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The enigma of sclera-specific autoimmunity in scleritis

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ABSTRACT

Scleritis is a severe and painful ophthalmic disorder, in which a pathogenic role for collagen-directed autoimmunity was repeatedly suggested. We evaluated the presence of sclera-specific antibodies in a large cohort of patients with non-infectious scleritis. Therefore, we prospectively collected serum samples from 121 patients with non-infectious scleritis in a multicenter cohort study in the Netherlands. In addition, healthy (n = 39) and uveitis controls (n = 48) were included. Serum samples were tested for anti-native human type II collagen antibodies using a validated enzyme-linked immunosorbent assay (ELISA). Further, sclera-specific antibodies were determined using indirect immunofluorescence (IIF) on primate retinal/scleral cryosections. Lastly, human leukocyte antigen (HLA) typing was performed in 111 patients with scleritis. Anti-type II collagen antibodies were found in 13% of scleritis patients, in 10% of healthy controls and in 11% of uveitis controls ($p = 0.91$). A specific reaction to scleral nerve tissue on IIF was observed in 33% of patients with scleritis, which was higher than in healthy controls (11%; $p = 0.01$), but similar to uveitis controls (25%; $p = 0.36$). Reactivity to the scleral nerve tissue was significantly associated with earlier onset of scleritis (48 versus 56 years; $p < 0.001$), bilateral involvement (65% versus 42%; $p = 0.01$), and less frequent development of scleral necrosis (5% versus 22%; $p = 0.02$). HLA-B27 was found to be twice as prevalent in patients with scleritis (15.3%) compared to a healthy population (7.2%). In conclusion, scleral nerve autoantibody reactivity was more common in scleritis and uveitis patients in contrast to healthy controls. Further research is needed to characterize these scleral-nerve directed antibodies and assess their clinical value.

1. Introduction

Scleritis is a severe and painful ophthalmic disorder, with largely unknown pathogenesis, but a role for autoimmunity in its pathogenesis has repeatedly been suggested [1–5].

Circumstantial evidence for autoimmunity in scleritis is present from various viewpoints [6]. For example, specific autoantibodies are clearly involved in the pathogenesis of the systemic autoimmune diseases associated with scleritis, such as rheumatoid arthritis (RA), granulomatosis with polyangiitis, and relapsing polychondritis [7–9]. Lymphocytic infiltrates and human leukocyte antigens (HLA) class II expression are abundantly found in affected scleral tissue by scleritis [10]. Further, B-cell targeting treatment with rituximab has a beneficial effect in scleritis [11–13]. Finally, scleritis was induced after double

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immunization with type II collagen antigens in mouse models [14,15]. Defining criteria for autoimmune diseases, namely the presence of specific autoantibodies and/or the presence of auto-reactive T-cells, that after transfer lead to a similar disease in another individual, are not yet fulfilled in scleritis [6,16].

Candidate antigens in scleritis include type II collagen and other collagen types, which are abundantly present in the scleral stroma, as well as in cartilage and synovium, the affected tissues in RA and relapsing polychondritis [3,5,17,18]. High levels of anti-collagen type II antibodies were found in synovial fluid of patients with RA, and were suggested to initiate arthritis [9,19–21]. Likewise, these antibodies were also reported in relapsing polychondritis [22,23]. Research into the autoantigens and autoantibodies involved in scleritis is scarce.

We evaluated the presence of type II collagen antibodies, and investigated other potential sclera-specific autoantibodies in a large cohort of patients with non-infectious scleritis. Further, we tested for an association with HLA-B27, and searched for other HLA-associations in this cohort.

2. Methods

2.1. Study population

We prospectively performed a multicenter cohort study in the Netherlands of consecutive patients with scleritis. Patients were included from: Erasmus Medical Center (EMC), Amsterdam University Medical Center (AUMC), University Medical Center Groningen (UMCG), University Medical Center Utrecht (UMCU), and Maastricht University Medical Center (MUMC). We included a total of 121 patients with scleritis and 2 control groups: healthy individuals (n = 39) and ocular disease controls (specifically HLA-B27 positive anterior uveitis and birdshot chorioretinopathy, n = 48). The uveitis control group was proposed as such to include two distinct uveitis locations and pathogenic entities. The study was approved by the Medical Ethics Research Committee of the Erasmus MC (MEC-2019-0777), and all local Ethics Committees from the other participating medical centres. All patients signed a written informed consent to participate in the study. The research was performed according to the Tenets of the Declaration of Helsinki. The diagnoses of non-infectious scleritis and specific uveitis entities were made by an experienced ophthalmologist after a full evaluation according to national guidelines [24]. Signs and symptoms specific for scleritis were deep scleral redness, persistent redness after phenylephrine eye drops, and/or T-sign/scleral thickening/sub-scleral fluid on ultrasound B-scan, and/or signs of scleritis on MRI scan. Active scleritis or uveitis was defined by the presence of acute symptoms at presentation or during relapse despite treatment. Inactive scleritis was defined by the absence of active scleritis symptoms.

