Intact Calibers of Retinal Vessels in Patients with Systemic Sclerosis

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ABSTRACT. ***Objective.*** A primary endothelial cell dysfunction is thought to be involved in systemic sclerosis (SSc)-associated fibroproliferative vasculopathy of the microcirculation and small arterioles, even in sites not affected by fibrosis. Because the role of fibroblasts in pathologic modifications and vascular wall remodeling is relatively unclear, and because the retina provides a unique opportunity to assess microcirculation in the absence of resident fibroblasts, we systematically evaluated retinal vessels in patients with SSc.

Methods. Digital retinal images were obtained from both eyes of 93 consecutive patients with fully characterized SSc and 29 healthy controls matched 1:1 for age and sex with selected patients without diabetes, hypertension history, or antihypertensive treatment. Internal microvascular calibers (erythrocyte column width in μm) by central retinal arteriolar and venular equivalents and arteriolar to venular ratio were measured using validated software.

Results. Arteriolar and venular calibers were similar in patients and their matched controls (mean ± SEM; 187 ± 2 vs 184 ± 3, p = 0.444, and 211 ± 2 vs 216 ± 3, p = 0.314, respectively). Both arteriolar and venular calibers and their ratio in patients with SSc were not associated with disease duration, extent of skin involvement, pulmonary fibrosis, digital ulcers or pitting scars, amputations, digital capillaroscopic findings, inflammatory indices, or autoantibodies.

Conclusion. The evidence that retinal microcirculation is spared in SSc suggests that fibroproliferative vasculopathy may depend on specific cellular or soluble factors not present in the retinal environment. (J Rheumatol First Release Feb 1 2015; doi:10.3899/jrheum.141425)

Key Indexing Terms:
SYSTEMIC SCLEROSIS VASCULOPATHY RETINAL ARTERIOLES RETINAL VENULES

Systemic sclerosis (SSc) is the prototypic fibrotic disorder affecting the skin and the internal organs. Evidence suggests that a diffuse fibroproliferative vasculopathy of the microvasculature and small arterioles may already be present in the earliest phases of the disease, even in sites not clinically affected by fibrosis[1]. The evolution of microvascular involvement is clinically characterized by early manifestations such as the vasospastic condition Raynaud phenomenon (RP), followed during disease progression by digital ulcers, skin and gastric telangiectasias, pulmonary arterial hypertension, and renal crises. Structural alterations of the microvasculature are visible at nailfold capillaries and include enlargement of capillaries, decrease in their density, other capillary ramifications, and microhemorrhages, as detected by capillaroscopy[1,2]. Moreover, involvement of the macrocirculation may also be detected in SSc, as shown by the increased stiffness of the aorta regardless of the extent of the inflammatory fibrotic process in skin and lungs[3].

Fibroproliferative vasculopathy in SSc is thought to result from a primary endothelial cell dysfunction. As a consequence, an ischemia-reperfusion tissue injury occurs, along with vascular leakage of growth factors and tissue hypoxia, triggering progressive tissue fibrosis[1]. On the other hand, vascular disease is also thought to result from the fibrotic process *per se*, because increased migration of activated resident myofibroblasts into the vessel wall may

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augment extracellular matrix deposition, resulting in vascular stiffness and dysfunction[4]. Eventually, the interplay of several factors, including ischemia-reperfusion injury, endothelial dysfunction, impaired balance between nitric oxide and endothelin-1 synthesis, as well as a prominent deregulation of hemostatic and fibrinolytic mechanisms, culminates in a fibrotic vascular process, reducing the vessel lumen and resulting in a hypoxic effect in the skin and internal organs that leads to considerable morbidity and mortality in SSc[5,6]. A recent analysis of demographic, clinical, and outcome data for all 231 patients with SSc followed in our department (200 women) revealed that digital ulcers and renal crisis occurred earlier in men, and that survival was significantly worse in men than women, implying a difference in the development of vasculopathy between sexes[7].

In an analogy to capillaroscopy for nailfold microvasculature, fundoscopy can easily assess retinal microcirculation. Fundus changes are expected among hypertensive patients with SSc, but whether vascular retinal lesions that are associated with SSc *per se* exist in these patients remains uncertain. Along this line, in normotensive patients with SSc, retinal exudates and vascular tortuosity were found in 34%, compared with 8% of healthy controls[8]. In contrast, only 1 of 23 patients with SSc was found to have a superficial retinal hemorrhage in another study[9]. Recently, in a clinical and angiographic study, an acute retinal artery occlusion was detected in 3 patients with SSc; the histopathological studies showed severe retinal ischemic atrophy and concentric narrowing and fibrosis of small retinal vessels, suggestive of a fibroproliferative vasculopathy characteristic of SSc[10].

