ESC European Society of Cardiology

European Heart Journal (2018) **39**, 47–56
doi:10.1093/eurheartj/ehx565

Retinal microvascular dysfunction in heart failure

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Received 12 March 2017; revised 19 May 2017; editorial decision 24 August 2017; accepted 27 September 2017; online publish-ahead-of-print 21 October 2017

See page 57 for the editorial comment on this article (doi: 10.1093/eurheartj/ehx664)

Aims Retinal vessel analysis (RVA) represents a novel, non-invasive, and reliable method to study the microcirculation in the eye. The goal of this study was to assess the extent of retinal microvascular dysfunction in patients with chronic heart failure (CHF) compared to controls and established measures of vascular function.

Methods and results In this prospective, single-centre, observational study, 74 patients with compensated CHF (mean age 63.5 ± 11.2 years, 32% female, mean left-ventricular ejection fraction 37 ± 12.8%), 74 patients with cardiovascular risk factors (CVRF; 64.1 ± 12.7 years, 34% female), and 74 healthy controls (HC; 57.8 ± 14.2 years, 35% female) were included. The primary endpoint, flicker-induced dilatation of retinal arterioles (FID_{art}), was significantly reduced in patients with CHF compared to CVRF and HC (mean FID_{art} 0.9 ± 0.2 vs. 2.3 ± 0.3 and vs. 3.6 ± 0.3%, respectively, both $P < 0.001$ before and after propensity score-weighted analysis). Similar differences were seen for venular FID. FID_{art} was less impaired in patients with dilated compared to ischaemic cardiomyopathy. No significant differences were observed for arteriovenous ratio and flow-mediated dilatation. Impaired FID_{ven} was associated with echocardiographically estimated systolic pulmonary artery pressure and left atrial volume index.

Conclusion Retinal microvascular dilatation in response to flicker light is impaired in CHF. RVA may represent a new and useful method to non-invasively monitor microvascular abnormalities in heart failure in an easy and standardized way without the use of radiation.

Keywords Heart failure • Endothelial dysfunction • Microcirculation • Retinal vessel analysis

Introduction

Chronic heart failure (CHF) is a common cardiovascular disease with a significant economic and societal burden. In the last 20 years, significant progress has been made in medical and device treatment of the disease. Yet, long-term prognosis of heart failure patients remains poor and its pathophysiology is incompletely understood.

Endothelial cells play a central role in blood vessel physiology by regulating vessel wall integrity, vasodilatation and blood coagulation. Impairment of their function (endothelial dysfunction) is a hallmark feature of cardiovascular diseases,[1] including heart failure[2] and is considered an early marker of elevated cardiovascular risk.[3] Most studies on endothelial dysfunction in heart failure focused on evaluation of larger vessels such as brachial artery measurement of flow-mediated vasodilatation (FMD).[4–6] Recently, the role of the microvasculature in heart failure gained more attention.[7,8] Yet, non-invasive and radiation-free methods to evaluate microvascular dysfunction are still scarce. Retinal vessel analysis (RVA) is a new and unique method to evaluate the retinal microcirculation using flicker light-stimulation and high-resolution measurement of retinal vessels.[9] Flicker light-induced retinal dilatation has been found to involve nitric oxide NO signalling and thereby represents a potential new biomarker for microvascular endothelial dysfunction.[10]

This article has been handled by our Guest Editor Anthony DeMaria.

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Our study was designed to test whether retinal microvascular function as measured by flicker light-induced retinal arteriolar dilatation (FID_{art}) is compromised in stable and compensated heart failure patients compared to a cohort of healthy controls and patients with cardiovascular risk factors without established cardiovascular disease. Secondary objectives included the measurement of additional retinal microvascular parameters and established tests of vascular function (FMD and arterial stiffness) and correlation with heart failure-relevant clinical parameters.

Methods

Study design and protocol

The study is a cross-sectional observational investigation of retinal microvascular function in heart failure patients and controls in one university centre. The study protocol was approved by the local ethics committee (KEK-ZH-No. 2014-0329). All participants provided written informed consent. Three groups of patients were prospectively recruited for the study:

(i) Patients with a diagnosis of CHF according to the 2012 European Society of Cardiology guidelines in currently compensated clinical status and on stable pharmacologic therapy (CHF group). (ii) Patients currently treated for a modifiable cardiovascular risk factor (hypertension, dyslipidaemia, smoking, and/or diabetes) without any known or symptomatic cardiovascular disease (CVRF group). (iii) Healthy controls as defined by non-smoking volunteers, aged 35 years and older, without any known cardiovascular risk factor or any symptomatic or known cardiovascular disease (HC group). Exclusion criteria were pregnancy or breastfeeding, allergy against study drugs, photosensitive epilepsy, glaucoma, or other significant eye pathologies such as blindness, inability to fixate, progressive diabetic retinopathy, or prior retinal laser coagulation.

After signing informed consent, participants were invited to the main study visit which was conducted in the morning and included a medical history, measurement of clinical and laboratory parameters as well as vascular function tests (vascular stiffness and RVA followed by flow-mediated vasodilatation at the end of the examination). Patients were instructed to remain fasted for at least 8 h (except water), take their regular medication as planned (except for antidiabetic medications), refrain from coffee, alcohol and cigarette consumption for at least 12 h, avoid unusual exercise the day before the examination and only present in stable medical state (i.e. free of infections or acute illnesses).

