JVR
Journal of
Vascular Research

Research Article

J Vasc Res
DOI: 10.1159/000535618

Received: April 12, 2023
Accepted: November 30, 2023
Published online: March 6, 2024

Relationship of Warfarin and Apixaban with Vascular Function in Patients with Atrial Fibrillation

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Keywords
Atrial fibrillation · Flow-mediated dilatation · Shear stress · Warfarin · Apixaban

Abstract
Introduction: Atrial fibrillation (AF) is associated with endothelial damage/dysfunction. Herein, we tested the hypothesis that brachial artery flow-mediated dilation (FMD) is superior in AF patients taking apixaban compared to warfarin. ***Methods:*** AF patients on apixaban ($n = 46$; 67 [7] years; mean [standard deviation]; 15 women) and warfarin ($n = 27$; 73 [9] years ($p < 0.01$); 11 women) were recruited. Duplex Doppler ultrasound imaging was undertaken during baseline (2 min), cuff inflation (5 min), and following cuff deflation (3 min). FMD was defined as peak increase in brachial artery diameter following cuff deflation and analysed as percentage change in diameter, as a ratio of FMD, shear rate area under the curve (SR_{AUC}; FMD-to-SR_{AUC}), and using SR_{AUC} as a covariate (FMD_{SR}). ***Results:*** Baseline artery diameter (4.96 [1.14] vs. 4.89 [0.88] mm), peak diameter (5.12 [1.17] vs. 5.14 [0.93] mm), and FMD_{SR} (3.89 [3.62] vs. 4.80 [3.60] %) were not different between warfarin and apixaban ($p > 0.05$; analysis of covariance with age, CHA_2DS_2-VASc, years since AF diagnosis, number of diabetics, alcohol drinkers, and units of alcohol consumed per week as covariates). Stepwise multiple regression identified independent association of fibrillation, hypertension, and increased age with FMD. ***Conclusion:*** AF patients on warfarin and apixaban exhibit similar endothelium-dependent vasodilation. Increased blood pressure negatively impacts vasodilator capacity in AF patients.

Plain Language Summary
Atrial fibrillation (AF) is the most common sustained heart rhythm abnormality and is associated with blood vessel damage/dysfunction. AF patients also have a substantially increased risk of stroke from blood clots. As such, these

Gregory Y.H. Lip and James P. Fisher should be considered as joint senior authors.
The authors confirm that the Principal Investigators for this paper are Prof. Gregory Y.H. Lip and Dr. James P. Fisher. Prof. Gregory Lip had direct clinical responsibility for patients.

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patients are prescribed anti-coagulants, such as warfarin and apixaban. Herein, we compared the vascular function of the brachial artery in AF patients taking apixaban to those taking warfarin. Brachial artery's vasodilator capacity was not different between groups, indicating comparable vascular function of AF patients on warfarin and apixaban. Increased resting blood pressure in AF patients negatively impacted dilation capacity of the brachial artery in AF patients.

Introduction

Atrial fibrillation (AF) is a major risk factor for severe thromboembolic events, and the irregular cardiac rhythm and hypercoagulable states observed in AF are related to endothelial dysfunction [1]. Indeed, our group [2, 3] and others [4] have reported impaired brachial artery flow-mediated dilation (FMD), a marker of endothelial dysfunction, in AF. This is important because poor FMD is associated with adverse cardiovascular events [5], but strategies to enhance endothelial function in AF are limited.

There is a considerable interplay between coagulation, inflammation, and endothelial dysfunction. Endotoxins, C-reactive protein (CRP), and tumour necrosis factor α (TNFα) all promote tissue factor formation, and exposure of tissue factor to factor VIIa catalyses conversion of factor X to Xa, initiating thrombin formation and platelet activation [6]. In a self-perpetuating feedback cycle, coagulation proteases, such as factor Xa and thrombin, then contribute to the upregulation of the inflammatory response by increasing TNFα, interleukin-6 (IL-6), and IL-8 concentrations [6]. Various inflammatory molecules (e.g., CRP, TNFα, IL-2, IL-6, and IL-8) have been linked to the presence and outcome of AF, promoting endothelial damage/dysfunction [7]. Although positive linear correlations between plasma biomarkers of vascular dysfunction (e.g., von Willebrand factor) and coagulation factors (e.g., fibrinogen) have been reported in AF [8], whether oral anti-coagulation improves endothelial function remains unclear.

