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ORIGINAL ARTICLE

Eyes on amyloidosis: microvascular retinal dysfunction in cardiac amyloidosis

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Abstract

Aims Cardiac involvement in systemic amyloidosis is a marker of particularly poor prognosis. Cardiac amyloidosis (CA) is characterized by extracellular amyloid deposits inducing heart failure and symptoms of cardiac microvascular disease. While amyloid deposition is most common in the myocardium but also seen in pericardium and endocardium, atria, and vasculature, the role of (micro-)vascular dysfunction in CA pathophysiology remains still elusive. Because vascular function is associated with cardiovascular risk and severity of heart failure and represents a potential therapeutic target in CA, the present study investigated retinal vascular function, flow-mediated dilatation (FMD), and pulse-wave analysis and velocity (PWA/PWV) in patients with CA.

Methods and results Flicker-induced arterial dilatation (FIDa) was measured using dynamic retinal vessel analysis additionally to FMD and PWA/PWV. Thirty-three patients with CA [age 67 years [interquartile range, IQR, 62, 74], 14 with amyloid light-chain (AL) and 19 with transthyretin (ATTR) amyloidosis] were prospectively included in this cross-sectional, observational study and 70 healthy individuals (age 53 years [IQR 39, 67]) served as control. Potential confounders were balanced using entropy balancing propensity score analysis [inverse probability weighting (IPW)]. FIDa was reduced in CA patients (1.52 ± 1.73% vs. 3.09 ± 1.96%, $P < 0.001$, after IPW). While PWV was increased (8.74 ± 2.34 m/s vs. 7.49 ± 1.65 m/s, $P = 0.018$, after IPW), no difference in FMD was observed. FIDa was significantly associated with prognostic biomarkers of CA [estimated glomerular filtration rate ($r = 0.33$; $P < 0.001$), log-scaled troponin T ($r = -0.49$; $P < 0.001$), and N-terminal pro-B-type natriuretic peptide ($r = -0.51$; $P < 0.001$)].

Conclusions Retinal vascular function is impaired, associated with cardiac and renal biomarkers of CA severity, and may represent a potential therapeutic target in patients with amyloidosis.

Keywords Cardiac amyloidosis; Retinal vessel analysis; Vascular dysfunction; Endothelium; Heart failure

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Introduction

Cardiac amyloidosis (CA) is associated with high morbidity and increased mortality.[1,2] Amyloidosis is a complex multisystemic disease caused by the aggregation of amyloid fibrils in the extracellular space resulting from various precursor proteins.[3] Most patients with CA are either diagnosed with transthyretin amyloid (ATTR) or light-chain amyloid (AL) amyloidosis.[2] Aggregated intercellular ATTR and AL fibrils result in stiffening and thickening of the heart, a mechanical myocardial impairment characterized by signs and symptoms of restrictive heart failure. Additionally, toxic effects of amyloid fibrils, but also their soluble precursor oligomers, cause atrophy, degeneration, and cell loss in myocardial key compartments resulting in conduction delays and arrhythmias.[3] CA is a particular form of heart failure. In heart failure and cardiovascular diseases, endothelial dysfunction plays a critical role in development, progression, and prognosis of the

disease.[4,5] In amyloidosis, clinical symptoms such as angina in the absence of coronary stenosis,[6] pathologic evidence of amyloid deposits in intramural coronary vessels,[7,8] and functional imaging studies[9] suggest a significant involvement of the vasculature. Until now, data on vascular function, however, remain inconclusive due to the focus on conduit vessels and their possible affection by concomitant hypotension and autonomic dysfunction.[10,11] Dynamic retinal vessel analysis (DVA) is a new and validated method to assess microvascular function independently of autonomic nervous system control.[12] Recently, we demonstrated profound retinal vascular dysfunction in patients with cardiovascular risk factors, coronary artery disease (CAD), and heart failure.[5,13,14] Retinal vessel function in amyloidosis has not yet been explored. We hypothesized that microvascular dysfunction assessed via DVA is present in patients with CA in comparison with matched healthy controls (HC). Therefore, the aim of this prospective observational study was to investigate retinal vascular function in combination with comprehensive vascular and laboratory tests in amyloidosis patients.

