A greater burden of atrial fibrillation is associated with worse endothelial dysfunction in hypertension

Khan, Ahsan A.; Junejo, Rehan T.; Alsharari, Reem; Thomas, G. Neil; Fisher, James P.; Lip, Gregory Y.H.

Published in:
Journal of Human Hypertension

DOI (link to publication from Publisher):
10.1038/s41371-020-0383-8

Publication date:
2021

Document Version
Accepted author manuscript, peer reviewed version

A greater burden of atrial fibrillation is associated with worse endothelial dysfunction in hypertension

Short title: Endothelial function in AF and hypertension

Ahsan A Khan, MRCP[1]

Rehan T Junejo, PhD[2,3]

Reem Alsharari, MSc[4]

Graham N Thomas, PhD[1] *

James P Fisher, PhD[5] *

Gregory Y.H. Lip, MD[3,6] *

1. Institute of Applied Health Research, University of Birmingham, United Kingdom
2. School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, United Kingdom
3. Liverpool Centre for Cardiovascular Science, Institute of Ageing and Chronic Disease, University of Liverpool, United Kingdom
4. Institute of Cardiovascular Sciences, University of Birmingham, United Kingdom
5. Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, New Zealand
6. Aalborg Thrombosis Research Unit, Department of Clinical Medicine, Faculty of Health, Aalborg University, Denmark

*Joint senior authors

Correspondence to:

Professor Gregory Lip gregory.lip@liverpool.ac.uk

Full mailing address University of Liverpool, William Henry Duncan Building, 6 West Derby Street, Liverpool, L7 8TX

Telephone number 0151 794 9020

Word count: 4224 (not including abstract, figures, tables and references)

Abstract

Atrial fibrillation (AF) and hypertension often co-exist and both are associated with endothelial dysfunction. We hypothesised that AF would further worsen endothelium-dependent flow-mediated dilatation (FMD) in hypertension patients compared to those without AF. In a cross-sectional comparison, we measured brachial artery diameter at rest and during reactive hyperaemia following 5 minutes of arterial occlusion in two patient groups: AF (and hypertension) (n = 61) and hypertension control groups (n = 33). The AF (and hypertension) subgroups: permanent AF (n = 30) and paroxysmal AF (n = 31) were also assessed. The permanent AF patients received heart rate and blood pressure (BP) control optimisation and were then followed up after eight weeks for repeat FMD testing. There was no significant difference in FMD between AF (and hypertension) group and hypertension control group (4.6%, 95% CI [2.6 – 5.9%] vs 2.6%, 95% CI [1.9 – 5.3%]; p=0.25). There was a significant difference in FMD between permanent AF and paroxysmal AF groups (3.1%, 95% CI [2.3 – 4.8%] vs 5.9%, 95% CI [4.0 – 8.1%]; p=0.02). Endothelium-dependent FMD response showed a non-significant improvement trend following eight weeks of heart rate and BP optimisation (3.1%, 95% CI [2.3 – 4.8%] (baseline) vs 5.2%, 95% CI [3.9 – 6.5%] (follow up), p=0.09). Presence of AF generally does not incrementally worsen endothelial dysfunction in hypertension patients, although the duration and frequency of AF (paroxysmal AF to permanent AF) does lead to worsening endothelial function. Eight weeks of BP optimisation did not significantly improve endothelial dysfunction as measured by FMD.

Introduction

Atrial fibrillation (AF) is associated with increased morbidity including stroke, heart failure, thromboembolic complications and high mortality.[1] Hypertension accounts for more cases of AF than other risk factors, increasing the risk of AF two-fold.[2] In the Framingham study, for example, hypertension heralded an excess risk of AF by 50% in males and 40% in females.[3] Among individuals with a confirmed diagnosis of AF, hypertension is present in about 60% to 80% of these patients.[4] These 2 conditions often co-exist in the same patient, and their prevalence is increasing globally. It is widely perceived that the combination of these conditions confers a worse prognosis than either alone.[5]

Beat to beat variation in blood flow dynamics during AF has been related to presence of endothelial dysfunction.[6] It is well established that the endothelium plays a fundamental role in the regulation of vascular tone by releasing a variety of vasodilatory substances, particularly nitric oxide (NO). NO modulates vascular smooth muscle tone by exerting its effects at a cellular level. A key consequence of normal endothelial function in vivo is the ability to release NO in response to physiological stimuli, such as increased flow, reflecting endothelial flow-mediated dilatation (FMD).[7]

Impaired FMD is associated with cardiovascular risk factors and provides important prognostic information. FMD measurement using high-resolution ultrasound has become a reliable and reproducible technique for assessment of endothelial dysfunction.[8] When blood flow through a vessel increases, the resultant increase in shear stress on the vascular endothelium causes endothelium-dependent vasodilation. The magnitude of this vasodilatory response can be used as an index of endothelial function.

Several studies have previously shown impaired FMD as a marker of endothelial dysfunction in patients with various atherosclerotic risk factors, including advanced age, hypertension, hypercholesterolaemia, diabetes mellitus, tobacco use and postmenopausal status.[9-12] FMD is also found to be impaired in patients with AF.[13-16] Since AF and hypertension, commonly co-exist, we hypothesised that endothelium-dependent FMD will be reduced in patients with AF (and hypertension) compared to hypertensive controls and this may partly explain the poor prognosis in such patients.

We therefore aimed to assess whether presence of AF leads to worsening of endothelial dysfunction in hypertensive patients through assessment by FMD, to assess whether there are any differences in FMD between permanent AF and paroxysmal AF, and lastly whether improvement in blood pressure (BP) control can lead to improvement in FMD.

Methods

Participants were provided with detailed information sheets, and written informed consent was obtained from all participants, in accordance with the Declaration of Helsinki (2013). Eligible participants underwent screening against inclusion and exclusion criteria before being invited to take part in the study (see **supplementary material**). The study was approved by the Health Research Authority (HRA) and National Research and Ethics Service (NREC) Committee London – Camden & Kings Cross (18/LO/1064). Anonymized data and materials have been made publicly available at the Harvard Dataverse and can be accessed at https://doi.org/10.7910/DVN/QKG7DL.

A total of 94 participants were recruited from the atrial fibrillation and hypertension services at Sandwell and West Birmingham Hospitals NHS Trust between October 2018 –

March 2019. We recruited 2 groups of patients: AF (and hypertension) (n = 61) and hypertension control (n = 33). Patients with AF were stable on rate control and antithrombotic medication. The AF (and hypertension) group was further subdivided into permanent AF (n = 30) and paroxysmal AF (n = 31). Permanent AF was defined as an episode of AF in which efforts to restore normal sinus rhythm had either failed or been abandoned. Paroxysmal AF was defined as an episode of AF that terminates spontaneously or with intervention in less than seven days. The hypertension control group included patients with hypertension (defined as previous diagnosis of hypertension or clinic BP of ≥140/90 mmHg) but not AF. These patients had additional cardiovascular risk factors similar to the other two AF groups and acted as the control group.

