is associated with both AF and stroke[10,28].

There is conflicting evidence regarding the propensity of the LA to form thrombi. Some studiesimply that platelets are more activated within the LA. Unexpectedly, other studies suggest the contrary- that there are more pro-thrombotic factors,including elevated platelet activation markers, in the RA, as was shown in our study.

Yamamoto et al. have shown that the coagulation system is more activated in the LAin patients with mitral stenosis[29]. Willoughby et al. reportedelevated P-selectinlevels and elevated ADP-induced platelet aggregation within LA compared to RA in patients who underwent pulmonary vein isolation ablation for AF[10]. Lim et al.reportedhigher platelet activation (assessed by P-selectin levels) in patients with AF compared with anon-AF group, and an increase in thrombin generation in the LA compared with peripheral blood in patients with AF[27].

In contrast to these studies, Schultz et al. did not show any differences between P-selectin, CD 40 or endothelial markers in the right and the left atria in patients with either AF or supra-ventricular tachycardias[30]. Jesel et al. also did not show any atrial specific differences in the levels of pro-coagulant factors, including platelet derived microparticles.They did show, however, that endothelial-derived microparticles, tissue factor activity and collagen-induced platelet aggregation were slightly elevatedin the RA of patients with a history of AF[31]. Park et al. also did not show any differences between right and left atria regarding pro-thrombotic factors, such as E-selectin, in patients with valvular AF[32]. Additionally, Akar et al. showedin AF patients (spontaneously or following atrial pacing), thatP-selectin levels were elevated in the coronary sinus compared to the peripheral blood. They also showed increased local thrombin generation in the coronary sinus, decreased nitric oxide production and no change in in flammatory markers.This study suggests increased pro-thrombotic characteristics of platelets especially in the RA, although sampling from the left side of the heartwas not performed[20].

In our study we compared directly thrombogenic and inflammatory markersin RA vs. LA and also vs.peripheral blood in AF patients. Our results imply that thrombogenicityis higher in the RA than the LA in the setting of AF.We found an increase in platelet activation markers in the RA, as reflected by elevated P-selectin, E-selectin,IPC and IPF levels in the RA vs. LA, especially in patients with CHA_2DS_2-VASc≥2. In contrast, we did not find any significant difference between the atria in markers of inflammatory process (CD40 ligand, NLR or CRP levels).

Recently the approach to pathophysiology of AF focuses on atrial cardiomyopathy as the process leading to atrial dysfunction. The fibrillation of atrium is a symptom of this pathology. AF can affect both atria in atrial cardiomyopathy in patients with AF which is not related to rheumatic heart disease[33]. Bilge et al. showed that atrial thrombi are formed in the right atrium as well as in the left side in patients with non-valvular AF, as compared with valvular AF[34].Other echocardiographic studies have also shown thrombi formation in the RA as well as in LA[32-35]. In a study by Shahin et al. RA dysfunction was demonstrated in addition to left sided atrial dysfunction[35].

Another explanation could stem from the role of the lungs in this process: However, because the lungs are capable of absorbing microthrombi without any clinical significance, as opposed to the left system, the resulting neurological and peripheral consequence of the left side emboli are well expressed [36–39]. This explanation suggests that thrombi are formed in the right side of the heart as well as in the left side, with higher thrombogenicity in the RA, but with less clinical consequences. This is probably the reason for the higher soluble P-selectin in RA versus LA found in our study; soluble P-selectin is created by proteolytic degradation of P-selectin on platelets. It was higher in RA either due to secretion from higher IPF in RA (as stated above) or due to more protein sequestration in the lungs. Because of the scarce data available regarding the function and the role of RA in patients with AF, the data from our study that suggest that RA has thrombogenic properties, has a novel additive value to understandingthis controversial subject.

