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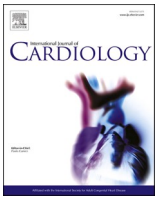

Impaired retinal micro-vascular function in patients with atrial fibrillation

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ABSTRACT

Background: Cardiovascular (CV) risk factors and CV diseases, in particular heart failure, are strongly associated with impaired microvascular retinal endothelial function. Whether atrial fibrillation (AF) contributes to vascular dysfunction is not clear. Therefore, the aim of this study was to investigate the impact of AF on retinal micro-vascular function.

Methods: In this study, vascular function was measured non-invasively with flicker-light induced vasodilatation of retinal arterioles (FIDart%). Patients with a history of AF and risk factors for heart failure (HF) or heart failure ($n = 69$; age 67.9 ± 9.2 years, 71% male, 35% HFrEF, 56% paroxysmal, 25% persistent, 19% permanent AF), as well as age, sex and ejection fraction matched patients with absent history of AF ($n = 66$; age 63.4 ± 10.6 years, 67% male, 47% HFrEF) were included. Patients with AF were further divided into those with paroxysmal AF (in sinus rhythm – AF_{SR}: $n = 38$, age 71.4 ± 9.2, 73% male), and those with AF at the time of the study visit.

Results: Retinal microvascular function was impaired in patients with AF compared to patients without AF (FIDart% 1.1% [0.3–2.8] vs. 2.7% [1.3–5.1], $p < 0.001$). Patients currently in AF have poorer retinal micro-vascular function (FIDart% 0.8% [0.1–1.9) compared to patients with a history of AF but currently in SR at the time of retinal function measurement (1.5% [0.6–4.9] $p = 0.017$). In patients with AF, impaired retinal vascular function was independently associated with larger left atrial volume (mean 49.8 ± 18.4), even after correction for confounding factors in different models (SCR = -0. 251 to −0.256, $p = 0.035$–0.01).

Conclusions: AF in patients with heart failure is associated with impaired vascular function, even if currently in sinus rhythm. The association of retinal microvascular dysfunction with left atrial volume, a surrogate for elevated cardiac filling pressures, may further highlight the important interplay between the vasculature and elevated filling pressures in the development of AF.

1. Introduction

Endothelial dysfunction represents the common denominator of cardiovascular (CV) risk factors and is a key component in the development of atherosclerotic diseases and its clinical sequelae finally leading to heart failure [1]. Endothelial function can be assessed non-invasively at the level of large-conduit arteries using flow-mediated dilation (FMD). Recently, dynamic retinal vessel analysis (RVA) was introduced to reliably measure endothelial microvascular dysfunction in small caliber arteries and venules. Dilatation of the retinal artery induced by flicker-light exposition is mediated by neurovascular-coupling and flow-mediated nitric oxide release, a key indicator of healthy endothelial function [2]. Impaired retinal vascular function has previously been demonstrated in patients with CV risk factors and heart failure [3,4]. Interestingly, it worsens gradually over the trajectory of cardiovascular disease, and might be an important marker for prognosis [3–6].

The lifetime AF risk estimate is 1 in 3 individuals at index age of 55 years and depends on age, genetic, and on clinical risk factor burden/multiple comorbidity [7]. Its incidence is particularly high in the setting of heart failure (HF) and, in particular, in HF with preserved ejection fraction (HFpEF). AF is considered to be a consequence of HF but may also perpetuate the disease [8]. As such, prevention, early diagnosis and treatment of modifiable risk factors is crucial to reduce the burden of AF [8].

The aim of this study is to investigate the association of AF with

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retinal microvascular function.

2. Methods

2.1. Study design and protocol

Patients within a single-center cohort study (RVA-Cardio study, KEK-ZH-No. 2014–0329) with heart failure ($n = 292$) or at risk for heart failure (at least 1 cvRF, $n = 349$) were screened for atrial fibrillation based on their clinical history. Patients with history of AF ($n = 68$) were further divided into those with paroxysmal AF, but in SR at the time of the study visit (AF_{SR}) and those in AF (AF_{AF}). Presence of AF was ascertained during clinical assessment, ECG, and arterial radial pulse wave characteristics on pulse wave analysis. Sixty-six age-, gender-, LVEF-matched patients with absent history of AF (control) were identified with a propensity score matching algorithm. Patients were enrolled between 01/2015 and 10/2022.