2.2. Sample and data collection

We collected serum samples from all patients and controls, which were stored at −80 °C before further processing. Clinical data of included patients were collected from medical charts and included demographics, associated systemic autoimmune disease (if present), characteristics of scleritis, presence of complications including scleral necrosis, and use of systemic immunosuppressive treatment at the moment of inclusion. Characteristics of scleritis included the onset and duration of disease, laterality, location of scleritis, i.e. anterior, posterior, panscleritis, or sclerouveitis, and subtype, i.e. diffuse, nodular, or necrotizing, in case necrosis was the most prominent feature.

2.3. Anti-type II collagen antibody enzyme-linked immunosorbent assay (ELISA)

Type II collagen antibodies were measured by ELISA using human native type II collagen (Chondrex, Redmond, Washington, USA) as antigen as previously described [25]. In short, maxisorp plates were incubated at 4 °C overnight with 100 μl of human native type II collagen diluted to 2.5 μg/ml in ice-cold phosphate-buffered saline (PBS) immediately before coating. Plates were blocked with 150 μl of 1% bovine serum albumin in PBS and subsequently incubated with 100 μl of serum diluted 1:100. Levels >95th percentile of blood donors (54 AU/mL) were considered positive.

2.4. Indirect immunofluorescence (IIF)

Serum anti-scleral and anti-retinal antibodies were determined as described previously, with slight optimization of the protocol [26]. In short, normal primate scleral/retinal tissue cryosection slides (Euroimmun, Lübeck, Germany) were incubated with patients' serum for 30 min at room temperature (RT) (dilution 1:100). After incubation, the slides were washed with PBS with Tween 20 (PBS-tween) for 15 min, and incubated with FITC-labelled goat anti-human IgA, IgG, and IgM (Euroimmun, Lübeck, Germany) for 30 min at RT. Slides were washed again with PBS-tween for 15 min, embedded in glycerol and covered with a coverslip. Slides were analyzed and photographed using an AXIO Scope.A1 fluorescence microscope (Zeiss, Thornwood, NY, USA). In case of extensive staining at a specific site the reaction is called positive. PBS-tween was used as negative reference. Rabbit polyclonal antibodies to types I, II and III collagens (ab34710; dilution 1:500; ab34712; dilution 1:100; and ab7778, dilution 1:100, respectively) with goat anti-rabbit IgG (ab150077; Alexa Fluor 488; dilution 1:500; all aforementioned antibodies from Abcam, Cambridge, UK) were used as positive references.

2.5. Proximity extension assay (PEA) technology

Serum of a subset of included patients (patients that were active; n = 39), was also tested for the presence of 18 inflammatory proteins using PEA technology (Inflammation panel, Olink Explore3072, Olink Proteomics, Uppsala, Sweden). These 18 proteins were found to be significantly up- or downregulated in scleritis in a previous study, i.e. nuclear migration protein nudC, hippocalcin like protein 1, cytosolic phospholipase A2, alpha-actinin-4, Heat shock protein 1A, eukaryotic translation initiation factor 4 gamma 1, glyoxylase domain-containing protein 4, CC motif chemokine ligand 7, C-type lectin domain family 4 member A, oncostatin M, BH3 interacting domain death agonist, peptidyl-propyl *cis*-trans isomerase FKBP1B, proteinase-activated receptor 1, hepatocyte growth factor, ras-related protein Rab-6A, cluster of differentiation 40, mitogen-activated protein kinase 9, GMP reductase 1 [27]. In PEA, matched pairs of specific antibodies both carrying a unique, complementary DNA tag will bind to the respective target protein. The DNA tags of both bound antibodies are able to hybridize when brought in proximity. The hybridized tags are extended to an amplicon, and subsequently detected and quantified using quantitative PCR (qPCR). The number of qPCR cycles is related to the expression of the protein in the tear sample, shown in log base-2 normalized protein expression (NPX) values [28].

