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28 **Abstract**

29

30 Atrial fibrillation (AF) and hypertension often co-exist and both are associated with  
31 endothelial dysfunction. We hypothesised that AF would further worsen endothelium-  
32 dependent flow-mediated dilatation (FMD) in hypertension patients compared to those  
33 without AF. In a cross-sectional comparison, we measured brachial artery diameter at rest  
34 and during reactive hyperaemia following 5 minutes of arterial occlusion in two patient  
35 groups: AF (and hypertension) (n = 61) and hypertension control groups (n = 33). The AF  
36 (and hypertension) subgroups: permanent AF (n = 30) and paroxysmal AF (n = 31) were also  
37 assessed. The permanent AF patients received heart rate and blood pressure (BP) control  
38 optimisation and were then followed up after eight weeks for repeat FMD testing. There  
39 was no significant difference in FMD between AF (and hypertension) group and  
40 hypertension control group (4.6%, 95% CI [2.6 – 5.9%] vs 2.6%, 95% CI [1.9 – 5.3%]; p=0.25).  
41 There was a significant difference in FMD between permanent AF and paroxysmal AF groups  
42 (3.1%, 95% CI [2.3 – 4.8%] vs 5.9%, 95% CI [4.0 – 8.1%]; p=0.02). Endothelium-dependent  
43 FMD response showed a non-significant improvement trend following eight weeks of heart  
44 rate and BP optimisation (3.1%, 95% CI [2.3 – 4.8%] (baseline) vs 5.2%, 95% CI [3.9 – 6.5%]  
45 (follow up), p=0.09). Presence of AF generally does not incrementally worsen endothelial  
46 dysfunction in hypertension patients, although the duration and frequency of AF  
47 (paroxysmal AF to permanent AF) does lead to worsening endothelial function. Eight weeks  
48 of BP optimisation did not significantly improve endothelial dysfunction as measured by  
49 FMD.

50

## 51 Introduction

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

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

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

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

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

#### 84 **Methods**

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

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

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

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

116

### 117 Experimental protocol

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

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

### 133 Measurements

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

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

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

161

162 Data analysis

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

176 Statistical analysis

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

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

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

197 To test specific hypothesis 2 ("Patients with permanent AF and hypertension will have  
198 worse parameters of vascular function compared to patients with paroxysmal AF and  
199 hypertension"), we recruited 61 patients in total, split between the 2 groups. This part of  
200 the study was powered based on an independent t-test, assessing the difference in FMD  
201 between permanent AF and paroxysmal AF. Mazaris *et al* reported a mean FMD of 4.09 (SD  
202 = 1.67) in permanent AF group compared to mean FMD of 6.83 (SD = 1.38) in paroxysmal AF  
203 group.<sup>16</sup> Assuming our SD is similar, the minimum sample size was computed as 8 patients  
204 per group at 90% power, 5% alpha and effect size of 1.79.

205 To test specific hypothesis 3 (“Eight weeks of intensive anti-hypertensive and  
206 anticoagulation therapy will improve vascular function in patients with permanent AF and  
207 hypertension”) we recruited 30 patients and tested them before and after intensification of  
208 their antihypertensive and anticoagulation treatment. This part of the study was powered  
209 based on a paired t-test, assessing the change in flow mediated dilation from pre- to post-  
210 treatment. It was assumed that the mean pre- intervention flow mediated dilation would be  
211 8.1 (SD=3.6), as per Skolidis et al, and that the effect size would be 1.06.<sup>6</sup> If this is the case,  
212 then the minimum number of patients required is 12 at 90% power and 5% alpha.

## 213 **Results**

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

215 Participants from AF (and hypertension) group and hypertension control group were  
216 matched for age and clinical characteristics (see table 1). Participants’ medication history is  
217 displayed in figure 1. There were no significant differences in age, sex, height, weight and  
218 BMI. Past medical history of all participants between the groups was similar except that  
219 participants in hypertension control group had significantly more patients with a  
220 background of chronic kidney disease (CKD) ( $p = 0.01$ ). The CHA<sub>2</sub>DS<sub>2</sub>-VASc score and HAS-  
221 BLED score were similar between the two groups. The mean heart rate was significantly  
222 lower in the hypertension control group ( $p = 0.02$ ). There were no significant differences in  
223 mean blood pressure (systolic and diastolic) between the two groups, baseline glycaemia  
224 control (HBA1c), kidney function (creatinine clearance) and left ventricular ejection fraction  
225 (EF (%)).