Retinal vessel analysis

RVA was conducted with an Imedos Dynamic Retinal Vessel Analyzer (Imedos, Jena, Germany) using a Zeiss FF450 plus fundus camera (Carl Zeiss Meditec AG, Jena, Germany) connected with two charge-coupled device cameras that provide digital images for a computerized vessel analysis software (Imedos, Jena, Germany). Previously established measurement protocols were used for the study.[9,11] In brief, one eye was randomly selected and mydriasis was induced using 0.5% tropicamide. After 20 min, dynamic RVA was conducted with measurement of retinal arteriolar and venular dilatation after provocation with 12.5 Hz optoelectronic flicker light. This dilatation is majorly mediated by the release of nitric oxide in response to increased oxygen consumption of retinal photosensitive cells and involves neurovascular coupling.[9,10] Nitric oxide diffuses to vascular smooth muscle cells where it mediates vascular relaxation via activation of guanylate cyclase and increase in cyclic guanosine monophosphate which activates protein kinase G. Analysis was performed on temporal segments of one retinal arteriole and venule 0.5–2 optic disc diameters away from the optic disc. The selected vessel segments had to be sharp, free of branching sites and reflex stripes and supplied at least one branch arteriole to the macula region. The protocol consisted of a 50 s baseline and three 20 s flicker stimulations each followed by a recovery period of 80 s. After acquisition, the results from the three flicker periods were averaged and percent dilatation of arteriole or venule from baseline (FID_{art} and FID_{ven}, respectively) was calculated automatically using the Imedos analysis software (*Figure 1A*). For static RVA, monochromatic 50° fundus photographs were obtained using Visualis and VesselMap 2 software (Imedos, Jena, Germany) and retinal arteries and vein diameters in the area 0.5–1 optic disc diameters distant from the optic disc were added with calculation of the central retinal artery and vein equivalent (CRAE and CRVE; *Figure 1B*).[11] CRAE and CRVE are plotted in relative measuring units (mu). Both values were used to calculate the arterio-venous ratio (AVR = CRAE/CRVE).

Flow-mediated vasodilatation

FMD was assessed using established protocols.[3] In brief, arterial diameter of one brachial artery was continuously measured using a 10 MHz linear array transducer (Siemens Acuson X300, Siemens AG) with automatic wall-tracking and analysis software (FMD-Studio, Pisa, Italy). One minute after its application to the lower arm, the blood pressure cuff was inflated 50 mmHg above systolic pressure for 5 min. After release, hyperaemia occurred and the change in arterial diameter was measured for further 10 min. The percent peak dilatation related to the baseline diameter was calculated and depicted as FMD (%). To ascertain endothelial-independent effects, pharmacological peak percent dilatation of the brachial artery was measured 6 min after one dose of sublingual glycerol trinitrate (GTN; Nitrolingual 0.4 mg, Pohl-Boskamp, Germany). The reproducibility of our laboratory's measurements was published.[12]

Arterial stiffness

Arterial stiffness was assessed by measuring pulse wave velocity (PWV) and augmentation index (AIX) with a SphygmoCor applanation tonometer system (AtCor Medical, Itasca, IL, USA) according to established protocols.[13,14] Briefly, patients rest in the supine position for 15 min and measurements are taken immediately after brachial blood pressure recording. AIX was measured at the level of the radial artery by obtaining ten high quality pulse wave measurements with automatic calculation of AIX using the manufacturer's proprietary software and after normalizing to a heart rate of 75 beats per minute. PWV (meter/second) was calculated from the pressure wave transit time and distance between carotid and femoral artery according to recent guidelines.[13,15] Transit time between arterial sites was determined in relation to the R wave of the electrocardiogram.

Echocardiography

Transthoracic echocardiographies were obtained during regular clinical outpatients visits using M-mode, 2D and color Doppler echocardiography by experienced cardiologists according to current guidelines.[16] All parameters were reanalysed by a single-blinded expert operator using stored images. Left-ventricular ejection fraction (LVEF) was calculated from biplane images using the Simpson's method. Right ventricular fractional area change (RVFAC) was defined as the ratio between the difference of the end-diastolic and end-systolic right ventricular areas and the end-diastolic area. The measurements were corrected for body surface area. Systolic pulmonary artery pressure (SPAP) was measured by addition of right atrial (RA) pressure, as determined by evaluation of inferior vena cava diameter and variability, to the right ventricular (RV)–RA peak systolic gradient as measured from the tricuspid regurgitant time-velocity integral.

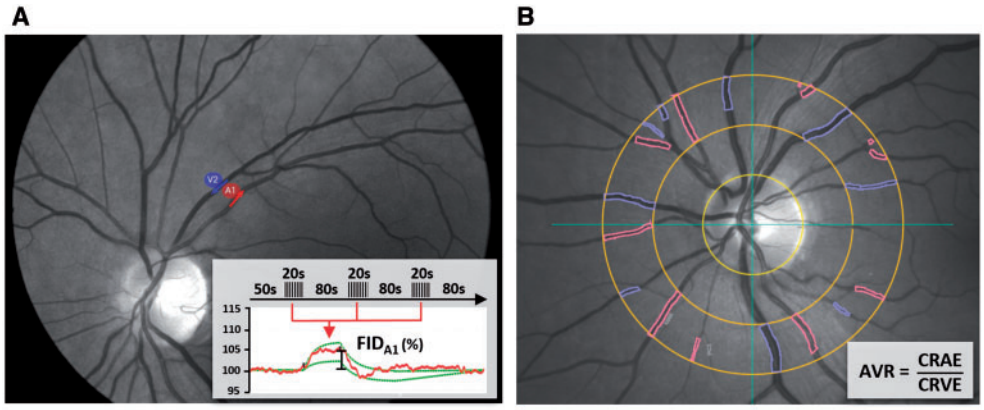

Figure 1 Retinal vessel analysis consists of a dynamic measurement of flicker-induced dilatation (FID) of retinal arterioles (red) and venules (blue) using a standardized flicker protocol (*A*) and a static measurement arteriolar and venular diameters (*B*). The arteriovenous ratio (AVR) is calculated from the central retinal vein and artery equivalent (CRAE and CRVE respectively).

Laboratory assessments

Blood samples were obtained in the fasted state using heparin plasma vials at the beginning of the study visit and analyzed on the same day at the Institute of Clinical Chemistry, University Hospital 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). Undetectable values were replaced by half the lower limit of detection.[17]

Statistical analysis

Statistical analyses were computed with R (Rproject) and JMP 12.1 (SAS Institute, Cary, North Carolina, USA). The difference in FID_{art} between heart failure patients, patients with cardiovascular risk factors and healthy controls represented the primary endpoint of the study. The other vascular measures (FID_{ven}, AVR, CRAE, CRVE, FMD, PWV, and AIX) served as secondary exploratory outcomes. The results are reported as is and after inverse probability weighting to achieve balance between groups. We aimed to carefully characterize our prospectively recruited cohort and reported associations of echocardiographic and laboratory findings with measures of vascular dysfunction. Results are expressed as mean ± standard deviation (SD) for baseline and predicted mean ± standard error (SE) for outcome statistics unless otherwise noted. Sample size was estimated based on data on FID_{art} of healthy controls by Mandecka *et al.*[18] Estimating a difference of FID_{art} of 1% (SD 2.1) with power of 80% and an alpha error of 5%, a group size of 70 patients was calculated.