Initially, a cross-sectional age and clinical characteristics-matched comparison of the two main groups, AF (and hypertension) versus hypertension control was carried out. This was followed by the two subgroups of AF (and hypertension) group. Lastly, the patient group with permanent AF (and hypertension) (n = 30) were studied longitudinally with a single follow-up interval of 8 weeks duration following optimisation of their heart rate (HR) and BP medication. The medication optimisation was carried out by a single clinician with experience in managing these conditions and involved either increasing the dosage of existing cardiovascular medication or addition of a new medication (for which the prescription was provided) according to participants' needs, allergy status, known contraindications and clinical indication. These patients underwent the same measurements as at their first visit.

Experimental protocol

Participants were expected to fast from food, water, caffeine and withhold their cardiovascular medications, except anticoagulation, for at least 12 hours prior to their appointment. They were advised to refrain from smoking for at least 4 hours, physical exercise for 12 hours and drinking alcohol for at least 24 hours prior to their appointment. At the experimental appointment, a detailed medical history was taken from the participants including medications history and a physical examination carried out. This included anthropometric measurements such as height and weight to determine BMI (weight/height^2; kg/m^2). An ECG was performed on all participants to determine rhythm.

Baseline blood samples to test for full blood count, renal, liver and thyroid function, fasting glucose, lipid, and clotting profile, were taken from participants from their left antecubital fossa if they have not had these tests taken within 6 months of their study appointment. A full transthoracic echocardiogram study was performed if a participant did not have a recent echocardiogram. Subsequent measurements were performed in a temperature-controlled room under uniform conditions with participants resting quietly in the supine position on a medical examination couch.

Measurements

Three serial BP readings were taken non-invasively from the left brachial artery using an automated sphygmomanometer over 5 minutes to determine an average. Vascular function was assessed by measuring brachial artery blood flow velocity and diameter. The measurements were obtained from the right arm positioned at heart level by Doppler ultrasound (CX50 CompactXtreme; Philips, Amsterdam, Netherlands) by a single

experimenter, using a 10-MHz multi-frequency linear-array transducer. B-mode imaging was used to measure arterial diameter, and peak blood velocity was simultaneously measured using the pulse-wave mode. Measurements were made in accordance with recent technical recommendations.[17] The ultrasound machine was connected via a HDMI AV.io (Epiphan Video Systems Inc, California, USA) video grabber to a laptop with a dedicated FMD software, QUIPU Cardiovascular Suite (Quipu srl, Pisa, Italy) with edge-detection capability and real-time processing and recording of B-mode ultrasound image sequence, removing the need for ECG gating.[18] This software utilises image based automated edge detection and wall tracking algorithms working independently of investigator influence. This system has been used and validated in other studies involving human participants.[18, 19]

Participants lay supine on the couch with their right arm extended out and had a narrow inflatable cuff (5-cm width; Hokanson, Bellevue, WA) placed 5 – 7 cm distal to the medial epicondyle. The arm was positioned in a comfortable position. The brachial artery was imaged 10-15 cm proximal to the medial epicondyle at 60^{o} insonation angle in the longitudinal plane. Duplex imaging was used to obtain a B-mode image of vessel diameter and pulse-wave mode for peak blood velocity. Ultrasound measurements were made in accordance with technical recommendations.[17] Following 1 minute of baseline diameter recording, the arterial occlusion cuff was inflated to 50 mmHg above systolic BP for 5 minutes. Following this, the cuff was rapidly deflated and arterial image recording continued for further 2 minutes. Recordings were screen captured and stored as video files and off-line analysis carried out with automated edge detection and wall tracking software (Cardiovascular Suite version 3.4.1; FMD Studio, Pisa, Italy).

Data analysis

Patients were matched for age and clinical characteristics to reduce chances of confounders. Body mass index (BMI) was expressed as the ratio of the participants' weight and their height squared. Digitally recorded data were extracted in an anonymized manner. Mean arterial pressure (MAP) was the mean blood pressure over each cardiac cycle. Brachial artery FMD was taken as the maximal change in brachial artery diameter following cuff deflation. The time to peak diameter was obtained between the cuff deflation and the maximal artery dilation, and the time to peak blood flow (reactive hyperaemia) was obtained between cuff deflation and maximal flow velocity. Shear rate (positive shear rate area to peak) was calculated as an integral between the cuff deflation and the maximal artery dilation. FMD was expressed as absolute (mm) and relative change (%) in diameter. Based on recent guidelines, covariate-corrected FMD was presented, adjusting for differences in baseline diameter between the two groups using analysis of covariance (ANCOVA).[20]

Statistical analysis

Descriptive statistics are presented as mean ± standard deviation (SD) or median with interquartile range, as appropriate for continuous variables. Categorical variables are expressed as numbers and percentages. Statistical analysis was performed using SPSS software (version 26.0; SPSS Inc., Chicago, Illinois). Continuous variables were tested for normality using the Shapiro-Wilk test. If passed, data was analysed using independent Student's t-test between the two groups. Data found to be not normally distributed were analysed with Mann-Whitney U test. For longitudinal comparison, continuous variables

were tested for normality using the Shapiro-Wilk test. If passed, data was analysed using Student's paired t-test. Data found to be not normally distributed were analysed with Wilcoxon Signed Rank test. A p value of < 0.05 was considered statistically significant. Associations between FMD and co-variates were assessed before and after adjustment for potential confounders (age, sex, BMI) using linear regression analysis.

To test specific hypothesis 1 ("Patients with AF and hypertension will have worse parameters of vascular function compared to hypertension control group"), we recruited 94 patients in total, split between 2 groups (a) AF and hypertension (b) hypertension control. This part of the study was powered based on independent t-test, comparing the flow-mediated dilatation values across the two groups. Skalidis *et al* reported a mean FMD of 8.1 (standard deviation (SD) = 3.6) in a pre-treatment (i.e. cardioversion) AF group.[6] Assuming our SD is similar, the minimum sample size was computed as 18 patients per group at 90% power, 5% alpha and effect size of 1.14.

To test specific hypothesis 2 ("Patients with permanent AF and hypertension will have worse parameters of vascular function compared to patients with paroxysmal AF and hypertension"), we recruited 61 patients in total, split between the 2 groups. This part of the study was powered based on an independent t-test, assessing the difference in FMD between permanent AF and paroxysmal AF. Mazaris *et al* reported a mean FMD of 4.09 (SD = 1.67) in permanent AF group compared to mean FMD of 6.83 (SD = 1.38) in paroxysmal AF group.[16] Assuming our SD is similar, the minimum sample size was computed as 8 patients per group at 90% power, 5% alpha and effect size of 1.79.

To test specific hypothesis 3 ("Eight weeks of intensive anti-hypertensive and anticoagulation therapy will improve vascular function in patients with permanent AF and hypertension") we recruited 30 patients and tested them before and after intensification of their antihypertensive and anticoagulation treatment. This part of the study was powered based on a paired t-test, assessing the change in flow mediated dilation from pre- to post-treatment. It was assumed that the mean pre- intervention flow mediated dilation would be 8.1 (SD=3.6), as per Skalidis et al, and that the effect size would be 1.06.[6] If this is the case, then the minimum number of patients required is 12 at 90% power and 5% alpha.