Platelets are removed from the circulation in the reticuloendothelial system after a short lifespan of 8-10 days. Much information exists regarding mechanisms of platelets activation. But less is knownabout the clearance of cells and their death [40]. Platelets undergo a

Table 3: A comparison of platelet and inflammatory markers between atria according to CHA_2DS_2-VASc Score

	CHA_2DS_2-VASc≥2 (N=48)			CHA_2DS_2-VASc<2 (N=19)		
	Right Atria	Left Atria	P value	Right Atria	Left Atria	P value
WBC(10^3/μL)	6.4 (5.2-8.3)	6.4 (5.3-8.2)	0.93	5.9 (4.8-6.8)	6.1	0.5
Neutrophils (10^3/μL)	4.1 (2.8-5.7)	4.0 (2.8-5.9)	0.99	3.5 (2.5-4.7)	3.5 (2.4-4.5)	0.5
Lymphocytes (10^3/μL)	1.6 (1.2-1.9)	1.6 (1.1-2.0)	0.73	1.7 (1.4-2.1)	1.6 (1.4-2.0)	0.4
Neutrophils (%)	64.0 (58.2-70.2)	64.2 (57,7-70.3)	0.74	60.6 (54.2-67.6)	60.3 (54.1-68.0)	0.4
Lymphocytes (%)	24.8 (18.9-30.5)	25.1 (18.5-31.0)	0.98	26.1 (23.7-36.3)	27.4 (23.0-35.6)	0.5
Platelets (103/μL)	192.5 (159.5-233.0)	193.0 (160.8-232.0)	0.72	207.0 (161.3-229.0)	197.5 (162.0-237.3)	0.8
IPC (103/μL)	7.4 (5.3-9.6)	7.0 (5.3-9.6)	0.02	7.9 (4.8-10.5)	7.3 (4.1-10.4)	0.08
IPF (%)	3.6 (2.7-9.6)	3.5 (2.7-5.2)	0.01	3.6 (2.5-4.7)	3.8 (4.1-10.4)	0.1
NLR (ratio)	2.5 (1.9-3.6)	2.6 (1.8-3.7)	0.5	2.3 (1.5-2.7)	2.5 (1.6-2.8)	0.5
CRP (mg/dL)	3.0 (1.6-5.5)	2.6 (1.6-5.5)	0.01	2.4 (1.0-5.0)	2.2(0.9-4.6)	0.08
Soluble P-Selectin (ng/mL)	69.3 (48.5-79.1)	59.3(49.0-75.6)	0.04	58.5 (49.8-69.8)	56.8 (40.1-74.3)	0.4
Soluble E-Selectin(ng/mL)	43.6 (30.5-52.8)	39.6(28.0-51.5)	0.08	40.0 (31.2-46.7)	37.4 (27.5-45.7)	0.8
CD-40 ligand (pg/mL)	512.5 (362.5-825.0)	500.0 (333.3-850.0)	0.4	512.5 (377.1-581.3)	496.9 (406.3-828.1)	0.4

Abbreviations: WBC= white blood count; NLR= neutrophils to lymphocytes ratio; CRP= C reactive protein; IPF= immature platelets fraction.IPC=immature platelets count.

process that is similar to intrinsic apoptosis in the liverthrough Ashwell-Morrell receptor (AMR) [41].Therefore, following this process of apoptosis the venous blood return from the liver likely contains more young platelets. The higher levels of IPF and IPC that we have found in the RA probably reflect the venous blood circulation after the apoptosis process of 'old' platelets, contributing to the higher proportion of immature platelets in the RA.According to this theory, it is notsurprising to find more young platelets in the RA and pulmonary vasculature.

In this study we found a small difference towards higher level of CRP in the RA compared to LA. CRP is produced in the liver and is distributed to the rest of the circulation through the venous return to the heart [42].This is probably the reason for the difference between the two atria. In our opinion, this difference is too small to have any clinical significance.