Apixaban is a direct oral anti-coagulant (DOAC) that inhibits factor Xa, thus decreasing thrombin generation and thrombus development. In the ARISTOTLE trial, apixaban was shown to be superior to warfarin, which non-specifically inhibits all vitamin K-dependent clotting factors, in preventing stroke or systemic embolism [9]. Further, apixaban treatment resulted in lower mortality in patients with AF and reduced incidence of major bleeding [9]. Indeed, apixaban has independently been shown to increase vasodilation and attenuate phenylephrine and 5-hydroxytryptamine-mediated vasoconstriction [10]. Moreover, in in vitro models of endothelial dysfunction resulting from uraemic toxins, apixaban has been shown to provide endothelial/vascular protection by decreasing ICAM-1, VCAM-1, and von Willebrand factor expression and upregulating expression of endothelial nitric oxide synthase (eNOS) [11]. In light of the emerging evidence for the malign trifecta of coagulation, inflammation, and endothelial dysfunction, we hypothesised that some of this benefit may be ascribable to superior FMD in patients taking apixaban compared to warfarin. This study was thus designed to investigate the influence of these two oral anti-coagulants on brachial artery FMD in AF.

Methods

Ethical Approval

All procedures were undertaken in accordance with the Declaration of Helsinki and were approved by the National Research Ethics Service Committee North West (17/NW/0714). Research funding support was provided by Bristol-Myers Squibb-Pfizer Alliance. Prospective participants were provided with a detailed verbal explanation of study procedures and an information sheet. Written informed consent was obtained from all participants.

Participant Characteristics

Seventy-three AF patients from predominantly white European ethnic background were recruited across 2 anti-coagulation groups based on their current anti-coagulant use: warfarin (n = 27) and apixaban (n = 46; Table 1). Patients were previously clinically diagnosed with at least paroxysmal AF (i.e., episodes are transient and spontaneously resolve within 48 h) and were recruited from dedicated cardiology clinics at City Hospital, Birmingham, at Liverpool Heart and Chest Hospital, Liverpool, and from West Midlands GP practices within the National Institute of Health Research Clinical Research Network. AF diagnosis was confirmed in nearly all patients at least a year prior to study recruitment, except 2 apixaban patients. Premenopausal women, individuals with valvular heart disease, left ventricular dysfunction, myocardial infarction, respiratory, hepatic, renal, inflammatory, connective tissue, neurological, malignant diseases, stroke, transient ischaemic attack (<3 years), uncontrolled thyroid disorders, and current smokers were excluded from participation. Prescribed and over-the-counter supplemental medications are listed in Table 2.

Experimental Measures

Medical history, medication use, smoking history, and current alcohol use were recorded. CHA_2DS_2-VASc scores were calculated using the medical history. Anthropometric measures of height, weight, waist (level of umbilicus), and hip (level of femoral trochanter) circumference were obtained. Brachial blood pressure (BP) and heart rate (HR) were determined non-invasively using an automated oscillometric device (M2,

Table 1. Participant characteristics

	Warfarin (n = 27)	Apixaban (n = 46)	p value (d, φ)
Age, years	73±9	67±7	0.002 (0.744)
Female, *n* (%)	11 (41)	15 (33)	0.484
Weight, kg	88±20	86±18	0.726
Height, m	1.72±0.09	1.72±0.09	0.959
BMI, kg m^{-2}	29±4	29±5	0.846
Waist, cm	98±10	97±12	0.709
Hip, cm	109±10	106±9	0.213
Waist-to-hip	0.88 [0.85–0.96]	0.91 [0.84–0.99]	0.827
Waist-to-height	0.57±0.05	0.56±0.07	0.670
Systolic BP, mm Hg	148±18	144±15	0.208
Diastolic BP, mm Hg	84±10	85±13	0.681
MBP, mm Hg	105±11	105±11	0.811
HR, b min^{-1}	67 [59–70]	69 [64–74]	0.665
Years since AF diagnosis	10±8	5±4	0.009 (0.791)
Non-fibrillating, *n* (%)	17 (63)	24 (52)	0.370
Hypertension, *n* (%)	18 (67)	23 (50)	0.166
Diabetes, *n* (%)	4 (15)	0	0.016 (0.314)
CHA_2DS_2-VASc score	3 [2–3]	2 [1–2]	<0.001 (0.921)
Previous smoker, *n* (%)	17 (63)	22 (48)	0.211
Alcohol users, *n* (%)	16 (59)	40 (87)	0.026 (0.317)
Alcohol (units), $week^{-1}$	2 [0–8]	7 [2–12]	0.049 (0.450)