Results

Matched AF (and hypertension) group vs matched hypertension control group

Participants from AF (and hypertension) group and hypertension control group were matched for age and clinical characteristics (see table 1). Participants' medication history is displayed in figure 1. There were no significant differences in age, sex, height, weight and BMI. Past medical history of all participants between the groups was similar except that participants in hypertension control group had significantly more patients with a background of chronic kidney disease (CKD) ($p = 0.01$). The CHA_2DS_2-VASc score and HAS-BLED score were similar between the two groups. The mean heart rate was significantly lower in the hypertension control group ($p = 0.02$). There were no significant differences in mean blood pressure (systolic and diastolic) between the two groups, baseline glycaemia control (HBA1c), kidney function (creatinine clearance) and left ventricular ejection fraction (EF (%)).

Baseline diameter of brachial artery was significantly smaller in the AF (and hypertension) group compared to hypertension control group (4.6 mm, 95% confidence interval (CI) [4.4 – 4.9 mm] vs 5.2 mm, 95% CI [4.8 – 5.6 mm]; p = 0.02) (see table 2). Following 5 minutes of forearm ischaemia, there was no significant difference in absolute FMD between AF (and hypertension) group and hypertension control group (0.2 mm, 95% CI [0.1 – 0.3 mm] vs 0.2 mm, 95% CI [0.1 – 0.3 mm]; p = 0.61) or FMD percentage (4.6%, 95% CI [2.6 – 5.9%] vs 2.6%, 95% CI [1.9 – 5.3%]; p = 0.25) respectively. The FMD (%) means were adjusted for baseline diameter and showed no significant difference between the two groups (4.9%, 95% CI [3.8 – 6.0%] (AF (and hypertension) group) vs 4.3%, 95% CI [2.8 – 5.9%] (hypertension control group), p = 0.56).

The peak diameter was significantly different between the two groups (4.9 mm, 95% CI [4.6 – 5.2 mm] (AF (and hypertension) group) vs 5.4 mm, 95% CI [5.0 – 5.8 mm] (hypertension control group); p = 0.03). There were no significant differences in time to peak diameter and shear rate between the two groups (p = 0.07 and p = 0.41 respectively). No variables were identified on univariate and stepwise multivariate analysis as independent predictors of reduced FMD.

<u>Permanent AF (and hypertension) vs PAF (and hypertension) groups</u>

Participants in the two AF subgroups (permanent AF vs paroxysmal AF) were well matched for age, sex, clinical characteristics including height, weight, BMI, mean blood pressure, HBA1c, creatinine clearance and left ventricular EF (%) (see table 3). Participants' medication history is displayed in figure 1. There was a significantly higher incidence of

247 ischaemic heart disease in paroxysmal AF group (p<0.001) and mean heart rate was found
248 to be significantly slower in participants in paroxysmal AF group (p = 0.003).

249 On FMD measurement, there were no significant difference in baseline diameter between
250 the two groups (permanent AF (4.5 mm, 95% CI [4.2 – 5.0 mm]) vs paroxysmal AF (4.8 mm,
251 95% CI [4.6 – 5.1 mm]) p = 0.67) (see table 4). Following 5 minutes of forearm ischaemia,
252 there was a significant difference in absolute FMD change between permanent AF and
253 paroxysmal AF (0.1 mm, 95% CI [0.1 – 0.2 mm] vs 0.3 mm, 95% CI [0.2 – 0.4 mm]; p = 0.01
254 respectively). There was also a significant difference in FMD percentage between the two
255 groups (3.1%, 95% CI [2.3 – 4.8%] (permanent AF) vs 5.9%, 95% CI [4.0 – 8.1%] (paroxysmal
256 AF); p = 0.02). This difference persisted with correction for baseline diameter (3.9%, 95% CI
257 [2.8 – 5.0%] (permanent AF) vs 5.9%, 95% CI [4.8 – 7.0%] (paroxysmal AF); p = 0.01).

258 There was no significant difference in peak diameter (p = 0.49), time to peak diameter (p =
259 0.23) and shear rate (p = 0.40) between the two groups. Presence of permanent AF
260 (Spearman's rho 0.295; p = 0.02) and ischaemic heart disease (Spearman's rho 0.280; p =
261 0.03) were identified as independent predictors of reduced FMD on univariate analysis (p =
262 0.03) but only permanent AF was identified as an independent predictor of reduced FMD on
263 stepwise multivariate analysis (R^2 0.090; F 5.855; p = 0.02).

264 <u>Permanent AF (and hypertension) group – longitudinal comparison</u>

265 Following optimisation of HR and BP medication, patients with permanent AF (and
266 hypertension) were followed up after eight weeks and FMD repeated (see table 5). There
267 was significant improvement in mean heart rate (77 beats per minute (bpm) ± 18 (baseline)
268 vs 72 bpm ± 17 (follow up), p = 0.01), systolic BP (140 mmHg [128 – 148] (baseline) vs 131

mmHg [122 – 146] (follow up), p = 0.03), diastolic BP (81 mmHg ± 13 (baseline) vs 77 mmHg ± 12 (follow up), p = 0.02) and mean arterial pressure (MAP) (100 mmHg ± 9 (baseline) vs 97 mmHg ± 13 (follow up), p = 0.01).

Both groups had a similar baseline brachial artery diameter (p = 0.34). Endothelium-dependent FMD response was better following eight weeks of HR and BP optimisation but this 68% relative improvement did not reach statistical significance (3.1%, 95% CI [2.3 – 4.8%] (baseline) vs 5.2%, 95% CI [3.9 – 6.5%] (follow up), p = 0.09). The FMD (%) means were adjusted for baseline diameter and showed no significant difference between the two groups (4.0%, 95% CI [3.0 – 4.9%] (baseline) vs 5.1%, 95% CI [4.2 – 6.1%] (follow up), p = 0.09). The difference was also not significant in absolute change in diameter (0.14 mm, 95% CI [0.11 – 0.25 mm] (baseline) vs 0.20 mm, 95% CI [0.17 – 0.28 mm] (follow up), p = 0.15). The time to peak diameter, peak diameter and shear rate stimulus were similar between the two groups (table 5). No variables were identified on univariate or stepwise multivariate analysis as independent predictors of reduced FMD.

Discussion

This is the first study investigating whether the presence of AF worsens the endothelial dysfunction seen in patients with hypertension. The results are consistent with other studies looking at FMD in hypertension and AF individually and confirms that endothelial dysfunction is present.[9, 13-16, 21] Our findings extend previous work by demonstrating that the presence of AF generally does not incrementally worsen endothelial dysfunction, nor was AF an independent predictor of endothelial dysfunction on multivariate analysis. However, permanent AF compared to paroxysmal AF does have significantly worse FMD parameters

with permanent AF being an independent predictor on multivariate analysis. Lastly, we did not find any significant improvement in FMD following 8 weeks of HR and BP optimisation in permanent AF and hypertension patients.

There are potentially several reasons that may explain the lack of differences seen between AF (and hypertension) and hypertension control group in our study. These can be broadly categorised into oxidative stress, inflammation and the role of endothelial nitric oxide synthase (eNOS). Increase in systemic oxidative stress is thought to play a part in endothelial dysfunction seen in patients with hypertension, whereas a reduction has been shown to reverse endothelial dysfunction.[22] Risk factors for AF are similar to those of atherosclerosis and hypertension, diseases known to be perpetuated by oxidative stress. This can explain why the addition of AF does not significantly worsen endothelial dysfunction seen in patients with hypertension.

