Imaging the Retina in Alzheimer's Disease

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CHAPTER 6

Is retinal vasculature a biomarker in amyloid proven Alzheimer's disease?

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Abstract

The retina is a potential source of non-invasive vascular biomarkers for Alzheimer's disease (AD). We assessed retinal microvasculature in well-characterized AD cases, taking ophthalmological confounders into account. We included 48 amyloid-positive AD patients and 38 amyloid-negative cognitively normal controls. All participants underwent ophthalmological screening to exclude interfering ocular disease. Using a multimodal approach, we measured retinal vascular parameters, choroidal thickness, macular vascular density and foveal avascular zone size (FAZ). We found no disease effects on retinal vascular measures [all β's < |0.15|, all p>0.2], adjusted for confounders. Venular tortuosity was inversely associated with Fazekas-score in controls [β -0.56, p<0.01], while vessel density in the outer ring of the macula was inversely associated with Fazekas-score in AD cases [β -0.64, p<0.01]. In conclusion, retinal vasculature did not discriminate AD from controls, despite evident changes on clinical, neuro-imaging and CSF biomarkers, challenging the use of retinal vasculature measurements as AD biomarker.

Background

Alzheimer disease (AD) pathophysiology is characterized by amyloid-beta (Aβ) accumulation, tau hyperphosphorylation, and neurodegeneration, ultimately leading to cognitive decline[1]. In addition, vascular changes are involved in AD pathophysiology. Vascular changes, either intrinsic or as co-pathology, interact with AD pathology, neurodegeneration, and cognitive impairment[2,3], and are described with the term vascular cognitive impairment (VCI)[4,5]. Vascular pathology is prevalent, and described at autopsy in up to 75% of dementia cases[4,6]. Vascular changes include white matter hyperintensities (WMHs) as a result of chronic (subcortical) ischemia of small vessels, large vessel infarcts, lacunar infarcts (e.g. thalamus), arterio-and atherosclerosis and cerebral amyloid angiopathy (CAA)[7,8]. CAA, is an intravascular pathology in which Aβ is deposited in the vessel wall, and is associated with micro-hemorrhages and micro-infarcts[9]. Its prevalence is between 20-40% in non-demented elderly and 50-60% in demented elderly in autopsy studies[9,10]. A strong correlation between AD and CAA exists, as in post mortem AD brains, in 85-95% of the cases CAA is found[9]. Moreover, recent studies also showed that decreased cerebral blood flow and blood-brain barrier alterations might be involved in AD pathophysiology, possibly playing a role in Aβ clearance[2,3]. Currently used biomarkers for vascular changes are limited to MRI[8]. To better understand and measure vascular changes in AD, new biomarkers for vascular changes might increase pathophysiological insight, and could complement the currently used ATN system for amyloid(A), tau(T) and neurodegeneration(N)[11].

The retina is a possible source of vascular biomarkers as retinal vasculature can be imaged non-invasively at the micro-meter level using different imaging modalities, including fundus photography, measurements of choroidal thickness (using Enhanced Depth Imaging Optical Coherence Tomography (EDI-OCT)) and OCT angiography (OCTA). Vascular changes in the retina have been described in AD, including changes in vascular parameters from fundus photography analysis, using Singapore Vessel I analysis (SIVA) software, such as increased venous diameter, decreased arterial diameter and decreased fractional dimension[12]. In contrast, a recent report showed an absence of group differences between AD and control participants, while showing decreased total and arteriolar fractal dimensions in VCI cases[13]. In addition, choroidal thinning in AD is reported in several studies using EDI-OCT[14-17]. Recent reports on retinal vasculature with OCTA reported increased foveal avascular zone (FAZ) size and decreased vessel density and flow in AD[18,19] and preclinical AD[20].

As ophthalmological comorbidity could potentially influence retinal vascular measurements and is often asymptomatic, a thorough ophthalmological screening is warranted. Secondly, assessing retinal biomarkers in patients with a confirmed AD

diagnosis by established biomarkers such as cortical atrophy, amyloid and tau in CSF or amyloid-PET supports the clinical diagnosis on the one hand and allows to compare the new biomarkers to the gold standard on the other hand.

The aim of this study was to identify retinal vascular biomarkers as possible non-invasive biomarkers in AD. Following previous publications we hypothesize to find increased CRAE, decreased CRVE, decreased FD, thinner choroidal thickness, smaller vessel density and larger foveal avascular zone in AD compared to control cases. We therefore measured retinal vascular parameters using fundus photography, EDI-OCT and OCTA in well characterized amyloid positive AD cases while taking ophthalmological confounders into account. In addition, we assessed relationships between retinal vascular parameters and WMHs on MRI.

Methods

Subjects

We assessed 50 AD patients and 38 controls from the Amsterdam Dementia Cohort (ADC) that were enrolled into our retinal imaging cohort as described earlier (all Mini-Mental State examination ≥17, capable of giving informed consent)[21]. In brief, all patients and controls underwent a standardized screening protocol including (medical) history, neuropsychological examination, blood draw for APOE genotype, blood pressure measurements, neuro-imaging and lumbar puncture[22]. All patients met NIA-AA criteria of AD and had evidence of amyloid pathology based on cerebrospinal fluid (CSF) analysis or amyloid-positron emission tomography (amyloid-PET)[11]. Controls were subjects with cognitive complaints that showed no evidence of objective cognitive impairment, neurodegeneration or amyloid pathology based on amyloid-PET or CSF analysis.

