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Microvascular changes of the retina in ankylosing spondylitis, and the association with cardiovascular disease – the eye for a heart study

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ABSTRACT

Objective: Ankylosing spondylitis (AS) is associated with an increased risk of cardiovascular disease (CVD). Microvasculature changes can precede overt CVD, but have been studied poorly in AS. The retinal vasculature is easily accessible and changes are associated with CVD (e.g. arteriolar narrowing, venular widening, loss of tortuosity). This proof of concept study compared the retinal microvasculature of AS patients with healthy controls, and the influence of gender.

Methods: Cross-sectional case-control study comparing AS patients with healthy controls. Main inclusion criteria were: age 50–75 years, no diabetes mellitus and, for AS, fulfillment of the modified New York criteria. All subjects underwent fundus photography, analyzed with Singapore I Vessel Assessment software, and Optical Coherence Tomography Angiography (OCTA). Subjects were compared with generalized estimating equations (GEE). Multivariable analyses were adjusted for demographics and cardiovascular risk, and stratified for gender.

Results: Fifty-nine AS patients and 105 controls were included (50% women). Controls were significantly older than patients (68 versus 60, $p<0.01$), but did not differ in cardiovascular profile. Patients had a lower retinal arteriolar tortuosity (β -0.1, 95%CI [-0.2; -0.01], p = 0.02), and higher vessel density (β 0.5, 95% CI [0.1; 0.9], p = 0.02). In addition, male AS patients showed a lower arteriovenular ratio compared to male controls (β -0.03, p = 0.04, 95%CI [-0.05; -0.001]). There were no differences found between women with and without AS.

Conclusion: This study detected several retinal microvascular changes, in AS patients compared to controls, which have been associated with CVD. Retinal imaging might be an interesting tool for future CVD screening.

Introduction

Patients with ankylosing spondylitis (AS) have an 1.5 times increased risk of cardiovascular comorbidity and mortality [1–3]. Current research suggests that chronic systemic inflammation contributes to the development of cardiovascular risk factors, such as hypertension and dyslipidaemia, and ischemic heart disease [1,4-6]. Changes in the microvasculature can precede clinically overt cardiovascular disease (CVD), but have been studied poorly in AS. The retinal vasculature is easily accessible and may provide an unique opportunity to timely recognize microvascular changes in AS patients.

Microvascular abnormalities, such as endothelial dysfunction and vascular remodeling, can precede macrovascular CVD and systemic endothelial dysfunction can reveal CVD [7,8] In addition, microvascular disease itself is increasingly recognized as an important contributor to myocardial ischaemia [9,10]. Mounting evidence has pointed out the importance of microvascular abnormalities in relation to CVD in women, aside from macrovascular disease [11,12]. Interestingly,

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this pathophysiology was also suggested for patients with rheumatic diseases [4,9].

In AS, several studies have demonstrated increased signs of macrovascular abnormalities (atherosclerosis) but the literature on microvascular changes is limited [13]. The few studies available so far, have shown an impairment of the microcirculation in structure and function [13–15]. These studies mostly use experimental methodology, such as capillary microscopy and laser Doppler fluxmetry, which are influenced by blood pressure and temperature [14,15]. In contrast, the retinal blood flow is mostly auto-regulated and is easily accessible for non-invasive visualization, which is part of standard care [16,17].

Retinal vascular parameters, measured through fundus photography and ocular coherence tomography angiography (OCT-A), could provide interesting biomarkers of (early) CVD in AS. Several large population studies have described associations between retinal vascular changes and CVD. In particular, changes of vascular diameter (narrower arterioles and wider venules), a lower tortuosity of the arterioles, a lower fractal dimension and a lower vessel density were reported in this context [16,18–22]. Some studies describe the abovementioned diameter changes to be especially associated with CVD in women, independent of other risk factors [11,16,23]. In the few studies in rheumatic diseases (not AS), similar vascular diameter changes were found, with widening of the venules being particularly associated with systemic inflammation [24–28]. The vascular tortuosity, -fractal dimension and vessel density have not yet been studied in patients with rheumatic diseases.

In AS, many patients visit the ophthalmologist multiple times in their lives, due to Acute Anterior Uveitis attacks (this does not involve the posterior part of the eye, which contains the retinal vasculature). Consequently, using ophthalmic evaluation, such as retinal imaging, for early recognition of CVD might be a good opportunity. However, currently, there are no reports available on the retinal vasculature in AS specifically. The primary aim of this study was to investigate the differences between the retinal vessels of AS patients compared with healthy controls, and whether this differs for men and women. Secondary, it evaluates the association of the retinal vasculature with disease activity and other cardiovascular risk factors in AS patients.