Exclusion criteria were photosensitive epilepsy, glaucoma or other relevant eye pathology, inability to fixate, progressive diabetic retinopathy, allergy to study drugs, current acute illness, pregnancy or breastfeeding.

The evaluation of vascular function was performed in the morning after fasting (for at least 8 h except water), with no coffee and alcohol consumption for at least 12 h, and avoiding intense physical activity the days prior to examination. Patients were allowed to take regular medications before examination, with the exception of antidiabetic medications. Clinical parameters, medical history, laboratory blood tests and evaluation of vascular function (starting with vascular stiffness assessment, followed by RVA and, finally, FMD) were performed. The study protocol was approved by the local ethic committee of canton Zurich (KEK-ZH-2014-0329). All participants signed a written informed consent prior to inclusion.

2.2. Assessment of microvascular endothelial function: retinal vessel analysis

Static and dynamic RVA as a marker of microvascular endothelial function was conducted using an Imedos Dynamic Retinal Vessel Analyzer (Imedos, Jena, Germany). Dynamic vessel analysis (DVA) measures dilatation of retinal vessels after provocation with flicker light [4]. FIDart% and FIDven%, i.e. respectively the arteriolar and venular vasodilatation in response to flickering-light, were analyzed and expressed as variation in percentage compared to baseline. To calculate these parameters, the maximal vascular response during last 10 s of each flickering-light episode or during the 3 s following it was identified [9]. This maximal value was averaged with the 2 s before and after it. Episodes with <50% of valid measurements during this period were excluded from calculation. Furthermore, the areas under the FIDart and FIDven curves were calculated.

Static vessel analysis (SVA) was performed by obtaining polychromatic and monochromatic fundus photographs using the static CCD camera and VesselMap 2 software. A monochromatic image with good quality was selected and all retinal arteries and veins in the area 0.5 to 1 optic disc diameters away from the optic disc are measured. The software adds up the diameters of all arteries and veins using a predefined formula to calculate the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE). Both values are used to calculate the AVR (AVR = CRAE/CRVE).

2.3. Assessment of conduit artery endothelial function: flow-mediated dilation

FMD was measured in supine position according to established protocols [10]. A 10 MHz linear array transducer (Siemens Acuson X300 and Juniper, Siemens AG) was used to assess the arterial diameter of one brachial artery in baseline condition for 1 min. A blood pressure cuff was placed distally and inflated 50 mmHg above systolic pressure for 5 min. After release, the brachial artery diameter was measured for further 4 min. An automatic wall-tracking and analysis software (FMD-Studio, Pisa, Italy) provided continuous wall-to-wall measurements [11]. The percent peak dilatation during the hyperemic phase in relation to the baseline resting diameter was calculated (FMD%). To evaluate for endothelial-independent effects, the percentage peak dilation of the brachial artery 6 min after one dose of sublingual glycerol trinitrate (GTN nitrolingual 0.4 mg) was performed.

2.4. Arterial stiffness

A SphygmoCor applanation tonometer system (AtCor Medical, Itasca, IL, USA) was used to measure augmentation index (AIX) and pulse wave velocity (PWV) according to established protocols [12]. AIX was measured at the level of the radial artery with patients resting in supine position for 15 min. AIX was automatically calculated based upon ten high-quality pulse wave measurements and normalized to a heart rate of 75 beats per minute. PWV (meter/s) was calculated based on the pressure wave transit time synchronized with the R wave of the electrocardiograms after assessment of distance between carotid and femoral artery [12].

2.5. Transthoracic echocardiography

Transthoracic echocardiography was obtained during regular clinical outpatient visits using 2D, M-mode and color Doppler echocardiography by experienced cardiologists. All examinations were performed in a time frame of ±12 months within vascular function measurements. Left ventricular ejection fraction (LVEF) was available in all patients with the exception of cumulative 16 patients with cardiovascular risk factors but no diagnosis of heart failure. A normal LVEF >50% was assumed.

2.6. Laboratory assessment

Blood samples were drawn in fasted state and analyzed on the same day at the Institute of Clinical Chemistry at the University Hospital of Zurich using standard methods. High sensitivity troponin T and NT-proBNP were quantified using electrochemiluminescence-immunoassays and the COBAS8000 autoanalyzer of Roche Diagnostics (Mannheim, Germany).