Inflammation has also been implicated in the pathophysiology of hypertension as well as initiation and perpetuation of AF and AF-related adverse effects.[23, 24] Endothelial dysfunction seen in hypertension relates to local vascular inflammation and systemic inflammation.[25] Also, inflammation contributes to the pathophysiology of AF, both directly and through AF-promoting cardiovascular conditions that have an inflammatory aetiology.[26] FMD has been shown to be inversely associated with serum C-reactive protein (CRP) levels in chronic AF patients, implying disruption by inflammation.[27] Since inflammation plays an important role in causing endothelial dysfunction in both conditions, it is perhaps unsurprising that we did not see a significant difference in the FMD response between the groups, suggesting that endothelial perturbation seen in AF may reflect underlying comorbidities rather than AF per se. Interestingly, endothelial dysfunction itself enhances

oxidative stress and leads to increase in recruitment of proinflammatory agents promoting a vicious cycle.[28] The complex interplay involving oxidative stress and inflammation seen in both conditions is summarised in figure 2.

eNOS, a key regulator of vascular tone is found to be reduced or dysfunctional in both hypertension and AF.[29, 30] eNOS produces NO to mediate relaxation of blood vessels and preservation of vascular function. When eNOS is deprived of its critical cofactor tetrahydrobiopterin or its substrate L-arginine, it results in synthesis of large volumes of reactive oxygen species such as peroxynitrite (superoxide) instead of NO, leading to nitric oxide synthase (NOS) uncoupling. Superoxide production by uncoupled eNOS further sustains oxidative stress in the vasculature, resulting in endothelial dysfunction, impaired endothelium-dependent vasorelaxation and elevated BP.[29] This inadvertently leads to tissue damage that promotes pathological remodelling of the myocardium contributing to initiation and propagation of AF.[31] Since the aetiology and pathophysiology of endothelial dysfunction are similar in both hypertension and AF, this supports our finding that AF and hypertension had similar effect on the FMD with no significant difference seen between the two groups. Our study also suggests that AF, as opposed to hypertension, is perhaps the dominant condition responsible for endothelial dysfunction in these patients as permanent AF group showed a worse FMD compared to paroxysmal AF group and 8 weeks of intensive hypertensive therapy revealed a non-significant improvement trend in FMD.

Interestingly, we were able to see a significant difference in FMD between permanent AF and paroxysmal AF groups with more impaired FMD noted in permanent AF group. This suggests that frequency and duration of AF episode or type of AF may be important in progression of endothelial dysfunction. Our findings are similar to other studies showing

that patients with permanent AF have worse FMD compared to patients with paroxysmal AF.[15, 16, 32] However, unlike previous studies, our study included patients with AF and hypertension, which has not been looked at before.

Although, our study did show that improvement in HR and BP can lead to improvement in FMD in hypertensive patients despite the presence of AF, however this 68% improvement did not reach statistical significance ($p = 0.09$). These results are similar to the study performed by Modena and colleagues who looked at hypertensive patients (without AF) and showed that 6 month of BP optimisation led to improvement in FMD and was associated with a more favourable prognosis.[33] Thus, longer-term improvement in FMD may have a prognostic implication.[34] Furthermore, it supports previous work showing modulation of endothelial function is possible and that endothelial dysfunction is a reversible condition.[25]

Our study has several important clinical implications. We have been able to show that endothelial dysfunction is present in patients with AF and hypertension. This may explain the increased risk of stroke and heart attack in these patients as endothelial function may be involved in the pathophysiology of these conditions, in addition to the prothrombotic state seen in AF. We have been able to show that increased frequency and duration of AF leads to worsening of endothelial function and thus these patients may benefit from closer monitoring and perhaps consideration for AF ablation. We have also shown that improvement in HR and BP leads to improvement in FMD, although it was not significant in our study. Nevertheless, it does suggest that endothelial function may be a reversible condition if risk factors such as blood pressure are controlled and optimised.

Strengths and limitations

We did not use nitrate to assess for endothelium-independent vasodilation as this has been studied previously in both AF and hypertension.[9, 13, 15, 21] Furthermore, use of intra-arterial acetylcholine would have been advantageous to investigate brachial artery endothelial function but FMD is a well established surrogate.[17] Given the widespread prevalence of AF in hypertensive patients, the inclusion of separate hypertension groups with and without AF, is a strength of our study. There have been limited studies looking at vascular function in patients with AF arrhythmia and therefore this makes our study unique. Participants in our group were well-matched for age, sex composition, comorbidities, CHA_2DS_2-VASc score, HAS-BLED score, BMI, BP, glycaemic control and LV systolic function. Nonetheless, the hypertension control group did have a significantly higher number of patients with CKD which may have been a source of bias. We accommodated for this and other potential confounders by utilisation of linear regression analysis. The longitudinal comparison of permanent AF (and hypertension) group in assessing FMD response to intervention has not been looked at before. The utilisation of edge detection software, assessment of shear rate and correcting for differences in group baseline diameters shows robustness of our methodological approach.

In contrast, our study has some limitations. Endothelial function was examined using the well-established brachial artery flow mediated dilatation technique in accordance with recent technical recommendations, however we acknowledge that this may not provide an optimal assessment of endothelial dysfunction.[17] Second, it would have been useful to compare our findings with a healthy control group and/or a group with AF but no hypertension as the relation between hypertension and AF is bi-univocal. Third, the use of

anti-hypertensives and other concomitant medications may have influenced endothelial function long term which cannot be excluded. Additionally, whilst we were able to show reduction in HR and BP in our longitudinal study, the short duration of 8 weeks may not be enough to reveal significant improvement in endothelial function. Fourth, we did not measure other potential causes for endothelial dysfunction such as changes in free fatty acids, inflammatory cytokines, inflammatory markers such as c-reactive protein (CRP), nitric oxide synthase expression and endothelin. However, this real world cohort has ecological validity and makes our observations more representative of the clinic. Future studies should look at whether and how endothelial function progresses in patients with AF over time and compare it to patients with hypertension to assess if there are any differences.

Conclusions

The presence of AF generally does not incrementally worsen endothelial dysfunction in hypertension, nor was AF an independent predictor of endothelial dysfunction on multivariate analysis. However, duration and frequency of AF leads to worsening endothelial function as demonstrated in our study. Eight weeks of BP optimisation did not give a significant improvement in endothelial dysfunction as measured by FMD.

399 Summary Table

What is known about topic?
• Atrial fibrillation and hypertension commonly co-exist and the combination of these two conditions confers a worse prognosis than either alone. • Endothelial dysfunction is present in both atrial fibrillation and hypertension. • Flow-mediated dilatation is a reliable tool to assess endothelial function.
What this study adds?
• Presence of AF generally does not incrementally worsen endothelial dysfunction in hypertension patients • The duration and frequency of AF (paroxysmal AF to permanent AF) does lead to worsening endothelial function. • There is potential for endothelial dysfunction to improve following optimisation of BP suggesting modulation of endothelial function is possible in patients with permanent AF and hypertension.

400

Acknowledgements

The time and effort expended by all the participants is greatly appreciated.

Conflict of Interest

Authors declare no conflict of interests for this article.

GYHL has served as a consultant for Bayer/Janssen, BMS/Pfizer, Biotronik, Medtronic, Boehringer Ingelheim, Microlife and Daiichi-Sankyo; and a speaker for Bayer, BMS/Pfizer, Medtronic, Boehringer Ingelheim, Microlife, Roche and Daiichi-Sankyo. No personal fees received.