Materials and methods

Design and study population

This cross sectional, case-control study is a proof of concept study which applied two retinal imaging modalities: fundus photography (vascular morphology) and optical coherence tomography angiography (OCT-A; retinal vessel density). Both AS patients and healthy control subjects were included, with an equal male-to-female ratio, and aged between 50–75 years, to increase the chance of detecting CVD-associated vascular changes.

AS patients were consecutively recruited from the Rheumatology outpatient clinics of the Amsterdam Rheumatology and immunology Centre (ARC) locations Reade and Amsterdam UMC-VUmc, the Netherlands. Patients had to fulfil the 1984 modified New York criteria, and auto-immune disorders other than AS-related (e.g. psoriasis, inflammatory bowel disease) were excluded. [29]

Healthy control subjects were selected from the two year follow up visit of the Dutch EMIF-AD PreClinAD cohort of the Amsterdam UMC-VUmc, that contains cognitively healthy, monozygotic twins of ≥60 years, from the “Netherlands Twin Registry” [30]. Control group data were collected previously [30]. Controls were eligible if they did not have a history of any rheumatic disease (including inflammatory bowel disease or psoriasis) and had undergone at least one ophthalmologic examination (fundus photography or OCT-A).

Exclusion criteria for all subjects were conditions interfering with ocular evaluation (diabetes mellitus, current anterior uveitis, glaucoma, significant cataract or eye surgery ≤6 months ago) and cerebrovascular events resulting in permanent disabilities (pre-existing exclusion criterion of the control group). Current use of non-steroidal anti-inflammatory drugs (NSAIDs) and biologicals was allowed, but corticosteroids (systemic/topical ocular corticosteroids, or injections in the previous three months) were not permitted.

All subjects underwent fundus photography and OCT-A, preferably of both eyes. Study procedures were performed consecutively on the same day, at the Amsterdam University Medical Centre (UMC) VUmc. The protocol (*NL66784.048.18*) was approved by the medical ethics committee of the Slotervaart hospital & Reade, Amsterdam, the Netherlands. All patients gave written informed consent according to the Helsinki Declaration.

Study parameters

Retinal vasculature assessment

Imaging was performed after application of tropicamide 0.5% eye drops for pupil dilation.

Retinal vessel morphology

Fundus photos (50° field of view, centred on the optic nerve head; Topcon TRC 50DX type IA) were analysed with Singapore I Vessel Assessment (SIVA) software (version 3.0; National University of Singapore, Singapore). SIVA automatically identifies retinal arterioles and venules, in the zone 0.5–2 disc diameters around the optic nerve head (Fig. 1). An experienced grader examined the traced vessels and made manual corrections if necessary (same grader for all subjects; intra-observer intra-class correlation, absolute agreement, of >0.80 [31]). Ungradable images were excluded. SIVA analyses resulted in: vascular diameter (central retinal arterioles, venules; and arteriovenous ratio), vascular curvature tortuosity (of the arterioles and venules) and fractal dimension. The latter is a measure of the branching complexity of the retinal vessels. SIVA skeletonizes the retinal vessels and uses a box-counting method to calculate the fractal dimension.

Retinal vessel density

OCT-A (Zeiss Meditec, Inc, Germany) registers the retinal microvascular network through movement detection of blood cells, enabling calculation of the vessel density (vessel area versus total area). Macula images (6 × 6 mm area around the fovea) were analysed automatically with accompanying software (Cirrus 5000 Angioplex, version 11), applying an ETDRS grid (three rings of 1 mm, 3 mm and 6 mm centred around the fovea). This divides the macula in a central foveal region, an inner and outer region, to calculate two parameters: macular inner- and outer region vessel density (mm/mm^2). The image quality was evaluated based on the software-reported quality score (0–10; ≥8=potentially eligible) and visual inspection. Images of insufficient quality, and ocular diseases interfering with OCT-A quality or vessel density (e.g. severe cataract/ametropia, glaucoma, epiretinal membrane) were excluded.

Cardiovascular history

Cardiovascular risk factors were recorded: Body Mass Index (BMI), current smoking, and history of dyslipidemia and hypertension. In addition, the history of previous CVD (myocardial infarction, coronary disease, peripheral artery disease, transient ischemic attack) was recorded.