226 Baseline diameter of brachial artery was significantly smaller in the AF (and hypertension)  
227 group compared to hypertension control group (4.6 mm, 95% confidence interval (CI) [4.4 –  
228 4.9 mm] vs 5.2 mm, 95% CI [4.8 – 5.6 mm];  $p = 0.02$ ) (see table 2). Following 5 minutes of  
229 forearm ischaemia, there was no significant difference in absolute FMD between AF (and  
230 hypertension) group and hypertension control group (0.2 mm, 95% CI [0.1 – 0.3 mm] vs 0.2  
231 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%,  
232 95% CI [1.9 – 5.3%];  $p = 0.25$ ) respectively. The FMD (%) means were adjusted for baseline  
233 diameter and showed no significant difference between the two groups (4.9%, 95% CI [3.8 –  
234 6.0%] (AF (and hypertension) group) vs 4.3%, 95% CI [2.8 – 5.9%] (hypertension control  
235 group),  $p = 0.56$ ).

236 The peak diameter was significantly different between the two groups (4.9 mm, 95% CI [4.6  
237 – 5.2 mm] (AF (and hypertension) group) vs 5.4 mm, 95% CI [5.0 – 5.8 mm] (hypertension  
238 control group);  $p = 0.03$ ). There were no significant differences in time to peak diameter and  
239 shear rate between the two groups ( $p = 0.07$  and  $p = 0.41$  respectively). No variables were  
240 identified on univariate and stepwise multivariate analysis as independent predictors of  
241 reduced FMD.

#### 242 Permanent AF (and hypertension) vs PAF (and hypertension) groups

243 Participants in the two AF subgroups (permanent AF vs paroxysmal AF) were well matched  
244 for age, sex, clinical characteristics including height, weight, BMI, mean blood pressure,  
245 HBA1c, creatinine clearance and left ventricular EF (%) (see table 3). Participants'  
246 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 Permanent AF (and hypertension) group – longitudinal comparison

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)  $\pm$  18 (baseline)  
268 vs 72 bpm  $\pm$  17 (follow up),  $p = 0.01$ ), systolic BP (140 mmHg [128 – 148] (baseline) vs 131

269 mmHg [122 – 146] (follow up),  $p = 0.03$ ), diastolic BP ( $81 \text{ mmHg} \pm 13$  (baseline) vs  $77 \text{ mmHg}$   
270  $\pm 12$  (follow up),  $p = 0.02$ ) and mean arterial pressure (MAP) ( $100 \text{ mmHg} \pm 9$  (baseline) vs  $97$   
271  $\text{mmHg} \pm 13$  (follow up),  $p = 0.01$ ).

272 Both groups had a similar baseline brachial artery diameter ( $p = 0.34$ ). Endothelium-  
273 dependent FMD response was better following eight weeks of HR and BP optimisation but  
274 this 68% relative improvement did not reach statistical significance (3.1%, 95% CI [2.3 –  
275 4.8%] (baseline) vs 5.2%, 95% CI [3.9 – 6.5%] (follow up),  $p = 0.09$ ). The FMD (%) means  
276 were adjusted for baseline diameter and showed no significant difference between the two  
277 groups (4.0%, 95% CI [3.0 – 4.9%] (baseline) vs 5.1%, 95% CI [4.2 – 6.1%] (follow up),  $p =$   
278 0.09). The difference was also not significant in absolute change in diameter (0.14 mm, 95%  
279 CI [0.11 – 0.25 mm] (baseline) vs 0.20 mm, 95% CI [0.17 – 0.28 mm] (follow up),  $p = 0.15$ ).  
280 The time to peak diameter, peak diameter and shear rate stimulus were similar between the  
281 two groups (table 5). No variables were identified on univariate or stepwise multivariate  
282 analysis as independent predictors of reduced FMD.

## 283 Discussion

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

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

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

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

314 oxidative stress and leads to increase in recruitment of proinflammatory agents promoting a  
315 vicious cycle.<sup>28</sup> The complex interplay involving oxidative stress and inflammation seen in  
316 both conditions is summarised in figure 2.

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

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

337 that patients with permanent AF have worse FMD compared to patients with paroxysmal  
338 AF.<sup>15, 16, 32</sup> However, unlike previous studies, our study included patients with AF and  
339 hypertension, which has not been looked at before.