Values expressed as the mean ± SD for normally distributed data, as median [interquartile range] for non-normally distributed data, and as frequency (percentage) for discrete variables. Statistical differences were tested using an independent t test or Mann-Whitney U test for continuous variables and χ^2 test for categorical data. If significant, the effect size was reported as Cohen's d or Phi for continuous and categorical variables, respectively. Significance: $p < 0.05$. φ, Phi; BMI, body mass index; d, Cohen's d; MBP, mean blood pressure.

Omron, Kyoto, Japan). Duplex Doppler ultrasound (GE LOGIQ e, GE Healthcare China Corporation, Jiangsu, China) was used to simultaneously obtain right brachial artery diameter and flow velocity measurements while the arm was supported at heart level. The artery was insonated at 60° ~10–15 cm proximal to the medial epicondyle. A 5–13 MHz multi-frequency linear-array transducer (GE 12L-RS, GE Healthcare Japan Corporation, Tokyo, Japan) was used for B-mode imaging of vessel diameter and the pulse wave mode used for assessment of peak blood velocity. Recordings were screen captured and stored as video files for offline analysis with automated edge detection and wall tracking software (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy). Vascular measurements were made in accordance with the recent technical recommendations [12], as described in detail below.

Experimental Protocol

Patients were requested to abstain from vigorous exercise, food, and caffeine for 12 h and alcohol for 24 h prior to their experimental sessions. Further, patients refrained from taking their usual morning medications on the study day, except for anti-coagulants. Participants were asked to lie supine on a medical examination couch with their head on a pillow, while all assessments took place. Three resting measures of right brachial BP and HR were taken, separated by ~ 2 min and averaged. A narrow inflatable tourniquet cuff (Hokanson, Bellevue, WA, USA) was then wrapped around the forearm ~7 cm distal to the medial epicondyle. The FMD protocol comprised a 2-min baseline, a 5-min supra-systolic cuff inflation (>240 mm Hg), and a 3-min recovery period following cuff deflation. Simultaneous measurements of brachial artery diameter and flow velocity were undertaken throughout.

Data Analysis

Waist-to-hip and waist-to-height ratios were calculated, along with body mass index (ratio of weight and height squared). Brachial artery measures were calculated as a 2 min average for the baseline period and on a second-by-second basis during the post-cuff deflation period. FMD was taken as the maximal change in brachial artery diameter following cuff deflation and expressed as absolute (mm) and relative (%) change. Time-to-peak diameter was obtained between the cuff deflation and the maximal artery dilation. Shear rate (SR) was calculated as follows:

$$\text{Shear rate} = \frac{\text{Brachial artery flow velocity} \cdot 4}{\text{Brachial artery diameter}}$$

Shear rate area under the curve (SR_{AUC}) was calculated as an integral between the cuff deflation and the maximal artery dilation. In accordance with guidelines [12], SR_{AUC} was used as a covariate to calculate SR-corrected FMD (FMD_{SR}) and

Table 2. Medication use

	Warfarin (n = 27)	Apixaban (n = 46)	p value
Antiplatelets, *n* (%)	0	2 (4)	0.527
α inhibitor, *n* (%)	2 (7)	2 (4)	0.623
β inhibitor, *n* (%)	21 (78)	26 (57)	0.067
Cardiac glycoside, *n* (%)	2 (7)	10 (22)	0.190
Anti-arrhythmia, *n* (%)	5 (19)	6 (13)	0.522
ACE inhibitor, *n* (%)	10 (37)	10 (22)	0.157
Ca^{2+} channel inhibitor, *n* (%)	4 (15)	11 (24)	0.353
ARB, *n* (%)	6 (22)	7 (15)	0.532
Loop diuretic, *n* (%)	2 (7)	2 (4)	0.623
Thiazide diuretic, *n* (%)	1 (4)	0	0.370
Biguanide, *n* (%)	3 (11)	0	0.047 (0.270)
PP inhibitor, *n* (%)	10 (37)	11 (24)	0.232
Statin, *n* (%)	16 (59)	22 (48)	0.345
SSR inhibitor, *n* (%)	0	4 (9)	0.290
Tricyclic antidepressant, *n* (%)	2 (7)	1 (2)	0.551
SNR inhibitor, *n* (%)	0	1 (2)	1.000
NSAID, *n* (%)	3 (11)	4 (9)	0.705
Folic acid, *n* (%)	0	1 (2)	1.000

