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Clinical Research

Prothrombotic State in Atrial Fibrillation Patients With One Additional Risk Factor of the CHA_2DS_2-VASc Score (Beyond Sex)

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See editorial by Nattel and Lip, pages 547–549 of this issue.

ABSTRACT

Background: It is unclear whether a prothrombotic state occurs in atrial fibrillation (AF) with low stroke risk.

Methods: We studied 118 patients with AF with the **C**ongestive Heart Failure, **H**ypertension, **A**ge (≥ 75 years), **D**iabetes, **S**troke/Transient Ischemic Attack/Systemic Embolism, **V**ascular Disease, **A**ge (65-74 years), **S**ex (Female) (CHA_2DS_2-VASc) score of 1 in men or 2 in women vs 52 patients with AF with the CHA_2DS_2-VASc score of 0 in men or 1 in women. Plasma clot permeability (K_s), a measure of fibrin clot density, and clot lysis time (CLT), endogenous thrombin potential (ETP), von Willebrand factor antigen, and plasminogen activator inhibitor-1 were evaluated in nonanticoagulated subjects.

Results: Patients with the CHA_2DS_2-VASc score of 1 (beyond sex), compared with those with 0, had lower K_s, prolonged CLT, increased ETP, von Willebrand factor antigen, and plasminogen activator inhibitor-1 (all $P < 0.001$), without any sex-dependent differences. Heart failure (odds ratio [OR]: 10.28; 95% confidence interval [CI]:

RÉSUMÉ

Contexte : La question de savoir si un état prothrombotique survient chez les patients atteints de fibrillation auriculaire (FA) à faible risque d'accident vasculaire cérébral (AVC) n'a pas été élucidée.

Méthodologie : Nous avons étudié 118 patients atteints de FA présentant un score CHA_2DS_2-VASc (insuffisance cardiaque congestive, hypertension, âge ≥ 75 ans, diabète, accident vasculaire cérébral/ischémie cérébrale transitoire, maladie vasculaire, âge compris entre 65 et 74 ans, sexe féminin) de 1 chez les hommes ou de 2 chez les femmes comparativement à 52 patients atteints de FA et présentant un score CHA_2DS_2-VASc de 0 chez les hommes ou de 1 chez les femmes. La perméabilité du caillot plasmatique (K_s), une mesure de la densité des caillots de fibrine, ainsi que le temps de lyse du caillot (TLC), le potentiel endogène de thrombine (PET), et la concentration d'antigène du facteur de von Willebrand et d'inhibiteur 1 des activateurs du plasminogène ont été évalués chez les patients non traités par un anticoagulant.

Atrial fibrillation (AF) is associated with an annual risk of stroke up to 20%.[1] The **C**ongestive Heart Failure, **H**ypertension, **A**ge (≥ 75 years), **D**iabetes, **S**troke/Transient Ischemic Attack/Systemic Embolism, **V**ascular Disease, **A**ge (65-74 years), **S**ex (Female) (CHA_2DS_2-VASc) score is commonly used to assess the risk of stroke in this group of patients.[2] Patients with AF not on antiplatelet or anticoagulant therapy without additional clinical stroke risk factors have the ischemic stroke rate of 0.43% per year.[3] Although in those with 1 additional point in the CHA_2DS_2-VASc score, this risk is 1.18% to 3.50% per year.[3,4] The Canadian Cardiovascular Society (CCS), European Society of Cardiology (ESC), and American Heart Association/American College of Cardiology/Heart Rhythm Society guidelines are consistent and suggest not to introduce anticoagulant therapy in patients with the CHA_2DS_2-VASc score of 0.[5-7] However, in patients with the CHA_2DS_2-VASc score of 1 (beyond sex), the ESC guidelines (class IIa, level of evidence B) recommend that anticoagulant therapy should be considered.[5] On the other hand, the American Heart Association/American College of Cardiology/Heart Rhythm Society guidelines (IIb, level of evidence C), in

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2.32-45.41), age 65-74 years (OR: 4.37; 95% CI: 1.76-10.83), and hypertension (OR: 5.03; 95% CI: 1.81-13.94) were independently associated with low K_s (the lowest quartile, $\leq 6.4 \times 10^{-9}$ cm^2), whereas only age 65-74 years (OR: 3.33; 95% CI: 1.59-6.96) significantly predicted prolonged CLT (the top quartile, $\geq$ 108 minutes). Age 65-74 years (OR: 5.21; 95% CI: 2.12-12.80), heart failure (OR: 6.58; 95% CI: 1.49-29.06), and hypertension (OR: 4.33; 95% CI: 1.54-12.15) were independently associated with high ETP (the top quartile, $\geq$ 1681.3 nM $\times$ minutes).

Conclusions: A prothrombotic state (increased thrombin generation, denser fibrin clots, impaired fibrinolysis, and endothelial injury) characterizes patients with AF with 1 additional clinical stroke risk factor (beyond sex), with age 65-74 years being particularly associated with prothrombotic indices.

Résultats : Les patients ayant un score CHA_2DS_2-VASc de 1 (sans égard au sexe), comparativement à ceux chez qui ce score était de 0, affichaient un K_s moins élevé, un TLC plus long, et des valeurs plus élevées de PET et d'antigènes du facteur de von Willebrand et d'inhibiteurs 1 des activateurs du plasminogène ($p < 0{,}001$ dans tous les cas), sans aucune différence liée au sexe. L'insuffisance cardiaque (rapport des cotes [RC]: 10,28; intervalle de confiance [IC] à 95 % : de 2,32 à 45,41), l'âge compris entre 65 et 74 ans (RC : 4,37; IC à 95 % : de 1,76 à 10,83) et l'hypertension (RC : 5,03; IC à 95 % : de 1,81 à 13,94) étaient associés de manière indépendante à une faible valeur de K_s (le quartile inférieur, $\leq 6{,}4 \times 10^{-9}$ cm^2), tandis que seul l'âge compris entre 65 et 74 ans (RC : 3,33; IC à 95 % : de 1,59 à 6,96) était associé de façon significative à l'allongement du TLC (le quartile supérieur, $\geq$ 108 minutes). L'âge compris entre 65 et 74 ans (RC: 5,21; IC à 95 % : de 2,12 à 12,80), l'insuffisance cardiaque (RC : 6,58; IC à 95 % : de 1,49 à 29,06) et l'hypertension (RC : 4,33; IC à 95 % : de 1,54 à 12,15) étaient associés de manière indépendante avec un PET élevé (le quartile supérieur, $\geq$ 1681,3 nM $\times$ minutes).