Material and methods

After approval by the local ethics committee (Basec No. PB 2016-01517), patients diagnosed with CA were recruited for this cross-sectional, prospective exploratory, and observational study between 2016 and 2019. The study was conducted according to the Declaration of Helsinki. All included participants signed the written informed consent form. Patients with either AL or ATTR CA diagnosed within the *Zurich Amyloidosis Network* were included in the study and compared with healthy individuals (HC) without cardiovascular risk factors (dyslipidaemia, hypertension, CAD, active smoker, and diabetes). Exclusion criteria were pregnancy, breastfeeding, allergy against tropicamide, photosensitive epilepsy, glaucoma, or other significant eye pathologies such as blindness, inability to fixate, progressive diabetic retinopathy, or prior retinal laser coagulation.

Microvascular retinal vascular function (primary endpoint) was measured via DVA. Other vascular measurements included flow-mediated dilatation (FMD) and pulse-wave analysis and velocity (PWA/PWV). Blood pressure and heart rate were assessed using an automated validated upper arm blood pressure device in the sitting position after resting for minimally 5 min. Medical history included heart failure hospitalizations within the last 12 months.

Retinal vessel analysis

Dynamic retinal vessel analysis 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 software analysis by proprietary algorithms (Imedos, Jena, Germany). Previously established measurement protocols were used for the study.[15,16] For further details, see previous works.[5,13] Concisely, after induction of mydriasis (0.5% tropicamide), temporal segments of one retinal artery and one vein 0.5–2 optic disc diameters distant from the optic disc were marked for tracing. The protocol consisted of 50 s baseline and three 20 s flicker stimulations, each followed by a recovery period of 80 s. The optoelectronic flicker frequency of 12.5 Hz allowed for alternating dark frames in the 25 frames/s video. The results from the three flicker periods were averaged and per cent dilatation of arteriole or venole in (FIDa, FIDv) as well as constriction from baseline was calculated automatically using the stack analysis output of the Imedos software ('Research Extension'). For static retinal vessel analysis, monochromatic 50° fundus photographs were obtained using Visualis and VesselMap 2 software (Imedos, Jena, Germany). Vessel 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).[16] Both values were used to calculate the arterio-venous ratio, AVR (CRAE/CRVE).

Flow-mediated dilatation and arterial stiffness

Brachial artery FMD was assessed according to established protocols.[17] In brief, arterial diameter of one brachial artery was continuously measured by a 10 MHz linear array transducer (Siemens Acuson X300, Siemens AG). Wall contour tracking was implemented by proprietary analysis software (FMD-Studio, Pisa, Italy). After 1 min baseline diameter recording, a cuff affixed to the lower arm was inflated 50 mmHg above systolic blood pressure for 5 min. After release, hyperaemia occurred. The per cent peak dilatation related to the baseline diameter was calculated as FMD (%). To ascertain endothelial-independent effects, pharmacological peak per cent dilatation of the brachial artery was measured 6 min after one dose of sublingual glycerol trinitrate (GTN; Nitrolingual 0.4 mg, Pohl-Boskamp, Hohenlockstedt, Germany). The reproducibility of our laboratory measurements was published.[18]

Arterial stiffness was assessed by measuring PWV and PWA (AI, augmentation index) with an applanation tonometer system (SphygmoCor, AtCor Medical, Itasca, IL, USA) according to established protocols.[19] Briefly, the patient laid resting for 1 min with immediate brachial blood pressure recording before PWV and PWA. The carotid-femoral PWV (m/s) was calculated from electrocardiographic R-wave relative to pressure wave transit time and measured distance according to recent guidelines.[19] AI was measured at the level of the radial artery; 10 high-quality pulse-wave measure-

ments (standard deviation < 1%) were recorded with automatic calculation of AI by the manufacturer's proprietary software. AI was normalized to a heart rate of 75 b.p.m.

Laboratory biomarker assessment

Blood samples were obtained in fasted state (heparin plasma) at study visit and analysed on the same day at the Institute of Clinical Chemistry, University Hospital Zurich, using standard methods. High-sensitivity troponin T (hs-TnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were quantified by electrochemiluminescence immunoassays and the COBAS8000 autoanalyser by Roche Diagnostics (Mannheim, Germany). Estimated glomerular filtration rate (eGFR) was calculated using CKD-EPI formula for all ages. Undetectable values were replaced by half the lower limit of detection.[20]

Statistical analysis

All data analyses were performed in R 4.04[21]; correlations were visualized with JMP 15, SAS Institute Inc. (Cary, NC). Normality was assessed visually with quantile–quantile plots. Descriptive normal data are presented as means ± standard deviation, and non-normal data as median ± interquartile range (IQR); data derived from analyses are reported as means ± standard error of the mean.