Values expressed as frequency (percentage) for discrete variables. Statistical differences were tested using χ^2 test for categorical data. Significance: $p < 0.05$. α, alpha; β, beta; ACE, angiotensin converting enzyme, ARB, angiotensin receptor blocker; Ca, calcium; NSAID, non-steroidal anti-inflammatory drug; PP, proton pump; SNR, serotonin noradrenaline reuptake; SSR, selective serotonin reuptake.

reported as estimated margin means. In addition, FMD responses were also normalised for SR by calculating FMD-to-SR_{AUC} ratio. Further, in accordance with guidelines for the allometric scaling of baseline artery diameters [13], slope and upper bound 95% confidence intervals of the log-transformed baseline and peak diameters were calculated. As the slope (0.99) and 95% confidence intervals (1.03) were close to unity, allometric scaling was not performed.

Brachial velocity profiles obtained from the Doppler trace are reported as net velocity (positive minus negative velocity profiles). Time-to-peak velocity was obtained between cuff deflation and peak net brachial velocity. Brachial artery blood flow (mL min^{-1}) was calculated as follows:

$$\text{Brachial artery blood flow} = \left[\frac{\text{Peak Envelope Velocity}}{2} \cdot \left(\pi\ (0.5 \cdot \text{Diameter})^2\right)\right] \cdot 60$$

Peak hyperaemia was taken as the peak brachial artery blood flow following cuff deflation. Time-to-peak hyperaemia was obtained between cuff deflation and peak blood flow response.

Statistical Analysis

The distribution of continuous variables was assessed using a Kolmogorov-Smirnov test. Non-normally distributed data were transformed using the natural logarithm method. Data (original and log-transformed) were screened for extreme outliers (3 × interquartile range), and any such individual points were excluded from analysis. Normally distributed data were analysed using an independent two-tailed Student's t test. Age, years since AF diagnosis, CHA_2DS_2-VASc scores, number of diabetics, alcohol drinkers, units of alcohol consumed per week, and biguanide use were identified as being significantly different between groups (Tables 1, 2). Correlation between these variables was assessed using Pearson's r or Spearman's rho, as appropriate, before they were incorporated as covariates in a one-way analysis of covariance (ANCOVA) to control for their influence on further group comparisons/analysis. Correlation between number of diabetics and biguanide use was greater than 0.8; as such, biguanide use was excluded from ANCOVA. Levene's test was used to test equality of error variances between groups. Data that remained non-normally distributed were analysed using a Mann-Whitney U test. Categorical data were analysed using a either a Pearson χ^2 or Fisher's exact test as appropriate. If significant, Cohen's d (d) and Phi value (φ) were reported for continuous and categorical variables, respectively. When comparisons were conducted for log-transformed data, transformed values were used for d calculations. All continuous and dichotomous variables presented in Tables 1 and 2 (with >5 data points) were entered into stepwise multiple regression to determine their influence on brachial FMD and FMD-to-SR_{AUC} (log-transformed). Multicollinearity and autocorrelation were tested to reduce the risk of regression model violations: reported variables had tolerance of 0.86–0.97, variance inflation factor of 1.03–1.16, and Durbin-Watson statistic of 2.01–2.40.