Differences in baseline characteristics (Supplementary material online, *Table S1*) were tested. Categorical variables were analysed using χ^2 test or Fisher's exact test as appropriate. Continuous characteristics were tested with one-way analysis of variance as omnibus and Student's *t*-test or Welch's test for unequal variances *post hoc*. Non-normally distributed data were compared using the Wilcoxon test. Normality was assessed visually by quantile–quantile plots.

To achieve between-group balance for possible confounders,[19] generalized covariate balancing propensity scores (CBPS, R package)[20] and subsequent inverse probability weights (IPW) were calculated. The covariates patient age, sex, body mass index (BMI), systolic and diastolic blood pressure, heart rate, total cholesterol, and fasting plasma glucose were selected independently of our sample based on published associations with the outcome and consideration of all possibly confounding group differences.[21] As fasting plasma glucose concentration was highly positively skewed ($G_1 = 3.32$) and leptokurtic (excess kurtosis = 11.1), glucose is coarsened to a binary covariate with the World Health Organization threshold of glucose > 5.6 mmol/L equalling impaired fasting glucose and glucose ≤ 5.6 mmol/L corresponding to the category normal fasting glucose.

Briefly, the CBPS method estimates propensity scores generalized for multiple groups within the framework of generalized method of moments[22] by modelling group assignment while simultaneously optimizing covariate balancing. Stabilized and standardized IPW are consequently calculated (R, package CBPS). Linear contrasts (R, package multcomp) and robust 'sandwich' SEs were calculated for generalized linear models (R, survey package) based on the weighted design. The *P*-values of LSD-Fisher test results are reported. Predicted means are reported in the bar graphs.

Linear regression was used to test the association of relevant echocardiographic and laboratory parameters with vascular function parameters. NT-proBNP was linearized by taking the logarithm. To test the influence of covariates on these associations, multiple linear regression analysis was performed as a second step including the propensity score covariates into the model.

Results

Baseline characteristics

Seventy four patients with CHF (mean age 63.5 ± 11.2 years; 32% female), 74 patients with cardiovascular risk factors without cardiovascular disease (CVRF group; mean age 64.1 ± 12.7; 34% female), and 74 healthy controls (HC group; mean age 57.8 ± 14.2 years, 35% female) were eligible and included in the study between January 2015 and September 2016. Their clinical characteristics, laboratory parameters, and concomitant medication are presented in *Table 1*. Patients with CHF were symptomatic (median New York Heart Association class II), had a mean LVEF of 37 ± 12.8% and a median NT-proBNP of 706 ng/L (IQR 1161 ng/L) respectively. Patients with CHF were somewhat older, had a higher BMI and a lower diastolic BP compared to healthy controls. They had a higher rate of comorbidities, including

Table 1 Baseline characteristics

Parameter	HC ($n = 74$)	CVRF ($n = 74$)	CHF ($n = 74$)	$P_{HC\ vs.\ CHF}$	$P_{CVRF\ vs.\ CHF}$
Clinical characteristics					
•Age (years)	57.8 ± 14.2	64.1 ± 12.7	63.5 ± 11.2	0.007	0.77
•Female sex (*n*)	26 (35%)	25 (34%)	24 (32%)	0.73	0.86
•BMI (kg/m^2)	24.4 ± 3.5	25.9 ± 3.8	28.6 ± 5.3	<0.001	<0.001
•Systolic BP (mmHg)	124.1 ± 11.3	138.6 ± 19.4	119.9 ± 20.6	0.15	<0.001
•Diastolic BP (mmHg)	77.1 ± 8.1	83.6 ± 11.1	70.8 ± 12.5	<0.001	<0.001
•Heart rate (beats/min)	63.7 ± 10.1	65.3 ± 10.2	65.4 ± 10.5	0.32	0.95
Comorbidities					
•Current smoking	0 (0%)	9 (12%)	17 (23%)	<0.001	0.08
•Hypertension	0 (0%)	52 (70%)	43 (58%)	<0.001	0.12
•Dyslipidaemia	0 (0%)	37 (50%)	42 (57%)	<0.001	0.40
•Diabetes mellitus	0 (0%)	7 (9%)	29 (39%)	<0.001	<0.001
Laboratory parameters					
•Total cholesterol (mmol/L)	5.1 ± 0.6	5.1 ± 0.9	4.7 ± 1.5	0.02	0.03
•HDL cholesterol (mmol/L)	1.7 ± 0.4	1.6 ± 0.5	1.2 ± 0.4	<0.001	<0.001
•LDL cholesterol (mmol/L)	3.0 ± 0.6	2.9 ± 0.9	2.6 ± 1.2	0.009	0.07
•Fasting plasma glucose (mmol/L)	5.2 ± 0.5	5.5 ± 0.9	6.8 ± 2.2	<0.001	<0.001
•Impaired fasting plasma glucose (*n*)	17 (23%)	23 (31%)	25 (34%)	<0.001	<0.001
•CRP, high sensitivity (mg/L) [Median (IQR 25–75%)]	0.8 (0.4–1.55)	1.0 (0.5–1.55)	2.2 (1.2–4.7)	<0.001	<0.001
•Sodium (mmol/L)	140.9 ± 1.6	140.0 ± 2.6	139.2 ± 2.5	<0.001	0.03
•Potassium (mmol/L)	3.9 ± 0.3	3.9 ± 0.3	4.1 ± 0.5	0.006	0.001
•eGFR CKD-EPI (mL/min)	89.7 ± 17.1	83.7 ± 16.6	64.3 ± 21.4	<0.001	<0.001
•NT-proBNP (ng/L) [Median (IQR 25–75%)]	58 (35–98)	61 (31–100)	706 (238–1399)	<0.001[a]	<0.001[a]
•Troponin T, high sensitivity (ng/L) [Median (IQR 25–75%)]	2.5 (2.5–7)	5 (2.5–7.5)	13 (8–28)	<0.001	<0.001
Concomitant medication					
•ACE inhibitor/ARB (%)	0 (0%)	40 (54%)	65 (88%)	<0.001	<0.001
•Beta blocker (%)	0 (0%)	18 (24%)	67 (91%)	<0.001	<0.001
•Mineralocorticoid antagonist (%)	0 (0%)	1 (1%)	44 (59%)	<0.001	<0.001
•Loop diuretic (%)	0 (0%)	2 (3%)	55 (74%)	<0.001	<0.001
•Calcium channel blocker (%)	0 (0%)	13 (18%)	9 (12%)	0.003	0.35
•Thiazide diuretic (%)	0 (0%)	15 (20%)	12 (16%)	<0.001	0.52
•Aspirin (%)	3 (4%)	25 (34%)	42 (57%)	<0.001	0.005
•Anticoagulant (%)	0 (0%)	0 (0%)	27 (36%)	<0.001	<0.001
•Statin (%)	0 (0%)	34 (46%)	51 (69%)	<0.001	0.005
•Vitamin/mineral supplement (%)	23 (31%)	21 (28%)	27 (36%)	0.49	0.29
•Non-insulin antidiabetic drugs (%)	0 (0%)	6 (8%)	18 (24%)	<0.001	0.006
•Insulin (%)	0 (0%)	1 (1%)	11 (15%)	<0.001	0.001