Given the varying results on the presence of retinal vasculopathy in SSc described above[8,9,10] and because the role of fibroblasts in pathologic modifications and vascular wall remodeling is relatively unclear, and because the retina provides a unique opportunity to assess the microcirculation in the absence of resident fibroblasts, we aimed to systematically study the retinal vessel calibers in these patients.

MATERIALS AND METHODS

Patients. Ninety-three consecutive patients with SSc followed in our department were recruited between November 2012 and January 2014. A subpopulation of 29 patients free of antihypertensive treatment and without high blood pressure (BP; systolic BP/diastolic BP ≤ 139/89 mmHg), diabetes mellitus, and cardiovascular disease was matched 1:1 for age and sex with healthy controls. Those 29 matched patients with SSc were a representative sample of the whole population, because there were no differences between them in demographics and SSc- specific features (Table 1). The study protocol was approved by the ethics committee of Laikon Hospital (Athens, Greece), and informed consent was obtained from all participants.

Measurements. Both eyes of each participant were photographed with a 45° digital nonmydriatic camera (Topcon TRC-NW8) after 5 minute adaptation in the dark and following overnight fasting. One retinal image per eye was obtained, centered on the optic disc. Subsequently, all images were quantitatively graded by a physician (EKA) who was blinded to clinical data. Calibers of the 6 largest retinal arterioles and venules, from both eyes, passing through a zone between 0.5 and 1.0 disc diameter from the optic disc margin were measured and analyzed using a static retinal vessel analyzer and Vesselmap 2 software (Visualis, Imedos Systems UG; Figure 1)[11]. These measurements were then summarized using formulae described elsewhere[12] to compute the central retinal arteriolar equivalent (CRAE) and the central retinal venular equivalent (CRVE), representing the average internal caliber of retinal arterioles and venules, respectively. Reproducibility of retinal vascular measurements has been reported with intraobserver correlation coefficients > 0.9. The arteriolar-to-venular ratio was computed as the quotient of CRAE over CRVE.

All patients underwent global assessment of classic cardiovascular risk factors (Table 1). Data on medical and family history were obtained using a structured questionnaire. Laboratory test data were obtained from patients' medical records. Anthropometric measurements, including weight, height, and arm circumference were assessed using standard techniques. Arterial BP was measured in triplicate over the right brachial artery, following 10 min of rest in the supine position (Microlife WatchBP Pro device). Capillaroscopic pattern (normal, early, active, or late) was defined in 46 of the 93 patients with SSc using nailfold videocapillaroscopy (DS Medica, model TUDC12B4).

Statistical analysis. Statistical analysis was performed using the SPSS statistical package (IBM, version 21.0). Variables were tested for normality by the Kolmogorov-Smirnov test. Independent t test or chi-squared test was used to compare the clinical and demographic characteristics of SSc cases and healthy controls, as well as retinal vascular diameters of patients with or without specific SSc traits. ANCOVA was applied to adjust for potential confounders. The level of statistical significance was set at $p < 0.05$ for all comparisons.

RESULTS

From 93 consecutive patients with SSc (mean age: 54.1 ± 13.9 yrs, 86% women, 43.5% hypertensive, median disease duration: 7 yrs) and 29 controls we obtained 244 retinal images. We excluded overall 21 images (20 with SSc and 1 in control group) because of nongradable retinal photographs that resulted from cataracts in 4 eyes, inadequate pupil dilation in 16 eyes, and unilateral central retinal vein occlusion in 1 patient.

When compared to patients, healthy controls had higher levels of diastolic and mean BP and higher blood glucose. After adjustment for all confounding factors (dyslipidemia, heart rate, body mass index, diastolic BP, smoking, erythrocyte sedimentation rate, blood glucose, family history of coronary artery disease, and fellow vessel caliber), no differences were found between SSc and controls regarding retinal vessel calibers (CRAE, CRVE) and arteriolar to venular ratio (Table 1).

As shown in Table 2, there was no significant association between retinal microvascular diameters and extent of skin sclerosis or the presence of pulmonary fibrosis in 93 patients with SSc. Moreover, no differences were found regarding retinal arteriolar and venular calibers and their ratio between patients with or without vascular clinical manifestations such as digital ulcers or digital pitting scars, and capillaroscopic patterns (not shown). Even patients with amputations had intact retinal microcirculation. Also, there were no associations with disease duration, inflammatory indices, or presence of anti-SCL70 or anticentromere antibodies (Table 2).

