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TITLE: Gulf War Illness: Exploring the Eye-Brain Connection

PRINCIPAL INVESTIGATOR: Anat Galor, MD, MSPH

CONTRACTING ORGANIZATION: South Florida VA Foundation for Research, Miami, FL

REPORT DATE: August 2021

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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Distribution Unlimited

# REPORT DOCUMENTATION PAGE

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| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
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| August 2021 | Annual | 01Aug2020-31Jul2021 |

**4. TITLE AND SUBTITLE**

Gulf War Illness: Exploring the Eye-Brain Connection

**5a. CONTRACT NUMBER**
W81XWH-20-1-0579

**5b. GRANT NUMBER**

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

Dr. Anat Galor

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

South Florida VA Foundation
for Research
1201 NW 16th St
Miami FL 33125-1624

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

This study will examine potential clinical biomarkers in Gulf War Illness (GWI) that are easily obtained in the eye clinic and apply them to disease stratification.
We are conducting a case control study at the Miami VA with the plan to enroll 100 subjects (50 cases with GWI and 50 age and gender matched controls without GWI). We will conduct an eye examination, including imaging of the corneal nerves (peripheral nervous system) and retina and optic nerve (central nervous system) We will also assess for ocular and systemic inflammatory markers. Imaging will be performed in triplicates (to assess reliability) at baseline and again at 1 year (to assess sensitivity to change over time). We will enroll an additional 40 subjects, 20 cases and 20 controls, as a validation cohort. We have 120 enrolled subjects who have completed the first visit, 72 (60%) were deployed to the Gulf during 1990-1991. Of those who deployed, 33 were cases with GWI and 39 were controls.

**15. SUBJECT TERMS**

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
| Unclassified | Unclassified | Unclassified | Unclassified | 136 | 19b. TELEPHONE NUMBER *(include area code)* |

TABLE OF CONTENTS

## 1. INTRODUCTION:

A significant number of veterans who actively participated and returned from Desert Storm and Desert Shield developed a range of symptoms that included cognitive/mood disorders, skin conditions, musculoskeletal disorders and chronic fatigue. The purpose of this research is to explore potential ocular biomarkers that associate with Gulf War Illness and/or its symptom clusters by observing baseline eye parameters and their changes over time in the eyes of Gulf War Era veterans with and without Gulf War Illness. This research may help with disease stratification and diagnosis as well as introduce new therapeutic approaches.

## 2. KEYWORDS:

Gulf War Illness; chronic fatigue syndrome, pain, inflammation, musculoskeletal disorder, confocal microscopy, corneal nerves, optical coherence tomography, neuro-inflammation,

## 3. ACCOMPLISHMENTS:

**What were the major goals of the project?**

**Specific Aim 1:** Determine whether peripheral and central biomarkers within the neurological system (corneal and retinal nerves) are indicative of GWI and identify symptom clusters (e.g. fatigue; mood and cognition disorders; musculoskeletal disorders).

**Specific Aim 2:** Identify interactions between peripheral and central biomarkers of the neurological system and immune system (ocular surface and corneal inflammation, systemic inflammation).

**Specific Aim 3:** Determine whether gender and ethnic differences impact peripheral and central biomarkers of the neurological system in GWI.

| Major Tasks | Timeline | % of Completion |
|---|---|---|
| **Major Task 1: Set up** | Months | |
| Refine eligibility criteria, exclusion criteria, screening protocol | 0-3 | 100% |
| Finalize consent form & human subjects protocol | 0-3 | 100% |
| IRB protocol submission | 0-3 | 100% |
| Submit amendments and protocol deviations to IRB, as needed | As Needed | |
| Submit annual IRB report for continuing review | Annually | |
| *Milestone Achieved: Local IRB approval* | | 100% |
| **Major Task 2: Coordinate Study Staff** | | |
| Subtask1: Hiring and Training of Study Staff | | |
| Coordinate with Sites for job descriptions design | 0-6 | 100% |
| Advertise and interview for project related staff | 0-6 | 100% |
| Coordinate for space allocation for new staff | 0-6 | 100% |

| | | |
|---|---|---|
| Coordinate training to maintain compliance with protocol | 0-6 | 100% |
| *Milestone Achieved: Research staff trained* | | 100% |
| Subtask 2: Facilitate and Coordinate with Sites for hiring, training, supervision and fidelity checks, as needed for attrition | 0-36 | 100% |
| Coordinate training to maintain compliance with protocol | 0-36 | 100% |
| *Milestone Achieved: Maintained trained staff throughout study* | | 100% |
| Coordinate flow chart for all study steps, web data collection and database requirements | 0-6 | 100% |
| Finalize assessment measurements | 0-6 | 100% |
| *Milestone Achieved: 1st participant consented, screened and enrolled* | | 100% |
| *Milestone Achieved: Study begins* | | 100% |
| Begin subject recruitment | 6 | 100% |
| Assess all participants | 6-36 | 40% |
| Assess and report all SAEs to IRB, DSMB; enact and see approval for protocol amendments to ensure patient safety, as needed. | As needed | 100% |
| *Milestone Achieved: Subjects enrolled, data collected and stored properly.* | | 40% |
| **Major Task 3: Data Analysis** | | |
| Subtask 1: Coordinate with Biostatistician for data checks and quality | 6-36 | 0% |
| Perform all analyses according to specifications, share output and finding with all investigators | 6-36 | 0% |
| Dissemination of findings (abstracts, presentation, publications, DOD, VA) | 6-36 | 0% |
| *Milestone Achieved: Report results from data analyses* | | 0% |
| **Major Task 4: BBRAIN Contributions** | | |
| Coordinate with BBRAIN, develop SOP for blood collection, handling, processing, and storage | 0-6 | 100% |
| Collect and handle all specimens, per protocol, in collaboration with BBRAIN | 6-36 | 40% |
| *Milestone Achieved: All samples collected and processed* | | 45% |

**What was accomplished under these goals?**

1) Major Activities:
   - Miami VA approved the study on 7/23/2020.
   - First subject visit was completed on 8/8/2020.
   - Continuing Review was approved on 6/3/2021 (IRB Human Subjects Subcommittee) and 5/12/2021 (IRB Chemical Hygiene and Biosafety Subcommittee)
   - As of 8/30/2021, 122 participants were enrolled in the study with 2 completing a second visit.
   - The HRT3 confocal machine was sent out for service maintenance 2/16/2021 and then shipped to Germany 6/4/2021, a loaner arrived 8/27/2021.

2) Specific Objectives:
   - Aim 1: Determine whether peripheral and central biomarkers within the neurological system (corneal and retinal nerves) are indicative of GWI and identify symptom clusters (e.g. fatigue; mood and cognition disorders; musculoskeletal disorders).
   - Aim 2: Identify interactions between peripheral and central biomarkers of the neurological system and immune system (ocular surface and corneal inflammation, systemic inflammation).
   - Aim 3: Determine whether gender and ethnic differences impact peripheral and central biomarkers of the neurological system in GWI.

3) Key Outcomes:
   - First subject visit was completed on 8/8/2020.
   - Continuing Review was approved on 6/3/2021 (IRB Human Subjects Subcommittee) and 5/12/2021 (IRB Chemical Hygiene and Biosafety Subcommittee).
   - As of 8/30/2021, 122 participants were enrolled in the study with 2 completing a second visit. Out of 120 completed first visits, we have 72 (60%) who were deployed to the Gulf between 1990-1991. Of those deployed to the Gulf, 33 (46%) were cases with GWI and 39 (54%) were controls.
   - We have analyzed 105 blood samples for cytokines.
   - We first examined for symptoms and signs of dry eye in the population and noted that individuals with GWI have higher dry eye and ocular pain scores compared to controls. However, ocular surface signs, including inflammation, was similar between both groups. This points to other etiologies to symptoms beyond nociceptive sources of ocular surface inflammation and tear film disruption. We are now in the process of examining relationships between GWI and measures of corneal nerves (via confocal microscopy), central nerves (via ocular coherence tomography), and systemic markers of inflammation.