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

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

358

359 *Strengths and limitations*

360 We did not use nitrate to assess for endothelium-independent vasodilation as this has been  
361 studied previously in both AF and hypertension.<sup>9, 13, 15, 21</sup> Furthermore, use of intra-arterial  
362 acetylcholine would have been advantageous to investigate brachial artery endothelial  
363 function but FMD is a well established surrogate.<sup>17</sup> Given the widespread prevalence of AF  
364 in hypertensive patients, the inclusion of separate hypertension groups with and without  
365 AF, is a strength of our study. There have been limited studies looking at vascular function in  
366 patients with AF arrhythmia and therefore this makes our study unique. Participants in our  
367 group were well-matched for age, sex composition, comorbidities, CHA<sub>2</sub>DS<sub>2</sub>-VASc score,  
368 HAS-BLED score, BMI, BP, glycaemic control and LV systolic function. Nonetheless, the  
369 hypertension control group did have a significantly higher number of patients with CKD  
370 which may have been a source of bias. We accommodated for this and other potential  
371 confounders by utilisation of linear regression analysis. The longitudinal comparison of  
372 permanent AF (and hypertension) group in assessing FMD response to intervention has not  
373 been looked at before. The utilisation of edge detection software, assessment of shear rate  
374 and correcting for differences in group baseline diameters shows robustness of our  
375 methodological approach.

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

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

## 392 **Conclusions**

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

398

399 Summary Table

<b>What is known about topic?</b>
<ul style="list-style-type: none"><li>• Atrial fibrillation and hypertension commonly co-exist and the combination of these two conditions confers a worse prognosis than either alone.</li><li>• Endothelial dysfunction is present in both atrial fibrillation and hypertension.</li><li>• Flow-mediated dilatation is a reliable tool to assess endothelial function.</li></ul>
<b>What this study adds?</b>
<ul style="list-style-type: none"><li>• Presence of AF generally does not incrementally worsen endothelial dysfunction in hypertension patients</li><li>• The duration and frequency of AF (paroxysmal AF to permanent AF) does lead to worsening endothelial function.</li><li>• 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.</li></ul>

400

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403 **Conflict of Interest**

404 Authors declare no conflict of interests for this article.

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508 **Legends**

509 **Figure 1**

510 Medication use by class of drugs

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

512 **Figure 2**

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

515 **Table 1**

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

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

527

528 **Table 2**

529

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

535 AF = atrial fibrillation; FMD = flow-mediated dilatation; FMDc = FMD % mean [95% CI]  
536 adjusted for baseline diameter