Statistical analysis of continuous variables: One-way ANOVA with Student's *t* post-test or Welch's test as appropriate. Wilcoxon test was used for non-parametric data. Statistical analysis of categorical variables: χ^2 test or Fisher's exact test as appropriate. Significant post tests are only reported when overall ANOVA was significant.
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; CHF, Patients with chronic heart failure; CRP, C-reactive protein; eGFR CKD-EPI, estimated glomerular filtration rate as calculated by chronic kidney disease epidemiology collaboration formula; HC, healthy controls; HDL, high-density lipoprotein; IQR, inter-quartile range; LDL, low-density protein; NT-proBNP, N-terminal pro brain natriuretic peptide; CVRF, Patients with cardiovascular risk factors; ANOVA, analysis of variance.
[a]Analysis of NT-proBNP performed using logarithmic data.

smoking, hypertension, dyslipidaemia, and diabetes compared to healthy controls. No significant differences were found for age, hypertension, and dyslipidaemia compared to the patients with CVRF. Clinical characteristics of the CHF group are shown in *Table 2*. The aetiology of CHF was ischaemic cardiomyopathy (ICM; $n = 34$), dilated CM (DCM; $n = 26$), and other CM ($n = 14$).

Supplementary material online, *Table S1* and *Figures S1* (in summary) and *Figure S2* (for each covariate) show balance before and after inverse probability weighting, whereas Supplementary material online, *Figure S3* shows weighted propensity score overlap.

Retinal vascular function and structure

RVA was available for all participants except one healthy control due to intolerance of the camera light. The primary endpoint (FID_{art}) was

Table 2 Baseline characteristics of heart failure subgroups

Parameter	DCM ($n = 26$)	ICM ($n = 34$)	Other CM[a] ($n = 14$)
Clinical characteristics			
•Age (years)	62.2 ± 10.9	62.4 ± 11.7	68.4 ± 10.0
•Female sex (*n*)	8 (31%)	8 (24%)	8 (57%)
•BMI (kg/m^2)	29.2 ± 5.7	27.5 ± 5.4	30.1 ± 4.3
•Diastolic BP (mmHg)	75.8 ± 10.5	66.9 ± 12.3	71.1 ± 14.2
•Current smoking (*n*)	9 (35%)	8 (24%)	0 (0%)
•Diabetes mellitus (*n*)	5 (19%)	16 (47%)	8 (57%)
•Coronary artery disease (*n*)	5 (19%)	34 (100%)	2 (14%)
•Atrial fibrillation (*n*)	4 (15%)	3 (9%)	11 (79%)
•ICD (*n*)	13 (50%)	17 (50%)	5 (36%)
•CRT (*n*)	8 (31%)	7 (21%)	3 (21%)
•NYHA-class (1–4) [Median (IQR 25–75%)]	2.0 (2–2)	2.0 (2–2.25)	2.0 (2–3)
Laboratory parameters			
•eGFR CKD-EPI (mL/min)	66.6 ± 20.5	67.6 ± 20.7	51.9 ± 22.4
•NT-proBNP (ng/L) [Median (IQR 25–75%)]	444 (159–1157)	784 (364–1399)	910 (192–3300)
•Troponin T, high sensitivity (ng/L) [Median (IQR 25–75%)]	10 (7–20)	14 (8–29)	21 (12–36)
Echocardiography[b]			
•LVEF (%) ($n = 74$)	41 (31–48)	30 (26–40)	41 (23–54)
•EDVI (mL/m^2) ($n = 72$)	65 (53–108)	85 (66–95)	64 (51–127)
•LAVI (mL/m^2) ($n = 72$)	35 (28–38)	37 (32–42)	50 (38–71)
•MR (Grade 0–3) ($n = 72$)	1 (0–1)	1 (0–1)	1 (0–1.25)
•TAM (mm) ($n = 70$)	18 (17–19)	17 (13–20)	14 (13–18)
•RVFAC (%) ($n = 72$)	40 (37–45)	39 (33–44)	35 (23–42)
•SPAP (mmHg) ($n = 71$)	27.5 (23–44)	28 (25–44)	39.5 (27–49)

BMI, body mass index; CRT, cardiac resynchronization therapy; DCM, dilated cardiomyopathy; EDVI, end-diastolic volume index; ICD, implantable cardioverter defibrillator; ICM, ischaemic cardiomyopathy; IQR, interquartile range; LAVI, left atrial volume index; LVEF, left ventricular ejection fraction; $mPAP_{calc}$, calculated mean pulmonary artery pressure; MR, Grade of mitral regurgitation (Grade 0 none/minimal, Grade 1 mild, Grade 2 moderate, Grade 3 severe); NYHA, New York Heart Association; eGFR CKD-EPI, estimated glomerular filtration rate as calculated by Chronic Kidney Disease Epidemiology Collaboration formula; TAM, tricuspid annulus motion; RVFAC, right ventricular fractional area change.

[a]Other CM group includes valvular CM ($n = 8$), heart failure with preserved ejection fraction ($n = 4$), non-compaction CM ($n = 1$), chemotherapy-induced CM ($n = 1$).