MRI scanning

MRI scans were reviewed by an experienced and blinded rater (FB) before the multidisciplinary meeting of the ADC, where a clinical diagnosis was made by consensus. Visual rating scores for atrophy on MRI were determined based on T1-weighted images and included medial temporal lobe atrophy (MTA), global cortical atrophy (GCA), and parietal cortical atrophy (PCA)[23-25]. Vascular assessment included Fazekas score for white matter hyperintensities (FLAIR sequence), assessment of lacunar infarcts (FLAIR, T2-weighted sequences) and microbleeds (T2*-sequence)[26].

Cerebrospinal fluid analysis

CSF was analysed using Innotest ELISA and measured amyloid-beta$_{1-42}$(Aβ_{1-42}), tau$_{181}$ and phosphorylated Tau (pTau). A tau$_{181}$/Aβ_{1-42} ratio ≥.52 was considered an AD profile[27].

Amyloid-PET analysis

A subset of cases was enrolled in research programs that included amyloid-PET scanning with the following tracers: ^{18}F-Florbetaben (NeuraCeq)(n=24), ^{18}F-Florbetapir (Amyvid)(n=9) and Pittsburgh compound (^{11}C-PIB)(n=3). Parametric Standardized Uptake Value (SUV) images of amyloid-PET scans were assessed by an experienced rater (BvB) and visually interpreted as amyloid positive or amyloid negative following guidelines for individual tracers.

Ophthalmological assessment

Subjects were included within a year after the ADC diagnostic screening program and underwent the following eye examinations to exclude possible confounding ophthalmological pathology: best corrected visual acuity, intraocular pressure (IOP) using non-contact tonometry (if IOP >20 mmHg, we used contact applanation tonometry), slit-lamp examination of the anterior and posterior segment, Heidelberg Retinal Tomography (HRT) optic nerve head analysis, and Frequency Doubling Technology (FDT) for visual fields. Tropicamide 0.5% was administrated for pupil dilation to facilitate optimal ophthalmic examination. We followed the fourth European Glaucoma Guideline criteria: glaucoma was diagnosed when two of the three following measurements were abnormal: ocular pressure (>21 mmHg), structural glaucomatous changes (examined with HRT using the Moorfields Regression Analysis), and functional changes (examined with Frequency Doubling Technology)[28]. All examinations were interpreted by an experienced ophthalmologist (FDV). Exclusion criteria were ophthalmological conditions interfering with imaging or retinal vasculature, such as severe cataract, age-related macular degeneration and glaucoma or systemic conditions such as diabetes mellitus.

Retinal vascular imaging

Retinal vasculature was measured by personnel blinded for diagnosis, using three imaging modalities: 1) fundus photography, 2) enhanced depth imaging OCT (EDI-OCT) and 3) OCT angiography (OCTA)(Figure 1).

Digital fundus images of 50° field of view of the macula and optic nerve head (ONH) were obtained using a Topcon TRC 50DX type IA (Topcon Medical Systems, Inc., Oakland, CA, USA). Macular photographs were assessed for incident pathology by an experienced ophthalmologist (FDV), while ONH photographs were used for Singapore

Vessel I (SIVA) analysis (version 3.0; National University of Singapore, Singapore)[29-31] by two trained raters (JdH, JAvdK). Values from both eyes of every participant were averaged; if only one suitable image was present, only this eye was included (n=3). We assessed vascular parameters that were earlier found to have high intra-observer intra-class correlation (>0.80)[32], namely: central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), arteriole–venule ratio (AVR), fractal dimension of the arteriolar network (FDa), fractal dimension of the venular network (FDv), curvature tortuosity of the arterioles (cTORTa), and curvature tortuosity of the venules (cTORTv). We averaged vascular parameters of two raters. Intra-observer intra-class correlation coefficients of the current study are shown in Supplement Table 1.