2.6. Human leukocyte antigen (HLA) typing

DNA was isolated from whole blood using a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany), following manufacturer protocol. HLA class I and II typing, medium resolution, 1st field, was performed on all patients in which DNA was available (N = 111). HLA typing for HLA-A, -B, –C, -DRB1, DRB3/4/5, DQB1, DQA1 was performed by the reverse sequence specific oligonucleotide (SSO) method on a suspension array platform using microspheres as a solid support to immobilize oligonucleotide probes (Luminex bead technology; Immunocor Transplant Diagnostics, Stamford, CT). This results in low-to-medium resolution typing, as further described by van Sonderen et al. [29] The HLA type of the study subjects was compared to the frequency in 5604 Dutch healthy

blood donors.

2.7. Statistical analysis

Continuous data were reported as mean and standard deviation (SD), and categorical data were reported as number with percentage. A Student's t-test test, one-way ANOVA with Tukey's post-hoc test, Chi-square, and Fisher exact test were performed to analyze the results. A multivariate linear regression was used to assess the association between scleral nerve positivity and age of onset, and multivariate logistic regression was performed to study the association between scleral nerve positivity and the presence of scleral necrosis, which is shown as the unstandardized beta and 95% confidence interval (CI). To test for statistical significance in the level of inflammatory proteins between groups a Mann-Whitney U test was used with multiple testing correction using the Benjamini-Hochberg procedure, also known as false discovery rate (FDR) correction. Differences in HLA frequencies were analyzed with Fisher exact test, while probability values were corrected for multiple testing (Sidak's method), except for HLA-B27 which was previously reported to be associated with scleritis [30]. Odds ratios (ORs) were calculated using Haldane's modification of Woolf's method. R (v4.2.2, R Core Team 2021) or IBM SPSS Statistics version 25.0 was used for statistical analysis, and a corrected P value below 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

The baseline characteristics of our study cohort are shown in Table 1. Mean age at inclusion was similar in all included study groups ($p = 0.19$). Forty out of 121 patients with scleritis (33%) were active at the moment of inclusion. Systemic diseases were diagnosed in 46 patients (38%). A considerable percentage of scleritis patients had complications (62 out of 121; 51%), such as scleral necrosis (20%), cystoid macular edema or papillitis (15%). At the moment of inclusion, 89 patients (74%) received systemic treatment.

3.2. Type II collagen antibodies

In a validated ELISA for type II collagen antibodies only 13% of scleritis patients were found to have serum antibodies (Table 2). No significant differences were seen with healthy controls (10%) and a subset of the uveitis controls (11%; n = 19). Within active patients 3 out of 40 patients (7.5%) showed type II collagen antibodies, while in inactive patients 12 out of 80 (15%) showed these antibodies ($p = 0.380$; Chi-squared test). We did not identify a subset of scleritis patients that had exceptionally high levels of these antibodies. Patients with type II collagen antibodies had a significantly earlier onset of disease than scleritis patients lacking these antibodies (45 (16) versus 54 (13) years; $p = 0.02$). No further associations were found with type II collagen positivity and clinical characteristics of scleritis.

3.3. Other sclera-specific autoantibodies

IIF on retinal/scleral tissue showed specific staining patterns of type I, II and III collagen positive control antibodies, including staining of scleral nerve components, choroidal and retinal vascular walls, and inner retinal structures (type II collagen) (Fig. 1A, B, and 1C). Sera of scleritis patients did not reproduce any of the above patterns of staining, however, 40 patients (33%) showed a reaction to scleral nerve components (Fig. 1D). This percentage was significantly higher than that in healthy controls (11%; $p = 0.01$), but similar to that in uveitis controls (25%; $p = 0.36$) (Table 2). No differences in occurrence of scleral nerve reactivity was found between active (13/40; 33%) and inactive patients (27/81; 33%; $p = 1.00$; Chi-squared test). Fig. 2A shows a detailed image of this staining pattern with staining of the inner structures of the nerve fibers, the nerve axons. In contrast, Fig. 2B shows that type III collagen antibodies stains structures surrounding the nerve fibers.