Sources of Funding

None

References

1. Kannel WB, Wolf PA, Benjamin EJ and Levy D. Prevalence, incidence, prognosis, and predisposing conditions for atrial fibrillation: population-based estimates. Am J Cardiol. 1998; 82: 2n-9n.

2. Lau YF, Yiu KH, Siu CW and Tse HF. Hypertension and atrial fibrillation: epidemiology, pathophysiology and therapeutic implications. J Hum Hypertens. 2012; 26: 563-9.

3. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ and Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. JAMA. 1994; 271: 840-4.

4. Nabauer M, Gerth A, Limbourg T, Schneider S, Oeff M, Kirchhof P, et al. The Registry of the German Competence NETwork on Atrial Fibrillation: patient characteristics and initial management. Europace. 2009; 11: 423-34.

5. Dzeshka MS, Shantsila A, Shantsila E and Lip GYH. Atrial Fibrillation and Hypertension. Hypertension. 2017; 70: 854-861.

6. Skalidis EI, Zacharis EA, Tsetis DK, Pagonidis K, Chlouverakis G, Yarmenitis S, et al. Endothelial Cell Function During Atrial Fibrillation and After Restoration of Sinus Rhythm. Am J Cardiol. 2007; 99: 1258-1262.

7. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation. 1995; 91: 1314-9.

8. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. A report of the International Brachial Artery Reactivity Task Force. 2002; 39: 257-265.

436 9. Iiyama K, Nagano M, Yo Y, Nagano N, Kamide K, Higaki J, et al. Impaired endothelial
437 function with essential hypertension assessed by ultrasonography. Am Heart J. 1996; 132:
438 779-782.
439 10. Simons LA, Sullivan D, Simons J and Celermajer DS. Effects of atorvastatin monotherapy
440 and simvastatin plus cholestyramine on arterial endothelial function in patients with severe
441 primary hypercholesterolaemia. Atherosclerosis. 1998; 137: 197-203.
442 11. Lekakis J, Papamichael C, Anastasiou H, Alevizaki M, Desses N, Souvatzoglou A, et al.
443 Endothelial dysfunction of conduit arteries in insulin-dependent diabetes mellitus without
444 microalbuminuria. Cardiovasc Res. 1997; 34: 164-168.
445 12. Thomas GN, Chook P, Yip TW, Kwong SK, Chan TY, Qiao M, et al. Smoking without
446 exception adversely affects vascular structure and function in apparently healthy Chinese:
447 implications in global atherosclerosis prevention. Int J Cardiol. 2008; 128: 172-7.
448 13. Freestone B, Chong AY, Nuttall S and Lip GY. Impaired flow mediated dilatation as
449 evidence of endothelial dysfunction in chronic atrial fibrillation: relationship to plasma von
450 Willebrand factor and soluble E-selectin levels. Thromb Res. 2008; 122: 85-90.
451 14. Borschel CS, Rubsamen N, Ojeda FM, Wild PS, Hoffmann BA, Prochaska JH, et al.
452 Noninvasive peripheral vascular function and atrial fibrillation in the general population. J
453 Hypertens. 2019; 37: 928-934.
454 15. Komatsu T, Kunugita F, Ozawa M, Satoh Y, Yoshizawa R, Owada S, et al. Relationship
455 between Impairment of the Vascular Endothelial Function and the CHA2DS2-VASc Score in
456 Patients with Sinus Rhythm and Non-valvular Atrial Fibrillation. Intern Med. 2018; 57: 2131-
457 2139.

16. Mazaris S. TD, Siasos G., Zisimos K., Oikonomou E., Kokkou E., Konsola T., Lazaros G., Chrysohoou C., Stefanadis C. The role of endothelial function on paroxysmal and chronic atrial fibrillation. Circ J. 2014; 130.

17. Thijssen DHJ, Bruno RM, van Mil ACCM, Holder SM, Faita F, Greyling A, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. Eur Heart J. 2019; 40: 2534-2547.

18. Gemignani V, Bianchini E, Faita F, Giannarelli C, Plantinga Y, Ghiadoni L, et al. Ultrasound measurement of the brachial artery flow-mediated dilation without ECG gating. Ultrasound Med Biol. 2008; 34: 385-91.

19. Junejo RT, May S, Alsalahi S, Alali M, Ogoh S and Fisher JP. Cerebrovascular carbon dioxide reactivity and flow-mediated dilation in young healthy South Asian and Caucasian European men. American Journal of Physiology-Heart and Circulatory Physiology. 2020; 318: H756-H763.

20. Atkinson G and Batterham AM. Allometric scaling of diameter change in the original flow-mediated dilation protocol. Atherosclerosis. 2013; 226: 425-427.

21. Felmeden DC, Spencer CGC, Chung NAY, Belgore FM, Blann AD, Beevers DG, et al. Relation of thrombogenesis in systemic hypertension to angiogenesis and endothelial damage/dysfunction (a Substudy of the Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT]). The American Journal of Cardiology. 2003; 92: 400-405.

22. Kizhakekuttu TJ and Widlansky ME. Natural antioxidants and hypertension: promise and challenges. Cardiovasc Ther. 2010; 28: e20-32.

23. Schiffrin EL. The immune system: role in hypertension. Can J Cardiol. 2013; 29: 543-8.

24. Guo Y, Lip GY and Apostolakis S. Inflammation in atrial fibrillation. J Am Coll Cardiol. 2012; 60: 2263-70.

482 25. Dharmashankar K and Widlansky ME. Vascular endothelial function and hypertension:
483 insights and directions. Curr Hypertens Rep. 2010; 12: 448-455.
484 26. Harada M, Van Wagoner DR and Nattel S. Role of inflammation in atrial fibrillation
485 pathophysiology and management. Circulation journal : official journal of the Japanese
486 Circulation Society. 2015; 79: 495-502.
487 27. Tousoulis D, Zisimos K, Antoniades C, Stefanadi E, Siasos G, Tsioufis C, et al. Oxidative
488 stress and inflammatory process in patients with atrial fibrillation: the role of left atrium
489 distension. Int J Cardiol. 2009; 136: 258-62.
490 28. Guazzi M, Casali M, Berti F, Rossoni G, Colonna VD and Guazzi MD. Endothelium-
491 mediated modulation of ergoreflex and improvement in exercise ventilation by acute
492 sildenafil in heart failure patients. Clin Pharmacol Ther. 2008; 83: 336-41.
493 29. Li Q, Youn J-Y and Cai H. Mechanisms and consequences of endothelial nitric oxide
494 synthase dysfunction in hypertension. J Hypertens. 2015; 33: 1128-1136.
495 30. Cai H, Li Z, Goette A, Mera F, Honeycutt C, Feterik K, et al. Downregulation of
496 endocardial nitric oxide synthase expression and nitric oxide production in atrial fibrillation:
497 potential mechanisms for atrial thrombosis and stroke. Circulation. 2002; 106: 2854-8.
498 31. Fares F, Smith Y, Azzam N, Zafrir B, Lewis BS and Amir O. The 894G Allele of the
499 Endothelial Nitric Oxide Synthase 3 (eNOS) is Associated with Atrial Fibrillation in Chronic
500 Systolic Heart Failure. J Atr Fibrillation. 2012; 5: 757-757.
501 32. Siasos G, Mazaris S, Zisimos K, Oikonomou E, Kokkou E, Konsola T, et al. THE IMPACT OF
502 ATRIAL FIBRILLATION ON ENDOTHELIAL DYSFUNCTION. J Am Coll Cardiol. 2015; 65: A477.
503 33. Modena MG, Bonetti L, Coppi F, Bursi F and Rossi R. Prognostic role of reversible
504 endothelial dysfunction in hypertensive postmenopausal women. J Am Coll Cardiol. 2002;
505 40: 505-10.