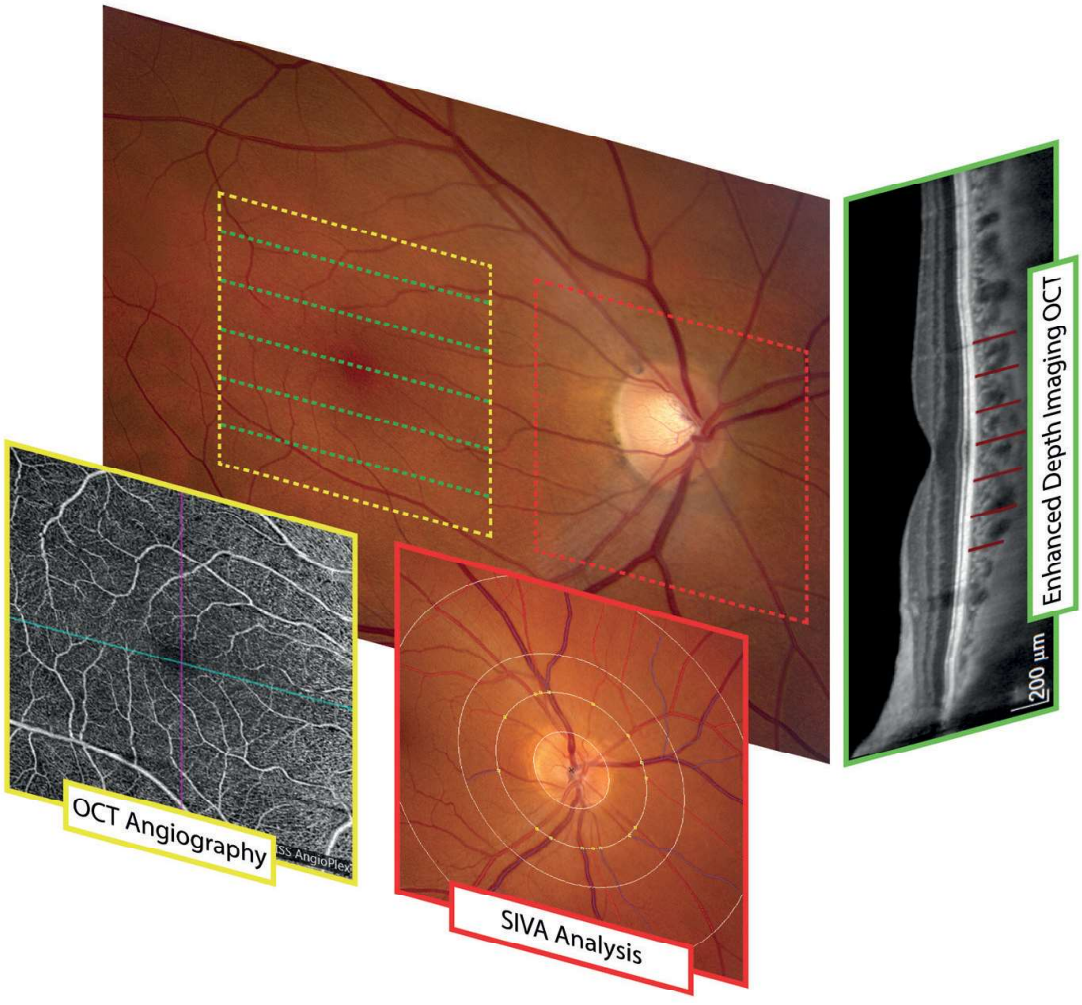

Figure 1 Overview of retinal modalities to assess microvasculature

Retinal vasculature was assessed using three imaging modalities: retinal vascular parameter analysis using SIVA analysis applied on fundus photographs (red), choroidal thickness measurements using Enhanced Depth Imaging Optical Coherence Tomography (EDI-OCT)(green) and vessel density and foveal avascular zone (FAZ) measured with OCT Angiography (OCTA)(yellow).

EDI-OCT scans were acquired with a Heidelberg Spectralis Spectral Domain (SD)-OCT, using the following protocol: central retina (macula) fast horizontal scanning; central 20°x20° area; 25 B-scans (averaging 9 frames per b-scan); 512 a-scans per b-scan. Manual measurements of choroidal thickness were performed in five evenly distributed b-scans per macular volume scan (Supplemental Figure 1)[14,16,17]. Five measurements per b-scan were performed: foveal, 1 mm nasal and temporal from the fovea and 2 mm nasal and temporal from the fovea. The anterior boundary of the choroid layer was defined as the hyper-reflective band corresponding to the Retinal Pigment Epithelium – Bruch's membrane complex. To define the posterior boundary, each measurement point was categorized based on the presence or absence of the choroidal-scleral interface (CSI) as a hyper-reflective band, the suprachoroidal space (SCS) as a hypo-reflecting band and/or a smooth line marking the posterior boundary on the OCT image. The posterior boundary was then defined as the outer limit of the CSI, the inner limit of the SCS or the smooth line. If no clear boundary was identifiable over the length of the scan, the image was defined as ungradable. The averages of the measurements (n=25) from both eyes were used for analysis of mean choroidal thickness.

OCTA was acquired using a Zeiss Model 5000 SD-OCT with Angioplex, and consisted of 6x6mm scans of the macula, 350 b-scans each. Vascular density measured in the inner ring [Ø 1-3 mm around fovea], and outer ring [Ø 3-6 mm around fovea] of the Early Treatment in Diabetes Retinopathy Study (ETDRS) grid. Foveal avascular zone surface area was measured. Scans were visually assessed for quality. All scans had quality factors ≥7/10 and could be considered 'good' quality.

2.7 Statistical analysis

2.7.1 Power calculation

Based on a previous meta-analysis[12], comparing 374 AD cases compared to 707 controls, a decrease of 7.52 µm in CRAE can be expected. Assuming a true effect of 7.52 µm and a standard deviation of ≈15 µm, 28 subjects in each group are needed to reject the null hypothesis of no difference between the disease and control group with a power of 0.80. In addition, from the same meta-analysis a decrease of 10.74 µm in CRVE can be expected. Assuming a true effect of 10.74 µm and a standard deviation of ≈15 µm, 15 subjects in each group are needed to reject the null hypothesis of no difference between the disease and control group with a power of 0.80. From a previous report[16], a decrease of ≈60 µm in choroidal thickness can be expected. Assuming a true effect of 60 µm and a standard deviation of ≈50 µm, 6 subjects in each group are needed to reject the null hypothesis of no difference between the disease and control group with a power of 0.80. We included >35 participants per disease group.