Other study parameters

During the study visit, data were collected on demographics (gender, age), hip- and waist circumference, blood pressure, medication (daily use of NSAIDs, nonsteroidal anti-inflammatory drugs; DMARDs, conventional disease modifying antirheumatic drugs; biologicals) and lipid profile. The latter was collected for AS patients within 12 months, and for healthy controls within 24 months prior to the study visit.

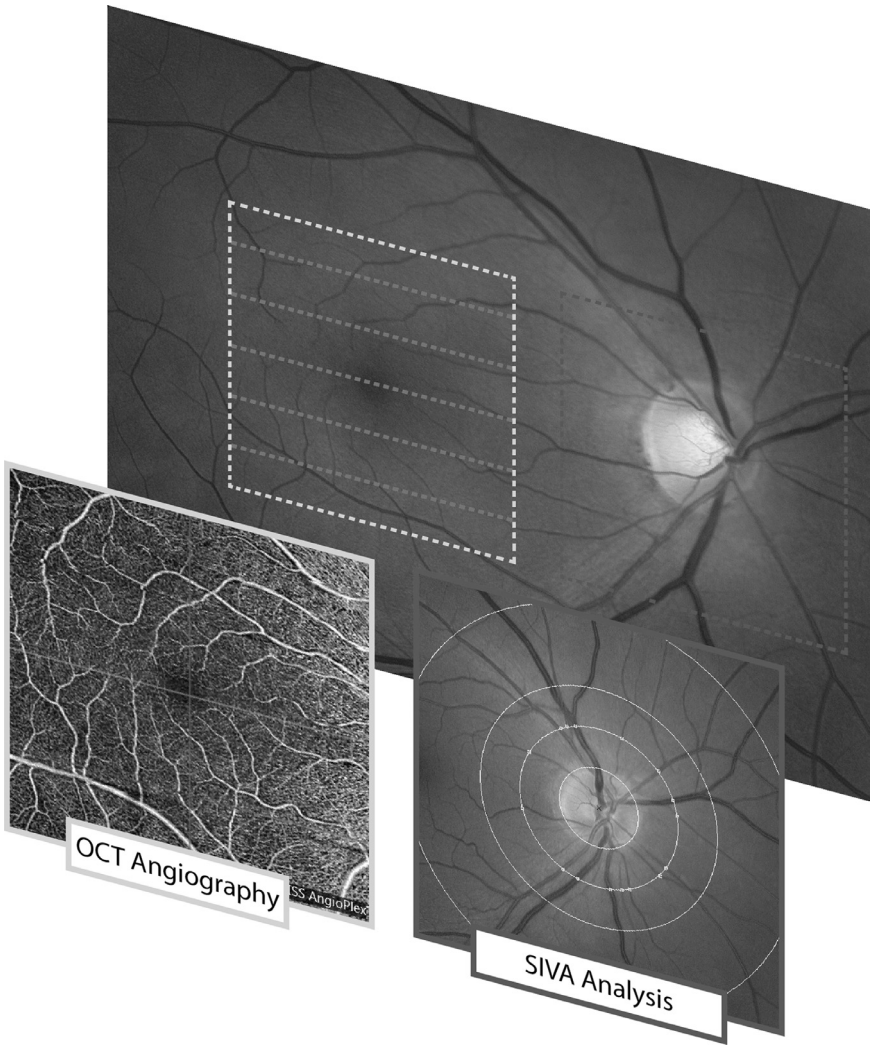

Fig. 1. Overview of the imaging modalities FOR THE EVALUATION OF the retinal vasculature*. Retinal vascular parameters were derived from fundus photography analysed with SIVA software (Singapore I Vessel analysis, resulting in vascular morphology parameters) and Optical Coherence Tomography Angiography (OCT-A; resulting in capillary density parameters).
*Image adapted, with permission, from Haan et al. (32).

In addition, for AS patients, data were collected on disease duration, HLA-B27, extra-articular manifestations, C-reactive protein (during or within 3 months prior/after study visit) and disease activity (Bath AS Disease Activity Index (BASDAI), AS Disease Activity Score (ASDAS). High disease activity was defined as an ASDAS-score of $\geq$2.1 or, if unavailable, a BASDAI of $\geq$4.