2.7. Statistical analysis

Statistical analyses and computations were performed using the SPSS software (v25, SPSS Inc., USA) and Python (v3.9.7, Python Software Foundation, Oregon, USA). Controls were identified with a propensity score matching algorithm (v3.9.7, Python Software Foundation, Oregon, USA, psmpy statistical package) and matched according to gender, age, and LVEF. A k-nearest neighbour matching stratification algorithm with 1:1 matching without replacement was used on the RVA-Cardio cohort study (matcher = 'propensity_logit', replacement = False, caliper = None). Patients with a ventricular assist device or heart transplantation as well as healthy probands were excluded from matching.

Continuous variables are presented as mean (± standard deviation) for normal distribution and median (± interquartile range) for skewed distribution. Categorical variables are expressed as percentage, unless otherwise stated. Differences in baseline characteristics between the groups were assessed by independent Student's *t*-test or ANOVA for parameters with parametric distribution, while Mann-Whitney *U* test or Kruskal-Wallis tests were used for parameters with a non-normal distribution. Pearson Chi-square test or Fisher's Exact test were calculated for dichotomic variables. Univariable analysis for relevant clinical covariates was performed using Pearson or Spearman's test, as appropriate. Multivariable regression analyses were performed with a

stepwise approach, and the strength of relationships was tested with F-test ANOVA. Standard regression coefficients (SRC) are reported for single parameters. To avoid overfitting, 2 different multivariable models with FIDart% as dependent and left atrial volume index (LAVI) as independent variable were driven. Cofounders were chosen based on correlation and/or on clinical relevance: 1. Model included correction for age, serum creatinine, systolic blood pressure, LDL-cholesterol, high-sensitivity C-reactive protein, and E/e'; model 2 included correction for age, LVEF, and NT-proBNP. A two-sided *p*-value of <0.05 was considered to be statistically significant.

3. Results

3.1. Study population

3.1.1. AF vs. matched controls

Baseline characteristics of the AF and control groups are shown in Table 1. EF was inserted as matching criterion between the two groups with and without history of AF. No differences in clinical characteristics were found between the two groups. AF patients were more likely to be treated with oral anticoagulation ($p < 0.001$), and were more likely to be prescribed with loop diuretics as well as amiodarone ($p = 0.008$ and $p = 0.003$, respectively). In the AF group, 56% had a paroxysmal AF, 25% had a persistent AF, and 19% had a permanent AF.

Flicker-light induced arteriolar dilatation (FIDart%) was significantly lower (1.1% [0.3–2.8]) in patients with a history of AF as compared to matched controls with no history of AF (2.7% [1.3–5.1], $p < 0.001$, Fig. 1a). Contrarily, no differences were found in endothelial function measured with FMD at the level of conduit arteries (Table 2).

3.1.2. AF patients currently in SR versus those in AF at the time of the study visit

Baseline characteristics of the AF_{SR} and AF_{AF} cohorts are shown in Table 3. Both groups were well-matched with respect to their clinical characteristics and no relevant differences in therapy were found. Among the patients who were in AF at time of analyses, $n = 11$ (37%) had a known paroxysmal AF, $n = 8$ (27%) had a permanent AF, and $n = 11$ (37%) had a persistent AF.

In patients with a history of AF, those currently in AF have further impaired FIDart% (0.8% [0.1–1.9]) compared to those with a history of AF but currently in SR (1.5% [0.6–4.9], $p = 0.017$, *Fig. 1b*). Again, no differences were found between the AF_{AF} and the AF_{SR} subgroups with respect to endothelial function measured by FMD% at the level of conduit arteries (Table 2). Patients in the AF_{AF} subgroup presented with an increased pulse-wave velocity as compared to the AF_{SR} subgroup (9.1 [7.9–12.6] vs. 8.2 [6.7–10.1] m/s, $p = 0.023$).

FIDart% was inversely associated with increased left atrial volume indexed for body surface area (LAVI, $r = -0.318$, $p = 0.001$), with LV ejection fraction (LVEF, $r = 0.310$, $p = 0.001$), with LV filling pressures (E/e') ($r = -0.216$, $p = 0.042$), and natriuretic peptide values (NT-proBNP: $r = -0.194$, $p = 0.025$). In different regression models, LAVI remained as an independent predictor of an impaired FIDart% after correction for cofounding factors (model 1: age, serum creatinine, systolic blood pressure, LDL-cholesterol, high-sensitivity C-reactive protein, and E/e; model 2: age, LVEF, NT-proBNP) (Model 1: SCR = -0. 251, $p = 0.035$, model 2: SCR = -0.256, $p = 0.01$).