2.7.1 Data analysis

Data were visually tested for a normal distribution using histograms and Q-Q plots. Measures that were normally distributed were tested with an independent t-test, non-normally distributed measures with a Mann-Whitney U test, and binary variables with a Chi-Squared test. Linear regression models were used to assess if changes in retinal vasculature (dependent) were attributable to diagnosis (independent) or age, sex, spherical equivalent, quality factor and/or hypertension (covariates). All betas reported are standardized betas. Stratified analysis for APOE genotype carriers versus non-carriers was performed for retinal vascular parameters. Intra observer intra class correlation (absolute agreement) was calculated to test intra-rater variability for SIVA analysis. Data analysis was performed with IBM SPSS Statistics (version 22.0).

Results

Table 1 shows cohort characteristics. We included 50 AD patients and 38 controls from our retinal imaging[21] cohort with retinal vasculature imaging available. One AD case was excluded because of glaucoma. In addition, we excluded one AD case that was later found to have a progranulin mutation, a mutation known to directly affect retinal integrity[33]. As a result, fundus photographs were available in 48 AD and 38 controls participants. EDI-OCT and OCTA became available in the course of the study and were obtained in 41 AD and 31 control participants for EDI-OCT and 26 AD and 31 control participants for OCTA. AD cases were slightly older (65.4y vs 60.6y, $p<0.01$) and had MMSE- and MRI atrophy scores indicative of an AD diagnosis. There was no difference between groups for WMH and lacunes indicating similar cerebrovascular loading. By design, all AD cases were amyloid positive and all control participants were amyloid negative based on CSF(n=50), amyloid-PET(n=11) or both(n=25). As expected APOE-ε4 carriers were more prevalent in AD cases (70.2% vs. 29.4% in controls)[34]. Systolic blood pressure (SBP), pulse pressure (PP) and mean arterial pressure (MAP) were higher in controls (independent t-test, $p=0.02$, $p<0.01$, $p=0.01$ respectively).

		Alzheimer's disease	Controls	p-value
Number		48	38	
Sex (m/f)		25/23	24/14	0.24*
Age		65.4(±8.1)	60.6(±5.0)	**<0.01****
MMSE		23(±3)	29(±1)	**<0.01****
Body Mass Index (kg/m^2)		24.4(±3.1)	26.2(±4.1)	0.32**
APOE genotype[1]	E4 homozygous (n (%))	11(23.4)	1(2.9)	**<0.01***
	E4 heterozygous (n (%))	22(46.8)	9(26.5)	0.05*
	E4 negative (n (%))	14(29.8)	24(70.6)	**<0.01***
Blood Pressure measures	Systolic Blood Pressure (mmHg)	148.9(±17.5)	139.0(±18.4)	**0.02****
	Diastolic Blood Pressure (mmHg)	84.2(±10.1)	82.8(±9.1)	0.55**
	Pulse Pressure (mmHg)	64.7(±12.6)	56.2(±12.5)	**<0.01****
	Mean Arterial Pressure (mmHg)	84.4(±64.1)	59.3(±83.5)	**0.01****
MRI[2]	Global Cortical Atrophy (GCA)	1(0-2)	0(0-1)	**<0.01****
	Medial Temporal Lobe Atrophy (MTA)	1.5(0-2.5)	0(0-2)	**<0.01****
	Parietal Cortical Atrophy (PCA)	1(0-3)	0(0-1)	**<0.01****
	Fazekas score	1(0-3)	1(0-2)	0.08***
	Microbleeds (n)	0.1(±0.4)	2.7(±14.6)	0.35***
	Lacunar infarcts (n)	0(±0)	0.2(±0.6)	**0.02*****
CSF[3]	$A\beta_{1\text{-}42}$ (ng/L)	555.1(±106.2)	1162.3(±200.0)	**<0.01*****
	Tau_{181} (ng/L)	711.5(±304.5)	242.2(±85.7)	**<0.01*****
	pTau (ng/L)	89.0(±28.3)	43.0(±11.8)	**<0.01*****
	$Tau_{181}/A\beta_{1\text{-}42}$ ratio	1.3(±0.5)	0.2(±0.1)	**<0.01*****
Aβ-PET[4]	Positive/negative	17/0	0/19	**<0.01*****
Ophthalmological	Intra ocular pressure (mmHg)	16.3(±2.3)	16.0(±2.1)	0.39***
	Visual Acuity (LogMAR)	0.0(±0.1)	-0.1(0.1)	**<0.01*****

Table 1 Cohort Characteristics

*Chi-Square test, **independent-samples t-test, ***Mann-Whitney U test, [1]APOE genotype was available in 47 AD cases and 34 controls, [2]MRI was available in 47 AD cases and 35 controls, [3]CSF was available in 46 AD cases and 29 controls,[4]Amyloid-PET was available in 17 AD cases and 19 controls

Retinal vasculature parameters

Singapore I Vessel Analysis (SIVA)

Seven retinal vascular parameters, previously shown to have good reproducibility[32], showed no group differences between AD and control participants (Figure 2A). Linear regression models assessing relationships between retinal vascular parameters and diagnosis, adjusted for age, and spherical equivalent (SE), showed no disease effect (Table 2). CRVE and FDa were found to be associated with age [β -0.25, p=0.03] and [β -0.28, p=0.02] respectively (Table 2). Adjusting analysis for SBP, PP or MAP did not alter disease effects.