Conclusions : Un état prothrombotique (production accrue de thrombine, caillots de fibrine plus denses, perturbation de la fibrinolyse et lésions endothéliales) caractérise les patients atteints de FA qui présentent 1 facteur de risque d'AVC clinique additionnel (sans égard au sexe), l'âge compris entre 65 et 74 ans présentant une association particulièrement élevée avec les indices d'un tel état.

patients with a single clinical stroke risk factor, propose 3 options, that is, no antithrombotic therapy, oral anticoagulation, or aspirin treatment.[6] Recent CCS guidelines recommend long-term therapy with an oral anticoagulant in patients with the **C**ongestive Heart Failure, **H**ypertension, **A**ge, **D**iabetes, **S**troke/Transient Ischemic Attack ($CHADS_2$) score $\geq$ 1, whereas in the remaining patients with arterial vascular disease, they support the antiplatelet therapy ("CHADS-65").[7]

There is evidence suggesting that an increased risk of stroke and systemic thromboembolism in AF is at least in part related to a persistent prothrombotic state, as evidenced by increased platelet activation and thrombin formation, decreased fibrinolysis, enhanced inflammation, and endothelial dysfunction.[8-11] Prothrombotic alterations were observed even in young, very low-risk patients with AF.[12] Increased soluble P-selectin, von Willebrand factor (vWF), and plasma fibrinogen have been shown to be associated with thromboembolic events in patients with AF.[13-15] An additional feature of a prothrombotic state is the so-called prothrombotic fibrin clot phenotype, characterized by faster formation of a dense fibre meshwork composed of thinner fibres resistant to lysis.[16,17] Interestingly, fibrin clot density, reflected by its permeability measured *in vitro*, has been demonstrated to represent a predictor of ischemic stroke or transient ischemic attack in patients with AF with a median CHA_2DS_2-VASc score of 3, while on vitamin K antagonists (VKA).[18] Prothrombotic clot properties have also been reported in patients with AF in sinus rhythm.[15] Of note, several CHA_2DS_2-VASc score risk factors, including diabetes and heart failure (HF), have been found to be associated with unfavourable clot characteristics.[19-21] To our knowledge, patients with the CHA_2DS_2-VASc score of 1 in men and 2 in women vs those with the score of 0 have not been studied in terms of plasma clot properties and their determinants.

Given controversies around the optimal strategy in patients with AF with low stroke risk,[5,6] we investigated whether a prothrombotic state, including unfavourable clot properties, occurs in patients with AF with the CHA_2DS_2-VASc score of 1 compared with those with the CHA_2DS_2-VASc score of 0 regardless of sex.

Materials and Methods

Patients

In a prospective cross-sectional study, we investigated 170 consecutive patients with AF and a CHA_2DS_2-VASc score of 0 or 1 in men and 1 or 2 in women from 211 patients screened in an outpatient clinic between September 2016 and May 2017. Using a standardized questionnaire, we analysed demographics, information about comorbidities, risk factors, and treatment. Patients were eligible if they had documented AF episode diagnosed at least 3 months before enrollment and none or one additional CHA_2DS_2-VASc score risk factor in men and women (congestive HF/left ventricular dysfunction, hypertension, diabetes mellitus, vascular disease, age 65-74 years).[22] The exclusion criteria were acute coronary events within the preceding 12 months (n = 7), congenital heart disease (n = 2), significant valvular heart disease (n = 5), chronic kidney disease stage 4 or 5 (n = 3), known malignancy (n = 3), alanine aminotransferase 1.5 times above the upper limit of normal (n = 11), signs of acute infection (n = 4), C-reactive protein (CRP) > 10 mg/L (n = 2), and previous venous thromboembolism (n = 4). Patients with prior stroke, transient ischemic attack or systemic embolism as well as those 75 years old or older were ineligible. The final group comprised 170 patients with AF. Paroxysmal and persistent AF were defined as described.[5] Comorbidities and risk factors were defined as described[15,22] (Supplemental Data).

The study protocol was approved by the local ethics committee, and all participants gave informed consent. The data that support the findings of this study are available from the corresponding author on reasonable request.

Laboratory investigations

Fasting peripheral blood samples were drawn from an antecubital vein with minimal stasis. Patients on VKA discontinued anticoagulation up to 7-14 days and were switched to a low-molecular-weight heparin at therapeutic doses with blood collection > 12 hours since the last injection (the anti-Xa activity below 0.1 IU/mL). In patients on non-VKA oral anticoagulants, blood was taken > 24 hours since the last dose (drug concentrations below 30 ng/mL when measured using the Hemoclot Thrombin Inhibitor assay for dabigatran and the anti-Xa chromogenic assay, Biophen DiXaI, for rivaroxaban; both Hyphen BioMed, Neuville-sur-Oise, France). Plasma samples (9:1 of 3.2% trisodium citrate) were centrifuged (20 minutes at 2500 g). The aliquoted supernatant was stored at −80°C until analysis.

Complete blood count, glucose, creatinine, lipid profile, alanine transaminase, CRP, fibrinogen, activated partial thromboplastin time, and international normalized ratio were assessed by standard laboratory techniques. Plasminogen activator inhibitor-1 (PAI-1) antigen (American Diagnostica, Stamford, CT) and vWF antigen (Diagnostica Stago, Asnieres, France) in citrated plasma were determined using the enzyme-linked immunosorbent assays (ELISAs). N-terminal pro-B type natriuretic peptide (NT-proBNP) measurements were performed using electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). All measurements were performed by technicians blinded to the origin of the samples.

