HHS Public Access

Author manuscript

Ocul Surf. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

Ocul Surf. 2021 July ; 21: 186–192. doi:10.1016/j.jtos.2021.06.001.

Relationships between activated Dendritic Cells and Dry Eye Symptoms and Signs

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Abstract

Purpose: To examine whether “activated” dendritic cells (aDCs) could serve as a biomarker of systemic immune disorders in individuals with dry eye (DE) symptoms. Secondarily, to examine the impact of a topical anti-inflammatory agent on aDC number.

Methods: Retrospective analysis was conducted to identify individuals with DE symptoms who had in-vivo confocal microscopy (IVCM) imaging between October 2018 and July 2020 at the Miami Veterans Hospital. aDCs were manually quantified based on morphology. Receiver operating curve (ROC) analysis examined relationships between aDC number and systemic immune disease status. Individuals were then grouped by aDC number (≥2 versus <2) and demographics and DE parameters were examined. Paired t-test was performed to evaluated aDC number pre- vs post-initiation of an anti-inflammatory agent.

Results: 128 individuals were included. Their mean age was 57.1±15.0 years; 71.1% were male, 53.1% self-identified as White and 24.2% as Hispanic. The mean number of aDCs in the central cornea was 1.28±2.16 cells/image. The presence of ≥2 aDCs had a sensitivity of 60% and specificity of 77% for the diagnosis of a systemic immune disorder. Individuals with ≥2 aDCs were more likely to self-identify as Black, have Secondary Sjögren’s, and have higher nerve fiber area and fractal dimension. In 12 individuals, aDC number decreased from 2.69±2.36 to 0.58±0.73 cells/image after initiation of an anti-inflammatory agent, p=0.01.

Conclusions: The presence of ≥2 aDCs in the central cornea suggests a systemic immune disorder in individuals with DE symptoms. Topical anti-inflammatory therapy can reduce the number of aDCs in the central cornea.

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Declarations of Interest: None.

Disclaimer: The views expressed in this work are not an official position of the Veterans Health Administration.

INTRODUCTION:

Dry Eye (DE) is a common disease that affects over 16 million people in the United States (US).[1] DE can manifest with symptoms of pain, described as dryness, burning, and foreign body sensation; and/or visual disturbances, described as poor or fluctuating vision. Signs of DE are likewise varied and include decreased tear production, increased tear instability, epithet lial irregularities, and ocular surface inflammation. [2,3] Adding to the complexity, many studies have found a disconnect between symptoms and signs of disease.[4] Overall, DE symptoms interfere with activities of daily living (such as reading and computer work), have a negative effect on mental health, and decrease overall quality of life.[5, 6] The disease is more prevalent among women and its incidence increases with age.[4] Many factors can influence DE symptoms and signs including co-morbid systemic immune conditions, meibomian gland dysfunction (MGD), the use of topical and oral medications, and surgery.[4] A challenge in DE is to identify the specific abnormalities that contribute to symptoms and signs of disease in an individual patient.

One potential biomarker in DE is the presence of dendritic cells (DCs) in the cornea. DCs are antigen presenting cells (APCs) that play an important role in the innate and adaptive immune responses[7]. In a normal cornea, DCs are mostly found peripherally, within the anterior stroma and epithelium.[8,9] In response to inflammation, DCs undergo several changes that have been termed “activation”. These include changes in morphology (such as an increase in overall size, and increased number and length of dendrites[10]), location (migration into the central cornea[10]), and expression of surface markers (MHC-II, CD83, CD86 and CCR7).[11] Studies have demonstrated that “activated” Dendritic Cells (aDCs) migrate to the cervical lymph nodes and promote the differentiation of naïve T-cells, which then find their way to the cornea. [12,13] This process has been noted to occur in various animal models of DE.[14, 15] In a desiccating stress model, expression of maturation markers on DCs was accompanied by an increase of CD4+ T- cells within the cervical lymph nodes and cornea.[11] Similar findings were demonstrated in a mice model of Sjögren’s (TSP-1 null mice) where DE develops spontaneously.[16] In addition, the important role of DCs as APCs in DE was demonstrated by a higher percent of DCs in the cervical lymph node expressing an antigen placed on the cornea in TSP-1 null, compared to control mice.[16]

The morphology and location of DCs can also be evaluated in humans with in-vivo confocal microscopy (IVCM).[17] Specifically, metrics that can be captured using IVCM include number of DCs in the central cornea (usually represented as cells/mm^2 or cells/image), size of DCs, and number and length of dendrites. Overall, individuals with Sjögren’s-associated DE have been found to have a higher number of DCs (71.65±72.54 to 169±48 cells/mm^2) compared to individuals with non-Sjögren’s DE (40.33±31.63 to 89.8±10.8 cells/mm^2)[18–23], MGD (49.04±55.68 to 82±38 cells/mm^2)[24–26], and controls (21.46±21.74 to 53±34 cells/mm^2).[19–23, 25, 27–29] Beyond Sjögren’s, individuals with other autoimmune diseases have been found to have higher numbers of DCs in the central cornea, including those with rheumatoid arthritis (25.9±26.6 to 68.2±72.3 cells/mm^2),[21, 28] systemic lupus erythematosus (43.08±48.67 cells/mm^2),[30] and Behcet’s (19.6 [6.3–46.3] cells/mm^2),[31] compared to healthy controls (10.1 to 23.85 ± 33.81 cells/mm^2).[28, 30, 31]

Interestingly, the morphological characteristics of “activation” (size, number, and length of dendrites) has also been associated with immunohistochemistry (IHC) markers of “activation” (HLA-DR) in humans. In a study of 41 individuals that had an IVCM scan prior to penetrating keratoplasty due to different etiologies (herpetic keratitis, keratoconus, or prior graft rejection), the morphology of DCs on IVCM was compared to markers noted on IHC. “Immature” or “inactive” DCs (DC-SIGN+, HLA-DR-) had “bright cell bodies with shorter plumb dendrites” on IVCM and were predominant in individuals with keratoconus; while “mature” or “active” DCs (DC-SIGN+, HLA-DR+) had “longer interdigitating dendrites” and were more predominant in individuals with inflamed corneas (history of herpetic keratitis or graft rejection)[32].