Choroidal thickness analysis

Choroidal thickness in the macula showed no group differences between AD (246.4 μm, ±82.0) and control participants (251.3 μm, ±68.6)(independent t-test, p=0.588)(Figure 2B). Linear regression models assessing relationships between choroidal thickness and diagnosis, adjusted for age, and spherical equivalent (SE), showed no disease effect [β 0.14, p=0.24], but was associated with age [β -0.34, p=0.01] and SE [β 0.43, p=0.01] (Table 2). Adjusting analysis for different measures of blood pressure did not alter AD disease effects.

OCTA analysis

Vessel density in the inner and outer ring of the macula and the size of the foveal avascular zone (FAZ) showed no group differences between AD and control participants (Figure 2C). Linear regression models assessing relationships between OCTA measures and diagnosis, adjusted for age, SE and quality factor, showed no disease effect for vessel density in the inner ring [β 0.02, p=0.85] or outer ring [β -0.10, p=0.36], or FAZ [β -0.14, p=0.33]. Vessel density was however strongly associated with quality factor (Inner Ring [β 0.77, p<0.001], Outer Ring [β 0.65, p<0.001]), unlike FAZ [β -0.04, p=0.81] (Table 2). Adjusting analysis for different measures of blood pressure did not alter AD disease effects.

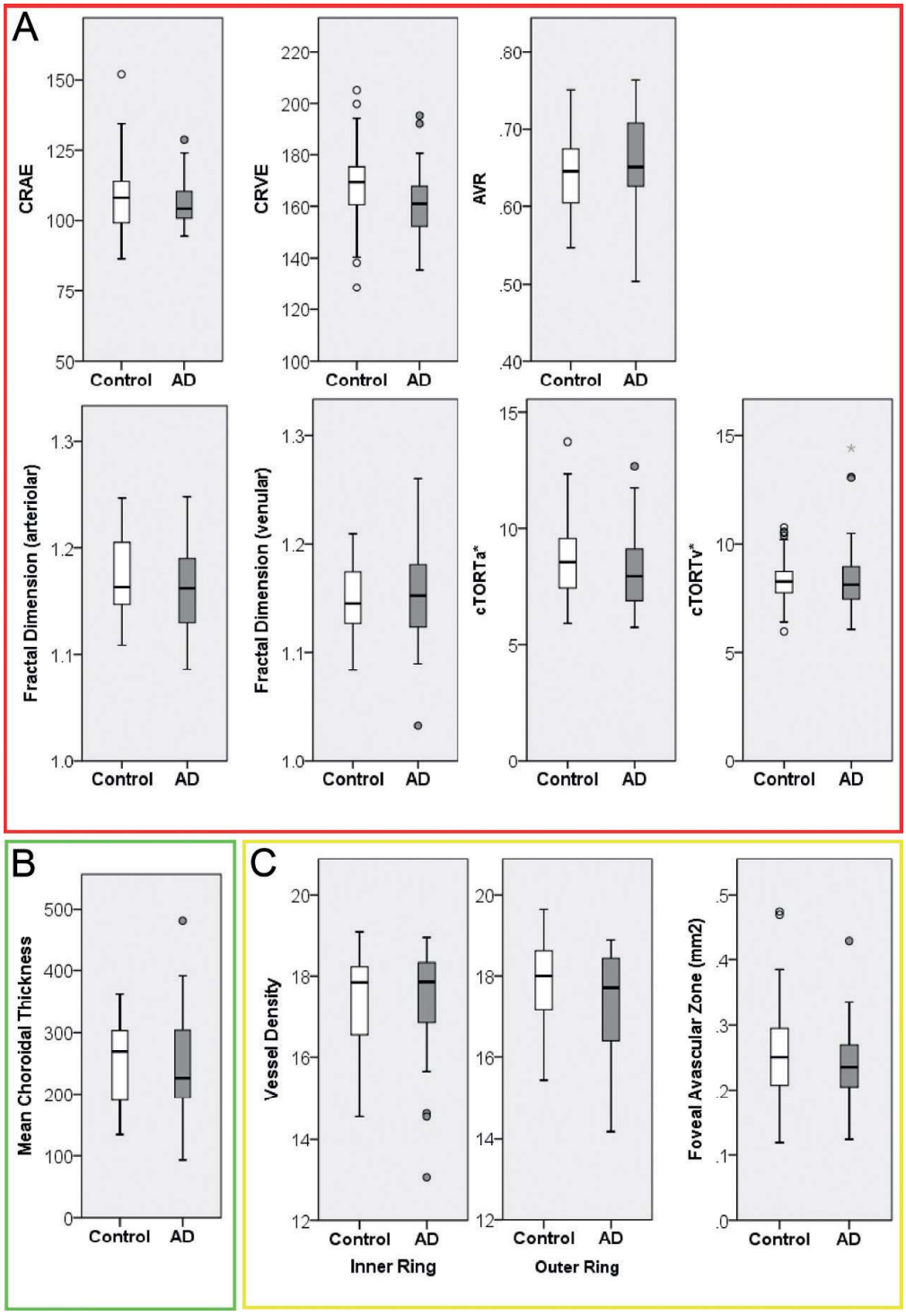

Figure 2 Vascular parameters measured with fundus photography, EDI-OCT and OCTA

A) Vascular parameters calculated using Singapore I Vessel Analysis (SIVA) software included central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), arteriole–venule ratio (AVR), fractal dimension of the arteriolar network (FDa), fractal dimension of the venular network (FDv), curvature tortuosity of the arterioles (cTORTa, values $*10^{-5}$), and curvature tortuosity of the venules (cTORTv, values $*10^{-5}$), compared between Alzheimer's disease participants (AD) and controls (HC).
B) Mean choroidal thickness measured in the macula (µm) in AD.
C) Vascular parameters from Optical Coherence Tomography Angiography (OCTA). Vessel density in the inner and outer ring of the ETDRS-grid and surface area (mm^2) of the foveal avascular zone, compared between AD and control participants.

Retinal vasculature parameter		Mean(sd)		Age		SE		AD		Quality Factor	
		AD	Controls	β	p-value	β	p-value	β	p-value	β	p-value