Thrombin generation

Endogenous thrombin potential (ETP) was measured in duplicate using calibrated automated thrombography (Thrombinoscope BV, Maastricht, The Netherlands) in a 96-well plate fluorometer (Ascent Reader; Thermolat Systems OY, Helsinki, Finland) at 37°C, as previously described.[22-24] Briefly, 80 μL of platelet-poor plasma was diluted with 20 μL of tissue factor (TF)-based activator (Diagnostica Stago) containing 5 pmol/L recombinant TF, 4 micromolar phosphatidylserine/phosphatidylcholine/phosphatidylethanolamine vesicles and FluCa solution (20 μL; Hepes, pH 7.35, 100 nmol/L $CaCl_2$, 60 mg/mL bovine albumin, and 2.5 mmol/L Z-Gly-Gly-Arg-amidomethylcoumarin).

Fibrin clot permeability

The assessment of fibrin clot permeability was performed as described previously.[15,25] Briefly, plasma was mixed with calcium chloride (20 mM) and human thrombin (1 U/mL; Sigma-Aldrich, St Louis, MO). Tubes that contained the clots were connected via plastic tubing to a reservoir of a buffer (0.05 M Tris-HCl, 0.15 M NaCl, pH 7.5). The volume flowing for 60 minutes through the gels was assessed. The permeation coefficient (K_s), which reflects the pore size, was calculated from the following equation: $K_s = Q \times L \times \eta / t \times A \times \Delta p$, where Q is the flow rate in percolating time (t), L is the length of a fibrin gel, η is the viscosity of liquid, A is the cross-sectional area (cm^2), and Δp is a differential pressure in dyne/cm^2. The intraindividual variability of results was 7%.

Clot lysis time

TF-induced clot lysis assay was used to assess clot lysis time (CLT), as described previously.[15,26,27] Briefly, citrated plasma was mixed with calcium chloride (15 mM), 10,000-diluted human TF (Innovin, Siemens, Marburg, Germany), 12 μM phospholipid vesicles (Avanti PolarLipids, Alabaster, AL), and 60 ng/mL recombinant tissue-type plasminogen activator (Boehringer Ingelheim, Ingelheim, Germany). Assessments of turbidity were performed at 405 nm at 37°C. CLT was defined as the time from the midpoint of the clear-to-maximum-turbid transition, to the midpoint of the maximum-turbid-to-clear transition. The intraindividual results variability was 6%.

Statistical analysis

Categorical variables were presented as numbers and percentages. Categorical variables were compared by Pearson's χ^2 test or Fisher's exact test as appropriate. Continuous variables were expressed as mean (standard deviation) or median (interquartile range). Normality was assessed by the Shapiro-Wilk test. Equality of variances was assessed using Levene's test. Differences between groups were compared using Student's or Welch's t-test depending on the equality of variances for normally distributed variables. The Mann-Whitney U test was used for non-normally distributed continuous variables. Spearman's rank correlation coefficient was calculated to measure the monotonic trend between 2 continuous variables. We performed logistic regression analysis to find out variables independently predicting high ETP (the top quartile), low K_s (the lowest quartile), and prolonged CLT (the top quartile). Potential predictors were risk factors of the CHA_2DS_2-VASc score. Variables that were associated with dependent variables with a significance level of $P < 0.25$ in univariate analysis served as the candidate factors for the multivariate backward stepwise regression model. $P < 0.25$ was chosen as the threshold for including variables in the multivariate model as suggested elsewhere.[28] Using the same rules, we performed additional analysis that included, besides the CHA_2DS_2-VASc score risk factors, persistent AF, AF time, body mass index, smoking history, chronic obstructive pulmonary disease, CRP, NT-proBNP, D-dimer, ETP (apart from prediction of high ETP), vWF, PAI-1, and CHA_2DS_2-VASc score. Multivariate models were fitted using backward stepwise regression with the Wald χ^2 $P < 0.05$ threshold stopping rule. The results of the logistic regression models are presented using odds ratios (OR) with 95% confidence intervals (CI). The predictive ability of the multivariate backward regression model was assessed using area under the curve (AUC). The study was powered to have a 90% chance of detecting a 10% difference in K_s values between groups using a P value of 0.05 and assuming standard deviation equal to 0.9. To demonstrate such a difference or greater, 31 patients were required in each group. A P value < 0.05 was considered significant. All calculations were performed with JMP, Version 13.1.0 (SAS Institute Inc, Cary, NC).

Table 1. Patient characteristics

Variables	Whole group, n = 170	CHA_2DS_2-VASc score of 0 in men and 1 in women, n = 52	CHA_2DS_2-VASc score of 1 in men and 2 in women, n = 118	*P* value
Age (y)	62.0 (58.0-67.0)	59.0 (56.0-62.0)	64.0 (59.0-70.0)	< 0.0001
Male, n (%)	62 (36.5)	22 (42.3)	40 (33.9)	0.29
BMI (kg/m^2)	27.95 (24.5-31.3)	25.65 (23.9-30.1)	28.40 (24.9-31.5)	0.09
Type of AF, n (%)				
Paroxysmal	119 (70.0)	35 (67.3)	84 (71.2)	0.61
Persistent	51 (30.0)	17 (32.7)	34 (28.8)	
AF time (mo)	9 (7.0-12.0)	8 (6.0-12.8)	9 (8.0-12.0)	0.15
Comorbidities and risk factors, n (%)				
Arterial hypertension	29 (17.1)	0 (0.0)	29 (24.6)	< 0.0001
Hypercholesterolemia	107 (62.9)	27 (51.9)	80 (67.8)	0.048
Current smoking	49 (28.8)	16 (30.8)	33 (28.0)	0.71
Diabetes mellitus	9 (5.3)	0 (0.0)	9 (7.6)	0.06
Previous MI	13 (7.7)	0 (0.0)	13 (11.0)	0.01
Heart failure	9 (5.3)	0 (0.0)	9 (7.6)	0.06
CKD	28 (16.5)	2 (3.9)	26 (22.0)	0.003
COPD	26 (15.3)	7 (13.5)	19 (16.1)	0.66
PAD	9 (5.3)	0 (0.0)	9 (7.6)	0.06
Past bleeding	6 (3.5)	1 (1.9)	5 (4.2)	0.67
Treatment, n (%)				
Aspirin	23 (13.5)	5 (9.6)	18 (15.3)	0.32
Statins	44 (25.9)	16 (30.8)	28 (23.7)	0.33
ACE-I	36 (21.2)	7 (13.5)	29 (24.6)	0.10
Beta-blockers	67 (39.4)	14 (26.9)	53 (44.9)	0.027
Anticoagulation	112 (65.9)	26 (50.0)	86 (72.9)	0.004
Anticoagulant, n (%)				
Rivaroxaban	42 (24.7)	11 (21.2)	31 (26.3)	0.005
Dabigatran	30 (17.7)	11 (21.2)	19 (16.1)	
Apixaban	21 (12.4)	3 (5.8)	18 (15.3)	
Warfarin	19 (11.2)	1 (1.9)	18 (15.3)	