Table 1. Characteristics of patients with systemic sclerosis (SSc) and healthy controls. Data are presented as mean (± SD) or median (interquartile range) for continuous and as percentage (%) for categorical variables.

Characteristics	All SSc, n = 93	SSc-matched, n = 29	Control-matched, n = 29
Age, yrs	54.1 ± 13.9	51.2 ± 11.1	50.4 ± 10.2
Female (%)	86.0	86.2	86.2
HTN (%)	43.5	0.0	0.0
CVD (%)	3.3	0.0	0.0
Diabetes type 2 (%)	4.3	0.0	0.0
Dyslipidemia (%)	22.8	10.3	27.6
Smoking (pack/yrs)	11.5 (5.0–21.0)	10.0 (5.0–21.8)	25.5 (14.6–29.8)
BMI, kg/m^2	24.5 ± 4.2	24.9 ± 4.1	26.0 ± 4.0
Glucose, mg/dl	88.0 (81.5–94.0)	86.5 ± 6.4*	91.6 ± 9.6*
White blood cells, 10^9/l	7151.8 ± 1977.5	7108.6 ± 1877.4	6888.8 ± 2368.5
ESR, mm/h	27.5 ± 18.0	31.1 ± 22.6**	12.3 ± 7.4**
CRP, mg/l	2.6 (1.2–6.3)	2.7 (1.4–7.5)	ND
Creatinine, mg/dl	0.79 ± 0.2	0.75 ± 0.2	0.78 ± 0.1
Systolic BP, mmHg	121.4 ± 17.0	114.5 ± 11.8	119.6 ± 12.0
Diastolic BP, mmHg	70.1 ± 7.2	68.1 ± 6.0**	74.7 ± 7.7**
Mean arterial pressure, mmHg	82.3 ± 10.4	78.8 ± 8.5**	87.0 ± 10.9**
Heart rate, bpm	72.3 ± 9.2	69.8 ± 8.5	65.9 ± 7.8
CRAE, μm	184.0 ± 16.1	187.1 ± 2.1†	184.0 ± 2.8†
CRVE, μm	211.3 ± 18.1	211.1 ± 2.3§	215.6 ± 3.0§
AVR	0.87 ± 0.1	0.88 ± 0.01	0.86 ± 0.01
Angiotensin II receptor inhibitors (%)	9.8	0.0	0.0
Angiotensin-converting enzyme inhibitor (%)	15.2	0.0	0.0
B-blockers (%)	8.7	0.0	0.0
Diuretics (%)	9.8	0.0	0.0
Calcium channel blockers (%)	47.8	0.0	0.0
SSc disease duration, yrs	7.0 (4.0–13.0)	7.1 ± 6.0	—
SSc subtype			
Limited (%)	61.5	60.7	
Diffuse (%)	38.5	39.3	—
Pulmonary fibrosis (%)	68.1	60.7	—
Pulmonary hypertension (%)	5.4	3.4	—
Renal crisis (%)	2.2	0.0	—
Digital ulcers (%)	52.7	42.9	—
Digital pitting scars (%)	11.8	3.4	—
Amputations (%)	2.2	0.0	—
Capillaroscopic pattern (n/N)			
Early (%)	15 (7/46)	12 (2/17)	—
Active (%)	52 (24/46)	47 (8/17)	—
Late (%)	33 (15/46)	41 (7/17)	—
Autoantibodies			
Anti–SCL-70 (%)	55.9	58.6	
ACA (%)	32.3	20.7	—
Steroid treatment (%)	53.3	48.3	—
MTX (%)	19.6	6.9	—
AZA (%)	4.3	6.9	—
CYC (%)	6.5	10.3	—

*p < 0.05, **p < 0.01, † p = 0.444, § p = 0.314, adjusted for dyslipidemia, heart rate, BMI, diastolic blood pressure, smoking, erythrocyte sedimentation rate, blood glucose, family history of coronary artery disease, and fellow vessel caliber. Data on retinal diameters (CRAE, CRVE, AVR) concern both left and right eyes (n = 244). CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; AVR: arteriolar to venular ratio; ND: nondetectable; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; MTX: methotrexate; AZA: azathioprine; CYC: cyclophosphamide; BMI: body mass index; ACA: anticentromere antibodies; CVD: cardiovascular disease; HTN: hypertension; BP: blood pressure.