SIVA-analysis	CRAE	106.1(±7.5)	107.9(±12.1)	-0.12	0.32	0.16	0.17	-0.08	0.49	n.a.	n.a.
	CRVE	161.6(±12.2)	168.1(±15.6)	-0.25	**0.03**	0.19	0.08	-0.13	0.27	n.a.	n.a.
	AVR	0.66(±0.05)	0.64(±0.06)	0.13	0.27	-0.03	0.78	0.05	0.68	n.a.	n.a.
	FDa	1.16(±0.04)	1.17(±0.04)	-0.28	**0.02**	0.15	0.17	-0.05	0.68	n.a.	n.a.
	FDv	1.15(±0.04)	1.15(±0.04)	-0.15	0.21	-0.02	0.83	0.14	0.23	n.a.	n.a.
	cTORTa[1]	8.1(±1.5)	8.7(±1.9)	-0.05	0.69	0.04	0.74	-0.15	0.22	n.a.	n.a.
	cTORTv[1]	8.5(±1.7)	8.4(±1.2)	-0.05	0.66	0.09	0.41	0.06	0.61	n.a.	n.a.
EDI-OCT	Choroidal Thickness (μm)	246.4(±82.0)	251.3(±68.6)	-0.34	**<0.01**	0.43	**<0.01**	0.14	0.24	n.a.	n.a.
OCTA	Vessel Density Inner Ring	17.3(±1.5)	17.4(±1.2)	0.07	0.49	0.08	0.37	0.02	0.85	0.77	**<0.01**
	Vessel Density Outer Ring	17.4(±1.3)	17.8(±1.1)	-0.07	0.50	0.16	0.11	-0.10	0.36	0.65	**<0.01**
	Foveal Avascular Zone (mm^2)	0.24(±0.06)	0.26(±0.08)	0.04	0.81	0.07	0.60	-0.14	0.33	-0.04	0.81

Table 2 Associations of vascular parameters with diagnosis, age, spherical equivalent, and quality factor

Associations between vascular parameters, age, spherical equivalent, AD-diagnosis, and quality factor from linear regression models. Means and standard deviations and uncorrected p-values are given. [1]Values $*10^{-5}$. Abbreviations: AD = Alzheimer's disease, β = standardized beta, CRAE = Central Retinal Artery Equivalent, CRVE = Central Retinal Vein Equivalent, AVR = Arteriole-Venule Ratio, cTORTa= Curvature Tortuosity of the Arterioles, cTORTv = Curvature Tortuosity of the venules, EDI-OCT = Enhanced Depth Imaging Optical Coherence Tomography, FDa = Fractal Dimension of the Arteriolar Network, FDv = Fractal Dimension of the Venular Network, OCTA = Optical Coherence Tomography Angiography, SE = Spherical Equivalent, SIVA = Singapore I Vessel Analysis.

Relationships between retinal vascular parameters and white matter hyperintensities

To assess relationships between retinal vasculature and intracerebral vascular changes, we correlated retinal vascular parameters with WMH scores on MRI (Fazekas-score), adjusting for age and sex. Vessel density in the outer ring was found to be inversely associated with Fazekas-score in AD participants [β -0.64, p<0.01] while curvature tortuosity of veins was inversely associated with Fazekas-score in controls [β -0.56, p<0.01]. No associations were found between other retinal vascular parameters and WMH scores (Table 3).