Statistical analyses

Data are presented as mean (standard deviation, SD), number (with percentage) or percentage only. Non-normally distributed variables were presented as median (interquartile range, IQR) and transformed with the natural logarithm (Ln) for further analyses. For all retinal parameters, the mean of both eyes was used. If only one eye was available (due to unavailability, failed imaging or ocular disease), only data of the available eye was used.

Differences between AS patients and healthy controls were tested with Generalized Estimating Equations (GEE) analyses, correcting for genetic relatedness within twin-pairs of the control group. AS patients and controls were compared in univariable and multivariable analyses, with stepwise addition of cardiovascular risk factors: demographics and lifestyle (model 1: age, gender, BMI and current smoking status), and factors that could also be part of the pathophysiological pathway between AS and CVD (model 2: model 1 plus hypertension and dyslipidaemia). In addition, OCT-A data were always corrected for software-reported scan quality, as scan quality can significantly influence the vessel density measurement. Next, the influence of gender on the differences between AS and controls in retinal parameters was evaluated by including a cross-product interaction term (gender*patient) as independent variable in the final multiple regression model (model 2). For parameters with an interaction term of $p < 0.10$, the gender-specific regression coefficients were reported as well. Last, within the AS population, the association between retinal parameters and disease parameters (ASDAS, high disease activity, biological use) or cardiovascular risk (hypertension, dyslipidemia, previous cardiovascular disease) was evaluated with linear or logistic regression, corrected for age and gender.

Results

Study population

Sixty-one AS patients were recruited, of whom two were excluded because of active anterior uveitis (Fig. 2). The control population consisted of 152 healthy subjects, of whom 47 did not meet the inclusion criteria, resulting in 105 eligible subjects.

Demographic characteristics are depicted in Tables 1 and 2. The control group was significantly older than AS patients (68 (SD4)

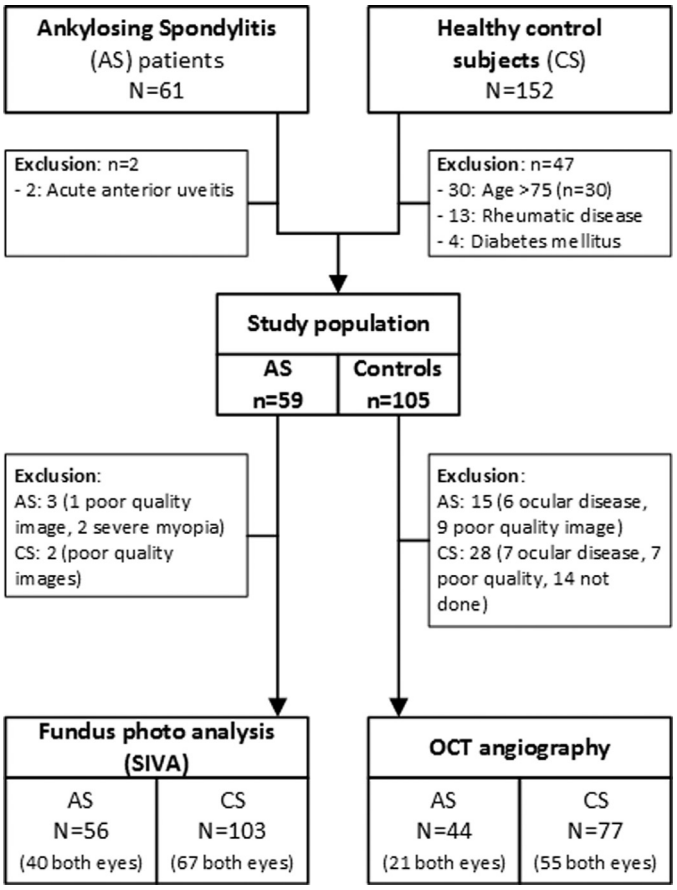

Fig. 2. Flowchart study population (AS patients and healthy controls). As, Ankylosing Spondylitis; CS, healthy control subjects; OCT, Optical Coherence Tomography; SIVA, Singapore I Vessel Assessment software.

years, versus 60 (SD6) years, $p<0.001$, respectively). However, there were no significant differences in the presence of cardiovascular risk factors or previous CVD (Table 2).

In AS patients, the average disease duration was 36 (SD 12) years, the mean ASDAS-CRP/ESR-score 2.1 (SD 0.9), 41% used NSAIDs, 10% a DMARD and 49% received biological treatment, mainly TNF inhibitors (Table 1). No subject used corticosteroids, as this was an exclusion criterion. Men and women with AS did not differ significantly in disease activity, medication use or disease duration (data not shown). among the healthy controls, only 6% used a NSAID, and no one used a conventional DMARD or biological agent.