Values are presented as median (interquartile range) or number (percentage).

ACE-I, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; BMI, body mass index; CHA_2DS_2-VASc, **C**ongestive Heart Failure, **H**ypertension, **A**ge (≥ 75 years), **D**iabetes, **S**troke/Transient Ischemic Attack/Systemic Embolism, **V**ascular Disease, **A**ge (65-74 years), **S**ex (Female); CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; MI, myocardial infarction; PAD, peripheral artery disease.

Results

Patient characteristics

A total of 118 patients with AF and the CHA_2DS_2-VASc score of 1 in men and 2 in women (n = 118; 69.4%) were compared with 52 patients with AF and the CHA_2DS_2-VASc score of 0 in men and 1 in women (Table 1). The median age of the patients was 62 years (58-67) years. The largest group represented women with paroxysmal AF. None of the patients underwent AF ablation.

The use of anticoagulation and beta-blockers was more common in patients with the CHA_2DS_2-VASc score of 1 in men and 2 in women than in the remainder ($P = 0.03$). Anticoagulation therapy was used in 112 (65.9%). The most common anticoagulant was rivaroxaban (n = 42, 37.5%), followed by dabigatran (n = 30, 26.8%).

Table 2. Laboratory parameters stratified by the CHA_2DS_2-VASc score

Variables	Whole group, n = 170	CHA_2DS_2-VASc score of 0 in men and 1 in women, n = 52	CHA_2DS_2-VASc score of 1 in men and 2 in women, n = 118	*P* value
Platelet count < 100 × 1000/μL, n (%)	9 (5.3)	2 (3.9)	7 (5.9)	0.72
C-reactive protein (mg/L)	2.0 (1.1-3.2)	1.85 (0.91-3.2)	2.05 (1.2-3.2)	0.29
NT-proBNP (pg/mL)	86.5 (72.0-101.3)	90.0 (70.5-106.3)	86.0 (72.0-99.3)	0.87
APTT (s)	28.7 (25.9-30.5)	29.7 (27.9-31.0)	28.0 (25.6-30.0)	0.0061
INR	1.0 (0.9-1.1)	1.0 (0.9-1.1)	1.0 (0.9-1.1)	0.91
Fibrinogen (g/L)	3.2 (2.6-3.7)	3.1 (2.8-3.7)	3.2 (2.6-3.7)	0.86
D-dimer (ng/mL)	295.0 (230.0-356.0)	260.0 (215.3-320.3)	300.5 (235.3-371.3)	0.0110
ETP (nM × min)	1575.0 (1498-1681.3)	1502.5 (1450.0-1577.5)	1622.0 (1532.8-1713.0)	< 0.0001
vWF (%)	194.0 (164.8-215.3)	166.5 (154.3-191.5)	201.5 (177.5-233.3)	< 0.0001
PAI-1 (ng/mL)	30.2 (27.4-37.0)	29.1 (26.6-30.9)	31.2 (27.9-37.9)	0.0005
K_s ($\times 10^{-9}$ cm^2)	6.8 ± 0.8	7.3 ± 0.6	6.6 ± 0.8	< 0.0001
CLT (min)	96.0 (82.8-108.0)	87.0 (75.0-95.0)	99.0 (89.0-109.0)	< 0.0001

Values are presented as mean ± SD or median (interquartile range).

APTT, activated partial thromboplastin time; CHA_2DS_2-VASc, **C**ongestive Heart Failure, **H**ypertension, **A**ge (≥ 75 years), **D**iabetes, **S**troke/Transient Ischemic Attack/Systemic Embolism, **V**ascular Disease, **A**ge (65-74 years), **S**ex (Female); CLT, clot lysis time; ETP, endogenous thrombin potential; INR, international normalized ratio; K_s, clot permeability; NT-proBNP, N-terminal pro-B type natriuretic peptide; PAI-1, plasminogen activator inhibitor-1 antigen; SD, standard deviation; vWF, von Willebrand factor.