[b]All parameters are median (IQR 25–75% percentile in brackets).

significantly impaired in CHF patients compared with both patients with CVRF and healthy controls (mean FID_{art} 0.9 ± 0.2 vs. 2.3 ± 0.3 and vs. 3.6 ± 0.2%, both $P < 0.001$ respectively, *Table 3* and *Figure 2*). The primary endpoint was significant before and after correction for baseline group imbalance by propensity score analysis with inverse probability weighting (Supplementary material online, *Table S2*).

Results of secondary outcomes are shown in *Table 3* and *Figure 3*. Similar differences were found for the exploratory endpoint FID_{ven} with significantly lower venular dilatation in response to flicker light in CHF patients compared to healthy controls ($P = 0.027$; *Figure 3A*).

CHF patients with DCM had a significantly higher FID_{art} compared to CHF patients with ICM (mean FID_{art} 1.5 ± 0.3 vs. 0.4 ± 0.2, $P = 0.0045$; *Figure 3B* and *Table 4*). There was no significant difference in FID_{art} and FID_{ven} between patients with HFpEF and HFrEF (Supplementary material online, *Figure S4*).

In further exploration, although no significant differences in AVR, CRAE, and CRVE were found between the groups (*Table 3* and *Figure 3C–E*), CRVE tended to be higher in CHF patients compared to HC ($P = 0.071$).

Other measures of vascular function

Both FMD and GTN-mediated vasodilatation of the brachial artery were not significantly different between CHF and CVRF patients as well as healthy controls (mean FMD 5.1 ± 0.3 vs. 5.6 ± 0.4 vs. 6.1 ± 0.7%, $P = 0.32$ and $P = 0.17$, respectively; *Figure 4A* and *B*). CHF patients had increased arterial stiffness as evidenced by increased PWV compared to healthy controls and patients with CVRF (mean PWV 9.8 ± 0.4 vs. 7.3 ± 0.2 vs. 8.5 ± 0.3 m/s, $P < 0.001$ and $P = 0.001$, respectively, *Figure 4C*). On the other hand, there were no significant differences in AIX_{HR75} between CHF patients and CVRF patients and healthy controls (*Figure 4D*).

Associations of vascular with clinical and echocardiographic heart failure parameters

Concerning the relationship between retinal function and structure, there was no significant correlation between FID_{art} and AVR ($r = 0.02$, $P = 0.70$). FMD correlated only weakly with FID_{art} ($r = 0.15$, $P = 0.03$). Patients with CHF and diabetes had significantly lower

FID_{ven} compared to CHF patients without diabetes (FID_{ven} 2.1 ± 0.29 vs. 3.2 ± 0.24, $P = 0.004$). No significant differences in FID_{art}, FMD, CRAE, CRVE, and AVR were seen between CHF patients with and without diabetes (data not shown).

With regard to laboratory markers related to heart failure and prognosis, FID_{ven} correlated negatively with log-scaled NT-proBNP ($r = -0.25$, $P = 0.04$) and high sensitivity troponin T ($r = -0.24$, $P = 0.04$) and positively with eGFR CKD-EPI ($r = 0.27$, $P = 0.02$; Supplementary material online, *Figure S5*). There was no significant correlation with FID_{art} and NT-proBNP, troponin T or eGFR CKD-EPI ($r = -0.1$, $P = 0.41$, $r = 0.15$, $P = 0.19$, and $r = 0.11$, $P = 0.33$, respectively).

Figure 5 depicts the correlations of retinal venular function with echocardiography measurements [on average 72 days (95% confidence interval 41–112 days) apart from each other]. Interestingly, FID_{ven} correlated negatively with SPAP and left atrial volume index (LAVI), a surrogate for chronically elevated left ventricular filling pressure ($r = -0.51$, $P < 0.001$ and $r = -0.33$, $P = 0.004$, respectively). Both correlations remained significant in the multiple linear regression model including the covariates used in the propensity score, excluding systolic BP due to collinearity with diastolic BP ($\beta = -4.84$, $P < 0.001$ and $\beta = -2.81$, $P = 0.03$, respectively). Weaker correlations were found for FID_{art} with SPAP and LAVI ($r = -0.29$, $P = 0.03$ and $r = -0.20$, $P = 0.09$ respectively; not significant after multivariate adjustment). There was no significant correlation of FIDart and FIDven with LVEF. No significant correlation between FMD and SPAP, LAVI, and LVEF was found ($r = 0.06$, $P = 0.68$; $r = -0.07$, $P = 0.54$ and $r = -0.04$, $P = 0.73$ respectively).

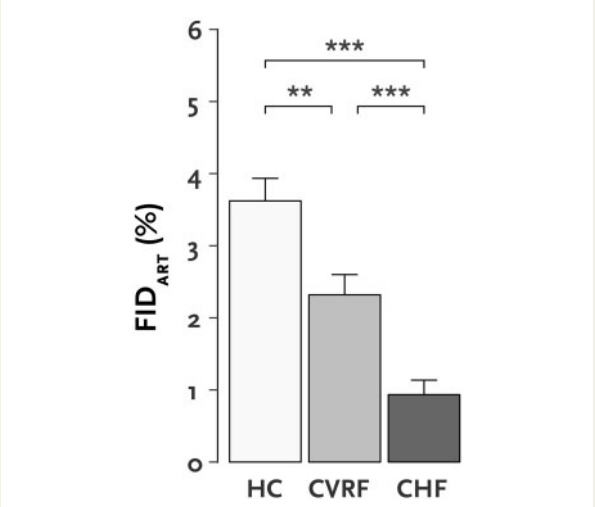

Figure 2 Primary endpoint of the study with comparison of flicker-induced dilatation of retinal arterioles (FID_{art}) between heart failure patients (CHF), patients with cardiovascular risk factors (CVRF) and healthy controls (HC). $^{**}P < 0.01$, $^{***}P < 0.001$.

Discussion

This systematic cross-sectional study on RVA in stable, compensated heart failure patients for the first time showed that CHF is characterized by a profound alteration in retinal microvascular function as evidenced by significantly reduced flicker-light induced dilatation of the arterial (primary endpoint) and venular vessels of the retina compared to controls. In further exploration considering the positive endpoint, retinal vascular responsiveness to flicker light was particularly impaired in patients with ICM compared to those with DCM. In addition, the extent of retinal venular dysfunction in CHF was correlated with echocardiographic markers of left ventricular filling pressures.