Based on the data above, the goal of this study was to examine whether DC number could be used clinically to identify individuals with DE in the setting of a co-morbid systemic immune condition. As there are no built-in software packages to quantify DC number, clinicians usually rely on manual counts. Given that aDC numbers are lower than total DC numbers (and therefore quicker to quantify manually) and that the two metrics are correlated[19, 21], our current study examined whether aDC number could be used as a biomarker for the presence of a systemic immune disorder in individuals with DE symptoms. Secondarily, we evaluated whether aDC numbers decreased after initiation of a topical anti-inflammatory agent, irrespective of the presence or absence of a co-morbid autoimmune disease.

METHODS:

2.1 Study population

Retrospective chart review was conducted to identify individuals seen at the Miami Veterans Administration Medical Center with DE symptoms (Dry Eye Questionnaire (DEQ-5) ≥6) and an IVCM scan between October 2018 and July 2020. Individuals were excluded if they had ocular co-morbidities that could confound the ability to detect aDCs on IVCM, such as corneal scarring or a history of corneal infection. [33,34] Data regarding demographics and pertinent medical information including ocular and systemic comorbidities, surgical history, and current medications were obtained. A total of 12 individuals with DE symptoms were started on topical 0.05% cyclosporine therapy twice a day combined with fluorometholone 0.1% for the first month and had a repeat scan ≥3 months after commencing therapy.

2.2 Clinical assessment

All individuals seen in the dry eye clinic underwent a standard evaluation which included filling out validated questionnaires for dry eye symptoms: Dry Eye Questionnaire-5 (DEQ-5; 0–22)[35], Ocular Surface Disease Index (OSDI; 0–100)[36], and 4 select questions from the NPSI-Eye[37] regarding intensity of burning pain, evoked pain to wind, light and changes in temperature (scale 0–10 for each question)[38]. The ocular surface examination included, in the order performed (1) matrix metalloproteinase 9 test (MMP-9; InflammaDry; Quidel, San Diego, CA; 0–3); (2) eyelid evaluation for anterior blepharitis (scale 0–3), vascularity (scale 0–3), Meibomian gland inspissation (scale 0–3); (3) fluorescein placed and conjunctivochalasis evaluated (temporal, middle and nasal; scale 0–2); (4) tear break-up time

(TBUT), 3 values measured in each eye and averaged in seconds; (5) corneal epithelial cell disruption graded to the NEI scale (0–15)[39]; (5) palpebral conjunctiva morphology graded (scale 0–2); (6) ocular pain assessment via numerical rating scale (NRS, 0–10)[40] before and after topical anesthesia; (7) basal tear secretion (Schirmer's test with anesthesia measured in mm of wetting at 5 minutes); and (8) meibum quality (scale 0–4). A repeated evaluation including DEQ-5, OSDI and MMP-9 measurements was conducted for individuals who received a second scan after commencing topical anti-inflammatory therapy.

2.3 In vivo Confocal microscopy

Laser scanning in vivo microscopic was conducted using the Rostock Cornea Module of the Heidelberg Retina Tomograph III (Heidelberg Engineering, Heidelberg, Germany) to capture images of the central cornea of all patients as previously described.[33] This confocal microscope utilizes a 670 nm wavelength Helium-Neon diode laser as the illumination source and a 63x objective immersion lens. Each patient received a drop of 0.5% proparacaine hydrochloride as topical anesthetic before the examination. Patients were properly positioned and instructed to fixate on a target light. An appropriate amount of 0.3% Hypromellose gel (Systane Lubricant Eye Gel, Alcon, Fort Worth, TX) was applied to the lens tip and a disposable sterile plastic cap (Tomo-Cap, Heidelberg Engineering, Heidelberg, Germany) for lubrication and to improve optical coupling. The lens was moved toward the eye until the gel contacted the central cornea. Maximum image acquisition time was set at 5 minutes per eye. A total of 5 sequence scans of non-overlapping areas of the central cornea with a target depth of 30–60 μm were recorded at a rate of 30 frames per second, yielding up to 100 images per scan for each patient. Each image represented a coronal section measuring 400 μm by 400 μm, with 1–2 μm lateral resolution and 4 μm axial resolution.

Representative sub-basal nerve plexus images were selected by reviewers masked to the clinical findings using the following selection criteria: 1) best focused complete image of a single plane located between Bowman's layer and the basal epithelial membrane; 2) absence of significant artifacts (such as motion or folds); 3) adequate contrast allowing appropriate nerve detection. A total of three non-overlapping images was obtained per patient and the averages of the obtained values were used for analyses.

2.4 Quantitative image analysis

Quantitative image analysis was performed using the automated ACCMetrics Corneal Nerve Fiber Analyzer software v.2 (University of Manchester, Manchester, United Kingdom)[41] which has been previously validated.[42] The parameters analyzed included: Corneal Nerve Fiber Density defined as the total number of major nerve fibers per squared millimeter (fibers/mm^2); Corneal Nerve Fiber Length defined as the total length of all nerve fibers per squared millimeter (mm/mm^2); Corneal Nerve Branch Density defined as the number of branches originating from major nerve trunks per squared millimeter (branches/mm^2); Corneal Total Branch Density defined as the total number of branch points per squared millimeter (branches/mm^2); Corneal Nerve Fiber Area defined as the total nerve fiber area in squared millimeters per squared millimeter (mm^2/mm^2); Corneal Nerve Fiber Width defined as the average nerve fiber width per squared millimeter (mm/mm^2); and Corneal Nerve Fractal Dimension that measures the structural complexity of corneal nerves.[43] The nerve

tracings of all images analyzed automatically by the software were reviewed and corroborated by the investigators.