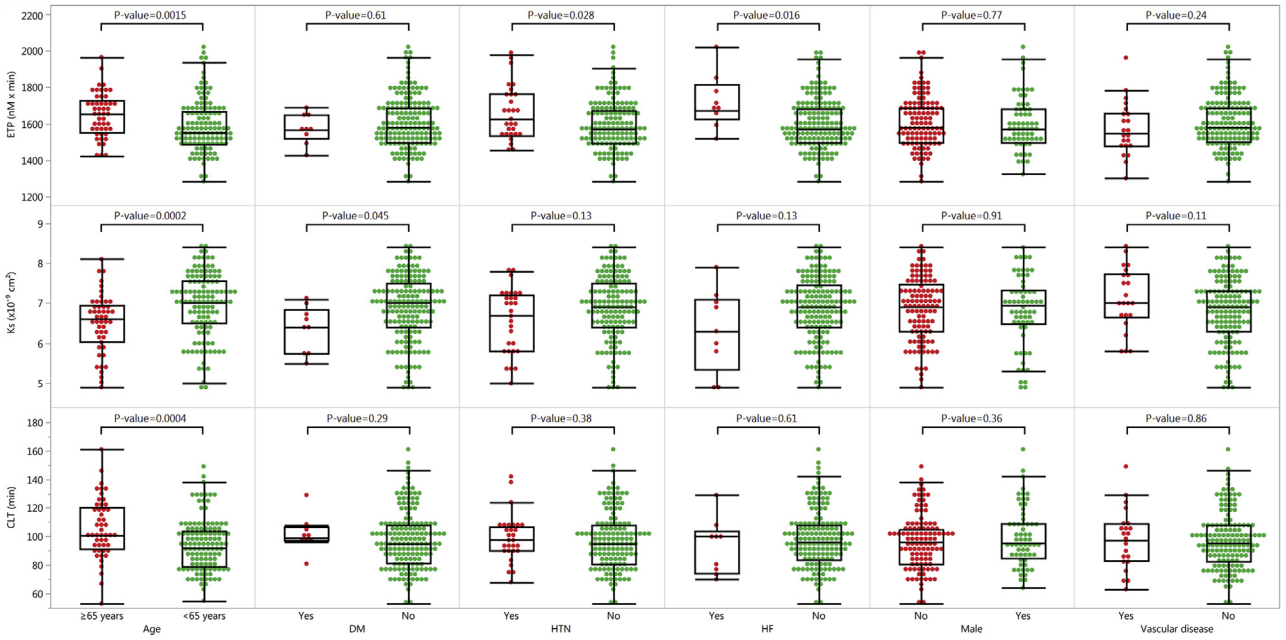

Figure 1. Associations between age 65-74 years, diabetes mellitus (DM), arterial hypertension (HTN), heart failure (HF), female sex, and vascular disease, and endogenous thrombin potential (ETP), clot permeability (K_s), and clot lysis time (CLT). Green dots indicate low-risk patients (CHA_2DS_2-VASc score of 0 in men and 1 in women), whereas red dots indicate patients at a higher thromboembolic risk (CHA_2DS_2-VASc score of 1 in men and 2 in women). Values are presented as medians, interquartile ranges ± 1.5 interquartile ranges; if the data points do not reach the computed ranges, then the whiskers are determined by the upper and lower data point values (not including outliers).

Plasma D-dimer was higher in the patients with the CHA_2DS_2-VASc score of 1 in men and 2 in women compared with the remainder, whereas plasma fibrinogen was similar in both groups (Table 2). Arterial hypertension was associated with the increased use of angiotensin-converting enzyme inhibitor ($P = 0.015$). Patients with vascular disease

Table 3. Associations between CHA_2DS_2-VASc score risk factors (assigned 1 point in the scale) and fibrin clot properties and endogenous thrombin potential

Variables	Whole group, n = 170	Risk factor		*P* value
		No	Yes	
Heart failure		n = 161	n = 9	
ETP (nM × min)	1575.00 (1498.00-1681.25)	1570.00 (1497.00-1679.50)	1670.00 (1626.00-1815.50)	0.016
K_s ($\times 10^{-9}$ cm^2)	6.90 (6.38-7.40)	6.90 (6.40-7.45)	6.30 (5.35-7.10)	0.13
CLT (min)	96.00 (82.75-108.00)	96.00 (83.50-108.00)	100.00 (74.50-104.00)	0.62
Arterial hypertension		n = 141	n = 29	
ETP (nM × min)	1575.00 (1498.00-1681.25)	1570.00 (1494.00-1674.50)	1627.00 (1535.50-1766.00)	0.028
K_s ($\times 10^{-9}$ cm^2)	6.90 (6.38-7.40)	6.90 (6.40-7.50)	6.70 (5.80-7.20)	0.13
CLT (min)	96.00 (82.75-108.00)	95.00 (81.00-108.00)	98.00 (90.00-106.50)	0.38
Diabetes mellitus		n = 161	n = 9	
ETP (nM × min)	1575.00 (1498.00-1681.25)	1578.00 (1498.00-1685.00)	1564.00 (1518.00-1650.00)	0.61
K_s ($\times 10^{-9}$ cm^2)	6.90 (6.38-7.40)	7.00 (6.40-7.50)	6.40 (5.75-6.85)	0.045
CLT (min)	96.00 (82.75-108.00)	95.00 (81.50-108.00)	99.00 (97.00-106.50)	0.29
Vascular disease*		n = 148	n = 22	
ETP (nM × min)	1575.00 (1498.00-1681.25)	1579.00 (1502.00-1686.50)	1546.50 (1476.75-1658.25)	0.24
K_s ($\times 10^{-9}$ cm^2)	6.90 (6.38-7.40)	6.90 (6.30-7.30)	7.00 (6.65-7.73)	0.11
CLT (min)	96.00 (82.75-108.00)	95.50 (82.25-107.75)	97.50 (83.25-109.00)	0.86
Age (≥ 65 and < 75 y)		n = 121	n = 49	
ETP (nM × min)	1575.00 (1498.00-1681.25)	1554.00 (1489.00-1667.00)	1655.00 (1554.00-1728.50)	0.0015
K_s ($\times 10^{-9}$ cm^2)	6.90 (6.38-7.40)	7.00 (6.50-7.55)	6.60 (6.05-6.95)	0.0002
CLT (min)	96.00 (82.75-108.00)	92.00 (79.00-103.50)	101.00 (91.50-120.00)	0.0004
Female sex		n = 62	n = 108	
ETP (nM × min)	1575.00 (1498.00-1681.25)	1571.50 (1497.75-1681.25)	1581.00 (1498.75-1686.00)	0.77
K_s ($\times 10^{-9}$ cm^2)	6.90 (6.38-7.40)	6.95 (6.48-7.33)	6.90 (6.30-7.48)	0.92
CLT (min)	96.00 (82.75-108.00)	95.50 (85.00-109.25)	96.00 (81.00-105.00)	0.20

Values are presented as median (interquartile range).

CHA_2DS_2-VASc, **C**ongestive Heart Failure, **H**ypertension, **A**ge (≥ 75 years), **D**iabetes, **S**troke/Transient Ischemic Attack/Systemic Embolism, **V**ascular Disease, **A**ge (65-74 years), **S**ex (Female); CLT, clot lysis time; ETP, endogenous thrombin potential; K_s, clot permeability.