34. Ghiadoni L, Taddei S and Virdis A. Hypertension and endothelial dysfunction: therapeutic approach. Curr Vasc Pharmacol. 2012; 10: 42-60.

Legends

Figure 1

Medication use by class of drugs

ACE inhibitor = Angiotensin converting enzyme; ARB = Angiotensin receptor blocker

Figure 2

Complex interplay between hypertension, AF, oxidative stress, inflammation and endothelial dysfunction

Table 1

Descriptive data are presented as numbers (with percentages). Normally distributed data are expressed as mean ± standard deviation. Non-normally distributed data are displayed as median with interquartile ranges. Statistical differences were tested for matched groups using an independent t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. Categorical data was compared using Chi-square test. Where Chi-square test was not valid, Fisher's Exact Test was used. Significance $p \leq 0.05$. - = unable to calculate p value as sample size too small/statistical test not valid
AF = atrial fibrillation; TIA = Transient Ischaemic Attack; COPD = Chronic Obstructive Pulmonary Disease; BMI = Body Mass Index; bpm = beats per minute; BP = blood pressure; HbA1c = Haemoglobin A1C; CrCl = Creatine Clearance (Cockroft-Gault method); TSH = Thyroid Stimulating Hormone; INR = International Normalised Ratio

Table 2

Normally distributed data are expressed as mean [95% confidence intervals (CI)]. Identified by superscript a. Non-normally distributed data are displayed as median [95% CI]. Identified by superscript b. Statistical differences were tested for matched groups using independent t-test (for parametric data) or Mann-Whitney U test (for non-parametric data). Significance $p \leq 0.05$.
AF = atrial fibrillation; FMD = flow-mediated dilatation; FMDc = FMD % mean [95% CI] adjusted for baseline diameter

Table 3

Descriptive data are presented as numbers (with percentages). Normally distributed data are expressed as mean ± standard deviation. Non-normally distributed data are displayed as median with interquartile ranges. Statistical differences were tested using an independent t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. Categorical data was compared using Chi-square test. Where Chi-square test was not valid, Fisher's Exact Test was used. Significance $p \leq 0.05$. - = unable to calculate p value as sample size too small/statistical test not valid

AF = atrial fibrillation; TIA = Transient Ischaemic Attack; COPD = Chronic Obstructive Pulmonary Disease; BMI = Body Mass Index; bpm = beats per minute; BP = blood pressure; HbA1c = Haemoglobin A1C; CrCl = Creatine Clearance (Cockroft-Gault method); TSH = Thyroid Stimulating Hormone; INR = International Normalised Ratio

Table 4

Normally distributed data are expressed as mean [95% confidence intervals (CI)]. Identified by superscript a. Non-normally distributed data are displayed as median [95% CI]. Identified by superscript b. Statistical differences were tested using independent t-test (for parametric data) or Mann-Whitney U test (for non-parametric data). Significance $p \leq 0.05$.

AF = atrial fibrillation; FMD = flow-mediated dilatation

Table 5

Normally distributed data are expressed as mean ± standard deviation for descriptive data and mean [95% confidence interval (CI)] otherwise. Identified by superscript a. Non-normally distributed data are displayed as median with interquartile ranges for descriptive data and median [95% CI] otherwise. Identified by superscript b. Normality test was performed using Shapiro-Wilk test. Statistical differences were tested using paired t-test (if passed) or Wilcoxon signed rank test (if failed). Significance $p \leq 0.05$. AF = atrial fibrillation; bpm = beats per minute; BP = blood pressure; FMD = flow mediated dilatation

Figure 1

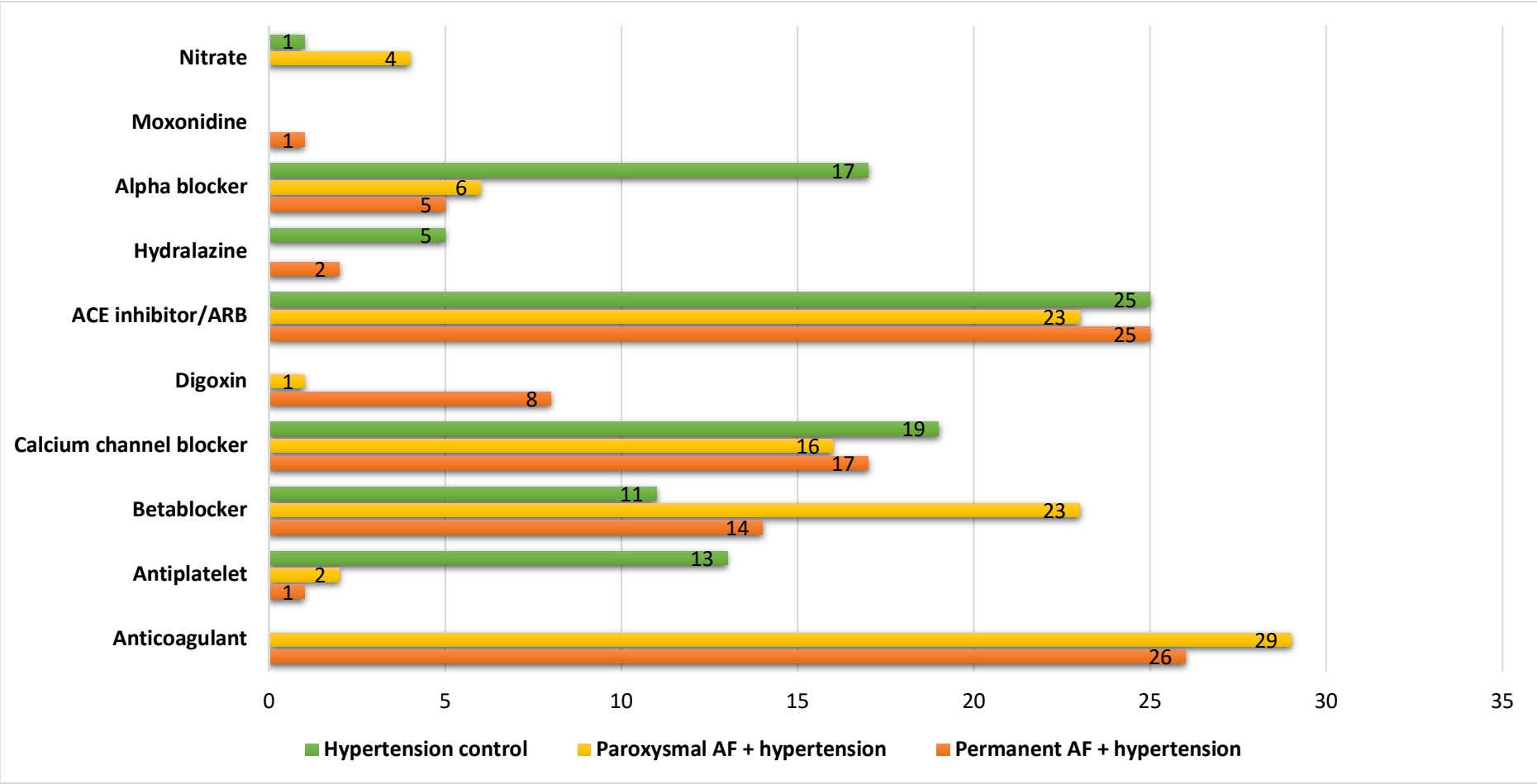
Nitrate
1
4
Moxonidine
1
Alpha blocker
17
6
5
Hydralazine
5
2
ACE inhibitor/ARB
25
23
25
Digoxin
1
8
Calcium channel blocker
19
16
17
Betablocker
11
23
14
Antiplatelet
13
2
1
Anticoagulant
29
26
0
5
10
15
20
25
30
35
Hypertension control
Paroxysmal AF + hypertension
Permanent AF + hypertension