Reactivity to scleral nerve tissue on IIF was significantly associated with an earlier onset of disease (48 (15) years versus 56 (12) years; $p < 0.001$), bilateral disease (65 vs 42%; $p = 0.01$), and less scleral necrosis (5 vs 22%: $p = 0.02$) (Table 3). No correlation was found between the presence of type II collagen antibodies and scleral nerve directed antibodies. In a multivariate linear regression analysis, the presence of scleral nerve directed antibodies remained significantly associated with age of onset (beta (95% CI) of −8.5 (−13.5 to – 3.5; $p < 0.001$)), after adjustment for gender, the presence of systemic disease, and the use of systemic treatment. The association between scleral nerve reactivity and the presence of scleral necrosis remained significant in a multivariate logistic regression analysis, with an odds ratio (95% CI) of 0.176 (0.04–0.86; $p = 0.032$), adjusted for the age of onset, gender, presence of systemic disease, bilateral disease, and the use of systemic treatment. No differences in etiology, i.e. idiopathic scleritis vs systemic underlying

Table 1
Baseline characteristics of study population.

	Scleritis (n = 121)	Healthy controls (n = 39)	Uveitis controls (n = 48)	p-value
Age at inclusion, mean (SD)	57 (14)	53 (15)	60 (14)	0.66
Age onset, mean (SD)	53 (14)		49 (14)	
Male, N (%)	39 (32)	18 (49)	21 (44)	0.12
Disease activity, N (%)				
Active	40 (33)		25 (53)	
Inactive	81 (67)		22 (47)	
Baseline characteristics scleritis				
Bilateral, N (%)	60 (50)			
Etiology, N (%)				
Systemic disease-associated	46 (38)			
GPA	10 (22)			
RA	8 (17)			
RP	6 (13)			
SLE	2 (4)			
Behcet	2 (4)			
Other	18 (39)			
Idiopathic	74 (61)			
Trauma	1 (1)			
Location, N (%)				
Anterior	65 (54)			
Posterior	15 (12)			
Sclerouveitis	23 (19)			
Panscleritis	18 (15)			
Subtype scleritis, N (%)				
Diffuse	71 (68)			
Nodular	21 (20)			
Necrotizing	12 (11)			
Complications, N (%)[a]	62 (51)			
Systemic treatment during inclusion, No (%)	89 (74)			
NSAIDs < CS < 3months	27 (30)			
DMARDs/CS > 3months	65 (73)			
Biologicals/ cytostatics	30 (34)			

N = Number; GPA = Granulomatosis with polyangiitis; RA = Rheumatoid arthritis; RP: Relapsing polychondritis; SLE =Systemic lupus erythematosus; NSAIDs = Non-steroidal anti-inflammatory drugs; CS = corticosteroids; DMARDs = Disease modifying anti-rheumatic drugs; SD = Standard Deviation. A One-way ANOVA was used to test for statistical significance.

[a] Including scleral necrosis (20%) cataract (22%), glaucoma (4%), cystoid macular edema or papilledema (15%), choroidal effusion, detachments or folds (11%), serous retinal detachment (8%), peripheral ulcerative keratitis (3%), temporary ocular hypertension (3%), temporary hypotension (1%), limbitis (1%), diplopia (1%), enucleation (1%), and synechiae (1%).

Table 2
Presence of scleral-specific reaction on indirect immunofluorescence and type II collagen antibodies on ELISA.

	Total (N = 205)	Scleritis (n = 121)	Healthy controls (n = 39)	Uveitis control (n = 148)	P-value
Scleral nerve staining on IIF, N (%)	56 (27)	40 (33)	4 (11)	12 (25)	0.032[b]
Type II collagen Ab, N (%)	21 (12)	15 (13)[a]	4 (10)	2 (11)[c]	0.91
Level type II collagen Ab (AU/ml), mean (SD)	27.1 (42)	26.0 (29)	20.8 (19)	47.4 (101)[c]	0.06

SD = Standard Deviation; Ab = Antibodies.
A Pearson Chi-Square test was used for categorical data, while a One-way ANOVA was used for continuous data.
[a] 1 scleritis sample is missing, data of 120 scleritis patients.
[b] The percentage of scleritis patients with scleral nerve positivity differs significantly versus the healthy controls (p = 0.01), however, not compared to the uveitis controls (p = 0.36).
[c] The presence and level of type II collagen Ab were determined in a subset of uveitis controls (n = 19).

disease, was seen in the patients with or without scleral nerve reactivity (p = 0.77). Scleral nerve positivity was significantly associated with a higher level of the protein oncostatin M in active patients (n = 39; p = 0.045; Fig. 3).