*Peripheral artery disease and/or previous myocardial infarction.

Table 4. Associations of the CHA_2DS_2-VASc score risk factors with high endogenous thrombin potential, low clot permeability, and prolonged clot lysis time

	Univariate model			Multivariate model		
Dependent variable	Variable	OR (95% CI)	*P* value	Variable	OR (95% CI)	*P* value
High ETP	Heart failure	2.59 (0.76-10.13)	0.18	Heart failure	6.58 (1.49-29.06)	0.013
	Arterial hypertension	1.79 (0.76-4.24)	0.19	Arterial hypertension	4.33 (1.54-12.15)	0.0054
	Diabetes mellitus	0.37 (0.04-3.01)	0.29			
	Vascular disease*	0.64 (0.21-2.02)	0.43			
	Age ≥ 65 and < 75 vs < 65 y	2.70 (1.30-5.61)	0.008	Age ≥ 65 and < 75 vs < 65 y	5.21 (2.12-12.80)	0.0003
	Female sex	1.04 (0.51-2.16)	0.91			
Low K_s	Heart failure	4.19 (1.07-16.41)	0.04	Heart failure	10.28 (2.32-45.41)	0.0021
	Arterial hypertension	2.17 (0.93-5.07)	0.08	Arterial hypertension	5.03 (1.81-13.94)	0.0019
	Diabetes mellitus	1.56 (0.37-6.55)	0.55			
	Vascular disease*	0.64 (0.21-2.02)	0.43			
	Age ≥ 65 and < 75 vs < 65 y	2.04 (0.98-4.25)	0.06	Age ≥ 65 and < 75 vs < 65 y	4.37 (1.76-10.83)	0.0015
	Female sex	1.38 (0.66-2.92)	0.39			
Prolonged CLT	Heart failure	0.84 (0.17-4.19)	0.83			
	Arterial hypertension	0.73 (0.28-1.94)	0.52			
	Diabetes mellitus	0.84 (0.17-4.19)	0.83			
	Vascular disease*	1.13 (0.41-3.09)	0.82			
	Age ≥ 65 and < 75 vs < 65 y	3.38 (1.63-7.01)	0.001	Age ≥ 65 and < 75 vs < 65 y	3.33 (1.59-6.96)	0.0014
	Female sex	0.50 (0.25-1.01)	0.05	Female sex	0.51 (0.25-1.06)	0.0719

High ETP (the top quartile, ≥ 1681.3 nM × min, n = 42), low K_s (the lowest quartile, ≤ 6.4×10^{-9} cm^2, n = 42), prolonged CLT (the top quartile, ≥ 108.0 min, n = 43).

CHA_2DS_2-VASc, **C**ongestive Heart Failure, **H**ypertension, **A**ge (≥ 75 years), **D**iabetes, **S**troke/Transient Ischemic Attack/Systemic Embolism, **V**ascular Disease, **A**ge (65-74 years), **S**ex (Female); CI, confidence interval; CLT, clot lysis time; ETP, endogenous thrombin potential; K_s, clot permeability; OR, odds ratio.

*Peripheral artery disease and/or previous myocardial infarction.

history and aged < 65 years were more commonly treated with aspirin ($P < 0.0001$ and $P = 0.022$, respectively). Laboratory parameters stratified by the CHADS-65 score are shown in Supplemental Table S1. As expected, we did not find differences between patients with oral anticoagulant therapy and those without such treatment before enrollment with regard to major prothrombotic state characteristics studied (Supplemental Table S2). However, we observed correlations between prothrombotic state features and standard laboratory parameters (Supplemental Table S3 and S4).

Thrombin generation

Patients with the CHA_2DS_2-VASc score of 1 in men and 2 in women, compared with the remainder had increased ETP ($P < 0.0001$; Table 2). Analysis of clinical stroke risk factors showed that age between 65 and 74 years, arterial hypertension, and HF were associated with increased ETP (all $P < 0.05$; Fig. 1). There was no difference in ETP related to sex, coexistence of vascular disease (Table 3), and medications (data not shown). Among patients with the CHA_2DS_2-VASc score of 0 in men and 1 in women, those aged below 60 years and between 60 and 64 years had similar ETP (1532.2 ± 130.8 vs 1512.0 ± 87.6 nM × minutes; $P = 0.52$). In the whole group, ETP correlated with fibrinogen ($R = 0.46$, $P < 0.0001$) and weakly with CRP ($R = 0.22$, $P = 0.004$).

In all 170 patients, age 65-74 years (OR: 5.21; 95% CI: 2.12-12.80), HF (OR: 6.58; 95% CI: 1.49-29.06), and hypertension (OR: 4.33; 95% CI: 1.54-12.15) were associated with high ETP (the top quartile, ≥ 1681.3 nM × minutes, n = 42; AUC = 0.692; Table 4). In a model that included biomarkers (AUC = 0.735), high ETP was predicted by hypertension (OR: 3.46; 95% CI: 1.16-10.29), age between 65 and 74 years (OR: 4.00; 95% CI: 1.57-10.23), NT-proBNP (OR for a 10 pg/mL increase: 1.19; 95% CI: 1.04-1.35), and vWF (OR for a 10% increase: 1.12; 95% CI: 1.02-1.22) (Supplemental Table S5).

Endothelial injury

A higher vWF level (by 21%) was observed in patients with the CHA_2DS_2-VASc score of 1 in men and 2 in women ($P < 0.0001$) compared with those without additional risk factors (Table 2). There were no differences in vWF between female and male patients in the whole group (195.5 [164.3-215.8] vs 191.5 [164.5-213.8]%; $P = 0.95$) and among those with the CHA_2DS_2-VASc score of 0 and 1 (162.0 [148.3-190.0] vs 173.0 [158.3-193.3]%; $P = 0.31$), and those aged below 60 years and between 60 and 64 years (167.0 [157.0-199.0] vs 162.0 [150.5-191.0]%; $P = 0.48$). Medications used had no effect on vWF (data not shown). Importantly, vWF correlated positively with ETP in the whole group ($R = 0.35$, $P < 0.0001$).