DISCUSSION

Herein we report that (1) no differences regarding retinal arteriolar and venular calibers were found between patients with SSc and their matched controls, and (2) retinal vascular calibers were not associated with any disease characteristics investigated. Our data clearly show that in SSc, a unique

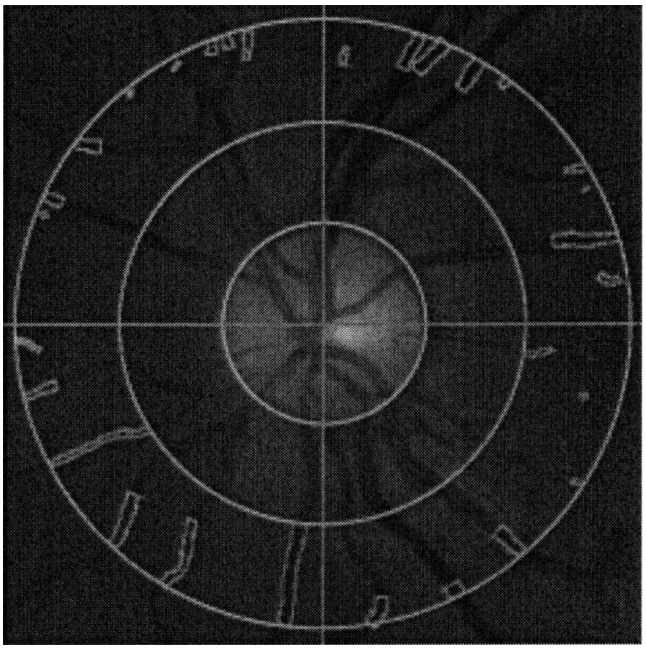

Figure 1. Internal vessel calibers analysis using a static retinal vessel analyzer and Vesselmap 2 software. Arterioles and venules are spread around the outer circle.

environment such as the retina does not present microvascular abnormalities that are commonly detected in other organs such as the heart, lung, gastrointestinal tract, and kidneys[1]. Notably, evidence from the literature on whether SSc is associated with the development of a primary retinopathy in humans, not related to the presence of arterial hypertension, was equivocal[8,9,13].

The method we used to evaluate retinal microcirculation has been validated in several large-scale clinical trials[14,15]. The basic working principle of our software is the measurement of the reflecting brightness derived from the erythrocyte column width[16,17]. We acknowledge that this may result in an overestimation of the internal diameter. However, any subintimal proliferative accumulation of mesenchymal cells in SSc would lead either to inward remodeling, producing a decrease of internal diameter, or to outward remodeling, without affecting the internal lumen. So, even if the retinal microcirculation is affected in SSc, our data suggest that the remodeling process is an outward remodeling, sparing the internal arteriolar diameter. It should be noted that histopathologic data from patients with SSc[18] show that microvascular arterioles display prominent endothelial fibroproliferative alterations, causing both thickening of vessel walls and severe narrowing of vessel lumen. Such narrowing or effacement of vessel lumen was not detected in our study.

We examined what makes the retinal microcirculation so unusual. One theory regarding vascular wall remodeling in SSc is that endothelial cells, undergoing endothelial-mesenchymal transition into myofibroblasts under pressure of offensive stimuli such as hypoxia, cytokines, or growth factors, contribute to the excessive extracellular matrix production[19]. Alternatively, other cell types, possibly vascular smooth muscle cells, pericytes, epithelial cells, resident fibroblasts, or circulating fibroblast precursors, migrate into the vessel wall (attracted by locally secreted chemokines) and transdifferentiate into collagen-producing myofibroblasts under the effect of transforming growth factor-β[4]. Therefore, a first explanation for the intact retinal microcirculation in patients with SSc could be the absence of resident (myo)fibroblasts[20]. Although intriguing, this explanation has limitations; one could argue that other circulatory beds, such as the liver, the spleen, the pancreas, the peritoneum, or the cardiac valves are also rarely, or never, affected in patients with SSc, despite the fact that all of them are rich in resident fibroblasts.

Another hypothesis to explain why retinal microcirculation is protected in patients with SSc could be its relative

Table 2. Retinal vascular measures (CRAE, CRVE, AVR) and disease characteristics in 93 patients with systemic sclerosis (SSc; n = 186 eyes). Data are presented as mean (± SD). Median is 7.0 years for disease duration, 2.6 mg/l for CRP, and 22 mm/h for ESR.