**What opportunities for training and professional development has the project provided?**

Training at the BBRAIN lab took place at the beginning of the study to allow for blood processing on the weekends. Research optometry technicians received training to capture specific areas of the eye using the HRT3 which led to improved image capture.

**How were the results disseminated to communities of interest?**

Nothing to Report

**What do you plan to do during the next reporting period to accomplish the goals?**

1) Finish recruitment of the initial 140 participants and continue with secondary visits.
2) Submit an amendment to expand the criteria for enrollment in order to ensure that the results from this study are due to specific exposure during the Gulf War and not just deployment to the Gulf theatre.
3) Analyze CRP levels within the blood to better understand systemic inflammation.

## *4.* IMPACT:

**What was the impact on the development of the principal discipline(s) of the project?**

As a result of the project, a new clinic was established, and new SOPs were created. This provided an opportunity for more comprehensive eye examinations to take place among the veteran community, specifically those individuals active during the Gulf War era.

The project has also provided an opportunity to liaison with the BBRAIN lab that will hopefully provide insight on Aim 2 to identify interactions between peripheral and central biomarkers of the neurological system and immune system (ocular surface and corneal inflammation, systemic inflammation).

**What was the impact on other disciplines?**

The findings of this project are likely to make an impact on disciplines related to the study of Gulf War Illness and its symptoms such as the area of immunology, neurology, pain etc. by potentially providing new biomarkers for diagnosis, a foundation for future research, and allowing for the development of innovative therapeutic approaches for Gulf War Illness and related conditions such as chronic widespread pain, etc.

**What was the impact on technology transfer?**

Nothing to report

**What was the impact on society beyond science and technology?**

Although the project is not complete, it has fostered discussion among veterans, healthcare providers, and caretakers. This has led to improvement in trust between veterans of the Gulf War era and the Miami VA Healthcare system and will potentially lead to an attitude shift within the community regarding Gulf War Illness.

## 5. CHANGES/PROBLEMS:

**Changes in approach and reasons for change**

Nothing to Report

**Actual or anticipated problems or delays and actions or plans to resolve them**

1) COVID-19 made recruitment slightly challenging in the beginning due to patient hesitation and hospital rules. This was resolved by offering Saturday clinic appointments to allow for social distancing and ensure the safety of participants.
2) The HRT3 confocal machine was sent to California for annual service maintenance and was delayed due to severe weather. Upon arrival, the service company could not complete the maintenance and the machine has been sent to Germany for repairs. An order has been placed for a new confocal machine and a loaner recently arrived.

**Changes that had a significant impact on expenditures**

Nothing to report

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to Report

**Significant changes in use or care of human subjects**

Nothing to report

**Significant changes in use or care of vertebrate animals**

Nothing to Report

**Significant changes in use of biohazards and/or select agents**

Nothing to Report

## 6. PRODUCTS:

- **Publications, conference papers, and presentations**

**Journal publications.**

1. Baksh BS, Zayan KL, Goldhardt R, Felix ER, Klimas N, Galor A. Ocular manifestations and biomarkers of Gulf War Illness in US veterans. *Sci Rep*. 2021;11(1):6548. Published 2021 Mar 22. doi:10.1038/s41598-021-86061-0
   Acknowledgement of Federal Support: yes

2. Baksh BS, Garcia JC, Galor A. Exploring the Link Between Dry Eye and Migraine: From Eye to Brain. *Eye Brain*. 2021;13:41-57. Published 2021 Mar 4. doi:10.2147/EB.S234073
   Acknowledgment of Federal Support: yes

3. Patel S, Mehra D, Cabrera K, Galor A. How Should Corneal Nerves Be Incorporated Into the Diagnosis and Management of Dry Eye?. *Current Ophthalmology Reports,* 2021. Published online 2021 May 20. doi: 10.1007/s40135-021-00268-y
   Acknowledgement of Federal Support: yes

4. Sanchez V, Baksh BS, Cabrera K, Choudhury A, Jensen K, Klimas N, Galor A. Dry eye symptoms and signs in US veterans with Gulf War Illness. Am J Ophthamol. 2021 Submitted for consideration 2021 July
   Acknowledgement of Federal Support: yes

**Books or other non-periodical, one-time publications.**

Nothing to Report

**Other publications, conference papers and presentations**.

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

Name: Anat Galor MD, MSPH
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-3026-6155
Nearest person month worked: 3.0

Contribution to Project: Created protocol (eligibility/exclusion criteria), clinic flow sheets, and source documents, worked on IRB submissions and reviews, assists with recruitment, data management, regulatory/essential documents management, collects human specimens, performs comprehensive eye examinations, provides guidance as an expert in ocular pain having regular attendance at pain meetings and knowledge of relevant literature.

Name: Nancy Klimas MD
Project Role: Co-PI
Research Identifier (e.g. ORCID ID): 0000-0003-1459-3268
Nearest Person month worked: 0.6

Contribution to Project: Assisted in creation of protocol, provides use of facilities, personnel, and equipment, provides guidance based on knowledge as an international expert on Gulf War Illness.

Name: Raquel Goldhardt MD
Project Role: Co-PI
Research Identifier (e.g. ORCID ID): 0000-0003-3140-6794
Nearest Person month worked: 0.6

Contribution to Project: Assisted in creation of protocol and clinic flow sheets, provides guidance on imaging, assists with recruitment, data management, regulatory/essential document management.

Name: Kimberly Cabrera MS
Project Role: Research Coordinator
Nearest Person month worked: 12

Contribution to Project: Responsible for recruitment, scheduling, and follow up of study participants, assists with patient consents, maintains all administrative binders and clinical databases, and ensures compliance with all IRB requirements, collects and processes human specimens.

Name: Mireya Hernandez
Project Role: Research Coordinator
Nearest Person month worked: 2.4

Contribution to Project: Assists with maintaining administrative binders and clinical databases and ensures compliance with all IRB requirements.

Name: Katherine Jensen, OD
Project Role: Research optometrist
Nearest Person month worked: 2.4

Contribution to Project: Performs comprehensive eye examinations, collects human specimens, assists in recruitment

Name: Molly Johnson, OD
Project Role: Research optometrist
Nearest Person month worked: 2.4

Contribution to Project: Performs comprehensive eye examinations, collects human specimens, assists in recruitment

Name: Andrew Jensen, OD
Project Role: Research optometrist
Nearest Person month worked: 2.4

Contribution to Project: Performs comprehensive eye examinations, collects human specimens, assists in recruitment

Name: Ramon Diaz
Project Role: Research optometrist technician
Nearest Person month worked: 2.4

Contribution to Project: Assists with performing all eye testing including acquisition of confocal and retinal nerve images.

Name: Madelyn Diaz
Project Role: Research optometrist technician
Nearest Person month worked: 2.4

Contribution to Project: Assists with performing all eye testing including acquisition of confocal and retinal nerve images.