Analyses comparing those patients who had a diagnosis of paroxysmal AF (paroxysmal AF, $n = 100$) to those who had a diagnosis of persistent or permanent AF (non-paroxysmal AF, $n = 34$) showed a positive trend for a more reduced FIDart% in patients with non-paroxysmal AF (1.6%±2) as compared to those with paroxysmal AF (2.5%±2.5, $p = 0.059$) was found. Similarly, flow-mediated dilation (FMD %) was tendentially more impaired in patients with non-paroxysmal AF (4.4%±2.4) as compared to those with paroxysmal AF (5.4%±3.2, $p = 0.096$).

Table 1
Patients with a history of atrial fibrillation compared to matched control patients without a history of atrial fibrillation (controls).

	History of AF $N = 68$	Absent history of AF $N = 66$	*p*-Value
Baseline characteristics			
Age years	67.2 (9.2)	63.4 (10.6)	0.12
BMI kg/m^2	33.9 (4.2)	26.7 (5.5)	0.165
Male	49 (71)	44 (67)	0.71
NYHA II	39 (55)	31 (47)	0.373
NYHA III-IV	12 (19)	9 (14)	0.078
Ejection fraction			0.063
EF < 40%	24 (35)	31 (47)	
EF 40–50%	11 (16)	9 (14)	
EF≥50%	34 (49)	26 (39)	
Arterial hypertension	36 (52)	38 (58)	0.605
Dyslipidemia	25 (36)	28 (42)	0.485
Diabetes	21 (30)	15 (23)	0.336
Coronary artery disease	22 (32)	27 (41)	0.289
Stroke	7 (10)	5 (8)	0.764
Chronic kidney disease	19 (28)	9 (14)	0.057
Dilatative cardiomyopathy	12 (17)	12 (18)	1
Amyloidosis	6 (9)	6 (9)	1
Atrial fibrillation:			
Paroxysmal	39 (56)		
Persistent	17 (25)		
Permanent	13 (19)		
Treatment			
OAK	60 (87)	13 (2)	**<0.001**
RAAS-antagonists	37 (54)	46 (70)	0.073
ARNI	7 (16)	2 (5)	0.16
B-blockers	49 (71)	46 (70)	0.59
Loop diuretics	49 (71)	30 (46)	**0.008**
MRA	30 (44)	22 (33)	0.307
Amiodarone	17 (25)	3 (5)	**0.003**
Statins	40 (58)	36 (55)	0.564
Cardiac resynchronization therapy	7 (10)	11 (17)	0.316
Echocardiography			
LVEF %	42.4 (14.7)	39.2 (12.8)	0.221
LVEDVI ml/m^2	73.1 (36.1)	80.2 (33.1)	0.283
LAVI ml/m^2	49.8 (18.4)	38.5 (12.7)	**<0.001**
E/e'	16.3 (7.9)	14.8 (9)	0.39
TAM mm	15.2 (4.6)	17.7 (5.2)	**0.011**
RV fac%	35.8 (9.5)	37.4 (8.2)	0.366
sPAP mmHg	34.2 (14.1)	31.3 (11.9)	0.305
Laboratory parameters			
Hämoglobin g/l	132.9 (19.1)	137.5 (17.8)	0.158
Thrombocytes G/l	200 (48.6)	228.7 (58.6)	**0.003**
Leucocytes G/l	6.6 (1.6)	6.5 (1.6)	0.659
Hs-CRP mg/l	8.6 (29.2)	2.9 (3.8)	0.117
Hs-troponin T ng/l	22 (12–43)	12 (7–27)	**0.002**
eGFR ml/min/1.73 m2	55 (41–74)	73 (57–90)	**<0.001**
NT-proBNP ng/l	1183 (341–2690)	441 (136–1422)	**0.002**
LDL cholesterol mmol/l	2.4 (1.2)	2.5 (0.9)	0.536

Data are mean (standard deviation), median (interquartile range) or number (percentage), as appropriate. Comparisons were performed with Student's t-test, Mann-U-Whitney, Fischer's exact, or Paerson Chi-square, as appropriate.
Bold = statistic significant.