Data are expressed as mean ± standard deviation (SD) for normally distributed data, median [inter-quartiles] for non-normally distributed data, and frequency (percentage) for categorical variables, unless stated in legends. Data presented are original and not log-transformed data or ANCOVA estimated

Table 3. FMD characteristics in AF patients anti-coagulated with warfarin or apixaban

	Warfarin	Apixaban	*p* value
Baseline diameter, mm	4.96±1.14	4.89±0.88	0.243
Baseline velocity, cm s^{-1}	8.93 (6.18)	9.86 (4.95)	0.876
Baseline blood flow, mL min^{-1}	38.09 [28.78–62.86]	51.28 [27.74–76.66]	0.445
Peak diameter, mm	5.12±1.17	5.14±0.93	0.272
Time-to-peak diameter, s	67±31	63±28	0.405
Peak velocity, cm s^{-1}	63.01±17.29	75.58±20.04	0.792
Time-to-peak velocity, s	10±4	10±3	0.299
Peak hyperaemia, mL min^{-1}	290.28 [227.31–444.12]	412.67 [301.84–542.96]	0.360
Time-to-peak hyperaemia, s	9 [7–11]	11 [8–13]	0.072
SR_{AUC}, s^{-1}	11,600±6,073	13,049±6,132	0.634
Absolute FMD, mm	0.16 [0.08–0.24]	0.23 [0.12–0.32]	0.825
FMD, %	3.29±2.99	5.15±3.83	0.715
FMD-to-SR_{AUC}, % s^{-1}	0.30 [0.16–0.46]	0.39 [0.26–0.63]	0.767
FMD_{SR}, %	3.89±3.62	4.80±3.60	0.642

Values expressed as the mean ± SD for normally distributed variables and as median [interquartile range] for non-normally distributed variables. Statistical differences were tested using one-way ANCOVA with age, CHA_2DS_2-VASc, years since AF diagnosis, number of diabetics, alcohol drinkers, and units of alcohol consumed per week as covariates; non-normally distributed variables were natural log-transformed before ANCOVA. FMD_{SR} ANCOVA was performed with SR_{AUC} as an additional covariate; values reported are estimated margin means. Significance: $p < 0.05$. FMD, flow-mediated dilatation; SR_{AUC}, positive shear rate area under the curve.

margin means, unless stated in legends. Correale et al. [14] reported an FMD of 7.5 ± 7.9% and 31.0 ± 15.1% between their AF and heart failure (HF) patients who had been on at least 4 months of either Warfarin or DOAC, respectively. This equates to an effect size of 1.95. Assuming a similar effect size in our AF groups, a sample size of only 8 patients in each group would have determined a statistically significant difference at 95% power and 5% alpha between warfarin and apixaban-treated AF patients. $p < 0.05$ was considered statistically significant. Analysis was performed using IBM SPSS Statistics, version 26 (IBM Corp., Armonk, NY, USA). Data that support the findings of this study are available on reasonable request from the corresponding author. Restrictions apply to availability of these data. Data are not publicly available due to privacy, ethical, and/or 3rd party restrictions.

Results

Participant Characteristics

Participant characteristics and medication use are presented in Tables 1 and 2, respectively. Aside from age, there were no significant differences in anthropometric and baseline cardiovascular (e.g., BP and HR) measures. Patients in warfarin group were diagnosed significantly earlier than those on apixaban (10 ± 8 vs. 5 ± 4 years, respectively, $p = 0.009$). Four diabetics were part of the warfarin group. Therefore, the proportion of diabetics was higher in the warfarin group compared to apixaban (15% vs. 0%, respectively, $p = 0.016$), as was the CHA_2DS_2-VASc score. Medication use was similar in the warfarin and apixaban groups, aside from biguanide use which was higher in the warfarin group (11% vs. 0%; $p = 0.047$). The proportion of individuals who consumed alcohol and the total units of alcohol consumed per week were greater in apixaban group compared to warfarin (Table 1).

Brachial Artery FMD

Baseline brachial artery diameter, velocity, and blood flow were not different between the two groups (Table 3; $p > 0.05$). FMD, whether measured as an absolute, percentage or normalised to SR (FMD-to-SR_{AUC} and FMD_{SR}), was not different between warfarin and apixaban (Table 3; $p > 0.05$). Time-to-peak FMD (i.e., peak arterial diameter) and SR_{AUC} responses were also not different between the two groups ($p > 0.05$; Table 3). Peak velocity, peak hyperaemia, and time-to-peak hyperaemia were also similar between groups ($p > 0.05$; Table 3).