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

**What other organizations were involved as partners?**

Organization Name: BBRAIN Lab
Location of Organization: Bruce W. Carter Miami VA Healthcare System
1201 NW 16th St Miami, FL 33125
Partner's contribution to the project: Facilities, personnel assistance, use of equipment

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:**

**QUAD CHARTS:**

## 9. APPENDICES:

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2023)

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Anat Galor

eRA COMMONS USER NAME (credential, e.g., agency login): Galor01

POSITION TITLE: Professor, University of Miami; Staff Physician, Miami VAMC

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| University of Miami School of Medicine, Miami, FL | MSPH | 5/2011 | Epidemiology |
| Carnegie Mellon University, Pittsburgh, PA | BS, BA | 6/1997 | Mech E, Biology |
| Washington University School of Medicine, St. Louis, MO | MD | 6/2002 | Medicine |

### A. Personal Statement

I am a cornea specialist with a clinical and research focus in ocular pain. Through a VA career development award (CDA), I began by studying the epidemiology of dry eye (DE) in the veteran population and obtained a Master's of Science in Public Health degree. I found that a diagnosis of DE is common in veterans and a source of significant morbidity. Most notably, I demonstrated that DE symptoms occurred independently of tear dysfunction and concluded that in many cases, DE symptoms are better represented as a pain condition, for at least a subset of patients. For example, I demonstrated that a significant proportion of patients with symptoms of dryness also endorse pain complaints similar to those seen in patients with non-ocular neuropathic pain (burning, sensitivity to light). This sub-group of patients also had more severe and chronic symptoms that were less likely to respond to artificial tears. Furthermore, I demonstrated that individuals with chronic overlapping pain conditions (COPC) were more likely to report DE symptoms. Through a VA merit award, I have worked with a world class multi-disciplinary team of clinical-scientists with expertise in DE (Dr. Galor), pain (Dr. Felix), and study design and execution (William Feuer). Over the past 5 years, we enrolled over 500 individuals into a study with the goal of determining whether ocular pain has a genetic component. Along with obtaining blood for genetic analysis, we evaluated non-ocular co-morbidities (pain, depression, anxiety), ocular and non-ocular somatosensory phenotypes (Belmonte aesthesiometry, quantitative sensory testing on the skin), and ocular surface status (tear film, inflammation). I currently run an oculofacial pain clinic and evaluate and treat many individuals with chronic ocular pain. As such, I am in an excellent position and well-qualified to oversee the implementation of this proposal. I will work with the all study investigators and will oversee all aspects of the study, including study design, IRB communications, regulatory documents, patient enrollment, safety monitoring, data analysis, and future study planning.

**B. Positions, Scientific Appointments, and Honors**

2002-2003 Intern in internal medicine, MetroHealth Medical Center, Cleveland, OH
2003-2006 Ophthalmology resident, Cleveland Clinic Cole Eye Institute, Cleveland, OH
2005-2006 Chief resident in ophthalmology, Cleveland Clinic Cole Eye Institute, Cleveland, OH
2006-2007 Uveitis clinical fellow, Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD
2007-2008 Cornea and refractive fellow, Bascom Palmer Eye Institute, University of Miami, Miami, FL
2008-2015 Assistant Professor of Clinical Ophthalmology, Bascom Palmer Eye Institute, University of Miami
2008- Staff physician, Miami Veteran Affairs Hospital, Miami, FL
2015- Associate Professor of Clinical Ophthalmology, Bascom Palmer Eye Institute, University of Miami
2018 Received tenure at the University of Miami
2018-2021 Promotion to Associate Professor of Ophthalmology, Bascom Palmer Eye Institute
Promotion to Professor of Ophthalmology, Bascom Palmer Eye Institute, University of Miami, FL

**Honors**

1997 Phi Beta Kappa, Carnegie Mellon University
1997 University honors and college research honors
1997 Mortar Board – national senior service honor society
2002 Alpha Omega Alpha, Washington University
2002 Glasgow Memorial achievement – for graduating in top10% of class
2002 Alumni scholarship – full tuition scholarship to student with highest class rank
2006 HEED fellowship recipient
2010 American Academy of Ophthalmology's *Achievement Award*
2010 Professor of the Year Award, Bascom Palmer Eye Institute
2012 AAO paper on DES in veterans highlighted in press release as "new and newsworthy"
2012 Guan H. 1st Place Young Investigator Clinical Services Presentation "Impact Of Ocular Surface Disease On Quality Of Life In Glaucoma Patients" Senior mentor
2013 McClellan, A. 1st Place Young Investigator Clinical Services Presentation "Epidemiology of OSSN in veteran population" Senior mentor
2013 AAO paper on DE and environment highlighted in press release as "new and newsworthy"
2014 AAO paper on DE and systemic symptoms awarded "best poster"
2015 Chhadva, P. Best paper award AAO "Post-LASIK Epithelial Ingrowth: Correction, Recurrence, and Long-term Follow-up. Meeting Presentation" Senior mentor
2016 Selected to participate in AAO Leadership Development Program
2017 American Academy of Ophthalmology's *Senior Achievement Award*
2018 Induction into the American Ophthalmology Society

**C. Contributions to Science**

1. **Dry eye (DE) is a common disease with significant morbidity.** DE is a prevalent disease both in the United States (US) and world-wide. I was the first to demonstrate that the disease affects approximately 1 in every 5 veterans and impacts veterans' quality of life. Furthermore, I demonstrated that DE signs and symptoms do not correlate; indicating that more research is needed to understand factors that drive symptoms, which are the main cause of DE morbidity. We found that a substantial proportion of those with DE symptoms report ocular pain and specifically features of neuropathic ocular pain (sensitivity to wind and light), independent of tear film abnormalities. These features were associated with DE symptom severity and persistence.
   a. **Galor A**, Feuer W, Lee DJ, Florez H, Carter D, Pouyeh B, Prunty WJ, Perez VL. Prevalence and Risk Factors of Dry Eye Syndrome in a United States Veterans Affairs Population. Am J Ophthalmol. 2011 Sep;152(3):377-384.
   b. **Galor A**, Feuer W, Lee DJ, Florez H, Faler AL, Zann KL, Perez VL. Depression, Post-traumatic Stress Disorder, and Dry Eye Syndrome: A Study Utilizing the National United States Veterans Affairs Administrative Database. Am J Ophthalmol. 2012 Aug;154(2):340-346.e2.
   c. Pouyeh B, Viteri E, Feuer W, Lee DJ, Florez H, Fabian JA, Perez VL, **Galor A.** Impact of ocular surface symptoms on quality of life in a United States Veterans Affairs population. Am J Ophthalmol. 2012 Jun;153(6):1061-1066.

d. **Galor A**, Feuer W, Lee DJ, Florez H, Venincasa VD, Perez VL. Ocular surface parameters in older male veterans. Investigative ophthalmology & visual science 2013;54:1426-33.

2. **We have the tools to assess for various aspects of DE, including novel techniques meant to evaluate for evidence of ocular and central somatosensory nerve dysfunction.** I have conducted several DE epidemiological studies and have standardized the approach to the DE examination. This has included developing questionnaires that assess both for DE symptoms and ocular pain, standardizing the ocular evaluation, and incorporating testing that assesses for the status of the ocular somatosensory nerves (confocal microscopy and modified Belmonte aesthesiometry). Furthermore, we have demonstrated our ability to look for sub-clinical markers of inflammation on the ocular surface including lipid derivatives, serotonin and MMP-9.
   a. Lanza NL, McClellan A, Batawi H, Felix ER, Sarantopoulos KD, Levitt RC, **Galor A**. Dry Eye Profiles in Patients with a Positive Elevated Surface Matrix Metalloproteinase 9 Point-of-Care Test Versus Negative Patients. Ocul Surf. 2016 Apr;14(2):216-23.
   b. Batawi H, Shalabi N, Joag M, Koru-Sengul T, Rodriguez J, Green PT, Campigotto M, Karp CL, Galor A. Sub-basal Corneal Nerve Plexus Analysis Using a New Software Technology. Eye Contact Lens. 2018 Sep;44 Suppl 1(Suppl 1):S199-S205
   c. Chhadva P, Lee T, Sarantopoulos CD, Hackam AS, McClellan AL, Felix ER, Levitt RC, **Galor A**. Human Tear Serotonin Levels Correlate with Symptoms and Signs of Dry Eye. Ophthalmology. 2015 Aug;122(8):1675-80.
   d. Walter SD, Gronert K, McClellan AL, Levitt RC, Sarantopoulos KD, **Galor A**. ω-3 Tear Film Lipids Correlate With Clinical Measures of Dry Eye. Invest Ophthalmol Vis Sci. 2016 May 1;57(6):2472-8.