4. Discussion

Both AF and endothelial dysfunction share several cardiovascular risk factors and, within this context, the presence of AF often indicates a more advanced stage of disease. Our results further demonstrate that AF is associated with microvascular dysfunction of retinal arterioles - more so in patients currently in AF than those currently in SR, but not with endothelial function in conduit arteries. Considering this, it is plausible

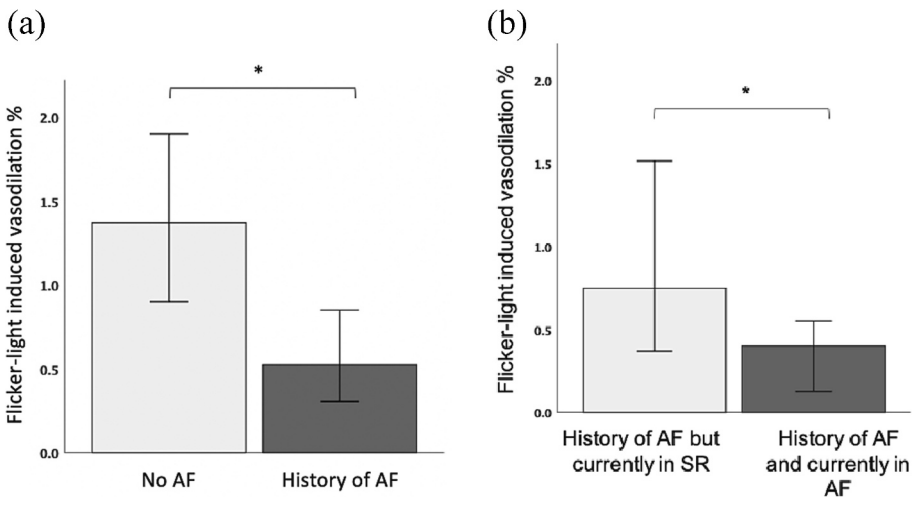

Fig. 1a. Flicker-light induced vasodilation % (FIDart%) performed in patients without (Non-AF group) as compared to patients with known history of atrial fibrillation (AF group). Values are median (95% confidence interval). *$p < 0.001$; 1b: Flicker-light induced vasodilation % (FIDart%) performed during sinus rhythm in patients with known atrial fibrillation as compared to FIDart% performed during atrial fibrillation. Values are median (95% confidence interval). *$p = 0.017$.

Table 2
Vascular function.

	History of AF $N = 68$	Absent history of AF $N = 66$	*p*-Value	AF_{SR} - Analysis during sinus rhythm $N = 38$	AF_{AF} - Analysis during AF $N = 30$	*p*-Value
FIDa %	1.1 (0.3–2.8)	2.7 (1.3–5.1)	**<0.001**	1.5 (0.6–4.9)	0.8 (0.1–1.9)	**0.017**
FIDv %	3.2 (1.8–5.8)	3.8 (2.4–4.9)	0.105	2.8 (1.5–5.6)	4.3 (2–8.1)	0.174
AVR	0.8 (0.8–0.9)	0.8 (0.8–0.9)	0.952	0.8 (0.8–0.9)	0.8 (0.8–0.9)	0.990
CRAE	180 (165.3–194.1)	182.4 (167.6–198)	0.525	177.6 (164.8–195.4)	180 (166.9–191.9)	0.617
CRVE	213.4 (202.3–228)	214 (204.4–226.6)	0.535	215.1 (200.5–225.3)	211.2 (202.8–234.3)	0.415
FMD %	4.2 (2.7–6.6)	5 (2.7–6.7)	0.391	4 (2.6–5.9)	5.2 (3.7–7)	0.608
GTN %	16.4 (10.2–18.9)	15.9 (9.6–20.6)	0.612	16.7 (10.9–19.2)	12 (9.5–18.6)	0.342
PWA	25.5 (18–31)	24.5 (16–31)	0.289	26 (18.5–29.5)	24 (15.5–32)	0.969
PWV m/s	8.8 (7.2–10.4)	8.5 (6.3–10.2)	0.103	8.2 (6.7–10.1)	9.1 (7.9–12.6)	**0.023**

Data are median (interquartile range). Comparisons were performed with Student's t-test or Mann-U-Whitney, as appropriate.
Bold = statistic significant.

that an underlying systemic process, such as chronic inflammation, contributes to the development of both atrial fibrillation and microvascular dysfunction, as supported by the evidence of reduced flow-mediated dilation (FID). Accordingly, patients with AF but currently in SR do have impaired RVA as well. The association of RVA with left atrial volume index and NT-proBNP may highlight the possibility of elevated filling pressures as an important mechanism. In support of this, we found that microvascular dysfunction is also independently driven by larger left atrial volume.