The primary endpoint was predetermined to be the comparison of FIDa between amyloidosis patients and HC. No formal sample size calculation was performed due to the exploratory nature of the study, but difference in means in FIDa as tested by an independent Student's *t*-test between healthy subjects and CA patients was calculated. Power of 90% was estimated to be achieved with an effect size of Cohen's $d = 0.7$ and a group size allocation of 1:2 with groups comprising 67 healthy subjects and 33 CA patients. Datasets with missing values were included in the analyses.

Several characteristics have previously been reported to potentially influence the primary and secondary outcomes (possible confounders). It was predetermined to use inverse probability weighting (IPW) for both primary and secondary outcomes to improve covariate balance and optimize estimate precision. We prespecified an entropy balancing propensity score model.[22] Age, blood pressure, LDL cholesterol, serum fasting glucose concentration, and body mass index (BMI) were included based on literature research. Due to the male pattern of wild-type ATTR amyloidosis, the effect of gender differences on FIDa[23] cannot be accounted for. This results in the lack of group overlap. Similarly, due to the definition of the healthy cohort as without comorbidities, concomitant diseases and medication were not balanced. IPW was calculated using R[21] with packages WeightIt[24] and doubly robust entropy balancing as previously described.[5] Statistical tests after IPW included Pearson's χ^2 test with second-order Rao–Scott correction for categorical data and generalized linear models with Wald regression term test ('survey *t*-test') for continuous outcomes. Unadjusted statistical tests used are Fisher's exact and Student's *t*-test.

Results

Baseline characteristics

Thirty-three patients with CA (14 AL amyloidosis and 19 ATTR amyloidosis) were inverse probability weighted (IPW) to match 70 HC. Baseline characteristics (unadjusted) are reported in *Table 1*. CA patients differed from HC in age, sex distribution, and heart rate as well as cardiovascular comorbidities and risk factors. After IPW sufficient balance (standardized mean difference < 0.1) in age, systolic and diastolic blood pressure, BMI, glucose, and LDL-cholesterol serum concentrations were achieved across covariates (Supporting Information, *Figure S1*). Supporting Information, *Table S1* contains details on baseline characteristics after IPW.

For characteristics of AL and ATTR CA patients, see *Table 1*. Patients with AL versus ATTR amyloidosis differ in age, baseline diastolic blood pressure, the presence of CAD, and atrial fibrillation. Use of loop diuretics was significantly more common in ATTR than AL patients. Wild-type ATTR amyloidosis was diagnosed in 10 and variant in 4 patients. In 5, genetic testing has not been performed. Six out of 19 ATTR patients were on treatment with transthyretin stabilizer tafamidis, 8 with green tea extract, and 1 patient received patisiran. In comparison, there were 10 lambda versus 4 kappa AL amyloidosis patients. Twelve of 14 AL patients had received immuno-chemotherapy before the study visit was conducted.

Eight CA patients reported heart failure hospitalizations within the last 12 months. There was no difference in FIDa (unadjusted) between CA patients with versus without hospitalization (data not shown).

Vascular measurements

Cardiac amyloidosis versus healthy controls

Vascular measurements (unadjusted) are summarized in *Table 2*. The primary endpoint FIDa was impaired in patients with CA compared with HC (1.52 ± 1.73% vs. 3.55 ± 1.82% unadjusted; $P < 0.001$). After IPW to account for intra-group differences in baseline characteristics, FIDa remained significantly reduced compared with controls (1.52 ± 1.73% vs. 3.09 ± 1.96% after IPW; $P < 0.001$; *Figure 1*). Similarly, PWV was increased in patients with CA (8.74 ± 2.34 m/s vs. 7.49 ± 1.65 m/s after IPW; $P = 0.018$; *Figure 1*). While FMD was lower in CA than in HC, this difference did not reach statistical significance (4.55 ± 2.50% vs. 5.52 ± 3.06%, $P = 0.167$,

Table 1 Baseline characteristics (unadjusted)