3. **We have demonstrated that individuals with DE symptoms have evidence of somatosensory dysfunction.** We evaluate for somatosensory dysfunction locally (on the cornea) and systemically (over the forehead and forearm). We have found that individuals with DE symptoms, especially those with symptoms of neuropathic ocular pain (i.e. burning, sensitivity to wind light), have increased sensitivity to mechanical stimulus on the cornea and increased sensitivity to thermal stimuli on the skin. Furthermore, these patients have evidence of central sensitization, assessed via surrogate markers such as increased temporal sensation and the presences of aftersensations.
   a. Spierer O, Felix ER, McClellan AL, Parel JM, Gonzalez A, Feuer WJ, Sarantopoulos CD, Levitt RC, Ehrmann K, **Galor A**. Corneal Mechanical Thresholds Negatively Associate With Dry Eye and Ocular Pain Symptoms. Invest Ophthalmol Vis Sci. 2016 Feb 1;57(2):617-25.
   b. **Galor A**, Levitt RC, McManus KT, Kalangara JP, Seiden BE, Park JJ, Covington DB, Sarantopoulos CD, Felix ER. Assessment of Somatosensory Function in Patients With Idiopathic Dry Eye Symptoms. JAMA Ophthalmol. 2016 Nov 1;134(11):1290-1298.

4. **We have found that DE symptoms associate more closely with non-ocular metrics than ocular parameters.** We found that DE symptoms associate more closely with depression, anxiety, and non-ocular pain than with tear film and ocular surface findings. In fact, ocular symptoms in individuals with a suspected neuropathic component are more closely aligned with non-ocular findings than in individuals with symptoms of dryness but without hot burning ocular pain and evoked pain to wind and light. We also found that individuals with chronic overlapping pain conditions (COPC) have an increased frequency and severity of DE symptoms. Putting this together, we hypothesize that in certain in individuals, DE symptoms represent a COPC with central sensitization underlying the finding of DE symptoms and other pain conditions (fibromyalgia, migraine, temporomandibular joint dysfunction, etc.)
   a. Crane AM, Feuer W, Felix ER, Levitt RC, McClellan AL, Sarantopoulos KD, **Galor A**. Evidence of central sensitisation in those with dry eye symptoms and neuropathic-like ocular pain complaints: incomplete response to topical anaesthesia and generalised heightened sensitivity to evoked pain. Br J Ophthalmol. 2017 Sep;101(9):1238-1243.
   b. Crane AM, Levitt RC, Felix ER, Sarantopoulos KD, McClellan AL, **Galor A**. Patients with more severe symptoms of neuropathic ocular pain report more frequent and severe chronic overlapping pain conditions and psychiatric disease. Br J Ophthalmol. 2017 Feb;101(2):227-231.
   c. **Galor A,** Felix ER, Feuer W, Shalabi N, Martin ER, Margolis TP, Sarantopoulos CD, Levitt RC. Dry eye symptoms align more closely to non-ocular conditions than to tear film parameters. 2015 Aug;99(8):1126-9.
   d. Levitt AE, **Galor A**, Chowdhury AR, Felix ER, Sarantopoulos CD, Zhuang GY, Patin D, Maixner W, Smith SB, Martin ER, Levitt RC. Evidence that Dry Eye Represents a Chronic Overlapping Pain Condition. Mol

Pain. 2017 Jan-Dec;13.

5. **We manage chronic ocular pain from a variety of insults using strategies that have been successfully applied to non-ocular pain.** We routinely use topical (autologous serum tears, anti-inflammatory agents) and systemic medications (gabapentin and pregabalin) to treat chronic ocular pain. In addition, we offer adjuvant therapies, such as non-invasive electrical stimulation, botulinum toxin, and cognitive behavior therapy in an open label fashion in our clinical practice to treat ocular pain that is resistant to traditional DE therapies and thought to have a neuropathic component.
   a. Sivanesan E, Levitt RC, Sarantopoulos CD, Patin D, **Galor A**. Noninvasive Electrical Stimulation for the Treatment of Chronic Ocular Pain and Photophobia. 2018 Dec;21(8):727-734. PMCID: PMC6023783
   b. **Galor A**, Moein HR, Lee C, Rodriguez A, Felix ER, Sarantopoulos KD, Levitt RC. Neuropathic pain and dry eye. Ocul Surf. 2018 Jan;16(1):31-44. PMCID: PMC5756672
   c. Small LR, **Galor A**, Felix ER, Horn DB, Levitt RC, Sarantopoulos CD. Oral Gabapentinoids and Nerve Blocks for the Treatment of Chronic Ocular Pain. Eye Contact Lens. 2020 May;46(3):174-181.
   d. Diel RJ, Kroeger ZA, Levitt RC, Sarantopoulos C, Sered H, Martinez-Barrizonte J, **Galor A**. Botulinum Toxin A for the Treatment of Photophobia and Dry Eye. Ophthalmology. 2018 Jan;125(1):139-140. PMCID: PMC5741464

**Complete List of Published Work in MyBibliography:**
http://www.ncbi.nlm.nih.gov/sites/myncbi/anat.galor.1/bibliograpahy/47572344/public/?sort=date&direction=ascending

**D. Additional Information: Research Support and/or Scholastic Performance**

**Ongoing Research Support**

Novartis Pharmaceuticals Anat Galor (Site PI)
3/1/2021 – 7/31/2023
Study of efficacy and safety of SAF312 eye drops in subjects with post-operative chronic ocular surface pain
This is a randomized, placebo-controlled study of a new treatment, SAF312 eye drops (5mg/ml and 15 mg/ml), for post-refractive ocular pain.
Role: Principal investigator

Regeneron Pharmaceuticals, Inc. Anat Galor (Site PI) 6/1/2020-5/31/2022
Observational Study of Conjunctivitis in the Setting of DUPIXENT® Treatment for Atopic Dermatitis
The goal of this study is to understand pathophysiological mechanisms underlying dupilumab associated conjunctivitis.
Role: Principal investigator

NEI R61EY032468 Anat Galor, Sue Aicher (MPI)
11/1/2020-10/31/2022
Tear protein biomarkers of refractive surgery pain
The goal of this study is to identify diagnostic and prognostic tear biomarkers for post-refractive pain.
Role: Co-principal investigator

DoD Vision Research Anat Galor (PI)
6/2020-5/2022
W81XWH-20-1-0820
Automated Assessment of Visual Photosensitivity in Traumatic Brain Injury
The goal of this study is to evaluate an objective device to measure photosensitivity in TBI
Role: Principal investigator

DoD GWI New Investigator Award Anat Galor (PI)
6/2020-5/2023
W81XWH-20-1-0579

The goal of this study is to evaluate whether imaging of peripheral and central nerves in the eye can serve as biomarkers for Gulf War Illness.
Role: Principal Investigator

VA Merit Award Anat Galor, Nawajes Mandal (MPI)
4/2020-3/2024
BX004893
Lipid mediators and their signaling in ocular surface inflammation and meibomian gland dysfunction.
The goal of this study is to evaluate the role of lipid mediators in meibomian gland dysfunction.
Role: Co-principal investigator

VA Merit Award Anat Galor (PI)
4/2020-3/2024
CX002015
Neural mechanisms of ocular pain and photophobia
The goal of this study is to evaluate the neural pathways of ocular pain in veterans.
Role: Principal investigator

NEI R01EY026174 Anat Galor, Naresh Kumar (MPI) 9/2016-8/2021
Dry Eye (DE) and Microenvironment
The goal of this study is to evaluate the contribution of the microenvironment to dry eye.
Role: Co-Principal Investigator

NEI Elizabeth Cohen (PI)
9/2016-8/2021
Zoster Eye Disease Study
This is a multi-center, randomized, double-masked, placebo-controlled clinical trial of suppressive valacyclovir for one year in immunocompetent study participants with an episode of dendriform epithelial keratitis, stromal keratitis, endothelial keratitis, and/or iritis due to Herpes Zoster Ophthalmicus (HZO) in the year prior to enrollment.
Role: Site co-investigator and steering committee member

**Research Support in Past 3 Years**

Sjögrens Foundation Anat Galor (PI)
9/2018-2/2020
Pilot study of fecal microbial transplant (FMT) in Sjögrens syndrome (SS).
The goal of this study was to evaluate the effect of FMT on immune profiles and dry eye in 10 individuals with Sjögrens
Role: Principal investigator

VA Merit award Anat Galor (PI)
1/2015-12/2019
Neuropathic pain: a critical missing piece in dry eye?
The goal of this study is to evaluate the epidemiology of neuropathic pain as a component of dry eye.
Role: Principal investigator

ARVO Collaborative Grant Anat Galor (co-PI)
4/2017-3/2018
Whole Exome Profiling of Ocular Surface Squamous Neoplasia (OSSN).
The goal of this study is to evaluate for genetic mutations within OSSN specimens and correlate these mutations to treatment response.
Role: Co-Principal Investigator
There is no scientific overlap between any of my current grants and this proposal.