2.5 Qualitative image analysis

Qualitative analysis was conducted using the same images to evaluate DCs based on their morphological appearance. Up to three images per individual were counted by reviewers masked to the clinical findings and DCs and aDCs were quantified. DCs were identified as hyperreflective cells with or without prominent processes emanating from them. DCs were categorized as "activated" if they had a slender body with at least 3 processes emanating from the cell trunk that were of the same size or longer than the cell body itself. [32, 44] Representative images demonstrating the selection parameters of aDCs are shown in Figure 1. Total DCs and aDCs were reported as the number per image (cells/image). For comparison to prior studies, this number was adjusted by a factor of 6.25 to report as cells/mm^2 given that each image measured 400μm by 400 μm. Prior to commencing the study, we first examined inter-rater reliability using the intra-class correlation coefficient (ICC). Two masked readers evaluated 20 images with an ICC of 0.981 ($p<0.001$) for DC number and 0.948 ($p<0.001$) for aDC number.

2.6 Statistical Analysis

Statistical analyses were performed using SPSS statistical package version 26.0 (IBM Corp, Armonk, NY). In this study, confocal data from the right eye of each individual were included. Receiver operator curve (ROC) analysis was conducted to examine the aDC cut-off that best discriminated between individuals with vs without a known systemic immune disease. Area under the curve, standard error (SE), 95% Confidence intervals (CI), and sensitivity and specificity were reported. Based on the ROC analysis, individuals were grouped based on the presence or absence of ≥2 aDC in the central cornea. Independent two-tailed Student t-test, Chi square and Fischer's exact tests were used, as appropriate, to compare variables of interest between the 2 groups. A forward stepwise binomial logistic regression was then conducted to evaluate the contribution of possible confounders to a diagnosis of autoimmune disease. For this analysis, aDC number was dichotomized (≥2aDCs vs <2aDC). Paired two-tailed Student t-test was utilized to compare variables before and after the initiation of a topical anti-inflammatory agent in a subset of individuals (n=12). P-value less than 0.05 was considered statistically significant.

2.7 Ethical statement

This study was approved by the Institutional Review Board (IRB) at the Miami Veterans Administration Medical Center and was conducted in accordance with the principles of the Declaration of Helsinki and the United States Health Insurance Portability and Accountability Act.

RESULTS

Study population:

A total of 128 individuals were included in this study. Demographic characteristics of the study population are presented in Table 1. Among the study population, the mean age was

57.1±15.0; 91 (71.1%) were male; 68 (53.1%) self-identified as White and 31 (24.2%) as Hispanic; 26 (20.3%) were current tobacco smokers.

Numbers of DCs and aDCs in the central cornea:

The mean number of DCs in the central cornea was 5.67±7.5 cells/image (35.4±46.9 cells/mm^2), range [0–48 cells/image; 0–300 cells/mm^2]. The mean number of aDCs in the central cornea was 1.28±2.16 cells/image (8.0±13.5 cells/mm^2), range [0–10.5 cells/image; 0–65.6 cells/mm^2] (Figure 2).

Receiver operator curve analysis:

13 of 128 individuals were diagnosed with a systemic immune disease including Sjögren's, Graft versus host disease (GVHD), autoimmune vasculitides (temporal arteritis, granulomatosis with polyangitis, Behcet's syndrome), sarcoidosis, rheumatoid or psoriatic arthritis, systemic lupus erythematosus, and/or psoriasis. ROC analysis was conducted to evaluate the sensitivity and specificity of aDC for grouping individuals into systemic immune disease categories (absence vs present) (Figure 3). The area under the ROC curve was 0.73 (SE 0.09; 95% CI 0.55–0.90; p-0.02). The ROC curve analysis suggested that the most useful cutoff was ≥2 aDCs per image, with a sensitivity of 60% and a specificity of 77%.

Demographics and co-morbidities by aDCs number:

Based on the ROC analysis, we then split individuals into two groups based on the presence or absence of aDCs≥2 in the central cornea. Demographics, systemic and ocular comorbidities, and medications split by aDC≥2 are presented in Table 2. Black individuals were more likely than White individuals to have ≥2aDCs in their central cornea (51.5% vs 27.7%, p=0.02). Age, sex, and ethnicity were similarly distributed between the two groups. Similar to the ROC analysis, individuals with vs without systemic immune diseases were more likely to have ≥2aDCs in their central cornea (23.3% vs 7.5%, p=0.04), with this relationship mostly driven by secondary Sjögren's (15.2% vs 3.2%, p=0.03). Individuals with ≥2aDCs in the central cornea were more likely to be treated with autologous serum tears (30.3% vs 9.5% p<0.01) and oral immunosuppressants (15.2% vs 2.1% p<0.01).

A binary logistic regression with forward stepwise analysis was performed to evaluate the contributions of demographics (age, sex, race and ethnicity), smoking status and comorbidities on the diagnosis of a systemic autoimmune disease. Female sex (OR: 7.62; 95% CI 2.07–28.09; p=0.002) and the presence of ≥2aDC (OR: 4.52; 95% CI 1.27– 16.04; p=0.02) remained significant predictors for the diagnosis of concomitant autoimmune disease and these variables accounted for approximately 24% of the variance in the model (R=0.49, p<0.001).