Stepwise Multivariate Linear Regression

Cardiac arrhythmia (presence of AF at time of investigation), hypertension, and age significantly predicted FMD ($F(3,66) = 6.054$, $p = 0.001$), accounting for ~22% of

Table 4. Stepwise multiple regression with FMD (%) and FMD-to-SR_{AUC} (% s^{-1}) as dependent variables

	β	B	95% CI for B	*p* value
Dependent variable: FMD, %				
$R = 0.465$; $R^2 = 0.216$; adjusted $R^2 = 0.180$; $p = 0.001$				
Cardiac arrhythmia (AF)	0.315	2.285	0.681–3.889	0.006
Hypertension	−0.360	−2.615	−4.244 to −0.986	0.002
Age, years	−0.232	−0.113	−0.220 to −0.005	0.040
Dependent variable: FMD-to-SR_{AUC} (%·s^{-1})				
$R = 0.422$; $R^2 = 0.178$; adjusted $R^2 = 0.151$; $p = 0.003$				
Waist, cm	0.435	0.013	0.005–0.020	0.001
MBP, mm Hg	−0.286	−0.008	−0.015 to −0.001	0.026

Data include warfarin and apixaban groups ($n = 73$). Variables reported in Tables 1 and 2 were entered in stepwise multiple regression with FMD (%) and FMD-to-SR_{AUC} (% s^{-1}) as dependent variables; independent variables with ≤5 data points were excluded from the model. Tolerance for each reported variable was 0.86–0.97, variance inflation factor 1.03–1.16, and Durbin-Watson 2.01–2.40. β, standardised coefficient; B, unstandardised coefficient; CI, confidence interval; *R*, correlation coefficient; R^2, coefficient of determination; MBP, mean blood pressure.

the variation (Table 4). Hypertension and yearly increase in age were negatively correlated with FMD, with a unique contribution of 2.61% and 0.11%, respectively. However, loss of normal cardiac rhythm (i.e., AF) was positively correlated with FMD with a unique contribution of 2.28%. Waist circumference and mean BP significantly predicted FMD-to-SR_{AUC} ($F(2,61) = 6.607$, $p = 0.003$), accounting for ~18% of the variation. Increase in waist size was positively correlated with FMD-to-SR_{AUC} providing a unique contribution of 1.31%.s^{-1}, while unit increase in mean BP was negatively correlated with FMD-to-SR_{AUC} by 0.80%.s^{-1}.

Discussion

The purpose of the present study was to investigate the hypothesis that the brachial artery FMD is superior in patients taking apixaban compared to warfarin. Contrary to our hypothesis, FMD was not significantly different in AF patients on either apixaban or warfarin. Given the physiological associations between FMD with endothelial/vascular function [15], and with cardiovascular outcomes [5], these findings support the concept that peripheral endothelial/vascular function in AF patients is similar with either apixaban or warfarin oral anti-coagulation.

Exposure of tissue factor to factor VIIa catalyses the conversion of factors IX and X to their enzymatic forms (IXa and Xa), initiating thrombin formation and platelet activation [6]. Evidence implicates inflammation in the process of thrombus formation and clot generation as well. Indeed, some inflammatory molecules (e.g., endotoxins, CRP, and TNFα) can promote tissue factor formation. Moreover, coagulation proteases (e.g., factor Xa, thrombin) may increase TNFα, IL-6, and IL-8 concentration [6], all of which are associated with the presence and outcome of AF, and promoting endothelial damage/dysfunction [7]. Mondillo et al. [8] reported that plasma markers of endothelial function, namely, von Willebrand factor and soluble thrombomodulin, were elevated in chronic non-rheumatic AF patients versus controls and were positively correlated with plasma fibrinogen. Moreover, Correale et al. [14] observed that FMD was improved when patients with both AF and chronic HF were switched from Warfarin to a DOAC (e.g., apixaban) for 4 months. This finding may be explained by a superior influence of DOACs on endothelial function [10] and their putative anti-inflammatory effects [16]. However, this could alternatively be attributable to the initial poor patient compliance with warfarin, given that this was the reason that patients were switched to a DOAC in their study. Long-term warfarin use has been associated with vascular calcification and endothelial dysfunction (i.e., increased pulse wave velocity and attenuated finger artery reactive hyperaemia) in AF [17], while changing AF patients from warfarin to the DOAC rivaroxaban for 6 months improves large artery stiffness (i.e., reduces estimated aortic pulse wave velocity and augmentation index) [18].