Clot permeability

Patients with the CHA_2DS_2-VASc score of 1 in men and 2 in women had lower K_s (−9.6%, $P < 0.0001$) compared with the remainder, indicating tighter fibrin network (Table 2).

Patients aged 65-74 years and diabetic subjects compared with the remainder had lower K_s (both $P < 0.05$, Fig. 1). Sex, vascular disease, arterial hypertension, or HF did not affect K_s (Table 3). Patients on angiotensin-converting enzyme inhibitor therapy were characterized by lower K_s when compared with the remainder (6.7 [5.9-7.2] vs 7.0 [6.5-7.5] × 10^{-9} cm^2; $P = 0.033$). There was no difference in this parameter related to other therapies (data not shown). Patients with the CHA_2DS_2-VASc score of 0 in men and 1 in women aged < 60 and 60-64 years had similar K_s (7.2 ± 0.6 vs 7.4 ± 0.5 × 10^{-9} cm^2; $P = 0.23$). In the whole group, K_s inversely correlated with fibrinogen, CRP, vWF, and ETP and

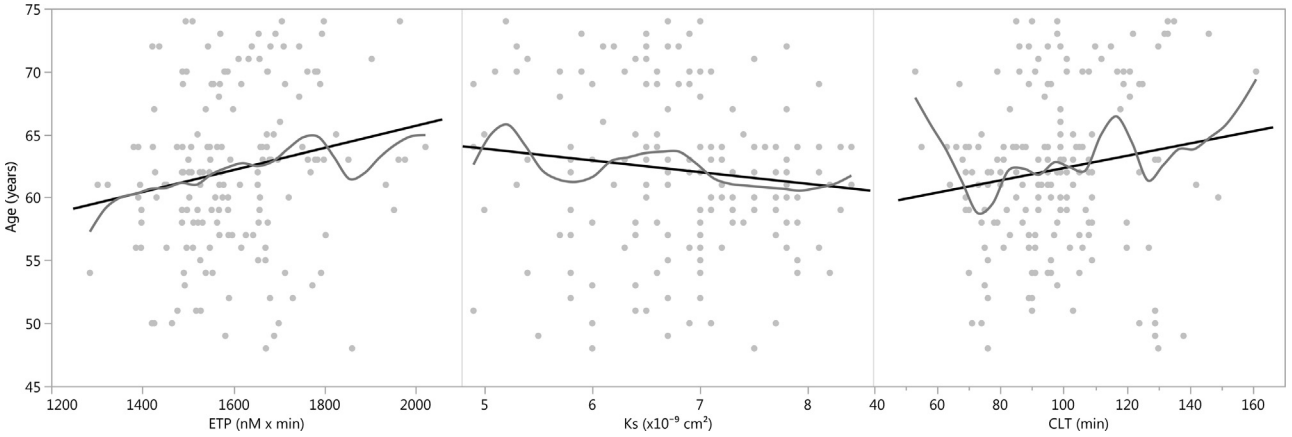

Figure 2. Associations between age, and endogenous thrombin potential (ETP), clot permeability (K_s), and clot lysis time (CLT).

tended to inversely associate with age (Fig. 2, Supplemental Table S3). In the whole patient group, HF (OR: 10.28; 95% CI: 2.32-45.41), age 65-74 years (OR: 4.37; 95% CI: 1.76-10.83), and hypertension (OR: 5.03; 95% CI: 1.81-13.94) were independently associated with low K_s (the lowest quartile, $\leq 6.4 \times 10^{-9}$ cm^2, n = 42; AUC = 0.699; Table 4). In another model (AUC = 0.833), low K_s was predicted by the CHA_2DS_2-VASc score of 1 in men and 2 in women (OR: 6.73; 95% CI: 1.45-31.16) and ETP (OR for a 100 nM/min increase: 2.38; 95% CI: 1.65-3.41) (Supplemental Table S5).

Fibrinolysis

Patients with the CHA_2DS_2-VASc score of 1 in men and 2 in women had prolonged CLT ($P < 0.0001$) and higher PAI-1 antigen ($P = 0.0005$) compared with the remainder (Table 2). Patients aged 65-74 years compared with the remainder were characterized by prolonged CLT ($P = 0.0004$; Fig. 1). There was no difference in CLT related to other CHA_2DS_2-VASc score risk factors (Table 3). Patients taking aspirin, compared with the remainder, were characterized by prolonged CLT (103.0 [90.0-124.0] vs 95.0 [81.0-107.0] minutes; $P = 0.044$). There was no difference in CLT related to other medications (data not shown). Among patients with AF with the CHA_2DS_2-VASc score of 0 in men and 1 in women, female and male patients, and those aged <60 years and between 60 and 64 years had similar CLT and PAI-1 (data not shown). In the whole group, CLT correlated with CRP, PAI-1, vWF, and ETP, but not with fibrinogen (Supplemental Table S4).

PAI-1 correlated positively with vWF ($R = 0.33$, $P < 0.0001$), whereas inversely with K_s in all patients (Supplemental Table S3). Age 65-74 years (OR: 3.33; 95% CI: 1.59-6.96) was the only significant CHA_2DS_2-VASc score risk factor associated with prolonged CLT (the top quartile, ≥108.0 minutes, n = 43; AUC = 0.634; Table 4). Taking into account biomarkers (AUC = 0.838), prolonged CLT was predicted by vWF (OR for a 10% increase: 1.15; 95% CI: 1.04-1.27), PAI-1 (OR for a 1 unit increase: 1.23; 95% CI: 1.14-1.34), and female sex (OR: 0.35; 95% CI: 0.14-0.82) (Supplemental Table S5).