Endothelial dysfunction depicts the "*risk factor of all cardiovascular risk factors*" [1]. Dynamic retinal vessel analysis is a non-invasive method allowing measurement of endothelial dependent dilatation of small retinal arterioles and venules in response to flicker-light. Retinal vascular function is mainly mediated by neurovascular-coupling, involving both vascular endothelial cells and pericytes, and flow-mediated nitric oxide release [2]. Endothelial dysfunction occurs systemically in the circulation – thus, the eye may serve as a window to the heart [13]. Nagele and Barthelmes have previously demonstrated that flicker-light induced arteriolar vasodilation is impaired in patients with cardiovascular risk factors and worsens gradually over the trajectory of cardiovascular disease [4,5].

Atrial disease has been increasingly acknowledged as a myopathy itself, which shares the same pathogenic mechanisms, cardiovascular risk factors and comorbidities with HF – with endothelial dysfunction as potential common denominator [1,14]. In the EHRA/HRS/APHRS/SOLAECE consensus paper, atrial cardiomyopathy has been defined as "any complex of structural, architectural, contractile or electrophysiological changes affecting the atria with the potential to produce clinically-relevant manifestations" [15].

Importantly, although still debated, AF is primarily a consequence of a pre-existing myocardial structural disease, endothelial dysfunction may be a key component of both entities. Of note, both HFA-PEFF and H_2FPEF scores were similarly in patients with preserved ejection fraction and a history of AF, as well as in patients without AF.

Endothelial dysfunction as well as increase in pro-inflammatory biomarkers have been linked to left atrial dilation and volume overload, which both represent the key feature of HF [16]. Endothelial dysfunction plays a key role in the development and subsistence of the arrhythmic substrate underlying AF and might embody the substrate underlying both AF and HF [17,18]. Indeed, endothelial dysfunction is driven by oxidative stress and inflammation, the presence of which lead to adverse electromechanical remodelling in atrial myocytes, thus promoting the onset and perpetuation of AF [19]. AF has been associated, both in vitro and in vivo models, with increased oxidative stress and a systemic depletion in nitric oxide via several pathways. At a local level, atrial oxidative injury alters myofibrillar energetics, leading to a myosin isoform switch, eventually related to the development of atrial mechanical dysfunction and to loss of electrical-mechanical coupling [20]. At a systemic level, AF-related tachycardia increases ADMA (asymmetric dimethylarginine) levels, an endogenous inhibitor of endothelial nitric oxide synthase (eNOS), thus depleting it [21]. Furthermore, AF directly promotes mitochondrial dysfunction via calcium-dependent pathways, thus leading to uncoupling of oxidative phosphorylation and, eventually, to oxidative damage [22]. Oxidative stress further activates NF-kB

Table 3
Patients with a history of atrial fibrillation currently in sinus rhythm vs. currently in AF.

	AF_{SR} - Analysis during sinus rhythm $N = 38$	AF_{AF} - Analysis during atrial fibrillation $N = 30$	*p*-Value
Baseline characteristics			
Age years	67.9 (9)	71.4 (9.2)	0.121
BMI kg/m^2	28.3 (5.0)	29.3 (5.0)	0.404
Male	27 (69)	22 (73)	0.460
NYHA 3–4	5 (14)	7 (30)	0.321
Arterial hypertension	21 (53)	15 (42)	0.811
Dyslipidemia	15 (39)	10 (33)	0.801
Diabetes	13 (33)	8 (27)	0.606
Coronary artery disease	11 (28)	11 (37)	0.740
Stroke	5 (13)	2 (7)	0.690
Chronic kidney disease	7 (18)	12 (40)	0.058
Dilatative cardiomyopathy	6 (15)	6 (20)	0.751
Amyloidosis	4 (6)	2 (7)	0.690
Therapy			
OAK	33 (85)	27 (90)	0.722
RAAS-antagonists	18 (46)	19 (63)	0.224
ARNI	4 (19)	3 (13)	0.693
B-blockers	25 (64)	24 (80)	0.186
Loop diuretics	24 (62)	25 (83)	0.063
MRA	15 (39)	15 (50)	0.463
Amiodarone	9 (23)	8 (27)	0.783
Statins	19 (49)	21 (70)	0.090
Cardiac resynchronization therapy	5 (7)	2 (7)	0.690
Echocardiography			
LVEF %	45 (14.8)	39.1 (14)	0.119
LVEDVI ml/m^2	69.9 (37)	77.7 (35.1)	0.413
LAVI ml/m^2	44.1 (15.1)	57.2 (19.9)	**0.006**
E/e'	15.7 (8.3)	17.3 (7.4)	0.508
TAM mm	16.1 (5.1)	14 (3.6)	0.096
RV fac%	36.5 (9.8)	34.8 (9.1)	0.525
sPAP mmHg	34.7 (15.8)	33.5 (11.9)	0.763
Laboratory parameters			
Hämoglobin g/l	137.2 (18.2)	127.5 (19.2)	0.036
Thrombocytes G/l	202.9 (50.7)	196.4 (46.4)	0.585
Leucocytes G/l	6.4 (1.4)	6.8 (1.8)	0.295
hs-CRP mg/l	2.8 (3.9)	16 (4.3)	**0.012**
hs-Troponin T ng/l	14.5 (10–33)	33 (22–47)	**0.006**
eGFR ml/min/1.73 m2	61.5 (44–80)	54.5 (34–70)	0.261
NT-proBNP ng/l	613 (174–1255)	2473.5 (1288–5158)	**<0.001**
LDL cholesterol mmol/l	2.1 (1)	2.6 (1.2)	0.062