Thus, RVA provides a unique window of opportunity to assess the microcirculation in a standardized manner without the use of invasive

Table 3 Vascular structure and function in heart failure patients and controls

Parameter	HC ($n = 74$)	CVRF ($n = 74$)	CHF ($n = 74$)	$P_{HC\ vs.\ CHF}$	$P_{HC\ vs.\ CVRF}$	$P_{CVRF\ vs.\ CHF}$
Retinal vessel analysis						
•FID_{art} (%)	3.6 ± 0.3	2.3 ± 0.3	0.9 ± 0.2	**<0.001**	**0.0019**	**<0.001**
•FID_{ven} (%)	4.2 ± 0.4	3.7 ± 0.2	3.2 ± 0.3	**0.027**	0.19	0.22
•AVR	0.84 ± 0.01	0.83 ± 0.01	0.82 ± 0.02	0.40	0.28	0.89
•CRAE	181.3 ± 2.0	182.7 ± 2.5	183.7 ± 2.1	0.42	0.68	0.75
•CRVE	216.3 ± 2.1	221.3 ± 2.4	224.6 ± 4.1	0.071	0.12	0.49
Flow-mediated dilatation						
•FMD (%)	6.1 ± 0.7	5.6 ± 0.4	5.1 ± 0.3	0.17	0.55	0.32
•GTN (%)	17.0 ± 0.8	15.2 ± 0.8	14.3 ± 1.1	0.052	0.13	0.50
Arterial stiffness						
•PWV (m/s)	7.3 ± 0.2	8.5 ± 0.3	9.8 ± 0.4	**<0.001**	**0.0011**	**0.0087**
•AIX_{HR75} (%)	23.5 ± 1.8	25.2 ± 1.2	25 ± 1.6	0.51	0.42	0.93

Results after IPW. IPW predicted means and 'sandwich' robust standard errors are shown. Significance reported from LSD-Fisher tests on contrasts from weighted generalized linear models of parameter regressed against CHF subgroup.

AIX, augmentation index normalized to heart rate of 75/min, AVR, arteriovenous ratio, CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; DCM, dilated cardiomyopathy; FID_{art}, Flicker-induced dilatation of retinal arterioles; FID_{ven}, Flicker-induced dilatation of retinal venules; FMD, flow-mediated vasodilatation; GTN, glycerol trinitrate-mediated vasodilatation; HC, healthy controls; ICM, ischaemic cardiomyopathy; CVRF, patients with cardiovascular risk factors; PWV, pulse wave velocity; IPW, inverse probability weighting.

$P < 0.05$ is significant (bold values).

or radiographic imaging modalities. Of note, the value of static changes in the retinal vasculature in predicting cardiovascular events, most prominently the relationship between retinal arteriole and venule diameters (AVR) has been well studied in larger prospective observational studies.[23–25] However, less is known about dynamic changes of retinal vessel reactivity in different forms of cardiovascular disease or after therapeutic interventions. Smaller studies indicate reduced arteriolar and venular dilatation in patients with cardiovascular risk factors, i.e. hypertension, hypercholesterolaemia, diabetes, and obesity.[9] We extend the findings to patients with heart failure, showing profoundly impaired retinal dilatation compared to controls, that remained robust after adjustment for cardiovascular risk factors and possible haemodynamic confounders. The results are in line with a smaller preliminary study showing significantly reduced FID_{art} in 16 patients with DCM compared to healthy controls.[26] Interestingly, retinal dilatation was even more profoundly impaired in ICM than in DCM in our study cohort which may be related to the higher systemic atherosclerotic burden in ICM patients compared to DCM.[27]

The pathophysiology of reduced flicker dilatation in patients with CHF is presently a matter of investigation. Defects of endothelial NO signalling in CHF are one possible mechanism. NO has been found to be involved in retinal dilatation[10] and, at the same time, endothelial NO production is known to be reduced in heart failure, possible due to inactivation by increased reactive oxygen species.[2] Due to its easy

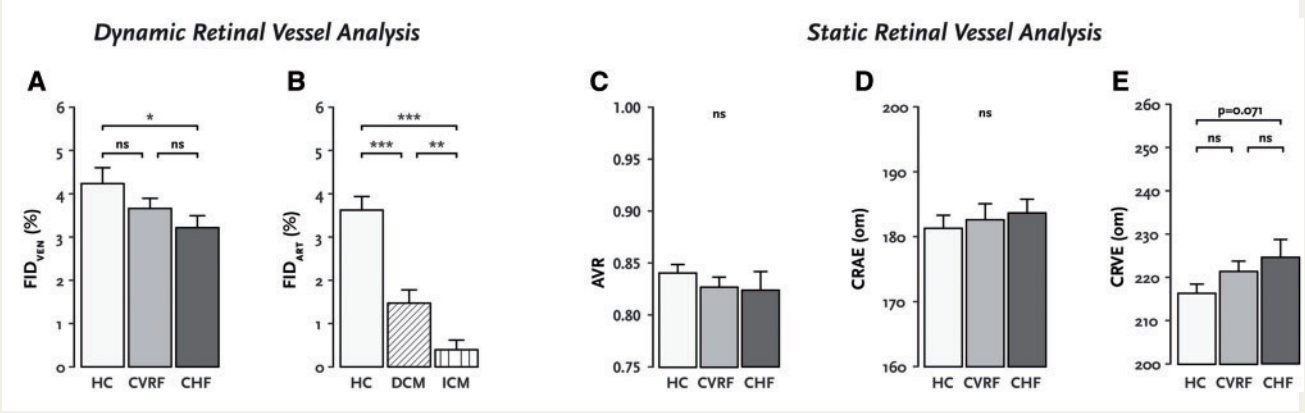

Figure 3 Secondary outcomes of retinal vessel analysis in heart failure patients and controls. Comparison of FID_{ven} between heart failure patients and controls (*A*) and FID_{art} between different heart failure subgroups (*B*). Static retinal vessel analysis with measurement of AVR (*C*) as calculated from CRAE (*D*) and CRVE (*E*). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. AVR, arteriovenous ratio; CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; DCM, dilated cardiomyopathy; FID_{art}, Flicker-induced dilatation of retinal arterioles; FID_{ven}, Flicker-induced dilatation of retinal venules; HC, healthy controls; ICM, ischaemic cardiomyopathy; CVRF, patients with cardiovascular risk factors.