Herein, we tested the hypothesis that in patients with AF, oral anti-coagulation with apixaban would be associated

with superior FMD than warfarin, but in contrast to expectation, FMD was not different between groups, perhaps on account of the better compliance with warfarin as an anti-coagulant by patients in our study. Indeed, warfarin inhibits multiple enzymatic factors within the intrinsic and extrinsic pathways of coagulation cascade [19], release of which has been linked to proinflammatory molecules [6]. However, we acknowledge that data on compliance with anti-coagulation regimens was not recorded in our patients, and this assumption is speculative; future studies in these study populations should record these data. Additionally, it is also possible that the improvements in FMD with DOAC in Correale et al. [14] are attributable to simultaneous presence of AF and HF, two cardiac conditions which are both independently associated with endothelial dysfunction [2, 20, 21]. Indeed, participants in our study were not diagnosed with HF. Lastly, statins also mitigate the release of inflammatory markers. Indeed, Lenart-Migdalska et al. [22] have noticed a DOAC-related increase in prothrombotic factors is mitigated by statins. Thus, it is possible that our results of similar macrovascular conduit artery endothelial FMD responses between the two anti-coagulation groups are in part affected by additional drug regimens (e.g., statins).

Time since AF diagnosis was recorded in our study. However, time since start of anti-coagulation was not obtained. In clinical practice, CHA_2DS_2-VAS_C criteria help determine need for anti-coagulation, and for most patients, anti-coagulation starts at the time of AF diagnosis. This important consideration was accounted for as a covariate in statistical analysis (i.e., duration of AF diagnosis), and brachial artery FMD was not different between warfarin and apixaban groups.

Endothelial dysfunction is observed in heavy [23] and even in some light alcohol consumers [24]. Given that alcohol use is also associated with AF [25] and both AF and alcohol are linked to endothelial dysfunction, it was possible that our findings of no differences in FMD in warfarin and apixaban groups was affected by significantly greater proportion of alcohol consumers and units of alcohol consumed per week in apixaban group. However, these factors were accounted for in statistical analysis as covariates, and brachial artery FMD was found to be not different between warfarin or apixaban groups.

Previous reports have identified impaired FMD in hypertension [26]. Further, FMD is not different between patients with hypertension versus those with both hypertension and AF [3]. Interestingly, in the current study, regression analysis suggests presence of AF is associated with a better FMD response which is contrary to expectation and previous observations [4]. However, Siasos et al. [27], Komatsu et al. [28], and Khan et al. [3] have shown that the frequency and duration of AF are better associated with attenuated FMD response compared to cardiac rhythm per se. Indeed, Siasos et al. [27] noted that in their cohort, the duration of AF events was the only factor that inversely affected FMD. This may be attributed to mechanosensitive endothelial cells responding to a consistently erratic shear response, leading to proinflammatory gene expression and reduced production of nitric oxide [29]. Conversely, the return to laminar shear is associated with upregulated eNOS gene expression [29]. Therefore, duration and frequency of AF appear to be better determinants of FMD than cardiac rhythm per se. Consistent with this is our observation of a marginally, but not significantly, larger FMD in our cohort who were diagnosed with AF <5 years ago (5.09 ± 4.07 vs. 3.74 ± 2.95, respectively; $p = 0.11$). Age is a well-known risk factor for endothelial dysfunction [30]. Therefore, poorer FMD in older participants was not an unexpected finding. Lastly, contrary to our expectation, waist size and FMD-to-SR_{AUC} were positively correlated. However, whether this reflects use of statins by some participants [31], presence of metabolically healthy obesity [32], or a combination of factors remains to be conclusively investigated. Indeed, Karelis et al. [33] have previously observed lower systemic inflammation, a factor associated with endothelial dysfunction [34, 35], in metabolically healthy individuals compared to insulin-resistant individuals.