3.4. HLA-genotype

HLA-B27 allele was present in 17 out of 111 (15.3%) patients with scleritis, which was significantly higher compared to 7.2% of healthy controls (OR 2.37; CI 1.41–3.99; p = 0.005). Six out of 17 HLA-B27 positive patients had a systemic disease, and no significant correlations to the scleritis location, subtype, or the occurrence of complications were found. The presence of scleral-nerve reactivity was not higher in HLA-B27 (35%) positive versus negative (36%; p = 1.00) patients. Further, no significant associations with specific HLA-A, HLA-B, HLA-C, HLA-DR or HLA-DQ types were found taking multiple testing into account (Supplementary Table 1). Some HLA-alleles, such as HLA-A31 and HLA-DR4, were more prevalent in scleritis patients than in healthy controls, but not statistically significant after correction.

4. Discussion

We found no evidence for a higher frequency of serum type II collagen antibodies in this large cohort of patients with scleritis. IIF showed absent scleral reactivity, but a common anti-scleral nerve responsiveness in patients with scleritis and uveitis controls. The presence of scleral nerve reactivity was observed in patients with early onset of disease, bilateral scleritis, and less occurrence of necrosis. The presence of HLA-B27 allele was found to be significantly higher in patients with scleritis compared to a healthy population.

A role for autoimmunity in the origin and pathogenesis of scleritis has been repeatedly proposed. However, following the Witebsky postulates no evidence of characterizing autoantibodies and/or autoreactive T-cells that are pathogenic is present yet [1–6,14,15,31]. The presence of antibodies against sclera-specific antigens was noted only once in a study by Aragaki et al., who found reactivity of two patients' sera against scleral antigens of 15 kDa and 45 kDa on Western blot [1].

Type II collagen was mentioned multiple times as a possible inciting antigen in scleritis, and in scarce mouse models scleritis could be initiated after repeated immunization with type II collagen antigens [3,5,14,15,17,18]. Also, in RA as well as relapsing polychondritis high levels of type II collagen antibodies were found, and a close resemblance between human cartilage and sclera is present [19–23,32]. We have tested the presence of anti-collagen type-II in the serum of our cohort of scleritis patients, but could not find any increased prevalence compared to healthy and uveitis controls. In RA, the prevalence of collagen type II antibodies is not high as well. However, they seem to be associated with