Comparison of the retinal vasculature of AS patients with healthy controls

Retinal vessel morphology (fundus photography)

Fundus photos of at least one eye were available in 56 (95%) AS patients and 103 (98%) healthy controls (Fig. 2). On a group level, in crude analyses, only fractal dimension differed between AS and healthy controls (higher in AS: β 0.01, 95% CI [0.0; 0.03], p = 0.04, Table 3), but this effect disappeared after correction for age (Table 4). Interestingly, crude analyses only showed a non-significant, lower arteriolar tortuosity in AS patients, but this association became stronger after correction for age and gender, and even more after correction for cardiovascular risk factors (Table 4; (Ln transformation of) arteriolar tortuosity: β −0.1, 95%CI [−0.2; −0.01], p = 0.02, Table 4; 11% lower). A positive history of anterior uveitis was not a confounder in these analyses.

The comparison of gender specific differences between AS patients and healthy controls, revealed that gender was only a significant effect modifier for the association between AS and vascular diameter (retinal arteriovenous ratio, AVR; $p<0.01$; Table 4). Stratified analyses showed that men with AS had a significantly lower AVR (β −0.03, 95%CI [−0.05; −0.001], p = 0.04) than men without AS, whereas this parameter did not differ significantly between women with and without AS.

Table 1
Disease characteristics Ankylosing Spondylitis patients (n = 59).

Women, n (%)	30	(51)
Age in years, mean (SD)	60	(6)
AS disease duration since diagnosis, in years, mean (SD)	24	(11)
HLA-B27 positive, n (%)	43	(78)
Extra-articular manifestations, n (%)		
Anterior uveitis	29	(49)
Inflammatory Bowel disease	3	(5)
Psoriasis	6	(10)
Current AS medication, n (%)		
Biologicals	29	(49)
DMARDs	6	(10)
NSAIDs	24	(41)
Disease activity parameters		
CRP in mg/L, median (IQR)	3	(2–4)
ESR in mm/hr, median (IQR)	11	(2–15)
ASDAS-CRP/BSE, mean (SD)	2.1	(0.9)
BASDAI, mean (SD)	4	(2)
High disease activity (ASDAS≥2.1)*, n (%)	27	(46)
BASFI score, mean (SD)	4	(2)

Legend: Values are depicted as number of patients (%), mean (standard deviation) or median (Q1-Q3). AS, Ankylosing Spondylitis; ASDAS, AS disease activity score; BASDAI, Bath AS disease activity index; BASFI, Bath AS functional index; CRP, C-reactive protein; DMARDs, disease modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; NSAIDs, nonsteroidal anti-inflammatory drugs. Biologicals: mostly TNF inhibitors (n = 27; secukinumab n = 2). *If ASDAS was unavailable, a BASDAI ≥4 was defined as high disease activity.

Retinal vessel density (OCT-Angiography)

OCT-A images were available for 75% of the AS patients (n = 44; both eyes in n = 21) and 73% of the healthy controls (n = 77; both eyes in n = 55; Fig. 2). In this subgroup, AS patients were also significantly younger than healthy controls (59 (SD 6) versus 68 years (SD 4) respectively, $p<0.01$). When comparing subjects with and without OCT-A: AS patients with OCT-A were significantly younger (58 (SD5) versus 66 (SD5) years, $p<0.01$), used more NSAIDs (66% versus 33%,

Table 2
Demographics and cardiovascular profile AS and healthy control subjects.

	AS patients *(n = 59)*		**Healthy controls** [a] *(n = 105)*		*p-value*
Women, n (%)	30	(51)	52	(50)	0.87
Age in years, mean (SD)	60	(6)	68	(4)	**<0.001**
Smoking currently, n (%)	11	(19)	8	(8)	0.06
Hypertension and/or dyslipidemia, n (%)	26	(44)	44	(42)	0.98
Hypertension	23	(39)	39	(37)	0.91
Dyslipidemia	9	(15)	18	(17)	0.78
History of cardiovascular disease, n (%)	9	(15)	15	(14)	0.89
Body mass index, mean (SD)	26	(4)	26	(3)	0.51
Waist-to-hip-ratio, mean (SD)	0.9	(0.1)	0.9	(0.1)	0.46
Systolic blood pressure, mean (SD)	143	(17)	143	(15)	0.98
Diastolic blood pressure, mean (SD)	83	(9)	81	(8)	0.30
Cholesterol-HDL ratio, mean (SD)	4	(1)	4	(1)	0.58

Legend: Values were depicted as mean (standard deviation) or number of patients (percentage of total). a. 43 twin pairs and 19 singletons. AS, Ankylosing Spondylitis.