	HC, *N* = 70	CA, *N* = 33	*P*	AL, *N* = 14	ATTR, *N* = 19	*P*
Male	37 (52.9%)	28 (84.8%)	**0.002**	10 (71.4%)	18 (94.7%)	0.14
Age (years)	53 [39, 67]	67 [62, 74]	**<0.001**	63 [60, 65]	70 [66, 74]	**0.04**
Systolic BP (mmHg)	122 (11)	121 (17)	0.74	127 (20)	117 (14)	0.12
Diastolic BP (mmHg)	77 (7)	77 (11)	0.66	82 (9)	74 (11)	**0.024**
Heart rate (b.p.m.)	64 (10)	73 (15)	**0.003**	79 (15)	69 (14)	0.065
Body mass index (kg/m^2)	23.9 [22.1, 26.1]	25.1 [22.6, 27.5]	0.24	24.6 [23.5, 27.0]	25.5 [21.9, 27.1]	0.69
NYHA						0.52
I	—	4 (12.1%)		2 (14.3%)	2 (10.5%)	
II	—	20 (60.6%)		7 (50.0%)	13 (68.4%)	
III	—	9 (27.3%)		5 (35.7%)	4 (21.1%)	
Diabetes mellitus type 2	0	1 (3.0%)	0.32	1 (7.1%)	0	0.42
Impaired fasting glucose	13 (18.6%)	9 (27.3%)	0.32	5 (35.7%)	4 (21.1%)	0.44
Coronary artery disease	0	7 (21.2%)	**<0.001**	0	7 (36.8%)	**0.013**
Dyslipidaemia	0	11 (33.3%)	**<0.001**	3 (21.4%)	8 (42.1%)	0.28
Hypertension	0	12 (36.4%)	**<0.001**	5 (35.7%)	7 (36.8%)	>0.99
Atrial fibrillation	0	12 (36.4%)	**<0.001**	2 (14.3%)	10 (52.6%)	**0.033**
Active smokers	0	1 (3.0%)	0.32	1 (7.1%)	0	0.42
Packyears smoking history	0 [0, 3.75]	0 [0, 7.5]	0.20	3 [0, 10]	0 [0, 3]	0.10
Acetylsalicylic acid	2 (2.9%)	7 (21.2%)	**0.005**	5 (35.7%)	2 (10.5%)	0.11
Oral anticoagulation	0	16 (48.5%)	**<0.001**	4 (28.6%)	12 (63.2%)	0.08
ACEI/ARB	0	9 (27.3%)	**<0.001**	2 (14.3%)	7 (36.8%)	0.24
Beta-blockers	0	10 (30.3%)	**<0.001**	3 (21.4%)	7 (36.8%)	0.46
MRA	0	7 (21.2%)	**<0.001**	2 (14.3%)	5 (26.3%)	0.67
Loop diuretics	0	18 (54.5%)	**<0.001**	12 (85.7%)	6 (31.6%)	0.004
Thiazides	0	2 (6.1%)	0.1	1 (7.1%)	1 (5.3%)	>0.99
CCB	0	2 (6.1%)	0.1	1 (7.1%)	1 (5.3%)	>0.99
Vitamin supplements	23 (32.9%)	10 (30.3%)	0.83	4 (28.6%)	6 (31.6%)	>0.99
Sodium (mmol/L)	141 [140, 142]	141 [139, 142]	0.67	141 [136, 143]	141 [140, 142]	0.51
Potassium (mmol/L)	3.90 [3.80, 4.00]	4.00 [3.70, 4.30]	0.12	3.75 [3.70, 4.20]	4.10 [3.90, 4.35]	0.13
Urea (mmol/L)	4.8 [4.3, 5.9]	8.4 [6.0, 10.6][1]	**<0.001**	8.6 [5.8, 13.0]	8.4 [6.2, 10.3][1]	0.77
Creatinine (μmol/L)	73 [64, 82]	104 [85, 139]	**0.001**	105 [78, 210]	104 [89, 131]	0.22
eGFR (mL/min/1.73 m^2)	92 [81, 103]	60 [44, 80]	**<0.001**	57 [30, 86]	65 [44, 78]	0.67
CRP (mg/L)	0.80 [0.40, 1.80]	1.60 [1.10, 3.60]	0.15	1.9 [1.3, 3.5]	1.6 [1.0, 2.9]	0.52
hs-troponin T (ng/L)	2 [2, 6]	47 [27, 66][2]	**<0.001**	31 [16, 60]	56 [31, 73][2]	0.12
NT-proBNP (ng/L)	56 [34, 91]	1598 [589, 3492]	**0.013**	810 [390, 4831]	1708 [890, 3016]	0.36
Glucose (mmol/L)	5.10 [4.82, 5.38]	5.30 [4.90, 5.70]	0.22	5.35 [4.90, 5.88]	5.10 [4.95, 5.55]	0.37
Total cholesterol (mmol/L)	5.00 [4.70, 5.30]	4.60 [3.80, 5.40]	0.35	5.10 [4.50, 5.88]	4.00 [3.20, 4.80]	**0.015**
HDL cholesterol (mmol/L)	1.71 [1.49, 2.05]	1.49 [1.24, 1.63]	**<0.001**	1.52 [1.37, 1.63]	1.43 [1.19, 1.68]	0.35
LDL cholesterol (mmol/L)	2.90 [2.40, 3.30]	2.70 [1.90, 3.10]	0.84	3.10 [2.47, 3.98]	2.10 [1.55, 2.85]	**0.03**
Triglycerides (mmol/L)	0.88 [0.65, 1.06]	0.89 [0.71, 1.15]	0.29	1.12 [0.98, 1.28]	0.79 [0.67, 0.92]	**0.037**
TSH (mU/L)	2.25 [1.62, 2.85]	2.10 [1.28, 3.05]	0.39	2.12 [1.34, 4.87]	1.67 [1.31, 2.97]	0.74
Haemoglobin (g/L)	141 [134, 153]	135 [122, 149]	**0.023**	128 [112, 147]	136 [128, 150]	0.18
Haematocrit	0.43 [0.41, 0.46]	0.41 [0.37, 0.46]	**0.021**	0.39 [0.33, 0.46]	0.41 [0.40, 0.46]	0.21
Leucocytes (10^9/L)	5.15 [4.52, 5.64]	5.98 [4.92, 7.19]	**0.005**	6.64 [5.52, 7.97]	5.68 [4.72, 6.88]	0.18