	CRAE			CRVE			AVR		
SSc Subtype	Limited	Diffuse	p	Limited	Diffuse	p	Limited	Diffuse	p
	183.0 ± 15.1	186.1 ± 17.7	0.234	211.2 ± 16.8	212.0 ± 20.5	0.795	0.87 ± 0.1	0.88 ± 0.1	0.264
Disease traits	Present	Absent	p	Present	Absent	p	Present	Absent	p
Pulmonary fibrosis	184.3 ± 16.9	184.0 ± 14.6	0.916	211.3 ± 19.8	212.2 ± 14.8	0.771	0.88 ± 0.1	0.87 ± 0.1	0.544
Digital ulcers	183.6 ± 17.6	185.0 ± 14.4	0.569	210.8 ± 18.7	212.5 ± 17.8	0.556	0.87 ± 0.1	0.87 ± 0.1	0.944
Digital pitting scars	181.7 ± 14.1	184.4 ± 16.3	0.479	206.7 ± 19.1	212.0 ± 18.0	0.214	0.88 ± 0.1	0.87 ± 0.1	0.494
anti–SCL-70	185.9 ± 16.5	181.3 ± 15.0	0.121	212.4 ± 17.2	209.1 ± 20.6	0.323	0.88 ± 0.1	0.87 ± 0.1	0.571
ACA	191.7 ± 11.2	183.7 ± 16.9	0.143	214.8 ± 15.2	210.3 ± 18.8	0.469	0.89 ± 0.1	0.88 ± 0.1	0.362
	Above Median	Below Median	p	Above Median	Below Median	p	Above Median	Below Median	p
Disease duration	189.3 ± 9.8	199.1 ± 6.5	0.119	225.1 ± 18.8	227.5 ± 8.4	0.817	0.85 ± 0.1	0.88 ± 0.0	0.539
ESR	183.3 ± 17.1	184.4 ± 16.3	0.672	209.7 ± 19.9	210.2 ± 15.0	0.455	0.88 ± 0.1	0.88 ± 0.1	0.796
CRP	184.8 ± 15.5	183.7 ± 15.8	0.668	213.2 ± 18.7	209.7 ± 15.3	0.243	0.87 ± 0.1	0.88 ± 0.1	0.456

CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; AVR: arteriolar to venular ratio; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; anti–SCL-70: anti–Scl-70 antibodies; ACA: anticentromere antibodies.

lack of exposure to vasospastic episodes and reperfusion injury. Unlike digital microcirculation, retinal vessels are not subject to intense temperature fluctuations; this could be why they are spared the deleterious effects of RP. The main argument against this hypothesis is that other internal organs such as the kidneys and lungs are also sheltered from temperature changes, yet have SSc-associated vasculopathy.

Other factors possibly accounting for the normal findings in the retinas of patients with SSc are pertinent to the immune-privileged environment of the eye. To protect vision, the eye needs to be sheltered from intense, potentially deleterious inflammation. Therefore, in addition to a strong blood-ocular barrier that prevents entry of pathogens, several other mechanisms modify immune responses in the ocular environment[21]. It is, therefore, possible that in the context of the immune-privileged environment of the eye the immune mechanisms that would lead to vascular damage in SSc[22] are neutralized, protecting the retinal microcirculation from the lesions that are observed in other vascular beds of the body.

Moreover, retinal blood vessels have no adrenergic vasomotor nerve supply[23]. Consequently, retinal blood flow is thought to be regulated mainly by local and myogenic changes[24] in arteriolar tone. Impairment of autonomic function in SSc[25,26] (parasympathetic dysfunction, sympathetic overactivity, and depression of the circadian rhythm of heart rate) has been implicated in esophageal dysmotility and RP. Therefore, the absence of adrenergic innervation of the retinal vessels might be an additional explanation for intact vascular calibers.

Interestingly, no involvement of the retina has been found in University of California at Davis line 200 chickens[27]. In addition, no endothelial cell apoptosis was identified in their posterior ocular segment, despite the fact that apoptosis was most prominent in the skin, esophagus, lungs, and kidneys of these animals. Based on this experimental model, the authors suggest that endothelial cell heterogeneity could account for the differential expression of SSc in specific organs and proposed that the endothelial cells of the posterior segment could be resistant to anti-endothelial cell antibody–mediated cytotoxicity[27].

The cross-sectional design of our study is obviously a limitation. Moreover, 20 images out of 186 obtained from the SSc group (11%) were excluded because of poor quality compared to only 1 of 58 images (2%) from the control group. This discrepancy could have led to selection bias, emphasizing or attenuating potential associations. Further, we cannot overlook that nearly 75% of our patients were receiving corticosteroids or potent immunosuppressive treatment, which could have affected our results.

Fibroproliferative vasculopathy, a cardinal feature of SSc, spares the retina, presumably because of the "fibroblast-free" and immune-privileged environment of the posterior ocular segment. This finding needs to be thoroughly studied because it could provide invaluable information regarding SSc pathogenesis and potential treatment approaches.

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