Table 3
Retinal vascular parameters of the AS patients and Healthy controls.

		AS Patients *(n = 59)*	**Healthy controls**[a] *(n = 105)*	*p-value*
Vascular morphology (Fundus photography)				
Number of patients		*56*	*103*	
Diameter	Arteriolar (μm)	125 (11)	124 (10)	0.37
	Venular (μm)	197 (20)	191 (14)	0.08
	Arteriovenous ratio (AVR)	0.64 (0.06)	0.65 (0.04)	0.43
Tortuosity	Arteriolar, x10^{-5} [a]	−9.8 (0.2)	−9.7 (0.2)	0.19
	Venular, x10^{-5} [a]	−9.7 (0.2)	−9.7 (0.2)	0.61
Complexity	Fractal dimension	1.19 (0.04)	1.18 (0.03)	**0.04**
Vessel density (OCT-angiography)				
Number of patients		*44*	*77*	
Vessel density	Inner ring macula, mm/mm^2	18.4 (0.7)	17.6 (1.1)	<**0.001**
	Outer ring macula, mm/mm^2	18.4 (0.6)	17.8 (1.1)	<**0.001**

Legend: Values are depicted as mean (standard deviation, SD). a. analyses were performed on log-transformed variable, because of a non-parametric distribution. a. 43 twin pairs and 19 singletons. AS, Ankylosing Spondylitis.

p = 0.03) and had less often hypertension (30% versus 67%, p = 0.01), than AS patients without OCT-A. Healthy controls in whom OCT-A was performed did not differ significantly from controls without.

On a group level, univariable analyses suggested that AS patients had a significantly higher retinal vessel density in both the inner- and outer region of the macula (Table 3), compared to healthy controls. After correction for demographics, lifestyle-factors, hypertension and dyslipidemia, this difference persisted for the inner macula region (higher in AS: β 0.5, 95%CI [0.1; 0.9], p = 0.02, Table 4).

Gender was only a significant effect modifier for the association between AS and capillary density of the outer macula (p<0. 01; Table 4). Gender stratified analysis showed that male AS patients had a significantly higher vessel density in the outer macula (β 0.6, 95%CI [0.1; 1.0], p = 0.03), compared to men without AS. Again, this parameter did not differ between women with and without AS.

Disease activity, cardiovascular history and retinal parameters in AS patients

Disease activity and retinal characteristics

The mean ASDAS was 2.1 (SD 0.9), with 27 AS patients (49%) reporting a high disease activity (Table 1). Biologicals were used less often by patients showing a high disease compared to patients with low disease activity, respectively 44% versus 50% (not significant).

AS patients with high disease activity showed a significantly higher venular diameter (β 10, 95%CI [0; 21], p = 0.05; corrected for age and gender), compared to patients with low disease activity. Interestingly, in contrast, in AS patients who used biologicals, a significantly higher arteriole diameter was found compared to patients without this treatment, regardless of disease activity, age and gender (β 8, 95%CI [2; 14], p<0.01).

Retinal parameters and cardiovascular history in AS patients

Cardiovascular risk factors were often present in AS patients: 39% had hypertension and 15% dyslipidemia. In addition, 15% reported a history of CVD. Hypertension and CVD were both significantly associated with a higher venule diameter in AS patients, also after correction for age and gender: hypertension, β 11 (95%CI [4; 25], p<0.01) and CVD: β 16 (95%CI [1; 31], p = 0.03). Other retinal parameters did not show any associations with CVD, hypertension or dyslipidaemia. In contrast, in controls, CVD were associated with a higher arteriolar diameter and AVR (data not shown).

Discussion

This study showed several retinal microvascular changes in AS patients, compared to healthy controls, of which some have been associated with cardiovascular risk. The most prominent changes in AS patients consisted of straighter arterioles and a higher vessel density. In addition, male AS patients showed a decreased arteriovenular ratio compared to male controls, whereas no specific changes were found in women with AS. In AS, high disease activity and previous CVD were associated with wider venules, whereas treatment with biologicals was related to wider arterioles.