DE profiles by aDC number:

DE symptoms, signs and IVCM parameters grouped by the presence or absence of ≥2 aDCs are presented in Table 3. DE symptoms and signs were similar between the groups. The exception was a lower frequency of conjunctivochalasis in individuals with ≥2 aDCs. Not surprisingly, individuals with ≥2 aDCs also had a higher number of overall DC compared to

those with <2aDCs. Furthermore, nerve fiber area and fractal dimension were also higher in the ≥2 aDCs vs < 2 aDCs group (0.006±0.002 vs 0.007±0.003 $\mu m^2/mm^2$, p<0.01 and 1.45±0.06 vs 1.47±0.04, p=0.05, respectively).

Change in aDCs with treatment:

A subset of the population with DE symptoms (n=12), independent of the presence or absence of a systemic immune disease, were started on topical anti-inflammatory therapy and had a repeat scan ≥3 months after commencing therapy. The average time interval between initiation of therapy and repeat scan was 5.1±1.5 months. The aDCs number in the central cornea decreased by 78% from 2.69±2.36 cells/image (16.81±14.75 cells/mm^2) to 0.58±0.73 cells/image (3.63±4.56 cells/mm^2), p=0.01. There were no significant changes in symptom scores over time (via the DEQ-5 and OSDI) in these individuals: however, the MMP-9 score decreased from 1.40±0.70 to 0.90±0.57, p=0.05.

DISCUSSION

In this study, we present the clinical utility of using aDC number in the central cornea as a biomarker of a co-morbid systemic immune disease in individuals with DE symptoms. This is needed, as an important consideration in DE is to determine whether a systemic immune disease contributes to symptoms and/or signs. Prior studies have demonstrated that 18–35% of individuals with an autoimmune disease, such as Sjögren's, rheumatoid arthritis, systemic lupus erythematosus or thyroid disorders, have DE symptoms.[45–49] Many individuals already carry a systemic diagnosis when presenting to the eye care provider, but in some cases, DE is diagnosed prior to a systemic autoimmune disease. This most commonly occurs with primary Sjögren's,[50] but can happen with other diseases, such as thyroid abnormalities.[51,52] In Sjögren's in particular, the systemic disease is often diagnosed years after the start of symptoms.[53]

Overall, we found that the presence of ≥2 aDC in the central cornea was more common in individuals with a diagnosed systemic immune disease, with a sensitivity of 60% and a specificity of 77%. This finding was mostly driven by secondary Sjögren's. While prior studies did not specifically comment on aDC number, the mean overall DC number in our population of individuals with a systemic immune condition (52.5±62.5 cells/mm^2) was similar to that previously described in Sjögren's (49.0±12.9 to 239.6±52.9 cells/mm^2),[22,54] rheumatoid arthritis (25.9±26.6 to 68.2±72.3 cells/mm^2),[21,28] ankylosing spondylitis[55] (75.5 [51.2–112.6] cells/mm^2), and thyroid-induced ophthalmopathy (47.5±38.6 to 76.4±67.8 cells/mm^2).[27]

Interestingly, we also found that Black individuals were more likely to have ≥2 aDC as compared to White individuals. The reason behind this association is uncertain, but previous studies have reported that Black individuals have higher serum levels of inflammatory markers, such as IL-6 and CRP, compared to their White counterparts.[56–59] Interestingly, a more robust proinflammatory state is thought to be a contributing factor to the higher COVID-19 hospitalization and mortality rates seen in Black individuals as compared to White individuals.[59–61] Furthermore, certain nerve parameters were also associated with aDC number, namely increased nerve fiber area and fractal dimension. Other studies in

diverse populations have also noted that increased aDC number in individuals with vs without DE was oftentimes accompanied by a higher nerve tortuosity grade (2.30±0.4 to 3.18±0.75 vs 1.09±0.54 to 1.50±0.52),[21, 62–64] and nerve beading (323±63 to 387±62 beadings/100μm vs 182±63 to 198±65 beadings/100μm).[62, 65] Nerves and DCs likely continuously interact and communicate with one another as was nicely demonstrated in mice, where corneal nerve regeneration after epithelial debridement was significantly impaired in corneas depleted of DCs compared to controls.[66] Interestingly, the addition of topical ciliary neurotrophic factor (normally secreted by DCs) restored nerve regeneration in DC depleted corneas after epithelial debridement.[66] Moreover, DC depleted corneas of mice exposed to desiccating stress had reduced paracentral corneal nerve density and reduced levels of neurotrophic factors, such as nerve growth factor, substance P and calcitonin gene related peptide, compared to non-DC depleted corneas[67], further suggesting an association between nerve health and corneal DCs.

Fortunately, independent of the presence of a systemic immune condition, aDC number decreased with topical anti-inflammatory therapy. This is in agreement with prior studies. In one study of 50 individuals with DE (TBUT <10s, Schirmer's <10mm/5min), DC numbers decreased after four weeks of therapy with topical loteprednol (61.2±16.7 to 46.5±13.3 cells/mm^2).[68] Similar data was noted in individuals with Sjögren's treated with topical cyclosporine for six months (250±108 to 93±58 cells/mm^2). Interestingly, DC numbers have also been found to decrease when systemic treatment with prednisone and methotrexate was initiated in individuals with rheumatoid arthritis and secondary Sjögren's (85.34±61.72 to 24.86±26.25 cells/mm^2).[21] While we do not have natural history data on the effect of persistent aDCs in the cornea, their presence is probably deleterious to the ocular surface health. In mice exposed to desiccating stress, depletion of APCs in the conjunctiva prevented the accumulation of infiltrating CD4+ T- cells within the ocular surface and preserved the number of goblet cells in the conjunctiva, suggesting that the inflammatory process seen in DE is dependent of APCs.[11] In humans, increased aDC number was associated with decreased conjunctival goblet cells number in individuals with Sjögren's associated DE.[69] Given their involvement in inflammation and T-cell activation, there is a biologic plausibility that reducing aDC number in the cornea may have a beneficial long-term effect on the ocular surface health.