Data are mean (standard deviation), median (interquartile range) or number (percentage), as appropriate. Comparisons were performed with Student's t-test, Mann-U-Whitney, Fischer's extact, oder Paerson Chi-square, as appropriate. Bold = statistic significant.

signaling pathway promoting the transcription of proinflammatory cytokines and finally impairing endothelial function at a systemic level [22]. This hypothesis is further supported by the finding that plasma levels of nitrites and nitrates are lower in patients with AF and are progressively restored over one month after cardioversion [23].

Our data thus support the hypothesis that AF is related to an increased systemic oxidative stress and endothelial dysfunction, and that microvascular dysfunction represents one of the first common steps behind the development of cardiovascular disease, being present in patients with HF, and is even more impaired in a *crescendo* in patients with a history of AF and in those with AF at the time of visit.

Impaired endothelial-dependent vasodilation as measured by flow-mediated dilation of the brachial artery has been observed in AF [13]. This observation was reproduced not only in patients with chronic AF as compared to controls in sinus rhythm, but also in subjects with persistent AF as compared to their counterparts with paroxysmal AF [13,24]. Furthermore, the presence of endothelial dysfunction of the large artery was found to be associated with a higher incidence of AF [25]. In contrast to other studies, we did not find significant differences in endothelial function at the level of large-conduit arteries as assessed by flow-mediated dilation. This is likely explained by the fact that our AF patients had comparable cardiovascular risk factors as compared to their matched controls, including no differences in diagnosis of arterial hypertension, dyslipidaemia or diabetes mellitus, which are known to influence endothelial function. Secondly, as previously demonstrated, FMD does not correlate with FIDart%, suggesting that endothelial function at the level of large conduit arteries may be better addressed by cardiovascular treatments than microvascular function [5]. On the other side, flicker-induced retinal dilatation might represent a better marker of vascular function to uncover residual cardiovascular risk in otherwise well-treated patients.

Barthelmes previously demonstrated impaired vascular function in the microcirculation only, a vascular bed less likely influenced by concomitant cardiovascular therapy [5]. This finding is particularly relevant, since atrial fibrillation has lately been associated with an increased risk of dementia independently from stroke or transitory ischemic attacks [26,27]. Indeed, the development of vascular dementia is strictly interconnected with dysfunction of the blood-brain barrier involving, among others, cerebral endothelial cells and pericytes [28]. Although only hypothesis-generating, the finding of an impaired retinal vascular function in AF patients suggests the presence of a common pathological pathways between AF and dementia and points towards considering AF as a component of a more complex systemic disease [27]. Indeed, several meta-analyses observed an increased risk of dementia in AF patients even after adjustment for stroke and transient ischemic attacks, suggesting that further mechanisms such as diffuse atherosclerosis, systemic inflammation and repetitive episodes of cerebral hypoperfusion and hypertension due to AF on a microvascular level might be involved. ([26,29–31]).