This proof of concept study is the first to report on the microvasculature of the retina specifically in AS patients. The results are mostly in line with the expectations based on other populations. Large population studies demonstrated CVD to be associated with arterial narrowing, venular widening (or a decreased arteriovenular ratio), reduced arteriolar tortuosity and reduced complexity [18,20,22,33–35]. The few studies in other rheumatic diseases, primarily including rheumatoid arthritis, psoriatic arthritis and systemic lupus erythematosus, found the same vascular diameter changes as reported for CVD (arteriolar narrowing, venular widening) in patients, compared to controls [24-28]. Only one study included a small number of AS patients (n = 26) together with other rheumatic diseases, and reported no differences with controls. However, the AS patients included in that study were younger compared to our study, and the majority of controls suffered from hypertension which is known to influence the retinal vasculature [36]. None of the studies in rheumatic patients evaluated the tortuosity or vessel density.

The current results are mostly in line with the expectations based on the abovementioned studies. Most prominently, the retinal arteriolar tortuosity was significantly decreased in AS patients, which is associated with an increased risk of CVD based on large population studies [22,33,35]. This decreased tortuosity appears to be due to AS itself as the association became stronger after correction for cardiovascular risk factors. In addition, there was a trend of an increased venular diameter and decreased arteriolar diameter and, consequently, decreased arteriovenular ratio (AVR), in AS patients. Although these diameter differences were not significant on a group level (only male AS patients had a significantly decreased AVR compared to controls), this combination of changes has been repeatedly reported to be associated with CVD [20,22]. Also in the current study, within the AS population, venular widening was associated with CVD. In contrast with other population studies, and the AS patients in this study, the controls in our study with CVD had wider arterioles [20,22,23].

OCT-A imaging, which had not yet been described for rheumatic diseases, revealed an increased retinal vessel density in AS patients. In addition, in male AS patients, this was found for a more extensive area. This is a novel finding. Until now, only one study studied the vessel density, in patients with coronary heart disease, and found a decreased density, compared to controls [21]. However, this study focused on macrovascular CVD, whereas an early disease state with primarily microvascular damage might have different hemodynamic

Table 4
Retinal vascular parameters, differences between AS versus Control subjects.

		Crude		Model 1 *Adjusted for: Demographics, lifestyle (age, gender, BMI, smoking)*[A]		Model 2 *Adjusted for: Demographics, lifestyle + hypertension, dyslipidemia*[A]		Effect modification by sex[B]
		β	**(95%CI)**	***p***	***β***	**(95%CI)**	***p***	***p***
Retinal vascular parameters		1.6	(−2.0, 5.2)	0.37	0.15	(−4.5, 4.8)	0.94	−0.2	(−4.8, 4.4)	0.92	0.60
Diameter	Arteriolar	5.4	(−0.66, 11.5)	**0.08**	2.9	(−5.0, 10.8)	0.47	2.5	(−5.4, 10.4)	0.53	0.17
	Venular	−0.01	(−0.03, 0.01)	0.43	−0.01	(−0.03, 0.01)	0.56	−0.01	(−0.03, 0.02)	0.65	**<0.01**
	Arteriovenular ratio	−0.05	(−0.12, 0.03)	0.19	**−0.08**	(−0.18, 0.00)	**0.05**	**−0.1**	(−0.2, −0.01)	**0.02**	0.14
Tortuosity	**Arteriolar**[a]	0.02	(−0.05, 0.08)	0.61	−0.01	(−0.09, 0.08)	0.88	−0.02	(−0.01, 0.1)	0.67	0.10
	Venular[a]	0.01	(0.00, 0.03)	**0.04**	0.00	(−0.02, 0.02)	0.78	0.0	(−0.02, 0.02)	0.88	0.16
Complexity	**Fractal dimension**	**0.8**	**(0.5, 1.1)**	**<0.001**	**0.5**	(0.03, 0.9)	**0.04**	**0.5**	**(0.1, 0.9)**	**0.02**	0.18
Vessel Density	**Inner ring**	**0.7**	**(0.4, 1.0)**	**<0.001**	0.2	(−0.3, 0.6)	0.49	0.2	(−0.2, 0.6)	0.42	**<0.01**
	Outer ring										

Legend: GEE analyses, with retinal parameters as the dependents. BMI, body mass index; CI, confidence interval; GEE, generalized estimating equation. a. analyses performed on log-transformed variable. A. Vessel density (OCT-A) analyses were corrected additionally for software-reported scan quality. B. Effect modification by sex was tested for model 2 and further described in the paper. *β*, regression coefficient; p, p-value.

states. An alternative hypothesis is that the increased vessel density found in the AS patients, is related with systemic inflammation, causing an increased capillary flow (in AS patients, a higher CRP was associated with a higher vessel density, although not significant; B0.01, 95%CI [−0.1; 0.13], p = 0.83). Nevertheless, the association between vessel density and microvasculopathy needs to be further explored.