Retinal vasculature parameter		White Matter Hyperintensities (Fazekas Score)					
		Total		AD		Controls	
		β	p-value	β	p-value	β	p-value
SIVA-analysis	CRAE	0.03	0.83	-0.05	0.79	0.01	0.98
	CRVE	-0.06	0.560	-0.09	0.57	-0.06	0.74
	AVR	0.07	0.57	0.01	0.97	0.09	0.61
	FDa	-0.11	0.40	-0.12	0.48	-0.14	0.47
	FDv	-0.01	0.94	0.02	0.92	-0.21	0.29
	cTORTa[1]	0.06	0.65	0.27	0.12	-0.29	0.14
	cTORTv[1]	-0.11	0.40	0.10	0.57	**-0.56**	**<0.01**
EDI-OCT	Choroidal Thickness (μm)	-0.07	0.58	-0.06	0.72	0.02	0.92
OCTA	Vessel Density Inner Ring	-0.29	0.09	-0.48	0.05	-0.01	0.98
	Vessel Density Outer Ring	**-0.32**	**<0.05**	**-0.64**	**<0.01**	0.18	0.40
	Foveal Avascular Zone (mm^2)	-0.18	0.29	-0.11	0.61	-0.08	0.69

Table 3 Association between vascular parameters and WMHs in the total cohort and stratified for diagnosis, adjusted for age and sex

Associations between vascular parameters and WMHs (Fazekas score) were assessed using linear regression, adjusted for age and sex. Standardized beta's and uncorrected p-values are given. Significant associations are indicated in bold. Abbreviations: AD = Alzheimer's disease, β = standardized beta, CRAE = Central Retinal Artery Equivalent, CRVE = Central Retinal Vein Equivalent, AVR = Arteriole-Venule Ratio, cTORTa= Curvature Tortuosity of the Arterioles, cTORTv = Curvature Tortuosity of the venules, EDI-OCT = Enhanced Depth Imaging Optical Coherence Tomography, FDa = Fractal Dimension of the Arteriolar Network, FDv = Fractal Dimension of the Venular Network, OCTA = Optical Coherence Tomography Angiography, SIVA = Singapore I Vessel Analysis.

Relationships between retinal vascular parameters and MMSE, CSF $A\beta_{1-42}$ and APOE-ε4 genotype

Both adjusted and unadjusted for age and sex, no relationships between retinal vascular parameters and MMSE or CSF $A\beta_{1-42}$ were observed. Comparing APOE-ε4 carriers (n=44) versus non-carriers (n=37), irrespective of diagnosis, no differences in retinal vascular measures were found (independent samples t-test, all p>0.16).

Stratified analyses for early- versus late onset Alzheimer's disease

Stratifying analyses of retinal vascular parameters, choroidal thickness and OCTA measures for early versus late-onset AD showed no group differences between the two disease group and controls, or between disease groups.

Discussion

In this cross-sectional study using three imaging modalities, we found that there were no group differences in retinal vasculature between well-phenotyped, amyloid confirmed, AD and control cases after correction for age and sex. Stratifying cases for early versus late-onset AD yielded similar results. Vessel density in the outer ring of the macula was found to be associated with WMH scores on MRI in AD participants, while curvature tortuosity of veins was associated with WMH scores in controls.

Our findings of unaltered retinal vascular caliber parameters on fundus photography in AD, confirms a recent study that included biomarker confirmed AD cases (n=29) based on amyloid-PET imaging[13]. In that same study, decreased FDa was observed in subcortical VCI patients[13]. In contrast, other studies found differences in various retinal vascular parameters in different directions, that include *decreased* CRVE[35,36], CRAE[36], FDv[35-37], FDa[35] and cTORTa[37] and *increased* cTORTa[35] and cTORTv[35] in AD compared to control participants. As these cohorts were larger (n>100 per patient group), it might indicate that the effect size of retinal vascular parameters is small, and possibly remained undetected in our study. Alternatively, as previous studies used clinical diagnosis, cohorts could have consisted of dementia cases with a primarily vascular etiology or of cases with relevant vascular co-pathology. In those cases, retinal (micro)vasculature changes may represent vascular (co)pathology rather than Alzheimer's related changes. In the current cohort of amyloid-proven AD participants, clinical, neuro-imaging and fluid biomarkers showed clear disease effects, while an intrinsic AD effect on retinal vasculature was not detected. This queries the clinical use of retinal vascular caliber measurements as AD biomarker.

Using EDI-OCT we did not observe choroidal thinning as previously described by a number of studies[14-17], despite comparable group size and methodology. Not all studies adjusted their analyses for confounders such as age and spherical equivalent, which could have influenced reported findings, as these confounders showed significant effects on choroidal thicknessin the current study contributing to choroidal thinning. Both uncorrected and corrected for age we found no group differences in choroidal thickness. In addition, as our study did not directly take measurement time point into account, we were not able to control for known diurnal variation of choroidal thickness, that

could have possibly influenced our findings, as variations in the order of 15-33 μm are observed over 24 hours[38-40]. However, distribution of measurement time over the day was comparable between groups in our study, with a mean around midday in both groups.

In this study, describing OCTA measurements in amyloid positive AD cases for the first time, we found no differences in vessel density and FAZ, while a strong effect of quality factor (QF) on vessel density measurements was observed [$\beta>0.65$, $p<0.001$] in scans with acceptable quality factors (between 7 and 10). In contrast, previous studies described a decrease in vessel density, and an increase in FAZ surface area in (preclinical) AD cases[18-20]. However, as ophthalmological confounders, age, SE and QF were not always taken into account, findings of those studies may represent an overestimation of true disease effects.