Table 4 Vascular structure and function in heart failure subgroups

Parameter	DCM ($n = 26$)	ICM ($n = 34$)	Other CM ($n = 14$)	$P_{DCM\ vs.\ ICM}$	$P_{DCM\ vs.\ OCM}$
Retinal vessel analysis					
•FID_{art} (%)	1.5 ± 0.3	0.4 ± 0.2	0.8 ± 0.2	**0.0045**	0.0578
•FID_{ven} (%)	3.2 ± 0.3	3.4 ± 0.5	2.9 ± 0.4	0.70	0.69
•AVR	0.85 ± 0.02	0.79 ± 0.03	0.83 ± 0.02	0.09	0.51
•CRAE	186.2 ± 3.1	182.5 ± 3.5	179.0 ± 4.0	0.42	0.16
•CRVE	220.2 ± 5.3	231.1 ± 5.6	220 ± 7.7	0.16	0.98
Flow-mediated dilatation					
•FMD (%)	5.1 ± 0.7	5.2 ± 0.4	4.8 ± 0.6	0.91	0.73
•GTN (%)	13.1 ± 1.5	15.2 ± 2.2	15.7 ± 0.9	0.43	0.13
Arterial stiffness					
•PWV (m/s)	10.7 ± 0.7	9.3 ± 0.5	8.2 ± 1.0	0.088	**0.041**
•AIX_{HR75} (%)	22.3 ± 3.1	26.7 ± 1.5	29.6 ± 1.5	0.20	**0.031**

Statistical analysis after IPW. IPW predicted means and robust 'sandwich' standard errors are reported. Significance reported from LSD-Fisher tests on contrasts from weighted generalized linear models of parameter regressed against CHF subgroup.
AIX, augmentation index, as normalized to heart rate of 75/min; AVR, arteriovenous ratio; CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; DCM, dilated cardiomyopathy; FID_{art}, Flicker-induced dilatation of retinal arterioles; FID_{ven}, Flicker-induced dilatation of retinal venules; FMD, flow-mediated vasodilatation; GTN, glycerol trinitrate-mediated vasodilatation; HC, healthy controls; ICM, ischaemic cardiomyopathy; OCM, other cardiomyopathy group; RF, patients with cardiovascular risk factors; PWV, pulse wave velocity; IPW, inverse probability weighting.
$P < 0.05$ is significant (bold values).

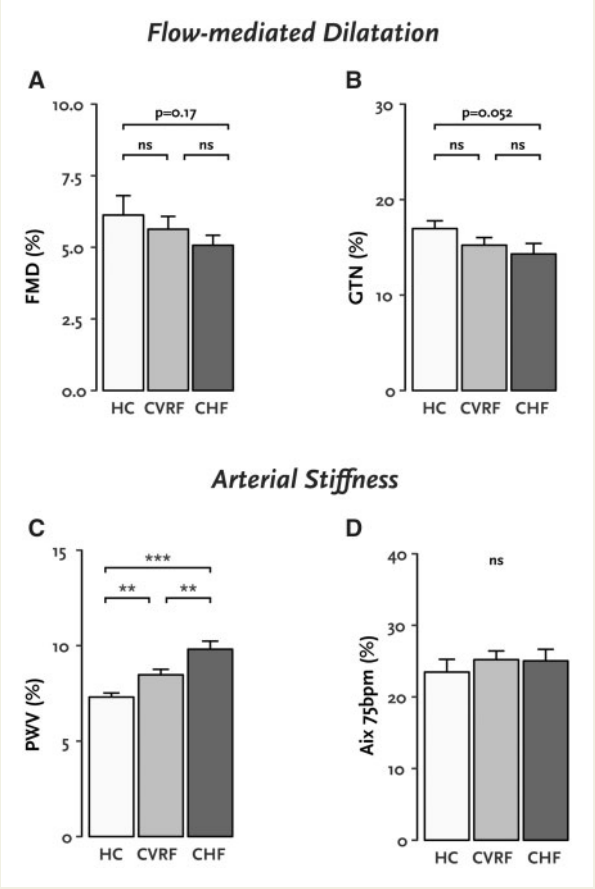

Figure 4 Secondary outcomes of established vascular measurements in heart failure patients and controls. Flow-mediated (*A*) and GTN-mediated (*B*) dilatation of the brachial artery in patients and controls. Markers of arterial stiffness including PWV (*C*) and AIX (*D*) among patients and controls. $^{**}P < 0.01$, $^{***}P < 0.001$. AIX, augmentation index, as normalized to heart rate of 75/min; FMD, flow-mediated vasodilatation; GTN, glycerol trinitrate-mediated vasodilatation; HC, healthy controls; CVRF, patients with cardiovascular risk factors; PWV, pulse wave velocity.

applicability, RVA may in the future be useful in clinical practice to assess risk in the context of cardiovascular prevention[28] and to quantify the effect of vascular targeted heart failure treatments as recommended by the European guidelines.[29] It is possible that such therapies work better in patients with more pronounced endothelial dysfunction and dynamic RVA may be helpful in selecting patients that benefit most from such treatments.

In our study, we observed in contrast to previous studies[4,30] no significant difference in FMD, an established measurement of NO-mediated vasodilatation of the brachial artery. One possible explanation of this finding might be the compensated clinical status and the baseline medical drug treatment of our patients with CHF. Indeed, FMD is known to be improved by treatment with ACE inhibitors, beta blockers, mineralocorticoid antagonists, or statins.[2,30] As the CHF patients in our study were well treated with respect to these drugs, FMD in these patients may have already been partially restored. Such restoration by disease-modifying drugs has not yet been shown for retinal FID. Until longitudinal studies are available, this explanation remains speculative and represents a limitation of the present study. We observed only a weak positive correlation between FMD and FID in our cohort, which was in line with a previous report.[31] There is a possibility that mechanisms beyond NO signalling may be involved in retinal microvascular dysfunction in heart failure. For example, there is evidence of close neurovascular coupling in the retina allowing for a regulation of retinal vessels in response to neuronal activation states and metabolic demands.[19] Cognitive impairment and both structural and functional neuronal alterations are common in CHF.[32] Hence, subclinical cellular dysfunction of retinal nerve cells, even without overt retinopathy, may also underlie retinal microvascular dysfunction in CHF.