Experimental Considerations

Group size remains uneven between warfarin and apixaban which can affect the statistical assumptions of equal variance between groups. However, significance values for Levene's test of equal variance remained >0.05 throughout between group ANCOVA comparisons which is reassuring for our statistical approach. Nonetheless, future studies comparing endothelial effects of warfarin and DOACs should aim for an evenly matched larger group size. FMD was used to non-invasively assess endothelial/vascular health of patients and guidelines [12, 13] were followed throughout. Future studies however could directly obtain arterial/endothelial tissue from AF patients to investigate the specific signalling pathways for our findings. Blood biomarkers of inflammation and endothelial health were not assessed. Previous studies have observed some differences in circulating biomarkers of vascular health and coagulation [36]. We acknowledge that future work should investigate the influences of anti-coagulants, circulating

blood biomarkers, and vascular function in same cohorts. Endothelium-independent dilatation was not assessed using administration of nitrate as has been previously undertaken in AF [2]. Participants in our group were well matched in terms of proportion of men and women, body mass index, BP, HR, and medication use (aside from biguanide and anti-coagulation). However, age, CHA_2DS_2-VASc score, years since AF diagnosis, number of diabetics, alcohol drinkers, and units of alcohol consumed per week were different between groups. Given that these might reasonably have been expected to affect FMD, these variables were used as covariates in the between-group assessment of FMD. The relatively stringent inclusion/exclusion criteria we used to limit comorbidities influencing the main outcome variables, the CHA_2DS_2-VASc score was relatively low in both groups, and this may limit the broader generalisation of our findings to AF patients with significant and common comorbidities (e.g., HF). Although there were only four diabetics in this study, future works should investigate the interplay between independent influences of hyperglycaemia and specific anti-coagulants on endothelial function in AF patients. While the brachial artery is commonly used in studies of peripheral vascular function due in part to it providing an accessible representation of the coronary arteries [37], further studies are required to extend our observations to other clinically relevant circulations (e.g., cerebral blood vessels) in which diminished functioning in AF has been identified [38, 39]. All patients were asked to withhold medications on the morning that the study was conducted; however, for clinical reasons, anti-coagulants were not withheld. For similar reasons, an AF control group that was anti-coagulation naive was not included. We employed a cross-sectional assessment of non-blinded AF patients taking either apixaban or warfarin, and we acknowledge that a more robust approach would have been to undertake a blinded, longitudinal study of the same patients. However, we believe that the real-world cohorts investigated make our observations representative of the clinic.

Conclusions

Despite the hypothesis of superiority of apixaban to provide endothelial protection, findings of this study provide important translational evidence that brachial artery FMD of AF patients is not different between those on the vitamin K inhibitor warfarin and the DOAC apixaban, indicative of a similar effects of these oral anti-coagulants on endothelial function. Higher BP was associated with decreased brachial artery vasodilator capacity in AF patients.

Acknowledgment

The authors thank Dr. Alana Cavadino (University of Auckland) and are grateful to her for her expert statistical support.

Statement of Ethics

All procedures were undertaken in accordance with the Declaration of Helsinki and were approved by the National Research Ethics Service Committee North West (17/NW/0714). Prospective participants were provided with a detailed verbal explanation of study procedures and an information sheet. Written informed consent was obtained from all participants.

Conflict of Interest Statement

G.Y.H.L. is a consultant and speaker for BMS/Pfizer, Boehringer Ingelheim and Daiichi-Sankyo. No fees are directly received personally. J.P.F. received funding from BMS/Pfizer for an investigator-led and competitively reviewed research project. R.-T.J. was employed by University of Birmingham as a research fellow to work and manage the BMS/Pfizer funded, investigator-led and competitively reviewed research project. This required regular contact with funders for continued project grant support. No fees/funds were directly received personally.

Funding Sources

Bristol-Myers Squibb-Pfizer Alliance provided the research funding support (investigator-led competitively reviewed research project grant [CV185-481]). The work was also supported by National Institute of Health Research Clinical Research Network (NIHR CRN).

Author Contributions

Gregory Y.H. Lip and James P. Fisher: conception and design; Rehan T. Junejo: data collection, extraction, analysis, figures, and tables; Rehan T. Junejo and James P. Fisher: manuscript draft; Rehan T. Junejo, Richard L. Snowdon, Dhiraj Gupta, Gregory Y.H. Lip, and James P. Fisher: interpretation, revision, critique, and final approval of the manuscript.

Data Availability Statement

Data which support the findings of this study are not publicly available due to funder restrictions but are available from the corresponding author, upon reasonable request.

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