ACEI, angiotensin-converting enzyme inhibitor; AL, amyloid light-chain; ARB, angiotensin receptor blocker; ATTR, amyloid transthyretin; BP, blood pressure; CA, cardiac amyloidosis; CCB, calcium channel blocker; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (CKD-EPI); HC, healthy controls; HDL, high-density lipoprotein; hs-troponin T, high-sensitive troponin T; LDL, low-density lipoprotein; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; NYHA, New York Heart Association; TSH, thyroid-stimulating hormone.

Statistics presented: *n* (%); median [interquartile range]; mean (standard deviation). Significant *P*-values are highlighted (bold).

Table 2 Vascular outcome parameters (unadjusted)

	HC, $N = 70$	CA, $N = 33$	P
FIDa (%)	3.55 (1.82)	1.52 (1.73)	**<0.001**
FIDv (%)	4.03 (2.00)	3.27 (1.58)	**0.045**
FICa (%)	−0.57 (1.13)	−0.36 (1.02)	0.370
AVR	0.86 (0.06)	0.87 (0.08)	0.358
CRAE (mu)	185.94 (14.13)	183.08 (18.04)	0.438
CRVE (mu)	216.95 (14.65)	210.04 (14.84)	**0.034**
FMD (%)	6.38 (3.22)	4.55 (2.50)	**0.003**
GTN (%)	18.33 (5.93)	15.66 (7.63)	0.136
PWV (m/s)	6.87 (1.43)	8.74 (2.34)	**<0.001**
AI (%)	21.10 (12.53)	19.37 (10.40)	0.476

AI, augmentation index; AVR, arterio-venous ratio; CA, cardiac amyloidosis; CRAE and CRVE, central retinal artery and vein equivalent; FICa, arterial flicker-induced constriction; FIDa, arterial flicker-induced dilatation; FIDv, venous flicker-induced dilatation; FMD, flow-mediated dilatation; GTN, dilatation in response to glycerol trinitrate; HC, healthy controls; mu, measuring units; PWV, pulse-wave velocity.
Parameters are presented as mean and standard deviation. Pairwise comparisons were carried out by Welch's *t*-test for heteroscedastic data. Significant *P*-values are highlighted (bold).

after IPW; *Figure 1*). Similarly, other DVA-derived vascular parameters were not significantly different after IPW (Supporting Information, *Table S2*).