Limitations: One of the limitations of the study is the non-relevance of the FV blood samples regarding thrombogenic factors. Given the sheaths (7F) were already introduced and blood was collected from the FV through the sheaths, it is possible that some of the observed values of the FV are due to introduction of potentially thrombogenic material into the vasculature. Anotherlimitation of the study is the lack of a control group of non-AF patients, in order to better understand the role of AF in these LA-RA differences. However, the above-mentioned previous studies did not find any differences between the atria in non-AF patients[30]. Another limitation is a non-uniform rhythm of the cohort during AF ablation: some patients underwent the procedure in sinus rhythm, others were in AF, and some patients started the procedure in AF and converted to sinus.

Conclusions

We found higher markers of thrombogenicity and platelet activation in the right compared to the left atrium in patients with AF. The results were even more pronounced in patients with CHA_2DS_2-VASc≥2.There does not appear to be a gradient of inflammatory properties between the two atria in these patients. These findings set the basis for further investigation to explain the higher stroke risk in AF patients.

References

1. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Heart J. 2016 Oct 7;37(38):2893–962.
2. Hirsh BJ, Copeland-Halperin RS, Halperin JL. Fibrotic atrial cardiomyopathy, atrial fibrillation, and thromboembolism: mechanistic links and clinical inferences. J Am Coll Cardiol. 2015 May 26;65(20):2239–51.
3. Brambatti M, Connolly SJ, Gold MR, Morillo CA, Capucci A, Muto C, et al. Temporal relationship between subclinical atrial fibrillation and embolic events. Circulation. 2014 May 27;129(21):2094–9.
4. Bernlochner I, Goedel A, Plischke C, Schüpke S, Haller B, Schulz C, et al. Impact of immature platelets on platelet response to ticagrelor and prasugrel in patients with acute coronary syndrome. Eur Heart J. 2015 Dec 1;36(45):3202–10.
5. Robinson MS, Mackie IJ, Khair K, Liesner R, Goodall AH, Savidge GF, et al. Flow cytometric analysis of reticulated platelets: evidence for a large proportion of non-specific labelling of dense granules by fluorescent dyes. Br J Haematol. 1998 Feb;100(2):351–7.
6. Grove EL, Hvas A-M, Kristensen SD. Immature platelets in patients with acute coronary syndromes. Thromb Haemost. 2009 Jan;101(1):151–6.
7. Cesari F, Marcucci R, Caporale R, Paniccia R, Romano E, Gensini GF, et al. Relationship between high platelet turnover and platelet function in high-risk patients with coronary artery disease on dual antiplatelet therapy. Thromb Haemost. 2008 May;99(5):930–5.
8. Lakkis N, Dokainish H, Abuzahra M, Tsyboulev V, Jorgensen J, De Leon AP, et al. Reticulated platelets in acute coronary syndrome: a marker of platelet activity. J Am Coll Cardiol. 2004;44(10):2091–3.
9. Pons I, Monteagudo M, Lucchetti G, Munoz L, Perea G, Colomina I, et al. Correlation between immature platelet fraction and reticulated platelets. Usefulness in the etiology diagnosis of thrombocytopenia. Eur J Haematol. 2010;85(2):158–63.
10. Willoughby SR, Roberts-Thomson RL, Lim HS, Schultz C, Prabhu A, De Sciscio P, et al. Atrial platelet reactivity in patients with atrial fibrillation. Heart Rhythm. 2010 Sep;7(9):1178–83.
11. Weymann A, Ali-Hasan-Al-Saegh S, Sabashnikov A, Popov AF, Mirhosseini SJ, Nombela-Franco L, et al. Platelets Cellular and Functional Characteristics in Patients with Atrial Fibrillation: A Comprehensive Meta-Analysis and Systematic Review. Med Sci Monit Basic Res. 2017;23:58–86.
12. Jennings JM, Ideker RE. Why do thrombi form in the left but not the right