Atrial fibrillation likely represents the clinical manifestation of an underlying atrial myopathy which is typically characterized by adverse atrial remodelling and enlargement, finally leading to electromechanical uncoupling and increased atrial fibrosis [32]. These alterations represents the electrical and structural substrate for AF onset and perpetuation and are generally expression of an underlying HF. Indeed, HF is characterized by increased LV filling pressures, which finally leads to left atrial dilation [33]. Furthermore, once AF develops, the loss of organized contractility leads to calcium overload, ischemia, and oxidative stress, thus further exacerbating adverse atrial remodelling processes, which structurally manifest with atrial enlargement [34,35]. As such, AF increases the likelihood of developing HF and worsen its prognosis [14]. In our study we found that larger left atrial volume was directly and independently associated with an impaired retinal vascular function in patients with a history of AF, thus highlighting the potential interplay role of AF and HF.

4.1. Limitations

This is a single-center, observational study with its inherent limitations. The number of patients was powered to show differences in the primary outcome (FIDart%), so the study might be underpowered to show relevant differences in FMD. As we had no reliable data about how long patients assigned to the AF_{AF} subgroup have been in AF prior to the study, we could not correct our analysis for this cofounder. However, all patients in the AF_{AF} subgroup underwent a first clinical investigation on average one hours prior to assessment of flicker-light induced retinal vasodilation and were checked once again prior to the examination with a one-lead electrocardiogram. As expected, AF patients were more likely to be under anticoagulation therapy as compared to their controls. Since anticoagulants might also have pleiotropic effects influencing systemic

endothelial function, this might have influenced our results.

5. Conclusion

This study provides evidence that AF is associated with vascular dysfunction in patients with risk factors or overt heart failure, despite optimal medical treatment. However, impaired endothelial functions cannot be solely attributed to the direct impact of atrial fibrillation (AF), as patients in sinus rhythm with a previous history of AF exhibit impaired function as well.

The association with elevated filling pressures, may further highlight the important interplay between the vasculature and elevated filling pressures in the development of AF.

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CRediT authorship contribution statement

Valentina A. Rossi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft. **Natallia Laptseva:** Investigation, Project administration. **Delia Nebunu:** Investigation, Project administration. **Thomas Haider:** Investigation. **Matthias P. Nägele:** Investigation, Project administration, Supervision. **Frank Ruschitzka:** Supervision, Writing – review & editing. **Isabella Sudano:** Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – review & editing. **Andreas J. Flammer:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of Competing Interest

VAR, DN and TH have no conflict of interest to declare. NL declares fees from Alnylam and Pfizer. IS declares in the last 10 years and until 2022 consulting fees, travel grant and honoraria from Amgen, Astra Zeneca, Daiichi Sankio, Medtronic, MSD, Novartis, Recordati, Sanofi und Servier unrelated to the topic of the paper. AJF declares fees from Alnylam, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Fresenius, Imedos Systems, Medtronic, MSD, Mundipharma, Novartis, Pierre Fabre, Pfizer, Roche, Schwabe Pharma, Vifor, and Zoll, as well as grant support by Novartis, AstraZeneca and Berlin Heart unrelated to this article. MPN declares speaker fees from AstraZeneca, Imedos Systems and Vifor Pharma, advisory board compensation from Boehringer Ingelheim and grant support by Amgen, unrelated to this article. FR has not received personal payments by pharmaceutical companies or device manufacturers in the last 3 years (remuneration for the time spent in activities, such as participation as steering committee member of clinical trials and member of the Pfizer Research Award selection committee in Switzerland, were made directly to the University of Zurich). The Department of Cardiology (University Hospital of Zurich/University of Zurich) reports research-, educational- and/or travel grants from Abbott, Amgen, Astra Zeneca, Bayer, Berlin Heart, B. Braun, Biosense Webster, Biosensors Europe AG, Biotronik, BMS, Boehringer Ingelheim, Boston Scientific, Bracco, Cardinal Health Switzerland, Corteria, Daiichi, Diatools AG, Edwards Lifesciences, Guidant Europe NV (BS), Hamilton Health Sciences, Kaneka Corporation, Kantar, Labormedizinisches Zentrum, Medtronic, MSD, Mundipharma Medical Company, Novartis, Novo Nordisk, Orion, Pfizer, Quintiles Switzerland Sarl, Roche Diagnostics, Sahajanand IN, Sanofi, Sarstedt AG, Servier, SIS Medical, SSS International Clinical Research, Terumo Deutschland, Trama Solutions, V- Wave, Vascular Medical, Vifor, Wissens Plus, ZOLL. The research and educational grants do not impact on his personal remuneration.

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