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**Miami VA Healthcare System Human Studies Subcommittee**
1201 Northwest 16thMiami, FL 33125-1693305-575-3179Fax: 305-575-3126

# Approval

| | |
|---|---|
| DATE: | June 4, 2021 |
| FROM | Miami VA Healthcare System Human Studies Subcommittee |
| To: | Anat Galor, MD |
| PROJECT TITLE: | Gulf War Illness: Exploring the Eye-Brain Connection |
| REFERENCE #: | 1570449-13 (3011.09) |
| SUBMISSION TYPE: | Continuing Review/Progress Report |
| REVIEW TYPE: | Full Committee Review |
| ACTION: | APPROVED |

The following items were reviewed and approved at the June 3, 2021 meeting:

- Conflict of Interest - Other - COI_Determination_Form_Galor 09 CR May 2021.pdf (UPDATED: 05/28/2021)
- Consent Form - ICF 09222020 - Clean Copy (UPDATED: 05/18/2021)
- Consent Form - ICF 09222020 (stamped) (UPDATED: 05/18/2021)
- Continuing Review/Progress Report - IRB Request for Continued Approval_Rev.12.2018._5.28.2021pdf.pdf (UPDATED: 05/28/2021)
- HIPAA Consent/Authorization - HIPAA Authorization - Clean Copy (UPDATED: 05/18/2021)
- HIPAA Consent/Authorization - HIPAA Authorization (stamped) (UPDATED: 05/18/2021)
- Other - VHA Research Protocol Privacy Review Checklist (UPDATED: 05/28/2021)
- Other - Tracking Log of Events (UPDATED: 05/28/2021)
- Other - Report of Staff (UPDATED: 05/18/2021)

The Miami VA Healthcare System Human Studies Subcommittee voted to approve your submission.

All research must be conducted in accordance with this approved submission.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation.

All apparent NON-COMPLIANCE issues or COMPLAINTS regarding this project must be reported promptly to this committee.

This approval is based on appropriate risk/benefit ration and a project wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

Please remember that informed consent is a process beginning with a description of the project and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the project via a dialogue between the researcher and research participant. Federal regulations require that each participant receives a copy of the consent document.

All UNANTICIPATED PROBLEMS involving risks to subjects or others and SERIOUS and UNEXPECTED adverse events must be reported promptly to this office. All FDA and sponsor reporting requirements should also be followed.

Approval is granted for a period of *(enter review frequency)* and will expire on . Your Continuing Review is scheduled for *(enter continuing review date)*.

The protocol was determined to have the following level of risk:
MINIMAL RISK

If you have any questions, please contact Eida Gomez at 305-575-7000, Ext 4278 or eida.gomez@va.gov. Please include your project title and reference number in all correspondence with this committee.

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**Miami VA Healthcare System Chemical Hygiene & Biosafety Subcommittee**
1201 Northwest 16th Miami, FL 33125-1693 305-575-3179 Fax: 305-575-3126

## Continuing Review Approval

| | |
|---|---|
| DATE: | May 12, 2021 |
| FROM | Miami VA Healthcare System Chemical Hygiene & Biosafety Subcommittee |
| To: | Anat Galor, MD |
| PROJECT TITLE: | Gulf War Illness: Exploring the Eye-Brain Connection |
| REFERENCE #: | 1570449-12 |
| SUBMISSION TYPE: | Response/Follow-Up |
| REVIEW TYPE: | Expedited Review |
| ACTION: | APPROVED |

The following items were reviewed and approved at the May 10, 2021 meeting, contingent upon stipulations in each item marked with as asterisk (*):

[1570449-11]

- Continuing Review/Progress Report - Progress Report for CHBS Continuing Review (UPDATED:05/6/2021)*
- Continuing Review/Progress Report - CHBS Continuing Review (UPDATED: 05/4/2021)*
- Other - CHBS Research Protocol Safety Survey (UPDATED: 05/6/2021)*
- Other - Report of Staff (UPDATED: 05/4/2021)*
- Other - ERDSP (UPDATED: 05/4/2021)
- Other - CHBS Lab-Specific Exposure Control Plan (UPDATED: 05/4/2021)

Stipulations:

The reviewers had the following concerns:

- **Please verify that all room numbers and lab personnel match in each form submitted. ***

The following items were reviewed and approved via expedited procedures on May 12, 2021 and will be presented at the May 26, 2021 meeting:

[1570449-12]

- Letter - CHBS modifications addressed letter (UPDATED: 05/10/2021)
- Other - CHBS Research Protocol Safety Survey (UPDATED: 05/10/2021)

All contingencies have been satisfied.

All research must be conducted in accordance with this approved submission.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation.

All apparent NON-COMPLIANCE issues or COMPLAINTS regarding this project must be reported promptly to this committee.

Approval is granted for a period of *12 months* and will expire on May 9, 2022. Your Continuing Review is scheduled for March 14, 2022.

If you have any questions, please contact Kaytrina Walker at (305) 575-7000x14395 or kaytrina.walker@va.gov. Please include your project title and reference number in all correspondence with this committee.

ID# _____ Date_____

## Appendix A

## DePaul Symptom Questionnaire

Please answer the following questions.

1. What is your height?_____

2. What is your weight?_____

3. What is your date of birth?_____

4. What is your gender?_____

5. To which of the following race(s) do you belong?

- ☐Black, African-American
- ☐White
- ☐American Indian or Alaska Native
- ☐Asian or Pacific Islander
- ☐Other race (*Please specify*)_____

6. Are you of Latino or Hispanic origin?

☐Yes ☐No

7. What is your current marital status?

- ☐Married or living with partner
- ☐Separated
- ☐Widowed
- ☐Divorced
- ☐Never married

8. Do you have any children?

☐Yes ☐No (*Skip to Question 9)*

8a. How many children do you have?_____

8b. How many of your children are under 18 years old?_____

9. How many people live in your home?_____

10. What grade or degree have you completed in school?

- ☐ Less than high school
- ☐ Some high school
- ☐ High school degree or GED
- ☐ Partial college (at least one year) or specialized training
- ☐ Standard college degree
- ☐ Graduate professional degree including masters and doctorate

11. What is your current work status? **(Check all that apply)**

- ☐ On disability
- ☐ Student
- ☐ Homemaker
- ☐ Retired
- ☐ Unemployed
- ☐ Working part-time
- ☐ Working full-time

11a. If you are on disability, for what condition do you receive disability compensation?

Please Specify_____

12. What is your current occupation?

Current_____

12a. If you are currently not working, what was your most recent occupation?