atrium in atrial fibrillation: differences in platelet P-selectin levels? Heart Rhythm. 2010;7(9):1184–5.
13. Krishnamoorthy S, Khoo CW, Lim HS, Lane DA, Pignatelli P, Basili S, et al. Prognostic role of plasma von Willebrand factor and soluble E-selectin levels for future cardiovascular events in a "real-world" community cohort of patients with atrial fibrillation. Eur J Clin Invest. 2013 Oct;43(10):1032–8.
14. Grosdidier C, Blanz KD, Deharo P, Bernot D, Poggi M, Bastelica D, et al. Platelet CD40 ligand and bleeding during P2Y12 inhibitor treatment in acute coronary syndrome. Res Pract Thromb Haemost. 2019 Oct;3(4):684–94.
15. Bath PM, May J, Heptinstall S. Clinical utility of remote platelet function measurement using P-selectin: assessment of aspirin, clopidogrel, and prasugrel and bleeding disorders. Platelets. 2018 Jul;29(5):425–30.
16. Ertaş G, Sönmez O, Turfan M, Kul S, Erdoğan E, Tasal A, et al. Neutrophil/lymphocyte ratio is associated with thromboembolic stroke in patients with non-valvular atrial fibrillation. J Neurol Sci. 2013 Jan 15;324(1–2):49–52.
17. Fukuda Y, Okamoto M, Tomomori S, Matsumura H, Tokuyama T, Nakano Y, et al. In Paroxysmal Atrial Fibrillation Patients, the Neutrophil-to-lymphocyte Ratio Is Related to Thrombogenesis and More Closely Associated with Left Atrial Appendage Contraction than with the Left Atrial Body Function. Intern Med. 2018 Mar 1;57(5):633–40.
18. Balta S, Ozturk C, Balta I, Demirkol S, Demir M, Celik T, et al. The Neutrophil-Lymphocyte Ratio and Inflammation. Angiology. 2016;67(3):298–9.
19. Yalcin M, Aparci M, Uz O, Isilak Z, Balta S, Dogan M, et al. Neutrophil-lymphocyte ratio may predict left atrial thrombus in patients with nonvalvular atrial fibrillation. Clin Appl Thromb Hemost. 2015 Mar;21(2):166–71.
20. Akar JG, Jeske W, Wilber DJ. Acute onset human atrial fibrillation is associated with local cardiac platelet activation and endothelial dysfunction. J Am Coll Cardiol. 2008;51(18):1790–3.
21. Barale C, Russo I. Influence of cardiometabolic risk factors on platelet function. Int J Mol Sci. 2020 Jan 17;21(2).
22. Myers D, Lester P, Adili R, Hawley A, Durham L, Dunivant V, et al. A new way to treat proximal deep venous thrombosis using E-selectin inhibition. J Vasc Surg Venous Lymphat Disord. 2020;8(2):268–78.
23. Pourtau L, Sellal JM, Lacroix R, Poncelet P, Bernus O, Clofent-Sanchez G, et al. Platelet function and microparticle levels in atrial fibrillation: Changes during the acute episode. Int J Cardiol. 2017 Sep 15;243:216–22.
24. Tarnowski D, Poitz DM, Plichta L, Heidrich FM, Wiedemann S, Ruf T, et al. Comparison of diverse platelet activation markers as indicators for left atrial thrombus in atrial fibrillation. Platelets. 2018 Jan;29(1):41–7.
25. Wysokinski WE, Cohoon KP, Melduni RM, Mazur M, Ammash N, Munger T, et al. Association between P-selectin levels and left atrial blood stasis in patients with nonvalvular atrial fibrillation. Thromb Res. 2018 Oct 10;172:4–8.
26. Kosiuk J, Uhe T, Stegmann C, Ueberham L, Bertagnolli L, Dagres N, et al. Morphological determinators of platelet activation status in patients with atrial fibrillation. Int J Cardiol. 2019 Mar 15;279:90–5.