Interestingly, impaired venular FID was associated with echocardiographically measured systolic pulmonary pressures and left atrial volume. Both are indirect markers of elevated left ventricular filling pressures in heart failure. Retinal microvascular dysfunction at the venous side may thus also be a consequence of myocardial and pulmonary stiffening resulting in increased retinal congestion in CHF. This is supported by the predominant correlation with venular dilatation and the tendency of higher baseline venular diameters in CHF patients as measured by CRVE. While the venular microvasculature might reflect high atrial pressure, the lack of association of arteriolar FID with high pulmonary pressures suggests that systemic mechanisms as discussed above are more dominant drivers of retinal dysfunction on the arterial side. An alternative explanation is that both retinal vascular and myocardial dysfunction are mediated by the same pathophysiologic changes, supported by the observation that pulmonary hypertension and diastolic dysfunction are both associated with alterations in NO signalling.[33,34]

Limitations

The study has some limitations. Due to the observational nature of the study, unmeasured confounding between CHF patients and controls is possible. The study was powered for FID_{art} and due to the systematic approach of measuring several vascular parameters the study may have been underpowered for detecting changes in secondary exploratory endpoints such as FMD. The CHF study cohort reflected the average distribution of patients in our heart failure outpatient unit, resulting in enrichment of HFrEF patients. Although we noted no significant differences, the study was not powered for meaningful subgroup analyses (such as of HFpEF patients). Future studies need to delineate whether retinal microvascular dysfunction is prevalent in this heart failure subtype, which appears likely as microvascular dysfunction is thought to play an important pathogenic role in HFpEF.[8] Another limitation is the lower rate of diabetes in the risk factor group compared to CHF patients. As we found no significant difference in arteriolar FID between CHF patients with and without diabetes, the observed differences in retinal microvascular dysfunction are likely to be still largely mediated by the presence of heart failure itself rather than the cumulative burden of individual risk factors.

The correlation of echocardiographic parameters is hypothesis generating. Echocardiograms were not performed on the same day of the vascular function measurements but collected in clinical routine. However, as LAVI is thought to reflect a slow anatomic change of the left atrium, we are convinced of the consistency of

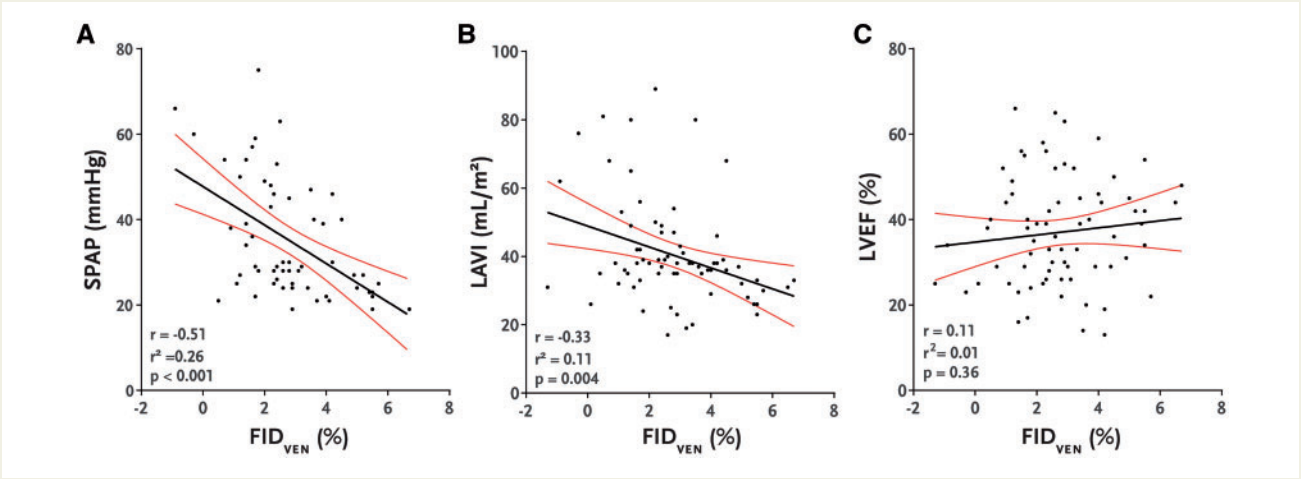

Figure 5 Correlation of the echocardiographic parameters systolic pulmonary artery pressure (SPAP; *A*), left atrial volume index (LAVI; *B*), and left-ventricular ejection fraction (LVEF; *C*) with flicker-induced dilatation of retinal venules (FID_{ven}).

our associations. Finally, our study was cross-sectional and focused on patients with symptomatic but compensated CHF on stable evidence-based therapy. We cannot extend our findings to patients with newly diagnosed, untreated or decompensated heart failure and the temporal longitudinal evolution of microvascular dysfunction in heart failure patients requires further study. Until longitudinal data on the prediction of heart failure outcomes by retinal microvascular dysfunction are available, the clinical applicability of this method is still limited.

Conclusion

In this first systematic study of both dynamic and static RVA in heart failure, CHF patients were characterized by a reduction of retinal microvascular dilatation in response to flicker light. This association remained significant after propensity score-weighted analysis. Impaired retinal vascular function in CHF was correlated with echocardiographic markers of diastolic dysfunction and pulmonary hypertension. Dynamic RVA may be a new and useful parameter for assessing microvascular dysfunction in heart failure. Future studies will need to delineate whether this method is useful for prognosis and prediction of treatment response of disease-modifying therapies.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Funding

Grants of the University Hospital Zurich; the Zurich Heart House; the LHW foundation; and the Swiss Heart Foundation.

Conflict of interest: M.P.N., J.B., V.L., S.C., A.v.E, F.E., T.F.L., I.S.: Nothing to declare. F.R.: Personal fees from Servier, personal fees from Cardiorentis, grants and personal fees from Biotronik, outside the submitted work. A.J.F.: Grants from LHW Foundation, grants from Swiss Heart Foundation, during the conduct of the study; personal fees from Novartis, grants and personal fees from Bayer, non-financial support from Orion Pharma, non-financial support from Amgen, personal fees from Bristol Myers Squibb, personal fees from Mepha, outside the submitted work.

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