Discussion

The present study shows that (1) patients with AF with the CHA_2DS_2-VASc score of 1 in men and 2 in women are characterized by prothrombotic alterations, including increased thrombin generation, formation of denser fibrin clots, and resistance to fibrinolysis, compared with patients without any CHA_2DS_2-VASc score risk factor (except female sex); (2) age 65-74 years, hypertension, diabetes, and HF are key stroke risk factors contributing to a prothrombotic state in patients with AF with single or without additional clinical stroke risk factor (beyond sex); and (3) increased endothelial injury (reflected by elevated vWF) in patients with the CHA_2DS_2-VASc score of 0-1 in men and 1-2 in women may be involved in hypofibrinolysis. The present study provides evidence for prothrombotic features, which might contribute to elevated thromboembolic risk among patients with AF with the CHA_2DS_2-VASc score of 1 regardless of sex. Compared with the results reported in patients with AF with high stroke risk,[15] the prothrombotic alterations were less pronounced in the low-risk AF group, but still patients with the CHA_2DS_2-VASc score of 0 had the most favourable coagulation profile. From a practical standpoint, the current findings could be interpreted as additional argument for initiating oral anticoagulation at least in a subset of patients with AF from the current low-risk category.

In search for CHA_2DS_2-VASc score risk factors contributing to high ETP, low K_s, and prolonged CLT in AF, we identified HF, arterial hypertension, and age 65-74 years as independently associated with the first 2 prothrombotic features, which correlated with each other. Our findings indicate that the most important clinical risk factor influencing ETP, K_s, and CLT in patients with AF who have the CHA_2DS_2-VASc score of 0-1 in men and 1-2 in women is age 65-74 years. This finding provides support for the emphasis on age 65 years and above in the CHADS-65 algorithm.

Previous papers suggested clinical utility of biomarkers in thromboembolic risk assessment in patients with AF with indications for anticoagulation.[22,29,30] Moreover, as reported recently by Hijazi et al.,[30] the ABC (age, biomarkers, clinical history) death risk score may be of value in the overall risk assessment in patients with AF. Our study supports the concept of a biomarker-based approach to risk assessment in

patients with AF by showing that increased NT-proBNP and vWF were independently associated with high ETP, a key measure of hypercoagulability. We confirmed previous observations demonstrating associations between NT-proBNP and ETP,[22] and that high thrombin concentrations are associated with the formation of less permeable plasma clots, composed of thin fibres, in human subjects.[31] It is known that thrombin also induces proinflammatory and profibrotic pathways.[32] Thus, increased thrombin generation in low-risk patients with AF, besides haemodynamic factors,[33] might also influence the development of chronic arrhythmia or occurrence of frequent AF episodes. It remains to be established whether ETP could be a valuable independent factor in stroke prediction in AF regardless of the CHA_2DS_2-VASc score.

Our observation that vWF and ETP correlate with CLT at a relatively low risk of stroke is consistent with previous observations in patients with AF at a higher risk of stroke.[15] We found that both vWF and PAI-1 predicted prolonged CLT. Given data on the association of vWF with stroke and bleeding in AF,[34] our finding is of importance supporting the significant role of this protein in disturbed haemostasis of patients with AF regardless of the clinical stroke risk.

The impact of sex on the measured parameters deserves a comment. In our study, male sex was independently associated with prolonged CLT, which might suggest a higher stroke risk compared with women in this risk category. No differences were observed for ETP or other prothrombotic markers between sexes. There are long-lasting controversies around female sex as a stroke risk factor in patients with AF at low thromboembolic risk.[35,36] Further studies on sex as a potential modulator of prothrombotic tendency in low-risk patients with AF are needed to optimize stroke prevention.

Regarding the medication use, we found that aspirin use had no influence on thrombin generation or clot density, but was linked to prolonged CLT. However, aspirin has been reported to increase both clot permeability and lysability in healthy subjects and patients with coronary artery disease,[37] but to our knowledge, there is no evidence for such effects in patients with AF. Our findings could be in part explained by the prothrombotic effect of atherosclerotic vascular disease, as a main indication to prescribe this drug in our patient group.

Most studies failed to report a significant impact of aspirin on fibrin properties in advanced vascular disease.[15] It might support growing evidence that aspirin cannot sufficiently prevent stroke in patients with AF,[38] as indicated in the current CCS and ESC guidelines.[5,7]

Unexpectedly, we failed to observe that diabetes and/or vascular disease concomitant with AF independently display prothrombotic alterations despite the fact that both diseases have been demonstrated to lead to hypercoagulability.[39,40] Because most diabetic patients were well controlled, without major cardiovascular complications, it might be speculated that in this clinical setting the prothrombotic impact of diabetes is rather weak. Similarly, vascular disease largely defined as previous myocardial infarction is often associated with older age, hypertension, and HF, which may hamper separate analysis of this factor as a determinant of clot properties in patients with AF. Nevertheless, our findings are consistent with the omission of vascular disease as a significant clinical stroke risk factor in CHADS-65.

Our study has several limitations. The sample size is relatively small, but the study was sufficiently powered to detect differences in fibrin clot properties. Subgroup analyses due to a sample size should be taken with caution and considered as hypothesis-generating observations. We assessed all variables at a single time point, and we cannot exclude that they may change with time. Because most patients on VKA received anticoagulant therapy and were switched to heparins, the inference might affect the parameters measured. However, blood was collected after $>$ 12 hours since the last injection resulting in undetectable or low heparin's anticoagulant effects, with no difference between patients treated with different anticoagulants. Associations reported here do not necessarily indicate a cause-effect relationship. Follow-up of the present low-risk patients to record thromboembolic events was beyond the scope of the current study.

In conclusion, the present study demonstrates prothrombotic alterations, including denser clot formation, impaired fibrinolysis driven by elevated PAI-1, and heightened thrombin generation that can be detectable in circulating blood in patients with AF at low risk of thromboembolic events. Especially, patients with AF aged above 65 years have been found to exhibit a prothrombotic state, implying potential benefits of oral anticoagulation in this group. Further studies are needed to elucidate clinical and laboratory factors that determine a hypercoagulability in patients with AF at low stroke risk.

Conclusions

Patients with AF and a single additional clinical stroke risk factor are characterized by increased thrombin generation, formation of denser fibrin networks, and impaired fibrinolysis. The greatest prothrombotic impact was observed for age above 65 years. These data support the current recommendation to use anticoagulation in patients with AF of both sexes with 1 additional clinical stroke risk factor.

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Disclosures

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Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Cardiology* at www.onlinecjc.ca and at https://doi.org/10.1016/j.cjca.2019.01.014.