27. Lim HS, Willoughby SR, Schultz C, Gan C, Alasady M, Lau DH, et al. Effect of atrial fibrillation on atrial thrombogenesis in humans: impact of rate and rhythm. J Am Coll Cardiol. 2013 Feb 26;61(8):852–60.
28. Jesel L, Abbas M, Toti F, Cohen A, Arentz T, Morel O. Microparticles in atrial fibrillation: a link between cell activation or apoptosis, tissue remodelling and thrombogenicity. Int J Cardiol. 2013 Sep 30;168(2):660–9.
29. Yamamoto K, Ikeda U, Seino Y, Mito H, Fujikawa H, Sekiguchi H, et al. Coagulation activity is increased in the left atrium of patients with mitral stenosis. J Am Coll Cardiol. 1995;25(1):107–12.
30. Schultz CD, Rangneker G, Lim HS, Fraudeau A, Young G, Roberts-Thomson K, et al. Characterization of thrombogenic, endothelial and inflammatory markers in supraventricular tachycardia: a study in patients with structurally normal hearts. Clin Exp Pharmacol Physiol. 2014 Aug;41(8):551–7.
31. Jesel L, Arentz T, Herrera-Siklody C, Trenk D, Zobairi F, Abbas M, et al. Do atrial differences in endothelial damage, leukocyte and platelet activation, or tissue factor activity contribute to chamber-specific thrombogenic status in patients with atrial fibrillation? J Cardiovasc Electrophysiol. 2014 Mar;25(3):266–70.
32. Park JH, Lee JS, Ko Y-G, Lee SH, Lee BS, Kang S-M, et al. Histological and Biochemical Comparisons between Right Atrium and Left Atrium in Patients with Mitral Valvular Atrial Fibrillation. Korean Circ J. 2014 Jul 25;44(4):233–42.
33. Bălăceanu A. Right atrium thrombosis in nonvalvular permanent atrial fibrillation. J Med Life. 2011 Nov 14;4(4):352–5.
34. Bilge M, Eryonucu B, Güler N, Erkoç R. Right atrial appendage function in patients with chronic nonvalvular atrial fibrillation. Jpn Heart J. 2000 Jul;41(4):451–62.
35. Sahin T, Ural D, Kilic T, Bildirici U, Kozdag G, Agacdiken A, et al. Right atrial appendage function in different etiologies of permanent atrial fibrillation: a transesophageal echocardiography and tissue Doppler imaging study. Echocardiography. 2010 Apr;27(4):384–93.
36. Barbati G, De Domenico R, Rossi S, Vecchiato E, Zeppellini R. [Right atrial appendage thrombosis during atrial fibrillation: an element to look for]. G Ital Cardiol. 2017 Mar;18(3):247–50.
37. de Divitiis M, Omran H, Rabahieh R, Rang B, Illien S, Schimpf R, et al. Right atrial appendage thrombosis in atrial fibrillation: its frequency and its clinical predictors. Am J Cardiol. 1999 Nov 1;84(9):1023–8.
38. Nemes A, Havasi K, Sághy L, Kohári M, Forster T. [Echocardiographic evaluation of the right atrial appendage and its clinical consequences]. Orv Hetil. 2019 Mar;160(12):443–7.
39. Sahin T, Ural D, Kilic T, Bildirici U, Kozdag G, Agacdiken A, et al. Right atrial appendage function in different etiologies of permanent atrial fibrillation: a transesophageal echocardiography and tissue Doppler imaging study. Echocardiography. 2010;27(4):384–93.
40. McArthur K, Chappaz S, Kile BT. Apoptosis in megakaryocytes and platelets: the life and death of a lineage. Blood. 2018 Feb 8;131(6):605–10.
41. Pluthero FG, Kahr WHA. The birth and death of platelets in health and disease. Physiology (Bethesda). 2018 May 1;33(3):225–34.
42. Black S, Kushner I, Samols D. C-reactive Protein. J Biol Chem. 2004 Nov 19;279(47):48487–90.