537 **Table 3**

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

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

549

#### 550 **Table 4**

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

555 AF = atrial fibrillation; FMD = flow-mediated dilatation

556

#### 557 **Table 5**

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

Figure 1

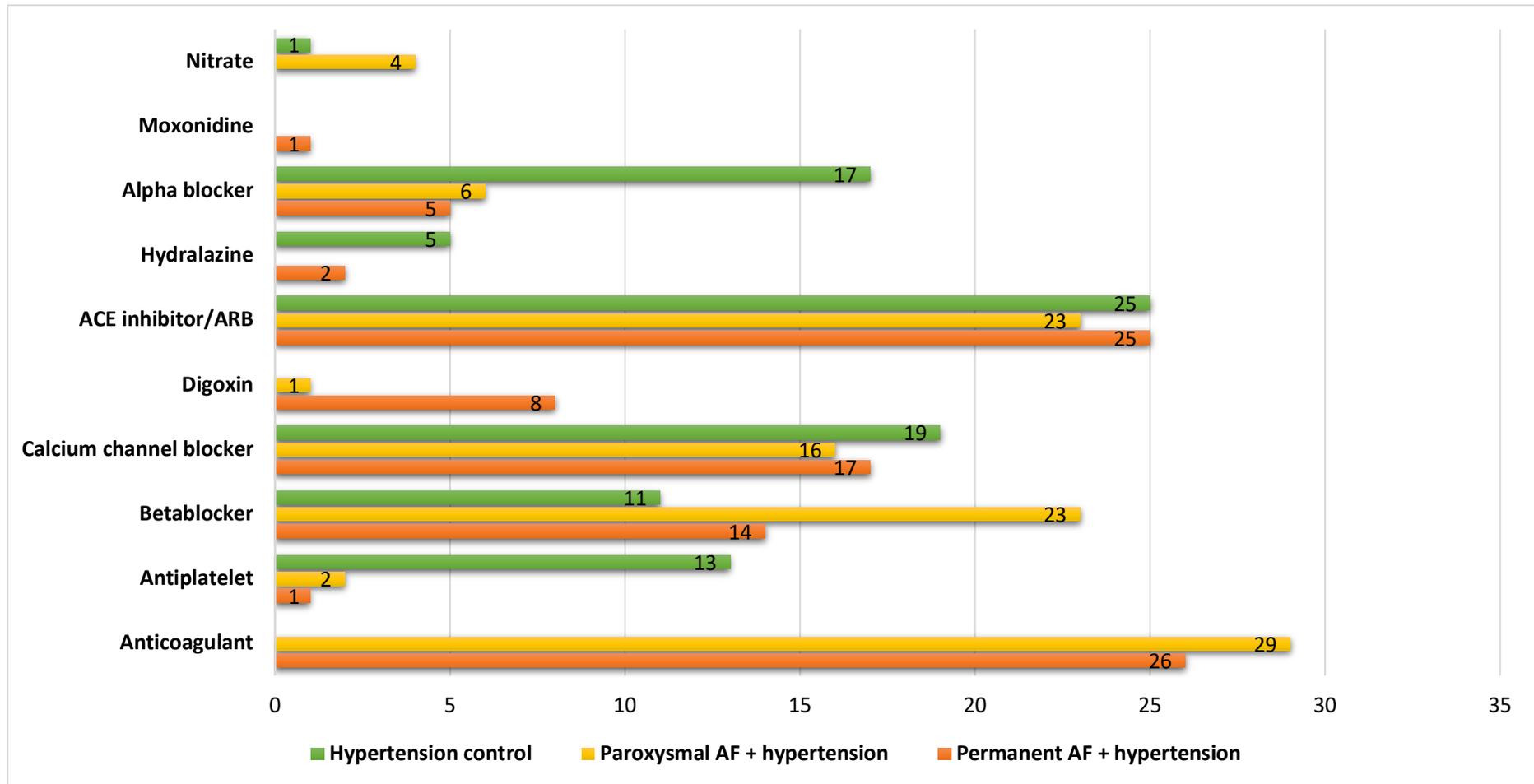


Figure 2

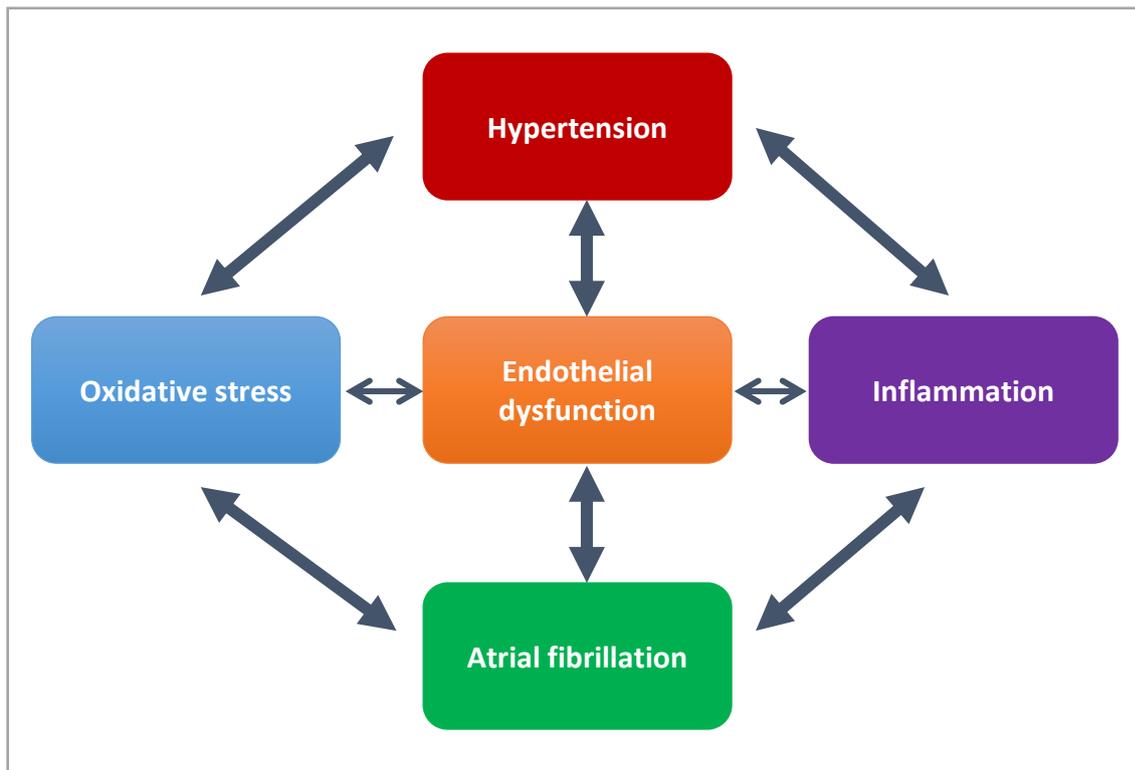


Table 1 – Demographics and clinical characteristics of matched AF (and hypertension) group and hypertension control group

	<b>AF + hypertension group (n = 40)</b>	<b>Hypertension control group (n = 20)</b>	<b>Matched groups p</b>
<b>Demographics</b>			
Age, years	66 ± 7	65 ± 7	0.71
Sex			
Male	29	15	0.84
Female	11	5	
Ethnicity			
Caucasians, n (%)	34 (85%)	10 (50%)	-
Blacks, n (%)	3 (7.5%)	6 (30%)	
Asians, n (%)	3 (7.5%)	3 (15%)	
Mixed, n (%)	0 (0%)	1 (5%)	
<b>Clinical characteristics</b>			
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%)	<b>0.01</b>
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 <sub>2</sub> DS <sub>2</sub> -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 (%)	19 (47.5%)	13 (65%)	-
Ex-smoker, n (%)	18 (45%)	7 (35%)	
Current, n (%)	3 (7.5%)	0 (0%)	
Alcohol			
None, n (%)	9 (22.5%)	5 (25%)	0.54
Recommended, n (%)	31 (77.5%)	15 (75%)	
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 <sup>2</sup> )	32.9 ± 5.2	32.1 ± 4.2	0.58
Heart rate (bpm)	70 [60 – 82]	63 [58 – 67]	<b>0.02</b>
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

	<b>AF + hypertension group (n = 40)</b>	<b>Hypertension control group (n = 20)</b>	<b>Matched groups p</b>