Several potential limitations should be noted when interpreting the results of this study. First, this study evaluated a population of older male US veterans seeking eye care services, and thus the findings may not be generalizable to other populations. Second, given the cross-sectional nature of this study, it is not possible to attribute causation, such as when considering relationships between DCs and nerve parameters. Third, DC "activation" status was evaluated morphologically and not using specific markers. However, IHC requires tissue which is not available in most individuals being evaluated for DE. Fourth, there are likely individuals in the group with an undiagnosed systemic immune disease and this would affect our sensitivity and specificity values. Moreover, information on the non-ocular clinical status of individuals with systemic immune diseases were not examined in this study. Fifth, our aDC count was performed manually on two-dimensional and as such, aDCs with dendrites positioned within the Z-axis may not have been counted. We chose this approach despite this limitation as we wanted to examine a strategy that could be immediately implemented in the

clinical setting without the need for postimaging processing. Fortunately, we demonstrated good inter-reader reliability in counting aDCs. The implementation of a built-in automated software to count aDCs would standardize this metric across centers and populations. Finally, only a limited number of individuals were started on a topical anti-inflammatory agent and underwent a repeat scan, and we do not have a comparable control group. Thus, we cannot comment on the natural history of change in aDC number in the central cornea.

Despite these limitations, our data suggest that aDC number could be a useful biomarker for a systemic immune disease in individuals with DE symptoms. While our current sensitivity and specificity are still not optimal for a diagnostic test, standardization of image interpretation with development of automated software could improve the numbers and facilitate the use of this test in the clinical setting. However, further longitudinal research is needed to validate our findings in more diverse populations, to confirm the effect of topical anti-inflammatories on aDC numbers and to evaluate how changes in aDCs number relate to changes in clinical symptoms and signs of disease.

Acknowledgments

Funding: Supported by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Clinical Sciences R&D (CSRD) I01 CX002015 (Dr. Galor) and Biomedical Laboratory R&D (BLRD) Service I01 BX004893 (Dr. Galor), Department of Defense Gulf War Illness Research Program (GWIRP) W81XWH-20-1-0579 (Dr. Galor) and Vision Research Program (VRP) W81XWH-20-1-0820 (Dr. Galor), National Eye Institute R01EY026174 (Dr. Galor) and R61EY032468 (Dr. Galor), NIH Center Core Grant P30EY014801 (institutional) and Research to Prevent Blindness Unrestricted Grant (institutional), Consejo Nacional de Ciencia y Tecnología (CONACYT) CVU810654 (H. Levine)

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Ethical statement

This study was approved by the Institutional Review Board (IRB) at the Miami Veterans Administration Medical Center and was conducted in accordance with the principles of the Declaration of Helsinki and the United States Health Insurance Portability and Accountability Act.

