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Impact of Atrial Fibrillation and Sinus Rhythm Restoration on Reticulated Platelets

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Abstract

Objective: To assess the impact of nonvalvular atrial fibrillation (NVAF) and sinus rhythm restoration on the distribution of reticulated platelets (RPs), which are known to be associated with thrombotic propensity and have a greater predilection for thrombus participation.

Participants and Methods: The RP content was assessed by flow cytometry (thiazole orange/CD61) in 110 consecutive patients with NVAF before and 3 to 4 months after catheter ablation of the pulmonary veins. Results were compared with those of 55 age- and sex-matched controls with normal sinus rhythm.

Results: The mean ± SD percentage of RPs was higher in patients with NVAF compared with controls (28.5%±7.3% vs 6.4%±5.3%; $P<.001$). The RP content did not vary by CHA_2DS_2-VASc score. After catheter ablation of the pulmonary veins, 63 patients were available for follow-up assessment. A significant reduction of RPs was observed compared with preintervention values (29.85%±7.1% vs 20.79%±7.6%; $P<.001$). During follow-up, 19% of patients (12 of 63) had confirmed AF recurrence. The mean ± SD percentage of RPs was higher in this group than in those without a recurrence (24.7%±6.5% vs 18.9%±7.5%; $P=.01$).

Conclusion: Nonvalvular atrial fibrillation affects the percentage of RPs, independent of the CHA_2DS_2-VASc score. After ablation, RP content dropped significantly. High RP content in patients with NVAF may explain the potential mechanism of thromboembolic complications and the lack of efficacy of currently available antiplatelet therapy for stroke prevention in this dysrhythmia.

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Cardioembolic stroke from nonvalvular atrial fibrillation (NVAF) begins with the development of left atrial appendage thrombus.[1-4] Thrombi in the fibrillating left atrial appendage contain platelet-rich regions surrounded by layers of fibrin.[4,5] The platelet content of embolized thrombi is significantly greater than that of thrombi that have not embolized. This morphologic observation implies a close relationship between platelets and the risk of stroke in AF. Trials of antiplatelet agents for stroke prevention in NVAF have been disappointing.[6] The pathophysiologic mechanism of this paradox is unclear.

Platelet populations are heterogeneous in terms of size, reactivity, circulatory experience, and susceptibility to antiplatelet therapies.[7-9] Platelets are anucleate buds from megakaryocytes.[7] Reticulated platelets (RPs) are young platelets that retain megakaryocyte-derived RNA.[10] Thiazole orange (TO) binds RNA selectively, enabling the detection of RPs by flow cytometry.[11,12] An increased percentage of RPs has been reported to be an independent predictor of cardiovascular death in acute coronary syndromes[13,14] and has been associated with embolic stroke.[15] Reticulated platelets have been found to be resistant (residual platelet reactivity) to antiplatelet therapy.[13,14,16] We hypothesized that NVAF would be associated with an increased percentage of RPs, which could partially explain the lack of efficacy of antiplatelet therapy in this disease.

The goal of this study was to assess the proportion of RPs in patients with NVAF

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compared with individuals with normal sinus rhythm (NSR). Moreover, to determine the impact of AF on this measure, RP content was assessed before and 3 to 4 months after catheter ablation of the pulmonary veins.

MATERIALS AND METHODS

Patient Recruitment

Consecutive patients undergoing catheter ablation of the pulmonary veins for symptomatic NVAF between March 25, 2009, and June 30, 2009, were approached for study participation unless they had (1) an acute illness, stroke, myocardial infarction, or surgery within 30 days; (2) chronic infections or inflammatory conditions, (3) more than moderate native heart valvular disease or heart valve protheses; (4) stage IV or V kidney disease; (5) more than mild liver disease; or (6) thrombocytosis, myeloproliferative disorders, myelodysplastic syndromes, or any other active malignancy. Every patient scheduled for catheter ablation of the pulmonary veins routinely underwent chest/cardiac computed tomography or magnetic resonance imaging to assess pulmonary venous anatomy and transesophageal echocardiography (TEE), and most patients also underwent transthoracic echocardiography. Moreover, during catheter ablation of the pulmonary veins, every patient routinely underwent intracardiac ultrasound.

After catheter ablation of the pulmonary veins, the clinical protocol included a follow-up visit in 3 to 4 months. This visit included ascertainment of recurrent symptoms of arrhythmia, electrocardiography (EKG), 24-hour Holter monitoring, computed tomography, or magnetic resonance imaging of the chest and heart to assess for pulmonary vein stenosis.

Control subjects consisted of patients in NSR with no history of AF. These individuals were recruited from the primary care clinic during their annual medical examination or from a preoperative assessment for elective minor outpatient procedures. The same exclusion criteria for patients with AF were applied to control subjects.

All the patients gave written permission to use their clinical data and biological specimens for research purposes. This protocol was approved by the institutional review boards of the Mayo Foundation, and all the research conduct was performed according to the ethical principles of the Declaration of Helsinki.

TABLE 1. Demographic and Clinical Data for Patients With Nonvalvular Atrial Fibrillation (Cases) and Individuals With Normal Sinus Rhythm (Controls)

Variable	Cases (n=110)	Controls (n=55)	*P* value
Age (y), mean ± SD	59.7±10.4	59.3±10.9	.81
Age category (No. [%])			.24
<40 y	7 (6)	3 (5)	
41-50 y	15 (14)	6 (11)	
51-60 y	33 (30)	25 (45)	
61-70 y	38 (35)	11 (20)	
>70 y	17 (15)	10 (18)	
Sex (No. [%])			.15
Male	80 (73)	34 (62)	
Female	30 (27)	21 (38)	
CHF (No. ([%])	18 (16)	0	.001
Hypertension (No. [%])	67 (61)	28 (51)	.22
Age ≥75 y (No. [%])	6 (5)	4 (7)	.64
Diabetes (No. [%])	9 (8)	7 (13)	.35
Stroke/TIA (No. [%])	7 (6)	2 (4)	.47
Vascular disease (No. [%])	12 (11)	6 (11)	.98
Age 65-74 y (No. [%])	37 (34)	15 (27)	.41
CHA_2DS_2-VASc score (mean ± SD)	1.8±1.4	1.6±1.4	.40
CHA_2DS_2-VASc category (No. [%])			.22
0	23 (21)	12 (22)	
1	24 (21)	18 (33)	
≥2	63 (58)	25 (45)	

CHA_2DS_2-VASc = congestive heart failure, hypertension, age ≥75 years (doubled), diabetes mellitus, stroke (doubled)—vascular disease, age 65 to 74 years, and sex category (female); CHF = congestive heart failure; TIA = transient ischemic attack.

Transesophageal Echocardiography

A TEE was performed as previously described.[17] Spontaneous echocardiographic contrast (SEC) was defined and graded as absent, mild, moderate, or severe according to the published criteria.[18] The left atrial appendage emptying velocity profiles were measured as previously described.[17] The left ventricular ejection fraction (LVEF) was visually estimated. Aortic atherosclerosis severity was defined as simple when atheroma thickness was less than 4 mm and immobile and as severe when it exceeded 4 mm or contained mobile components.[17,19] The left atrium volume index was assessed by transthoracic echocardiography performed within 1 week of TEE and calculated by the biplane area-length method.[19] All echocardiographic images were analyzed by the study cardiologist (N.A.), who was blinded to clinical and laboratory data.

Study Definitions and Event Adjudication

The CHA_2DS_2-VASc (congestive heart failure, hypertension, age ≥75 years (doubled), diabetes mellitus, stroke (doubled)—vascular disease, age