Cardiac amyloid light-chain amyloidosis versus amyloid transthyretin amyloidosis

Flicker-induced arterial dilatation and other vascular parameters were similar in both groups (Supporting Information, *Table S3*, unadjusted) even after IPW (FIDa for AL 1.76 ± 2.25% vs. ATTR 2.00 ± 1.72%; $P = 0.781$), except for potentially better AVR ($P = 0.04$) and AI ($P = 0.01$) (Supporting Information, *Table S2*) in ATTR patients.

Association of vascular function with biomarkers

Retinal vascular function was significantly associated with cardiac and renal biomarkers. FIDa negatively correlated with log-scaled troponin T (Pearson's $r = -0.49$; $P < 0.001$), log-scaled NT-proBNP ($r = -0.51$; $P < 0.001$), and positively for eGFR ($r = 0.33$; $P < 0.001$) (*Figure 2*).

Discussion

In this study, we demonstrate impaired retinal arterial flicker-induced dilatation in patients with CA compared with HC. Both AL and ATTR CA seem to be similarly affected. Patients with CA also showed signs of increased arterial stiffness (as evidenced by increased PVW), but no difference in endothelial function of larger vessels (FMD). The association of retinal microvascular dysfunction with cardiac and renal biomarkers for CA severity may be of further importance.

This study adds further evidence of vascular dysfunction in CA using DVA; this novel method assesses the microvascular function of the ocular fundus. In this cohort of CA patients, arterial flicker-induced dilatation was significantly impaired compared with HC indicating microvascular dysfunction. These results are in line with previous studies assessing DVA in patients with cardiovascular risk factors or established cardiovascular disease.[13,25] Recent studies emphasize the importance to differentiate between the microvasculature and macrovasculature, particularly in cardiovascular disease.[5,13] However, the currently available literature focusing on microcirculation in amyloidosis, even more specifically in CA, is

Figure 1 Primary and secondary outcomes adjusted for potential covariates. (A) Arterial flicker-induced dilatation (primary study outcome), (B) pulse-wave velocity, and (C) flow-mediated dilatation between healthy controls and patients with cardiac amyloidosis. Marginal means with 'sandwich'-robust standard errors are shown after inverse probability weighting as dynamite charts. Significant differences are starred ***$P < 0.001$; *$P = 0.018$.

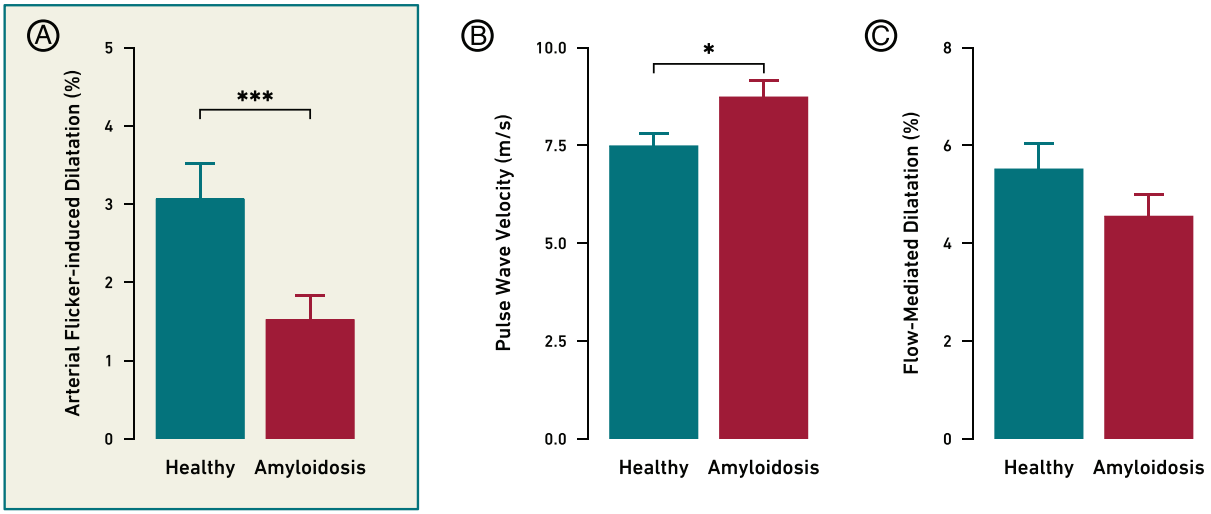
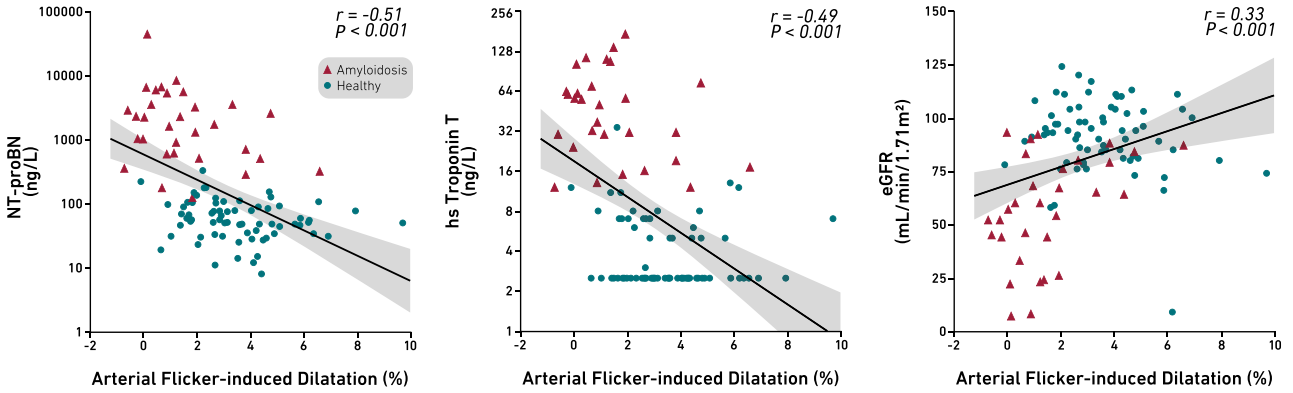