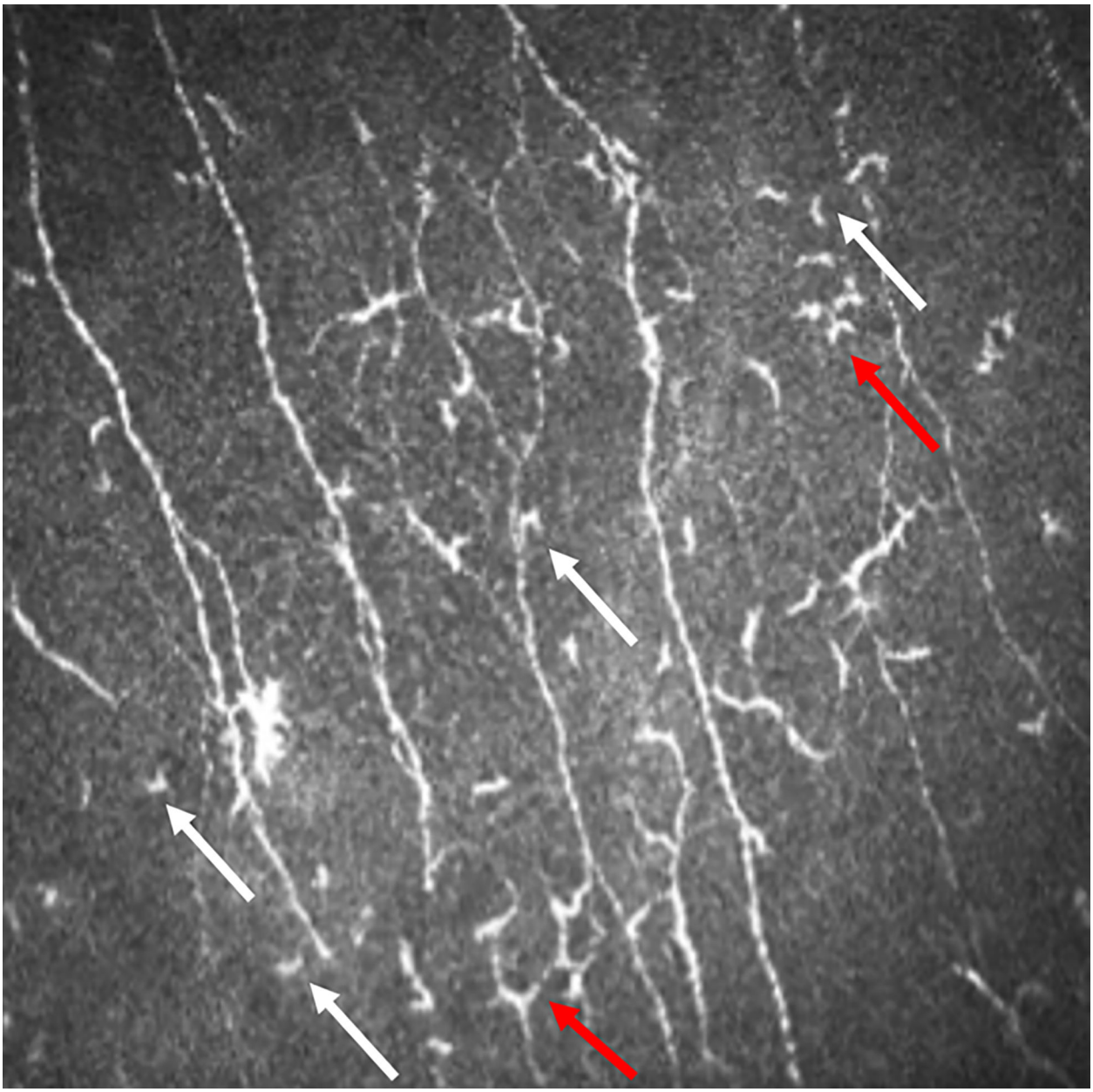

Figure 1.
Morphological evaluation of dendritic cells.
"Activated" dendritic cells are denoted by red arrows which were differentiated from non-activated dendritic cells (white arrows) by the number and size of their arms.

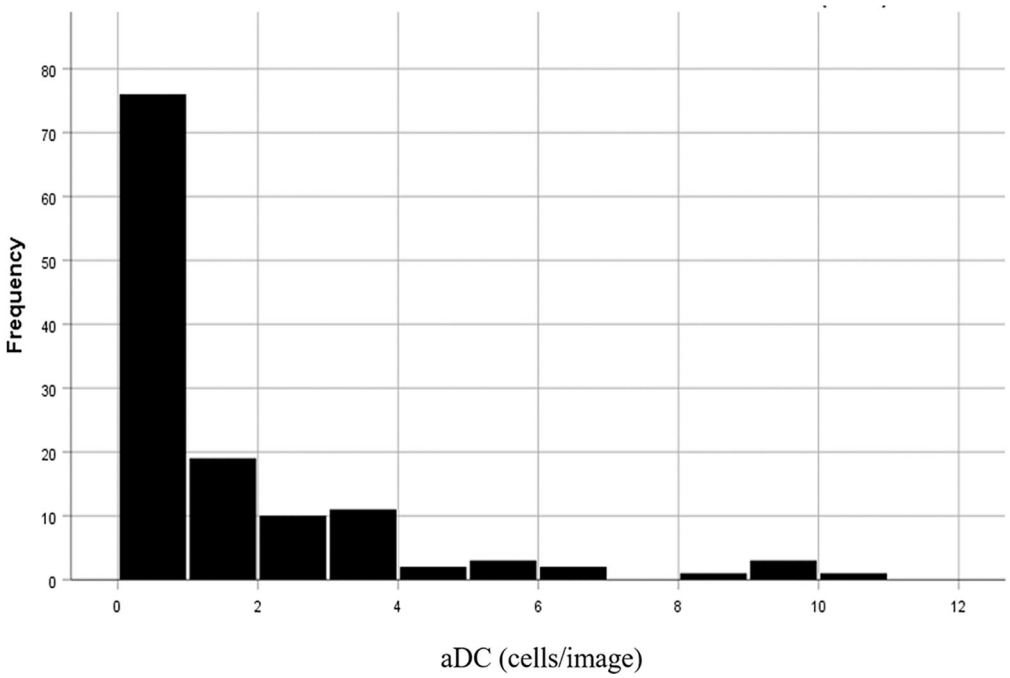

Figure 2:
Distribution of the number of activated dendritic cells (aDCs) in the central cornea of individuals with DE symptoms.