Most Recent_____

For the following questions (13-66), we would like to know **how often you have had each symptom** and **how much each symptom has bothered you over the last 6 months**. For each symptom please circle **one number for frequency and one number for severity**. Please fill the chart out from left to right.

| **Symptoms** | *Frequency:*<br>Throughout the **past 6 months**, **how often** have you had this symptom?<br>For each symptom listed below, circle a number from:<br>**0 = none of the time**<br>**1 = a little of the time**<br>**2 = about half the time**<br>**3 = most of the time**<br>**4 = all of the time** | | | | | *Severity:*<br>Throughout the **past 6 months**, **how much** has this symptom bothered you?<br>For each symptom listed below, circle a number from:<br>**0 = symptom not present**<br>**1 = mild**<br>**2 = moderate**<br>**3 = severe**<br>**4 = very severe** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **13) Fatigue/extreme tiredness** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **14) Dead, heavy feeling after starting to exercise** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **15) Next day soreness or fatigue after non-strenuous, everyday activities** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **16) Mentally tired after the slightest effort** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **17) Minimum exercise makes you physically tired** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **18) Physically drained or sick after mild activity** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **19) Feeling unrefreshed after you wake up in the morning** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **20) Need to nap daily** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **21) Problems falling asleep** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **22) Problems staying asleep** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **23) Waking up early in the morning (e.g. 3am)** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **24) Sleep all day and stay awake all night** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **25) Pain or aching in your muscles** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **26) Pain/stiffness/tenderness in more than one joint without swelling or redness** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **27) Eye pain** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |

| **Symptoms** | *Frequency:*<br>Throughout the **past 6 months**, **how often** have you had this symptom?<br>For each symptom listed below, circle a number from:<br>**0 = none of the time**<br>**1 = a little of the time**<br>**2 = about half the time**<br>**3 = most of the time**<br>**4 = all of the time** | | | | | *Severity:*<br>Throughout the **past 6 months**, **how much** has this symptom bothered you?<br>For each symptom listed below, circle a number from:<br>**0 = symptom not present**<br>**1 = mild**<br>**2 = moderate**<br>**3= severe**<br>**4 = very severe** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **28) Chest pain** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **29) Bloating** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **30) Abdomen/stomach pain** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **31) Headaches** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **32) Muscle twitches** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **33) Muscle weakness** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **34) Sensitivity to noise** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **35) Sensitivity to bright lights** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **36) Problems remembering things** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **37) Difficulty paying attention for a long period of time** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **38) Difficulty finding the right word to say or expressing thoughts** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **39) Difficulty understanding things** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **40) Only able to focus on one thing at a time** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **41) Unable to focus vision and/or attention** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **42) Loss of depth perception** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **43) Slowness of thought** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **44) Absent-mindedness or forgetfulness** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **45) Bladder problems** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **46) Irritable bowel problems** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |

| Symptoms | *Frequency:*<br>Throughout the **past 6 months**, **how often** have you had this symptom?<br>For each symptom listed below, circle a number from:<br>**0 = none of the time**<br>**1 = a little of the time**<br>**2 = about half the time**<br>**3 = most of the time**<br>**4 = all of the time** | | | | | *Severity:*<br>Throughout the **past 6 months**, **how much** has this symptom bothered you?<br>For each symptom listed below, circle a number from:<br>**0 = symptom not present**<br>**1 = mild**<br>**2 = moderate**<br>**3= severe**<br>**4 = very severe** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **47) Nausea** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **48) Feeling unsteady on your feet, like you might fall** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **49) Shortness of breath or trouble catching your breath** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **50) Dizziness or fainting** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **51) Irregular heart beats** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **52) Losing or gaining weight without trying** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **53) No appetite** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **54) Sweating hands** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **55) Night sweats** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **56) Cold limbs (e.g. arms, legs, hands)** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **57) Feeling chills or shivers** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **58) Feeling hot or cold for no reason** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **59) Feeling like you have a high temperature** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **60) Feeling like you have a low temperature** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **61) Alcohol intolerance** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **62) Sore throat** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **63) Tender/sore lymph nodes** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **64) Fever** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **65) Flu-like symptoms** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| **66) Some smells, foods, medications, or chemicals make you feel sick** | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |

67. Have you **always** had persistent or recurring **fatigue/energy problems**, even back to your earliest memories as a child? (By persistent or recurring, we mean that the fatigue/energy problems are usually ongoing and constant, but sometimes there are good periods and bad periods.)

☐Yes ☐No ☐Not having a problem with fatigue/energy

68. Since your **fatigue/energy related illness** began, do your headaches either happen more often, feel worse or more severe, or are they in a different place or spot?

☐Yes ☐No ☐Not having a problem with fatigue/energy

69. How long ago did your problem with **fatigue/energy** begin?

☐Less than 6 months

☐6-12 months

☐1-2 years

☐Longer than 2 years

☐Had problem with fatigue/energy since childhood or adolescence

☐Not having a problem with fatigue/energy

70. Have you been diagnosed with Chronic Fatigue Syndrome or Myalgic Encephalomyelitis?

☐Yes ☐No

70a. If yes, what year were you diagnosed?_____

70b. Do you currently have a diagnosis of Chronic Fatigue Syndrome or Myalgic Encephalomyelitis?

☐Yes ☐No

70c. Who diagnosed you with Chronic Fatigue Syndrome or Myalgic Encephalomyelitis?

☐Medical Doctor ☐Alternative Practitioner ☐Self-Diagnosed

70d. Have any of your family members been diagnosed with Chronic Fatigue Syndrome or Myalgic Encephalomyelitis?

☐Yes ☐No

If yes, please list their relation to you and current age_____

_____

71. Did you experience any of the following symptoms regularly and repeatedly in the months and years before your fatigue/energy problems began?

☐Sore throat

☐Tender/sore lymph nodes

☐Unrefreshing sleep

☐Impaired memory and concentration

☐Prolonged fatigue following physical or mental exertion

☐Muscle pain

☐Headaches

☐Joint Pain

☐Not having a problem with fatigue/energy

72. If you rest, does your problem with **fatigue/energy** go away? **(Check one)**

☐ Entirely

☐ Partially

☐ My fatigue/energy problem is not improved by rest *(Skip to Question 73)*

☐I am not having a problem with fatigue/energy *(Skip to Question 73)*

72a. How long do you have to rest for your problem with **fatigue/energy** to entirely or partially go away?

☐less than 30 minutes ☐30 to 59 minutes ☐1-2 hours ☐more than 2 hours

73. If you were to become exhausted after actively participating in extracurricular activities, sports, or outings with friends, would you recover within an hour or two after the activity ended?

☐Yes ☐No

74. Do you reduce your activity level to avoid experiencing problems with **fatigue/energy**?

☐Yes ☐No ☐Not having a problem with fatigue/energy

75. Do you experience a worsening of your **fatigue/energy related illness** after engaging in minimal physical effort?

☐Yes ☐No ☐Not having a problem with fatigue/energy

75a. Do you experience a worsening of your **fatigue/energy related illness** after engaging in mental effort?

☐Yes ☐No

75b. If you feel worse after activities, how long does this last? **(Check one)**

__1 hour or less __2 -3 hours __ 4-10 hours

__11-13 hours __14-24 hours __More than 24 hours

76. Are you currently engaging in any form of exercise?

☐Yes *(Skip to Question 77)* ☐ No

76a. If you do not exercise, why aren't you exercising? **(Check all boxes that you agree with)**

☐ Not interested

☐ No time

☐ Would like to but cannot because ofproblems with fatigue/energy

☐ Cannot because exercise makes symptoms worse

77. Over what period of time did your **fatigue/energy related illness,** develop? **(Check one)**

- ☐ Within 24 hours
- ☐ Over 1 week
- ☐ Over 1 month
- ☐ Over 2-6 months
- ☐ Over 7-12 months
- ☐ Over 1-2 years
- ☐ Longer than 2 years
- ☐ Had problem with fatigue/energy since childhood or adolescence
- ☐ I am not ill

78. How would you describe the course of your **fatigue/energy related illness? (Check one)**

- ☐ Constantly getting worse
- ☐ Constantly improving
- ☐ Persisting (no change)
- ☐ Relapsing & remitting (having "good" periods with no symptoms & "bad" periods)
- ☐ Fluctuating (symptoms periodically get better and get worse, but never disappear completely)
- ☐ No Symptoms/I am not ill

79. Which statement best describes your **fatigue/energy related illness** during the **last 6 months**? **(Check one)**

- ☐ I am not able to work or do anything, and I am bedridden.
- ☐ I can walk around the house, but I cannot do light housework.
- ☐ I can do light housework, but I cannot work part-time.
- ☐ I can only work part-time at work or on some family responsibilities.
- ☐ I can work full time, but I have no energy left for anything else.
- ☐ I can work full time and finish some family responsibilities but I have no energy left for anything else.

☐I can do all work or family responsibilities without any problems with my energy.