Figure 2

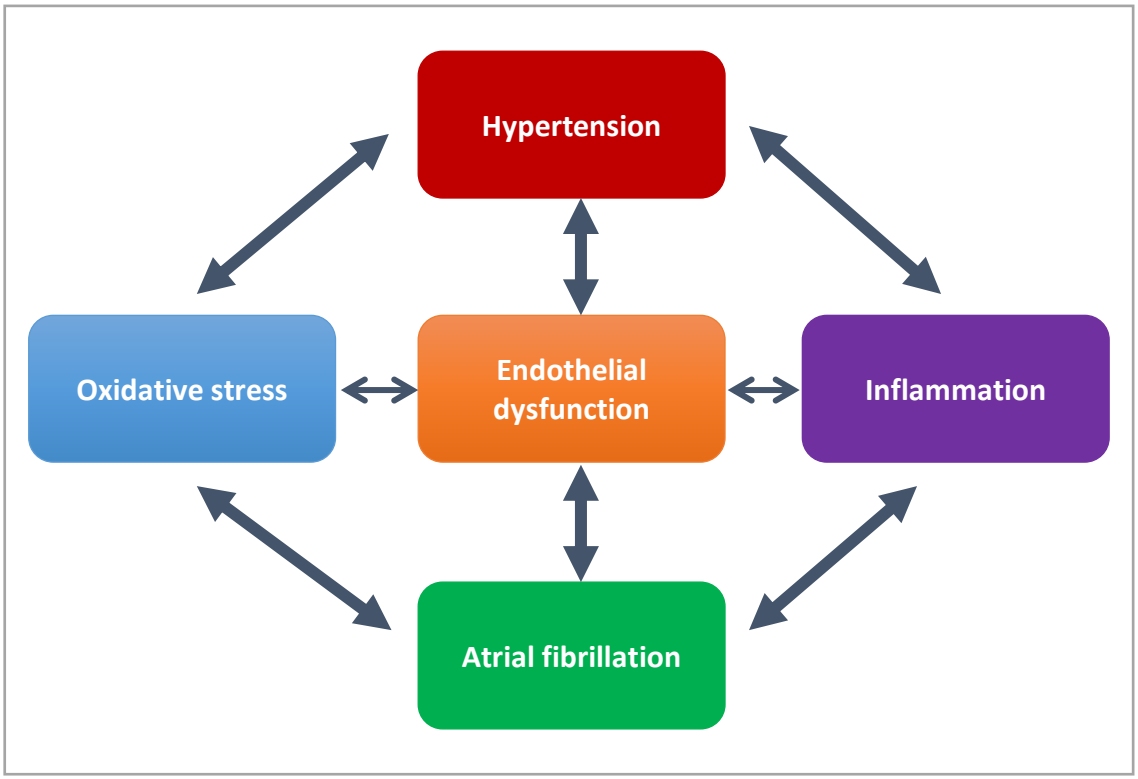
Hypertension
Oxidative stress
Endothelial dysfunction
Inflammation
Atrial fibrillation

Table 1 – Demographics and clinical characteristics of matched AF (and hypertension) group and hypertension control group

	AF + hypertension group (n = 40)	**Hypertension control group (n = 20)**	**Matched groups p**
Demographics			
Age, years	66 ± 7	65 ± 7	0.71
Sex Male Female	 29 11	 15 5	 0.84
Ethnicity Caucasians, n (%) Blacks, n (%) Asians, n (%) Mixed, n (%)	 34 (85%) 3 (7.5%) 3 (7.5%) 0 (0%)	 10 (50%) 6 (30%) 3 (15%) 1 (5%)	 -
Clinical characteristics			
Heart failure, n (%)	2 (5%)	0 (0%)	0.55
IHD, n (%)	5 (12.5%)	5 (25%)	0.28
Diabetes Mellitus, n (%)	10 (25%)	8 (40%)	0.23
Previous stroke/TIA, n (%)	5 (12.5%)	5 (25%)	0.28
Asthma/COPD, n (%)	5 (12.5%)	2 (10%)	0.57
Chronic liver disease, n (%)	0 (0%)	0 (0%)	-
Chronic kidney disease, n (%)	1 (2.5%)	5 (25%)	**0.01**
Anaemia, n (%)	0 (0%)	2 (10%)	0.11
Thyroid disorder, n (%)	3 (7.5%)	4 (20%)	0.21
Hypercholesterolaemia, n (%)	19 (47.5%)	11 (55%)	0.58
Arthritis, n (%)	24 (60%)	8 (40%)	0.14
CHA_2DS_2-VASc score	2 [2 – 4]	3 [1 – 4]	0.74
HAS-BLED score	1 [1 – 1]	2 [1 – 2]	0.06
Smoking status Never smoked, n (%) Ex-smoker, n (%) Current, n (%)	 19 (47.5%) 18 (45%) 3 (7.5%)	 13 (65%) 7 (35%) 0 (0%)	 -
Alcohol None, n (%) Recommended, n (%)	 9 (22.5%) 31 (77.5%)	 5 (25%) 15 (75%)	 0.54
Height (cm)	170.1 ± 8.9	169.4 ± 11.1	0.80
Weight (kg)	95.5 ± 18.4	92.3 ± 14.7	0.50
BMI (kg/m^2)	32.9 ± 5.2	32.1 ± 4.2	0.58
Heart rate (bpm)	70 [60 – 82]	63 [58 – 67]	**0.02**
Systolic BP (mm/Hg)	142 [133 – 152]	148 [135 – 175]	0.12
Diastolic BP (mm/Hg)	83 ± 14	85 ± 13	0.53
Mean Arterial Pressure (MAP) (mm/Hg)	103 ± 15	109 ± 16	0.23
HbA1c (mmol/mol)	41 [39 – 48]	45 [38 – 56]	0.32
CrCl (mL/min)	98.8 ± 29.6	85 ± 28.1	0.09
Ejection fraction (%)	58 ± 11	62 ± 7	0.14

Table 2 – Differences in flow mediated dilatation (FMD) between matched AF (and hypertension) and hypertension control groups – cross sectional comparison