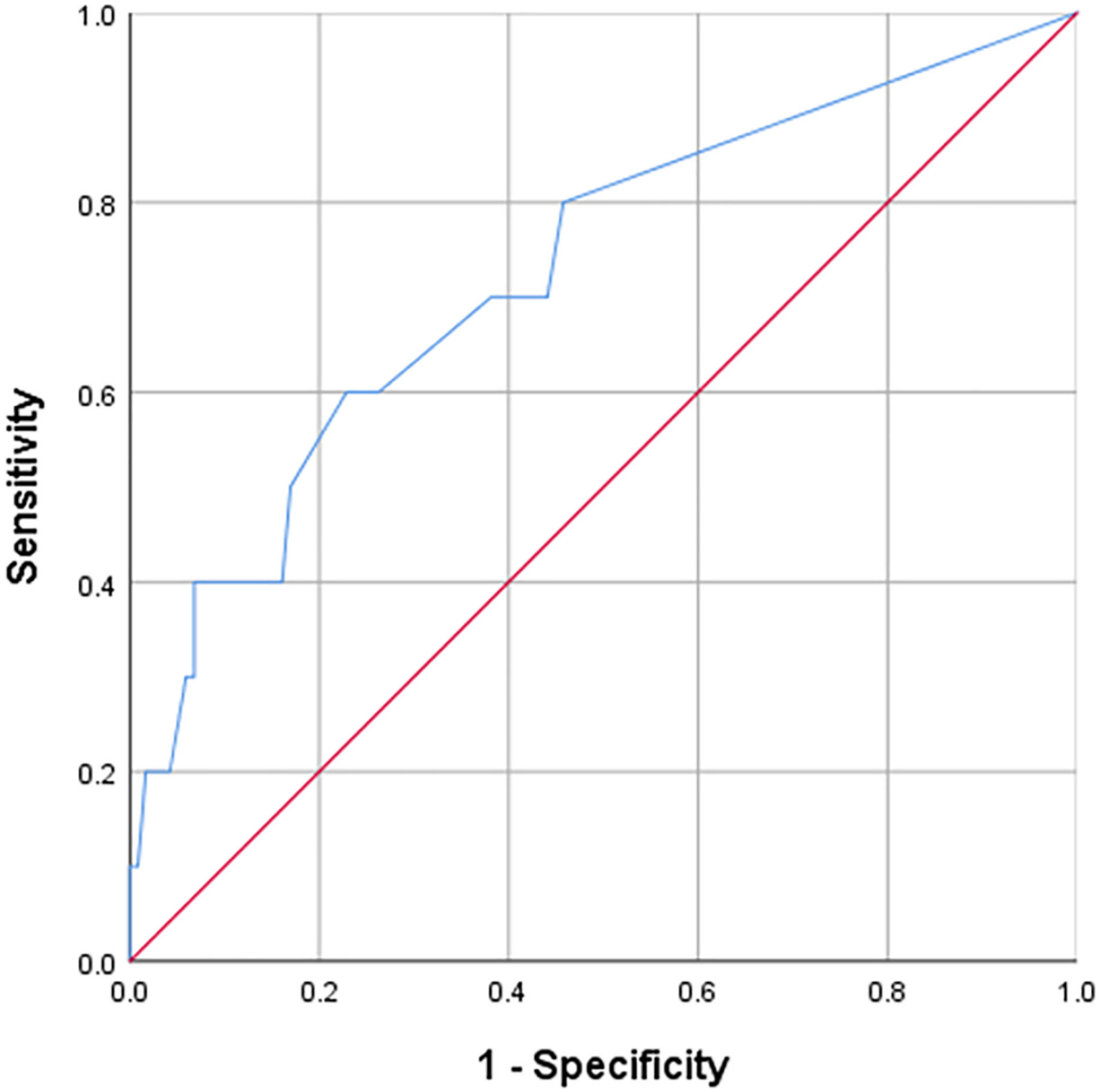

Figure 3:
ROC curve of the number of activated dendritic cells (aDCs) in the central cornea as an indicator of systemic immune disease.

Table 1:

Demographic information of the study population

Number	**128**
Age (years), mean ±SD [range]	57.1±15.0 [25–90]
Sex, male, n (%)	91 (71.1%)
Ethnicity, Hispanic, n (%)	31 (24.2%)
Race, n (%)	
White	68 (53.1%)
Black	43 (33.6%)
Asian	3 (2.3%)
American Indian/Alaska native	1 (0.8%)
Native Hawaiian/ Pacific Islander	4 (3.1%)
Current Smoker, n (%)	26 (20.3%)

SD=standard deviation; n=number in group

Table 2:

Descriptive statistics for individuals with dry eye (DE) symptoms grouped by the presence or absence of ≥2 activated dendritic cells (aDCs) in the central cornea.

Parameter	<2aDCs (n=95)	≥2 aDCs (n=33)	P-Value
Demographics			
Age (years), mean ±SD	57.5±15.5	56.1±13.5	0.62
Male sex, n (%)	68 (71.6%)	23 (69.7%)	0.83
Hispanic, n (%)	17 (23.4%)	9 (27.3%)	0.64
Race, White, n (%)	56 (59.6%)	12 (36.4%)	0.02
Black, n (%)	26 (27.7%)	17 (51.5%)	0.02
Medical history, n (%)			
Current Smoker	22 (23.2%)	4 (12.1%)	0.21
Major Depressive Disorder	37 (38.9%)	9 (27.3%)	0.29
Post-Traumatic Stress Disorder	16 (16.8%)	11 (33.3%)	0.08
Hypertension	49 (51.6%)	19 (57.6%)	0.69
Diabetes Mellitus	20 (21.1 %)	9 (27.3%)	0.48
Migraine	36 (37.9%)	14 (42.4%)	0.68
Fibromyalgia	10 (10.5%)	6 (18.2%)	0.36
Traumatic Brain Injury	4 (4.2%)	0 (0%)	0.57
Systemic immune Disease *	6 (7.5%)	7 (23.3%)	0.04
Primary Sjögren's	2 (2.1%)	1 (3.0%)	>0.99
Secondary Sjögren's	3 (3.2%)	5 (15.2%)	0.03
Positive early Sjögren's markers	18 (18.9%)	9 (27.3%)	0.33
Ocular History, right eye only, n (%)			
Glaucoma	9 (9.5%)	3 (9.1%)	>0.99
Glaucoma Surgery **	2 (2.1%)	1 (3.0%)	>0.99
Cataract Surgery	15 (15.8%)	7 (21.2%)	0.59
Refractive surgery	15 (15.8%)	1 (3.0%)	0.07
Systemic Medications, n (%)			
Anti-hypertensive	50 (47.4%)	19 (57.6%)	0.69
Glucose lowering medication	15 (15.8%)	7 (21.2%)	0.59
Inhaled corticosteroids	15 (15.8%)	2 (6.1%)	0.24
Oral corticosteroids	5 (5.3%)	2 (6.1%)	1.00
Immunosuppressive agents (tacrolimus, methotrexate, cyclosporine, azathioprine, mycophenolate)	2 (2.1%)	5 (15.2%)	0.01
NSAID	39 (41.1%)	14 (42.4%)	0.23
Acetaminophen	13 (13.7%)	7 (21.2%)	0.40
α2γ ligand (gabapentin or pregabalin)	32 (33.7%)	16 (48.5%)	0.15
Anti-migraine (triptan)	10 (10.5%)	6 (18.2%)	0.36
Anti-depressant (SSRI, SNRI, mirtazapine, TCA)	41 (43.2%)	15 (45.5%)	0.84