Baseline diameter (mm)	4.6 [4.4 – 4.9] <sup>a</sup>	5.2 [4.8 – 5.6] <sup>a</sup>	<b>0.02</b>
Peak diameter (mm)	4.9 [4.6 – 5.2] <sup>a</sup>	5.4 [5.0 – 5.8] <sup>a</sup>	<b>0.03</b>
Absolute FMD change (mm)	0.2 [0.1 – 0.3] <sup>b</sup>	0.2 [0.1 – 0.3] <sup>b</sup>	0.61
FMD (%)	4.6 [2.6 – 5.9] <sup>b</sup>	2.6 [1.9 – 5.3] <sup>b</sup>	0.25
FMDc (%)	4.9 [3.8 – 6.0] <sup>a</sup>	4.4 [2.7 – 6.0] <sup>a</sup>	0.60
Time to peak diameter (sec)	58 [40 – 90] <sup>b</sup>	36 [21 – 65] <sup>b</sup>	0.07
Shear rate (Positive shear rate area to peak) [sec.-1]	4421 [2800 – 6077] <sup>b</sup>	3300 [1296 – 6887] <sup>b</sup>	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
<b>Demographics</b>			
Age, years	70 ± 8	72 ± 11	0.64
Sex			0.46
Males	22	20	
Females	8	11	
Ethnicity			-
Caucasians, n (%)	28 (93.3%)	25 (80.6%)	
Blacks, n (%)	1 (3.3%)	3 (9.7%)	
Asians, n (%)	1 (3.3%)	3 (9.7%)	
Mixed, n (%)	0 (0%)	0 (0%)	
<b>Clinical characteristics</b>			
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 <sub>2</sub> DS <sub>2</sub> -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 (%)	13 (43.3%)	17 (54.8%)	
Ex-smoker, n (%)	15 (50%)	13 (42%)	
Current, n (%)	2 (6.7%)	1 (3.2%)	
Alcohol			0.85
None, n (%)	9 (30%)	10 (32.3%)	
Recommended, n (%)	21 (70%)	21 (67.7%)	
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 <sup>2</sup> )	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

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

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

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