Microvascular changes in women were of particular interest, as increasing evidence supports the importance of microvasculopathy, including retinal vascular diameter changes, in the risk of CVD [10,11,23,37]. It was expected that, in particular in AS women, microvascular changes would be observed. However, in this study, differences between AS and controls were found only on a group level, and additionally in men, when stratified for gender. The fact that microvascular changes were not specifically detected in women, cannot be explained by more systemic inflammation in men, because disease activity parameters and treatment (ASDAS, biologicals, NSAIDs) did not differ between AS men and women.

A higher AS disease activity was associated with wider retinal venules, in accordance with studies in other rheumatic diseases [14,25,28]. Although the pathophysiological process is not fully understood, wider venules are associated with metabolic disturbances (diabetes mellitus, obesity, dyslipidemia), inflammation, smoking and CVD, and is therefore considered unfavourable[16]. In contrast, biologicals were associated with a more favourable arteriolar morphology (wider diameter, instead of narrowing, which is associated with CVD), and this was independent of disease activity. The main target of the biological, tumour necrosis factor alpha, hampers nitro oxide release. Studies in AS patients initiating a TNFi have shown to improve the vascular status, such as the endothelial function, intima-media thickness and pulse wave velocity. [14,15,38] The current findings are in accordance with the theory that TNFi have a beneficial effect on the vessel wall.

As this was a proof of concept study, there were a few limitations. Firstly, the healthy control group was significantly older than the AS patients. Therefore, analyses were adjusted for age, limiting this influence, but also for other cardiovascular risk factors that are considered to influence the microvasculature. Secondly, 43 of the control subjects were genetically related to another subject (twin). Alternatively, only one of the twin pairs could have been selected, but this would have resulted in a much smaller control group. Therefore, to increase statistical power, all available subjects were included, with adjustment for genetic relatedness. Thirdly, it was not possible to link the retinal vasculature directly to the current cardiac vessels conditions, as cardiac imaging data were not available. However, as this was a secondary aim, retinal parameters were compared with the presence of CVD, a clinically important endpoint. Fourthly, it is unclear how NSAIDs (and biologicals) could have had a confounding influence on the differences between AS and controls, but the use of NSAIDs is very common in AS, and an AS group without NSAIDs would not be representative. In addition, the potential influence of exercise should be considered in future studies. Fifthly, 49% of the AS patients had a history of anterior uveitis (AAU), which is comparable with the prevalence reported for patients with a long disease duration (43%). [39] Unfortunately, whether a previous history of AAU influences these retinal parameters is unknown. However, AAU in AS is typically a short-term inflammation of the anterior eye segment, that generally does not involve the posterior segment and is, therefore, unlikely to affect the retinal vessels. In addition, patients with active AAU at the time of the study were excluded and a positive history of AAU was not a confounder in the analyses. Lastly, because this is a proof of concept study, the study population size was limited, rendering it potentially underpowered to detect more microvascular changes. In this perspective, no additional correction for multiple testing was applied. However, this is the first study focusing on AS and, furthermore, the first that also evaluated the vascular tortuosity

and density, which had not yet been described before for patients with rheumatic diseases in general.

In conclusion, this study was the first to report on the retinal microvasculature in a large group of AS patients, in comparison with healthy controls. It supports the hypothesis that AS causes microvascular changes, which were found more extensively in men. Importantly, the results might indicate a retinal vascular profile in AS patients that has also been demonstrated to be associated with CVD, whereas the value of a novel finding (increased vessel density) needs to be explored further. By all means, this proof of concept study indicates that retinal imaging techniques might be useful for the detection of microvascular changes in AS patients. Importantly, these techniques are non-invasive and easily accessible in daily clinical practice, in contrast with other microvascular assessments. If longitudinal follow up further demonstrates its opportunities and predictive value, these techniques might provide a great future opportunity for the early recognition of CVD in AS.

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Declaration of Competing Interest

The authors declare no conflicts of interest for this study.

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