	AF + hypertension group (n = 40)	**Hypertension control group (n = 20)**	**Matched groups p**
Baseline diameter (mm)	4.6 [4.4 – 4.9][a]	5.2 [4.8 – 5.6][a]	**0.02**
Peak diameter (mm)	4.9 [4.6 – 5.2][a]	5.4 [5.0 – 5.8][a]	**0.03**
Absolute FMD change (mm)	0.2 [0.1 – 0.3][b]	0.2 [0.1 – 0.3][b]	0.61
FMD (%)	4.6 [2.6 – 5.9][b]	2.6 [1.9 – 5.3][b]	0.25
FMDc (%)	4.9 [3.8 – 6.0][a]	4.4 [2.7 – 6.0][a]	0.60
Time to peak diameter (sec)	58 [40 – 90][b]	36 [21 – 65][b]	0.07
Shear rate (Positive shear rate area to peak) [sec.-1]	4421 [2800 – 6077][b]	3300 [1296 – 6887][b]	0.41

Table 3 – Demographics and clinical characteristics of permanent AF (and hypertension) group and paroxysmal AF (and hypertension) group

	Permanent AF + hypertension group (n = 30)	**Paroxysmal AF + hypertension group (n = 31)**	**p**
Demographics			
Age, years	70 ± 8	72 ± 11	0.64
Sex Males Females	 22 8	 20 11	 0.46
Ethnicity Caucasians, n (%) Blacks, n (%) Asians, n (%) Mixed, n (%)	 28 (93.3%) 1 (3.3%) 1 (3.3%) 0 (0%)	 25 (80.6%) 3 (9.7%) 3 (9.7%) 0 (0%)	 -
Clinical characteristics			
Heart failure, n (%)	3 (10%)	0 (0%)	0.11
IHD, n (%)	0 (0%)	10 (32.3%)	**<0.001**
Diabetes Mellitus, n (%)	7 (23.3%)	7 (22.6%)	0.81
Previous stroke/TIA, n (%)	5 (16.7%)	2 (6.5%)	0.26
Asthma/COPD, n (%)	9 (30%)	4 (12.9%)	0.10
Chronic liver disease, n (%)	0 (0%)	0 (0%)	-
Chronic kidney disease, n (%)	0 (0%)	1 (3.2%)	1.00
Anaemia, n (%)	1 (3.3%)	1 (3.2%)	1.00
Thyroid disorder, n (%)	1 (3.3%)	4 (12.9%)	0.35
Hypercholesterolaemia, n (%)	14 (46.7%)	15 (48.4%)	0.89
Arthritis, n (%)	14 (46.7%)	16 (51.6%)	0.70
CHA_2DS_2-VASc score	3 [2 – 4]	3 [2 – 4]	0.56
HAS-BLED score	1 [1 – 1]	1 [1 – 1]	0.18
Smoking status Never smoked, n (%) Ex-smoker, n (%) Current, n (%)	 13 (43.3%) 15 (50%) 2 (6.7%)	 17 (54.8%) 13 (42%) 1 (3.2%)	 -
Alcohol None, n (%) Recommended, n (%)	 9 (30%) 21 (70%)	 10 (32.3%) 21 (67.7%)	 0.85
Height (cm)	169.3 ± 8.4	167.3 ± 10.1	0.40
Weight (kg)	89.6 ± 19.1	87.2 ± 21.7	0.66
BMI (kg/m^2)	31.1 ± 5.1	31.0 ± 6.3	0.95
Heart rate (bpm)	77 [68 – 86]	62 [58 – 70]	**0.003**
Systolic BP (mm/Hg)	140 [128 – 148]	144 [134 – 153]	0.24
Diastolic BP (mm/Hg)	81 ± 13	76 ± 15	0.16
Mean Arterial Pressure (MAP) (mm/Hg)	101 ± 12	101 ± 16	0.87
HbA1c (mmol/mol)	41 [38 – 46]	41 [40 – 51]	0.94
CrCl (mL/min)	86.2 ± 30.8	75.9 ± 38.1	0.72
Ejection fraction (%)	55 [55 – 62]	62 [55 – 68]	0.22

Table 4 – Differences in flow mediated dilatation (FMD) between permanent AF and paroxysmal AF groups – cross sectional comparison

	Permanent AF + hypertension group (n = 30)	**Paroxysmal AF + hypertension group (n = 31)**	**P**
Baseline diameter (mm)	4.5 [4.2 – 5.0][b]	4.8 [4.6 – 5.1][b]	0.67
Peak diameter (mm)	4.7 [4.4 – 5.2][b]	5.2 [4.6 – 5.3][b]	0.49
Absolute FMD change (mm)	0.1 [0.1 – 0.2][b]	0.3 [0.2 – 0.4][b]	**0.01**
FMD (%)	3.1 [2.3 – 4.8][b]	5.9 [4.0 – 8.1][b]	**0.02**
FMDc (%)	3.9 [2.8 – 5.0][a]	5.9 [4.8 – 7.0][a]	**0.01**
Time to peak diameter (sec)	50 [29 – 85][b]	80 [36 – 93][b]	0.23
Shear rate (Positive shear rate area to peak) [sec.-1]	4592 [2278 – 5734][b]	4800 [2800 – 8102][b]	0.40

Table 5 – Haemodynamic and FMD data for longitudinal comparison of Permanent AF (and hypertension) group

	Permanent AF + hypertension group (Baseline) [n = 30]	**Permanent AF + hypertension group (Follow up) [n = 30]**	**p**
Clinical characteristics	Mean ± SD / Median [IQR]	Mean ± SD / Median [IQR]	
Weight (kg)	89.6 ± 19.1	90.1 ± 19.4	0.13
BMI (kg/m^2)	31.1 ± 5.1	31.2 ± 5.2	0.11
Heart rate (bpm)	77 ± 18	72 ± 17	**0.01**
Systolic BP (mm/Hg)	140 [128 – 148]	131 [122 – 146]	**0.03**
Diastolic BP (mm/Hg)	81 ± 13	77 ± 12	**0.02**
Mean Arterial Pressure (MAP) (mm/Hg)	100 ± 9	97 ± 13	**0.01**
CHA_2DS_2-VASc score	3 [2 – 4]	3 [2 – 4]	1.00
HAS-BLED score	1 [1 – 1]	1 [1 – 1]	1.00
FMD measurements	Mean [95% CI][a] / Median [95% CI][b]	Mean [95% CI][a] / Median [95% CI][b]	
Baseline diameter (mm)	4.5 [4.2 – 5.0][b]	4.4 [4.1 – 5.1][b]	0.34
Peak diameter (mm)	4.9 [4.5 – 5.3][a]	4.8 [4.5 – 5.2][a]	0.69
Absolute FMD change (mm)	0.14 [0.11 – 0.25][b]	0.20 [0.17 – 0.28][b]	0.15
FMD (%)	3.1 [2.3 – 4.8][b]	5.2 [3.9 – 6.5][b]	0.09
FMDc (%)	4.0 [3.0 – 4.9][a]	5.1 [4.2 – 6.1][a]	0.09
Time to peak diameter (sec)	50 [29 – 85][b]	72 [36 – 102][b]	0.29
Shear rate stimulus (Positive shear rate area to peak) [sec.-1]	4592 [2278 – 5734][b]	3961 [3526 – 8190][b]	0.54