Parameter	<2aDCs (n=95)	≥2 aDCs (n=33)	P-Value
Doxycycline	10 (10.5%)	2 (6.1%)	0.73
Topical Medications, n (%)			
Artificial Tears	72 (75.8%)	25 (75.8%)	>0.99
Antihistamine	12 (12.5%)	2 (6.1%)	0.52
Anti-inflammatory (cyclosporine, lifitegrast, corticosteroid, NSAID)	48 (50.5%)	17 (51.5%)	>0.99
Autologous Serum Tears (AST)	9 (9.5%)	10 (30.3%)	<0.01
IOP lowering	4 (4.2%)	3 (9.1%)	0.37

SD=standard deviation; n=number in group, GVHD=Graft versus host disease, NSAID=non-steroidal anti-inflammatory drug; SSRI=Selective serotonin reuptake inhibitor; SNRI=Serotonin-norepinephrine reuptake inhibitor; TCA=tricyclic antidepressants; IOP=intraocular pressure

*Includes autoimmune vasculitides (temporal arteritis, granulomatosis with polyangitis, Behcet's syndrome), sarcoidosis, rheumatoid or psoriatic arthritis, systemic lupus erythematosus, psoriasis, Sjögren's and GVHD.

**Excludes peripheral iridotomy.

Table 3:

Symptoms, signs and in-vivo confocal microscopy (IVCM) parameters of individuals with dry eye (DE) symptoms grouped by the presence or absence of ≥2 activated dendritic cells (aDCs) in the central cornea.

Parameter	<2aDCs (n=95)	≥2 aDCs (n=33)	P-Value
Dry eye specific questionnaires, mean (SD)			
Total DEQ-5 Score	15.1 (3.6)	16.1 (4.2)	0.19
Total OSDI Score	49.2 (25.3)	57.5 (21.0)	0.10
Ocular pain specific questionnaires			
Intensity of ocular pain, averaged over past	4.6 (2.9)	4.7 (2.7)	>0.99
week, mean (SD)			
NPSI-E sub-score *, mean (SD)	18.5 (11.6)	18.2 (10.0)	0.88
Intensity of burning, mean (SD)	4.5(3.3)	4.8(3.2)	0.71
Burning ≥ 8, n (%)	24 (25.3%)	8 (25.0%)	0.99
Wind sensitivity, mean (SD)	4.6 (3.3)	0.7 (2.6)	0.78
Wind sensitivity ≥ 8, n (%)	20 (24.2%)	4 (12.5%)	0.21
Light sensitivity, mean (SD)	5.1(3.4)	5.4(3.3)	0.62
Light sensitivity ≥ 8, n (%)	31 (33.0%)	11 (34.4%)	0.99
Temperature sensitivity, mean (SD)	4.4 (3.2)	3.9 (2.9)	0.41
Temperature sensitivity ≥ 8, n (%)	18 (19.1%)	3 (9.4%)	0.28
Persistent Pain After Anesthesia, n (%)**	53 (55.8%)	21 (63.6%)	0.54
Ocular surface finding			
MMP-9, mean (SD)	1.0 (0.9)	1.1 (1.0)	0.67
Anterior blepharitis, mean (SD)	0.6(0.8)	0.5 (0.7)	0.79
Eyelid vascularity, mean (SD)	1.0 (1.1)	0.7 (1.0)	0.10
Meibomian gland inspissation, mean (SD)	0.8 (0.7)	0.8 (0.7)	0.89
Temporal conjunctivochalasis, mean (SD)	0.6 (0.6)	0.4 (0.5)	0.19
Middle conjunctivochalasis, mean (SD)	0.1 (0.3)	0 (0)	<0.01
Nasal conjunctivochalasis, mean (SD)	0.3 (0.5)	0.1 (0.3)	<0.01
Tear break up time (s), mean (SD)	6.0 (4.0)	5.9 (4.0)	0.89
Corneal staining, mean (SD)	2.1 (2.7)	3.1 (4.2)	0.20
Schirmer wetting length (mm/5min)	9.6 (7.4)	8.1 (5.7)	0.30
Papillae, mean (SD)	0.5 (0.5)	0.4 (0.5)	0.82
Fibrosis, n (%)	5 (6.0%)	0 (0%)	0.32
Meibum quality, mean (SD)	1.1 (1.1)	1.1 (1.1)	>0.99
Central cornea IVCM Parameters, mean (SD)			
Nerve fiber density (fibers/mm^2)	19.8 (8.8)	18.3 (9.4)	0.42
Nerve fiber length (mm/mm^2)	12.0 (3.8)	13.0 (4.0)	0.18
Nerve branch density (branches/mm^2)	24.7 (17.4)	24.5 (16.3)	0.96
Total branch density (branches/mm^2)	38.3 (26.1)	45.8 (32.1)	0.24

Parameter	<2aDCs (n=95)	≥2 aDCs (n=33)	P-Value
Nerve fiber area (mm^2/mm^2)	0.006 (0.002)	0.007 (0.004)	<0.01
Nerve fiber width (mm/mm^2)	0.021 (0.002)	0.022 (0.002)	0.12
Nerve fractal dimension	1.45 (0.06)	1.47 (0.04)	0.05
Dendritic cells ($cells/mm^2$)	20 (25.6)	79.4 (63.1)	<0.01
Activated dendritic cells ($cells/mm^2$)	1.7 (2.8)	26.3 (15.0)	<0.01

SD = Standard Deviation; DEQ-5 = Dry Eye Questionnaire 5; OSDI = Ocular Surface Disease; aDC=activated Dendritic Cells; MMP-9= matrix metalloproteinase 9 test

*NPSI-E Sub-Score= Total score of four select questions from the Neuropathic Pain Symptom Inventory modified for the Eye referring to the intensity of burning, wind sensitivity, light sensitivity, temperature sensitivity; NRS=numerical rating scale

**NRS Pre-Anesthesia > 0 and NRS Post-Anesthesia > 0