80. Did your **fatigue/energy related illness** start after you experienced any of the following? **(Check one or more and please specify)**

☐An infectious illness_____

☐An accident_____

☐A trip or vacation_____

☐An immunization (shot at doctor's office)_____

☐Surgery_____

☐Severe stress (bad or unhappy event(s))_____

☐Other_____

☐I am not ill

81. Have you ever consulted a medical doctor or health professional about your **fatigue/energy** problem?

☐Yes ☐No *(Skip to Question 83)*

82. Do you currently have a medical doctor overseeing your **fatigue/energy** problem?

☐Yes ☐No

83. Do you have any medical illness (es) that might be causing your symptoms?

☐Yes ☐No *(Skip to Question 84)*

83a. What medical illnesses do you have?

Illness name(s) and year it began:_____

_____

_____

_____

83b. For which of these conditions are you currently receiving treatment?_____

_____

_____

_____

84. Are you currently taking any medications (over the counter or prescription)?

☐ Yes ☐ No *(Skip to Question 86)*

84a. What medications are you taking? _____

_____

85. Do you think any medication(s) is (are) causing your problem with **fatigue/energy**?

☐Yes ☐No *(Skip to Question 86)*

☐I do not have a problem with fatigue/energy *(Skip to Question 86)*

85a. Please specify which medications:_____

_____

86. Have you ever been diagnosed and/or treated for any of the following: **(Check all that apply and write year (s) experienced, years treated, and medication (if applicable) in the blank)**

☐Major depression_____

☐Major depression with melancholic or psychotic features_____

☐Bipolar disorder (Manic-depression)_____

☐Anxiety_____

☐Schizophrenia_____

☐Eating disorder_____

☐Substance abuse_____

☐Multiple chemical sensitivities_____

☐Fibromyalgia_____

☐Allergies_____

☐Other (*Please specify*)_____

☐No diagnosis/treatment

87. What do you think is the cause of your problem with **fatigue/energy**? **(Check one)**

☐Definitely physical

☐Mainly physical

☐Equally physical and psychological

☐Mainly psychological

☐Definitely psychological

☐No problem with fatigue/energy

88. Do you think anything specific in your personal life or environment accounts for your problem with **fatigue/energy**?

☐Yes ☐No *(Skip to Question 89)*

☐I do not have a problem with fatigue/energy (*Skip to Question 89)*

88a. Please specify:_____

89. In the **past 4 weeks**, approximately how many hours per week have you spent doing:

Household related activities?_____hours per week

Social/Recreational related activities?_____hours per week

Family related activities?_____hours per week

Work related activities?_____hours per week

90. In the **past 4 weeks**, have you had to reduce the number of hours you previously spent (prior to your illness) on occupational, social or family activities because of your health or problems with **fatigue/energy**?

☐ Yes ☐ No*(Skip to Question 91)* ☐Not having a problem with fatigue/energy

90a. **Before your fatigue/energy related illness**, approximately how many hours did you used to spend on:

Household related activities? _____hours per week

Social/Recreational related activities? _____hours per week

Family related activities? _____hours per week

Work related activities?_____hours per week

NOTE: For those people who are NOT having a problem with fatigue/energy, please answer questions 91-96 assuming that a score of 100= having abundant energy that allows one to work full-time and perform daily chores.

91. Please rate the amount of **energy** you had available **yesterday,** using a scale from 1 to 100 where 1 = no energy and 100 = your pre-illness energy level_____

92. Please rate the amount of **energy** you expended (used) **yesterday,** using a scale from 1 to 100 where 1 = no energy and 100 = your pre-illness energy expended_____

93. Please rate the amount of **fatigue** you had **yesterday,** using a scale from 1 to 100 where 1 = no fatigue and 100 = severe fatigue_____

94. For the **past week,** please rate the amount of **energy** you had available using a scale from 1 to 100 where 1=no energy and 100=your pre-illness energy level_____

95. For the **past week,** please rate the amount of **energy** you have expended (used) using a scale from 1 to 100 where 1 = no energy and 100 = your pre-illness energy expended_____

96. For the **past week,** please rate the amount of **fatigue** you have had using a scale from 1 to 100 where 1 = no fatigue and 100 = severe fatigue _____

# Kansas criteria for Gulf War Illness

**Instructions:** Symptoms (1) ***must have started during or after the Gulf War*** and (2) must have been present within the last year. Please score your symptoms in the past 6 months as either none, mild, moderate, or severe. **Only rate symptoms that began during or after the Gulf War.**

| Symptoms after the Gulf War | Severity of symptom in past 6 months | | | |
|---|---|---|---|---|
| **Fatigue / Sleep problems** | | | | |
| Feeling unwell after exercise or exertion | None | Mild | Moderate | Severe |
| Fatigue | None | Mild | Moderate | Severe |
| Moderate or multiple fatigue symptoms | None | Mild | Moderate | Severe |
| Problems staying asleep or falling asleep | None | Mild | Moderate | Severe |
| Not feeling rested after sleep | None | Mild | Moderate | Severe |
| **Pain symptoms** | | | | |
| Pain in muscles | None | Mild | Moderate | Severe |
| Body pain. Hurts all over | None | Mild | Moderate | Severe |
| Moderate or multiple pain symptoms | None | Mild | Moderate | Severe |
| Pain in joints | None | Mild | Moderate | Severe |
| **Neurologic / Cognitive / Mood symptoms** | | | | |
| Night sweats | None | Mild | Moderate | Severe |
| Feeling irritable or angry outbursts | None | Mild | Moderate | Severe |
| Problems remembering recent information | None | Mild | Moderate | Severe |
| Symptomatic response to chemicals, odors | None | Mild | Moderate | Severe |
| Difficulty concentrating | None | Mild | Moderate | Severe |
| Trouble finding words when speaking | None | Mild | Moderate | Severe |
| Moderate or multiple neurological symptoms | None | Mild | Moderate | Severe |

| | | | | |
|---|---|---|---|---|
| Low tolerance for heat or cold | None | Mild | Moderate | Severe |
| Feeling, dizzy, lightheaded, or faint | None | Mild | Moderate | Severe |
| Feeling down or depressed | None | Mild | Moderate | Severe |
| Headaches | None | Mild | Moderate | Severe |
| Eyes very sensitive to light | None | Mild | Moderate | Severe |
| Blurred or double vision | None | Mild | Moderate | Severe |
| Numbness or tingling in hands or feet | None | Mild | Moderate | Severe |
| Tremors or shaking | None | Mild | Moderate | Severe |
| **Gastrointestinal symptoms** | | | | |
| Nausea or upset stomach | None | Mild | Moderate | Severe |
| Abdominal pain or cramping | None | Mild | Moderate | Severe |
| Moderate or multiple gastrointestinal symptoms | None | Mild | Moderate | Severe |
| Diarrhea | None | Mild | Moderate | Severe |
| **Respiratory symptoms** | | | | |
| Difficulty breathing or catching your breath | None | Mild | Moderate | Severe |
| Moderate or multiple respiratory symptoms | None | Mild | Moderate | Severe |
| Wheezing | None | Mild | Moderate | Severe |
| Persistent cough without a cold | None | Mild | Moderate | Severe |
| **Skin symptoms** | | | | |
| Rashes | None | Mild | Moderate | Severe |
| Moderate or multiple skin symptoms | None | Mild | Moderate | Severe |

**Adapted from:**

Steele L. Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. *Am J Epidemiol.* 2000;152(10):992-1002.