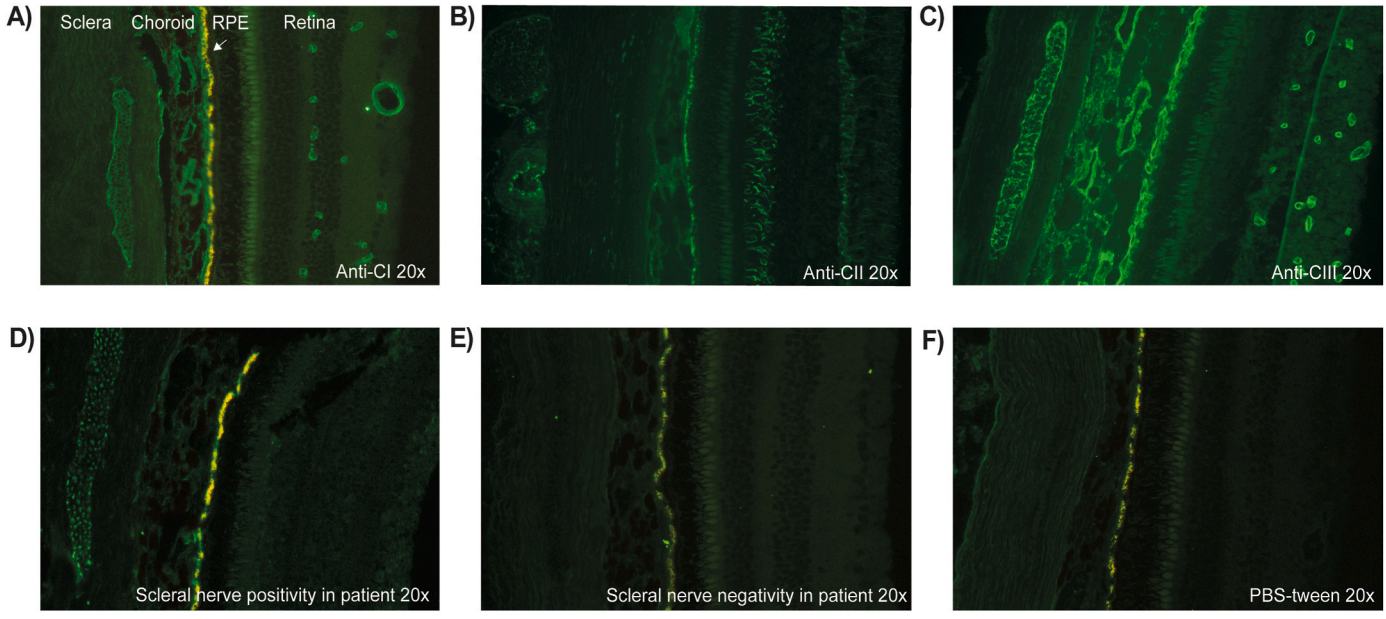

Fig. 1. Type I, II and III collagen antibodies and patient sera reactivity on retinal/scleral cryosections A) Pattern of type I collagen on scleral/retinal slide, the epineurium and perineurium of the scleral nerve, choroidal vessels, and inner retinal vessels are positive. B) Pattern of type II collagen on scleral/retinal slide with positivity of scleral vessel wall, RPE, retinal outer nuclear layer and retinal inner plexiform layer. C) Pattern of type III collagen on scleral/retinal slide, that is similar to type I collagen, with the epineurium and perineurium of the scleral nerve, and choroidal and retinal vessels that are positive. D) Serum of patient with scleritis incubated with scleral/retinal slide gives a positive reaction on scleral nerve. E) Serum of patient with scleritis incubated with scleral/retinal slide, without staining of scleral nerve, or other structures. F) PBS-tween as a negative control incubated with scleral/retinal slide, no staining visible. Anti-CI = Type I collagen antibody; Anti-CII = Type II collagen antibody; Anti-CIII = type III collagen antibody; PBS = phosphate buffered saline.

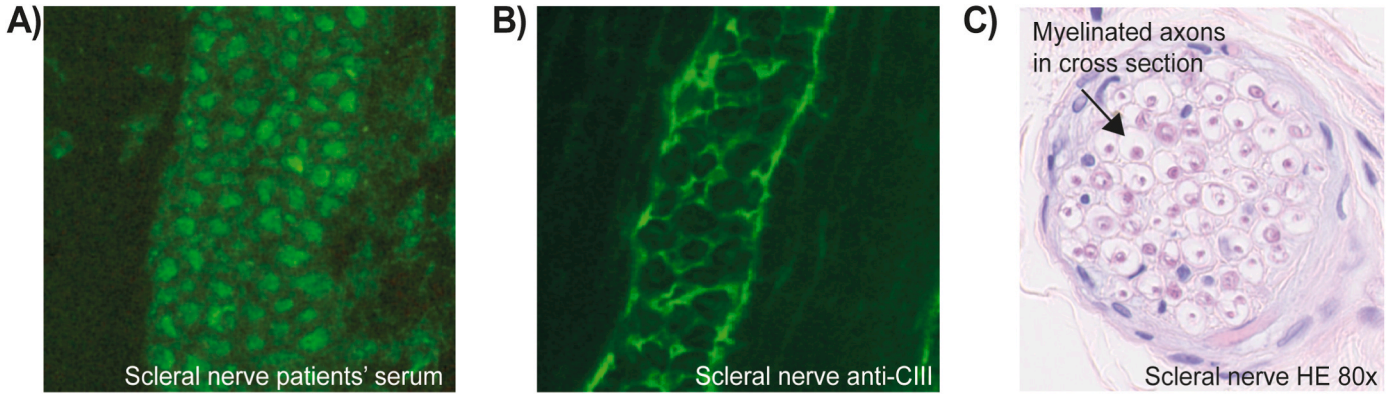

Fig. 2. Detailed view of patients' serum scleral nerve reactivity, type III collagen antibody scleral nerve reactivity, and HE image of scleral nerve A) Detailed image of patients' serum reactivity to the scleral nerve, seen in one-third of scleritis patients. Staining seems to be specific for the inner part of nerve fibers, I.e. axons. B) Detailed image of anti-CIII reactivity to the scleral nerve, wherein staining seems specific for fiber enclosing structures, i.e. the perineurium and endoneurium. C) HE stained image of a scleral nerve, wherein myelinated axons are seen in cross-section. Anti-CIII = anti-collagen type III; HE = Hematoxylin-Eosin staining.