Given the thorough characterization of our participants we were able to correlate retinal vascular measurements with WMH on MRI, $A\beta_{1-42}$, Tau_{-181} and pTau in CSF and MMSE. Venular tortuosity was inversely correlated with WMH scores on MRI in controls, while in contrast an earlier study reported a positive correlation between WMH and venular tortuosity in controls [32]. Confirming an earlier report, we found an inverse association between macular vessel density and WMH scores in AD, possibly reflecting microvasculature changes in chronic cerebral micro-infarction. Similarly, in a recent report a relation between WMH volume and fractal dimension was observed[13]. These findings, need confirmation in larger cohorts, including volumetric measures of WMHs. We found no associations between retinal vascular parameters and CSF biomarkers or MMSE.

A limitation of our study is its relatively small sample size and incomplete collection of all vascular markers, hampering sensitivity for small diseases effects on retinal vasculature. However, despite its relevance for understanding involvement of retinal vasculature in AD pathophysiology, the added value of these small effects for clinical use as biomarker remain doubtful. As our cohort consisted of cases with relatively little vascular comorbidity, studies in VCI and mixed pathology are warranted to assess the use of retinal vascular parameters to detect vascular (co)-pathology in these populations.

Conclusion

Using a multimodal retinal imaging approach in well-characterized amyloid status confirmed AD and control cases, we found no evidence that retinal vasculature can be used as non-invasive biomarker for AD.

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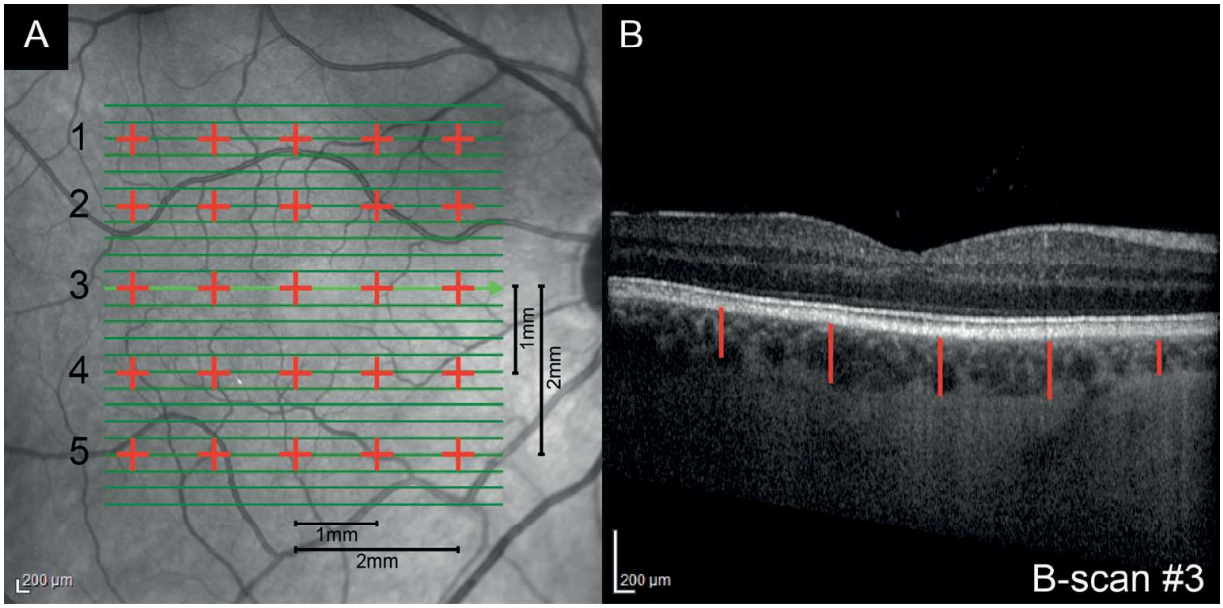
Supplementary Material



Supplementary Figure 1 Choroidal thickness measurements

Manual measurements of choroidal thickness were performed in five evenly distributed b-scans per macular volume scan (A). Five measurements per b-scan were performed: foveal, 1 mm nasal and temporal from the fovea, and 2 mm nasal and temporal from the fovea (B). The averages of the measurements (n=25) from both eyes were used for analysis of mean choroidal thickness.

Retinal Vascular Parameter		ICC	
		Single Measures	Average Measures
SIVA-analysis	CRAE	0.777	0.874
	CRVE	0.923	0.960
	AVR	0.809	0.894
	FDa	0.702	0.825
	FDv	0.639	0.780
	cTORTa[1]	0.888	0.941
	cTORTv[1]	0.762	0.865

Supplementary Table 1 Intraclass correlation

Intraclass correlations between two raters for retinal vascular parameters. CRAE = Central Retinal Artery Equivalent, CRVE = Central Retinal Vei Equivalent, AVR = Arteriole Venule Ratio, cTORTa= Curvature Tortuosity of the Arterioles, cTORTv = Curvature Tortuosity of the venules, FDa = Fractal Dimension of the Arteriolar Network, FDv = Fractal Dimension of the Venular Network.