Figure 2 Correlation. The primary outcome (arterial flicker-induced dilatation) correlates significantly with estimated glomerular filtration rate (eGFR), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and high-sensitivity (hs) troponin T concentrations. Circles represent healthy individuals, and triangles amyloidosis patients. Pearson's *r* and *P*-value are reported; Spearman's rho was similar (data not shown).



scarce. Of importance, CA patients can suffer from angina, without relevant coronary stenosis. In such patients, Dorbala *et al*.[9] demonstrated microvascular dysfunction via decreased myocardial blood flow and coronary flow reserve, using positron emission tomography. CA is considered the aetiology of a specific form of heart failure with preserved ejection fraction (HFpEF), for which microvascular endothelial dysfunction may represent a main mechanism.[26]

Notwithstanding, there can potentially be a variety of alternative or complementary explanations for the observed microcirculatory impairment in amyloidosis, it being a multisystemic disease. Amyloid fibril deposits are found beyond epicardial coronary vessels,[6,8] particularly affecting small arteries.[7] Histopathological evidence revealed, besides luminal obstruction, microvascular function is impaired further by the increase in fibrosis due to the modulation of the interstitial matrix resulting in external compression of the vessel.[27] The simple structural constraint of the fibrils on functional dilatation may result in reduced vascular elasticity. Nitric oxide-induced brachial artery dilatation (GTN), a marker of structural impairment, tended to be smaller in CA compared with HC. A cause of vascular dysfunction may also be a toxic effect of amyloid and its precursor proteins[2] that may directly damage the endothelium.[28]

In interpreting these results of a profound impairment of retinal vessel function, comprehensive testing of vascular function with other modalities and in other vascular beds is essential. Intriguingly, PWV was increased in CA compared with controls, indicating increased arterial stiffness that is well established to be associated with microvascular dysfunction.[29] Of note, FMD was not reduced in CA compared with HC under the condition of the present study that was powered to detect differences in retinal function. FMD has been assessed in two studies in amyloidosis before.[10,11] Whereas Modesto *et al*. showed decreased dilatation in 59 AL-amyloidosis patients,[10] similar to the trend observed in our cohort, results from a different study documented higher FMD in AL amyloidosis suggesting paradoxically abnormal vasoreactivity.[11] These results indicate that FMD may be challenging to interpret in amyloidosis due to autonomic dysfunction and hypotension.[11] The retinal vascular system is unique in this respect, as in contrast to brachial arteries, it is only minimally influenced by the autonomic nervous system.[12] Both FMD and DVA are based on the similar principle: reactive hyperaemia,[17,30] subsequent endothelial nitric oxide release, and vasodilation of the smooth muscle vascular cells.[15] There are likely several factors to why FIDa, but not FMD, is impaired in CA patients in the present study. Importantly, different vascular beds are assessed—with DVA focusing on the microcirculation and FMD on larger arteries. In addition, FMD is subject to high intra-observer and inter-observer variability.[17] Also, the present study was powered to detect differences in DVA and not FMD and could not adjust for concomitant renin-angiotensin-system inhibitors known to improve FMD.[17]