Table 3
Scleral nerve positivity and clinical characteristics of scleritis (n = 121).

	Scleral nerve positive (n = 40)	Scleral nerve negative (n = 81)	P -value
Age at onset, mean (SD)	48 (15)	56 (12)	<0.001
Active disease, N (%)	13 (33)	27 (33)	1.00
Etiology, N (%)			0.77
ystemic disease-associated	15 (38)	31 (38)	
Idiopathic	25 (63)	49 (61)	
Trauma	0 (0)	1 (1)	
Bilateral, N (%)	26 (65)	34 (42)	0.01
Location scleritis, N (%)			
Anterior	22 (55)	43 (53)	0.23
Posterior	8 (20)	7 (9)	
Sclerouveitis	5 (13)	18 (22)	
Panscleritis	5 (13)	13 (16)	
Subtype scleritis, N (%)			
Diffuse	29 (88)	42 (59)	0.01
Nodular	4 (12)	17 (24)	
Necrotizing	0 (0)	12 (17)	
Scleral necrosis, N (%)	2 (5)	18 (22)	0.02
Systemic treatment, N (%)	32 (80)	57 (70)	0.28
NSAIDs/CS < 3 months	9 (28)	18 (32)	0.81
DMARDs/CS > 3 months	22 (69)	43 (75)	0.62
Biologicals or cytostatics	11 (34)	19 (33)	1.00

N = Number; NSAIDs = Non-steroidal anti-inflammatory drugs; CS = Corticosteroids; DMARDs = Disease modifying anti-rheumatic drugs; SD = Standard Deviation.
A Pearson Chi-Square test was used for categorical data, while a Student's T-test was used for continuous data.

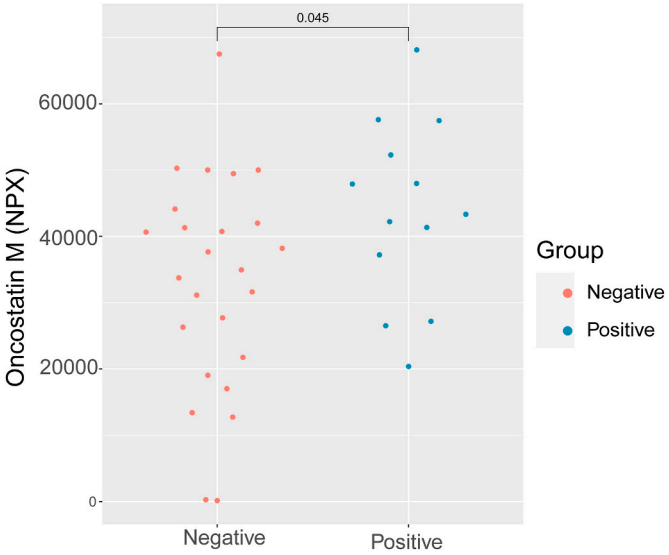

Fig. 3. Serum levels of Oncostatin M between scleral – nerve antibody positivity and negativity in active scleritis patients (n=39).
Serum levels of scleral-nerve antibody positive patients were significantly higher compared to scleral-nerve antibody negative patients (p = 0.045).

severe disease in the initial phase and favorable prognosis afterwards [21,25,33]. In our initial ELISA-based analysis, we did not specifically investigate alternative collagen types that are present within the scleral stroma (I, III, IV-XII, XIV-XVI, XVIII, XIX, XXII, XXIII, XXV, XXVI), and potentially could represent candidate antigens in scleritis [34–36].

Therefore, we used IIF on scleral tissue to screen a large cohort of scleritis patients' serum reactivity against other potential scleral antigens. IIF is a very sensitive and widely used technique for autoantibody discovery [37,38]. We did not discover scleral directed reactivity. However, we did find a specific reactivity against the scleral nerve in the sera of one-third of patients with scleritis. This specific scleral nerve reactivity seems to be directed to nerve axons. Further research is needed to further characterize the antigen, possibly by using immunoprecipitation and mass spectrometry [39]. Antibodies against peripheral nerve are known to be present in neuromyelitis optica, in which they are directed to the myelin sheet or astrocytes [7]. The role of these autoantibodies in disease causation is unclear. In contrast, in Guillain-Barre syndrome anti-ganglioside antibodies are believed to have a pathogenic role in nerve destruction [40]. To the best of our knowledge antibodies against peripheral nerve structures were not noted before in ocular diseases [41].

Even if the presence of antibodies directed against scleral nerve could be confirmed by subsequent studies, their pathogenicity remains questionable. We also found scleral-nerve specific reactivity in uveitis controls, reflecting low disease specificity. The sclera is a structure rich in nerve fibers and the intense pain that patients with scleritis generally suffer from could be a result of nerve inflammation [3,5]. However, intense pain is not typical for patients with uveitis. Unfortunately, we have not been able to investigate the association between the presence of antibodies and pain, as this was not recorded consistently. Also, in histopathological studies of few patients with scleritis, nerve damage was not reported [10,42–44]. Scleral-nerve directed antibody positivity

seems to be associated with a clinical phenotype in our study, that occurs reasonably early in life, is bilateral and mild (without necrosis). Furthermore, the level of oncostatin M, a cytokine belonging to the interleukin-6 group of cytokines, was enriched in patients with scleral-nerve directed antibody positivity. In an earlier study oncostatin M was associated with more prevalent bilateral disease [27]. Whether this reflects a higher state of innate immune activity, or a distinct pathogenesis is yet unclear. Furthermore, it remains to be determined whether scleral-nerve directed antibodies are pathogenic or an epiphenomenon of ocular inflammation and subsequent tissue damage. However, the potential prognostic value and clinical relevance of the scleral nerve directed antibodies may be further evaluated.

The presence of specific HLA-alleles has been implicated to play a role in the pathogenesis of various organ-specific autoimmune diseases including type I diabetes. Most likely, these HLA alleles contribute to the disease by enhanced presentation of disease-specific autoantigens [45]. HLA alleles are associated with the occurrence and severity of RA, relapsing polychondritis, and ANCA-associated vasculitis, all representing scleritis associated diseases [46–48]. In addition, an increased expression of HLA class II (HLA-DR) was seen in affected sclera in scleritis [3,36]. In our cohort we found a higher prevalence of HLA-B27 (15.3%) compared to a healthy population, which confirms the results of a small study by Anshu et al. [30] This association was found to be scleritis specific, as the minority of HLA-B27 positives had a systemic disease. However, HLA-B27 positivity was not associated to the presence of scleral-nerve directed antibodies. No further HLA association was observed in this large cohort of 111 patients with scleritis. The association with HLA-B27 is most known from spondylarthropathies, of which the pathogenesis is not fully elucidated. Optimalized antigen presentation, that is beneficial in the fight against viral infections, is generally thought to be responsible for the downside autoimmunity, or a chronic inflammatory response [49].

Our serological analysis is limited to IIF and a specific anti-collagen type II ELISA. We have chosen a validated type II collagen ELISA used in multiple studies in RA [9]. However, collagen remains a complex protein with a large native triple-helix form, that in tissues is cross-linked and sometimes post-translationally modified [50]. The used ELISA antigen is a native monomeric type II collagen, and no type II collagen antibodies against denatured or post-translationally modified epitopes will be found. Although IIF is a very sensitive technique, there is still a possibility of missing serum sclera-specific antibodies [37,38]. Firstly, we have used primate scleral/retinal cryosections, and small differences with human scleral/retinal tissue could be present. We attempted to test human scleral/retinal tissue, but the background staining was too intense for correct evaluation. Next, antigens might be inaccessible in the prepared tissue slides, as every form of fixation alters the tissue [51]. Finally, we focused on native antigens, while it might be possible that antibodies are present against damaged, or post-translationally modified antigens, as is the case in RA and anti-cyclic-citrullinated peptide antibodies [52]. In scleritis, it was hypothesized that at first a disruption of the proteoglycan-collagen structure by matrix metalloproteases takes place, that might induce neo-epitopes [5]. Next to research into autoantibodies, it would be interesting to study the presence of autoreactive T-cells using a cellular approach, as was done in RA [53]. Besides the described circumstantial evidence for an autoimmune process in scleritis, there is evidence for dysfunction of the innate immune system as well [12,54,55]. The distinction between a potential autoimmune and/or autoinflammatory origin of scleritis is consequently complicated and remains subject of discussion.

In conclusion, we found scleral-nerve reactivity of unknown antigenic origin in a subset of scleritis patients and in uveitis controls, but no evidence for a higher frequency of native, unmodified collagen type II antibodies in a large cohort of scleritis patients. Further research is needed to characterize the scleral nerve associated antigens and assess their clinical value.

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Appendix A. Supplementary data

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