In this study, we examined patients with AL and ATTR amyloidosis. While the distinction between AL and ATTR amyloidosis is clinically and pathophysiologically important and the similar general infiltrative mechanism of amyloid fibrils, AL CA, and ATTR CA is characterized by different clinical progression, therapy, and outcome,[2] the results of the present study indicate that vascular function did not differ between patients with AL and ATTR CA.

Endothelial function, a measurable endpoint of combined risk factors, has been suggested to characterize disease risk, severity, and prognosis.[4] Interestingly, in renal and heart disease, DVA gives valuable information on disease severity and prognosis.[13,25] In amyloidosis, one study predicted mortality using non-invasive FMD.[11] Stamatelopoulos *et al*. found abnormal vascular reactivity operationalized as high FMD correlates with increased mortality significantly even after correction for established prognosis markers. To date in CA,

prognostic value is estimated by scores that prominently incorporate the biomarkers troponin T, NT-proBNP, and estimated GFR in AL and ATTR CA.[2,31] In this regard, our observation of a strong association of these biomarkers with retinal vascular function is reaffirming: the vasculature of the eye may indeed reflect the extent of amyloid affection and, potentially, prognosis.

However, as outlined above, endothelial dysfunction may be impacted by various other cardiovascular diseases in this cohort, including kidney dysfunction and CAD. Although, and in contrast to CAD,[13] renal function has not been directly analysed with respect to retinal vessel function, its impact can most likely be assumed due to indirect data from our previous studies[5] and current reviews.[32] Due to the comparison with HC, both disease entities could not be accounted for in IPW, hence, may potentially weaken as possible confounders the current study results. Additionally, almost all AL patients received chemotherapy—agents known to be associated with cardiovascular toxicity.[33] In future studies, cross-sectional comparisons to patients with similar cardiovascular comorbidities and to patients with amyloidosis without cardiac manifestation are needed to specifically assign microvascular dysfunction to amyloidosis and CA, respectively. In addition, prospective longitudinal data are needed to establish DVA as utility tool for the impact of treatment, prognosis, and risk stratification in CA.

Limitations

The sample size of our prospective observational study was limited due to the rarity of the disease. Although IPW was used to account for potential confounders, residual confounding between CA patients and HC cannot be ruled out. However, because of both the very definition of HC and the multisystemic nature of amyloidosis, the overlap in cardiovascular risk factors between both groups is limited; consequently, not all confounders of endothelial dysfunction (including kidney dysfunction and CAD) could be accounted for. Our study also lacked power for additional analyses such as the degree of organ affection and cardiac involvement (e.g. ventricular vs. atrial affection), variant versus wild-type ATTR CA, or the degree of prior therapies. Of note, we only studied patients with CA, whereas amyloidosis patients without cardiac affection may differ from our population. Because this study is limited to a cross-sectional design, we could not provide data on the evolution of vascular dysfunction throughout the disease course.

Conclusions

In this study, patients with CA showed signs of significant retinal microvascular dysfunction and arterial stiffening indicating systemic vascular dysfunction. The association of impaired retinal dilatation with biomarkers of the disease corroborates that the vasculature of the eye may indeed mirror the extent of amyloid affection and, potentially, prognosis. Future studies focusing on longitudinal assessments before and after disease-modifying therapy may provide further evidence on the role of microvascular dysfunction in CA.

Conflict of interest

A.v.E., E.Z., D.N., L.K., and T.H. declare no conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. ***Love plots*** Standardized mean difference (SMD) before (red) and after (green) inverse probability weighting for potential confounders by doubly robust entropy balance. SMD of 0.1 or lower suggests balance has been achieved. Left Healthy vs. Cardiac Amyloidosis; Right AL vs. ATTR Amyloidosis; BMI: Body mass index; BP blood pressure. LDL low-density lipoprotein.

Table S1. Baseline characteristics after inverse probability weighting.

Table S2. Vascular outcome parameters after IPW.

Table S3. Vascular outcome parameters (unadjusted) for AL vs ATTR amyloidosis.

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