## MOS SURVEY

**INSTRUCTIONS:**

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Answer every question by marking the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is: ***(Please circle one)***

Excellent..... 1
Very good..... 2
Good ..... 3
Fair ..... 4
Poor..... 5

2. **Compared to one year ago,** how would you rate your health in general now? ***(Please circle one)***

Much better than one year ago ..... 1
Somewhat better now than one year ago ..... 2
About the same as one year ago ..... 3
Somewhat worse now than one year ago..... 4
Much worse now than one year ago ..... 5

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

| **Activities** | **Yes, Limited A Lot** | **Yes, Limited A Little** | **No, Not Limited At All** |
|---|---|---|---|
| **Vigorous activities**: running, lifting heavy objects, participating in strenuous sports | 1 | 2 | 3 |
| **Moderate activities**: moving a table, pushing a vacuum cleaner, bowling, playing golf | 1 | 2 | 3 |
| Lifting or carrying groceries | 1 | 2 | 3 |
| Climbing **several** flights of stairs | 1 | 2 | 3 |
| Climbing **one** flight of stairs | 1 | 2 | 3 |
| Bending, kneeling, or stooping | 1 | 2 | 3 |
| Walking **more than a mile** | 1 | 2 | 3 |
| Walking **several blocks** | 1 | 2 | 3 |
| Walking **one** block | 1 | 2 | 3 |
| Bathing or dressing yourself | 1 | 2 | 3 |

4. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities as a result of your **physical health**?

| **Problems** | **Yes** | **No** |
|---|---|---|
| Cut down on the **amount of time** you spent on work or other activities | 1 | 2 |
| **Accomplished less** than you would like | 1 | 2 |
| Were limited in the **kind** of work or other activities | 1 | 2 |
| Had **difficulty** performing the work or other activities (For example, it took extra effort) | 1 | 2 |

5. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

| **Problems** | **Yes** | **No** |
|---|---|---|
| Cut down the **amount of time** you spent on work or other activities | 1 | 2 |
| **Accomplished less** than you would like | 1 | 2 |
| Didn't do work or other activities as **carefully** as usual | 1 | 2 |

6. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, neighbors, or groups? ***(Please circle one)***

Not at all ..... 1
Slightly ..... 2
Moderately ..... 3
Quite a bit ..... 4
Extremely ..... 5

7. How much bodily pain have you had during the **past 4 weeks**?

None ..... 1
Very mild ..... 2
Mild ..... 3
Moderate ..... 4
Severe ..... 5
Very Severe ..... 6

8. During the **past 4 weeks**, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all ..... 1
Slightly ..... 2
Moderately ..... 3
Quite a bit ..... 4
Extremely ..... 5

9. These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time **during the past 4 weeks**-

| **Questions** | **All of the Time** | **Most of the Time** | **A Good Bit of the Time** | **Some of the Time** | **A Little of the Time** | **None of the Time** |
|---|---|---|---|---|---|---|
| Did you feel full of pep? | 1 | 2 | 3 | 4 | 5 | 6 |
| Have you been a nervous person? | 1 | 2 | 3 | 4 | 5 | 6 |
| Have you felt so down in the dumps that nothing could cheer you up? | 1 | 2 | 3 | 4 | 5 | 6 |
| Have you felt calm and peaceful? | 1 | 2 | 3 | 4 | 5 | 6 |
| Did you have a lot of energy? | 1 | 2 | 3 | 4 | 5 | 6 |
| Have you felt down-hearted and blue? | 1 | 2 | 3 | 4 | 5 | 6 |
| Did you feel worn out? | 1 | 2 | 3 | 4 | 5 | 6 |
| Have you been a happy person? | 1 | 2 | 3 | 4 | 5 | 6 |
| Did you feel tired? | 1 | 2 | 3 | 4 | 5 | 6 |

10. During the **past 4 weeks**, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time ..... 1
Most of the time ..... 2
Some of the time ..... 3
A little of the time ..... 4
None of the time ..... 5

11. How **TRUE** or **FALSE** is each of following statements for you?

| **Statements** | **Definitely True** | **Mostly True** | **Don't Know** | **Mostly False** | **Definitely False** |
|---|---|---|---|---|---|
| I seem to get sick a little easier than other people | 1 | 2 | 3 | 4 | 5 |
| I am as healthy as anybody I know | 1 | 2 | 3 | 4 | 5 |
| I expect my health to get worse | 1 | 2 | 3 | 4 | 5 |
| My health is excellent | 1 | 2 | 3 | 4 | 5 |

# PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

NAME:_____ DATE:_____

Over the last *2 weeks,* how often have you been bothered by any of the following problems?
*(use "✓" to indicate your answer)*

| | Not at all | Several days | More than half the days | Nearly every day |
|---|---|---|---|---|
| **1.** Little interest or pleasure in doing things | 0 | 1 | 2 | 3 |
| **2.** Feeling down, depressed, or hopeless | 0 | 1 | 2 | 3 |
| **3.** Trouble falling or staying asleep, or sleeping too much | 0 | 1 | 2 | 3 |
| **4.** Feeling tired or having little energy | 0 | 1 | 2 | 3 |
| **5.** Poor appetite or overeating | 0 | 1 | 2 | 3 |
| **6.** Feeling bad about yourself—or that you are a failure or have let yourself or your family down | 0 | 1 | 2 | 3 |
| **7.** Trouble concentrating on things, such as reading the newspaper or watching television | 0 | 1 | 2 | 3 |
| **8.** Moving or speaking so slowly that other people could have noticed. Or the opposite — being so figety or restless that you have been moving around a lot more than usual | 0 | 1 | 2 | 3 |
| **9.** Thoughts that you would be better off dead, or of hurting yourself | 0 | 1 | 2 | 3 |
| add columns | | + | + | |

*(Healthcare professional: For interpretation of TOTAL, please refer to accompanying scoring card).* TOTAL:

**10.** If you checked off *any problems,* how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?

- Not difficult at all _____
- Somewhat difficult _____
- Very difficult _____
- Extremely difficult _____

# *New Clinical Fibromyalgia Diagnostic Criteria – Part 1.*

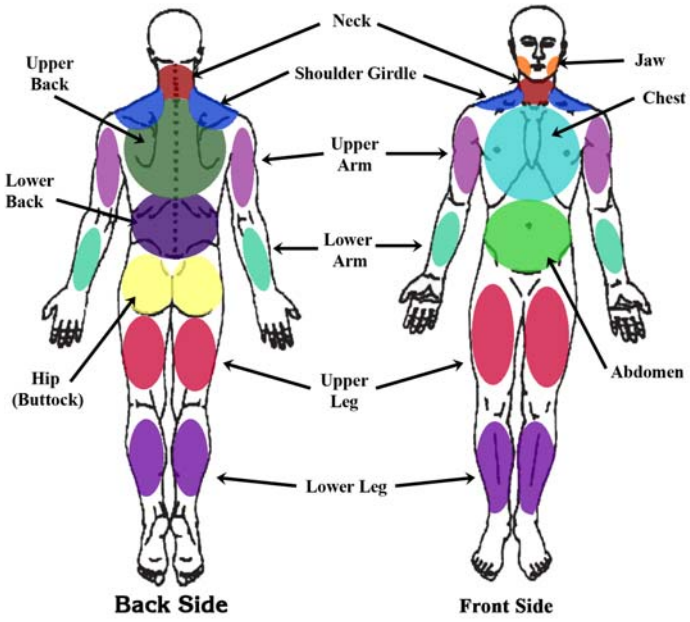
To answer the following questions, patients should take into consideration

- how you felt the **past week**,
- while taking your current therapies and treatments, and
- exclude your pain or symptoms from other known illnesses such as arthritis, Lupus, Sjogren's, etc.

**Check each area you have felt pain in over the past week.**

- ☐ Shoulder girdle, left
- ☐ Shoulder girdle, right
- ☐ Upper arm, left
- ☐ Upper arm, right
- ☐ Lower arm, left
- ☐ Lower arm, right
- ☐ Hip (buttock) left
- ☐ Hip (buttock) right
- ☐ Upper leg left
- ☐ Upper leg right
- ☐ Lower leg left
- ☐ Lower leg right
- ☐ Jaw left
- ☐ Jaw right
- ☐ Chest
- ☐ Abdomen
- ☐ Neck
- ☐ Upper back
- ☐ Lower back
- ☐ None of these areas

**Determining Your Widespread Pain Index (WPI)**
The WPI Index score from Part 1 is between 0 and 19.




**Count up the number of areas checked and enter your Widespread Pain Index or WPI score score here _____.**

# *Symptom Severity Score (SS score) - Part 2a.*

**Indicate your level of symptom severity over the past week using the following scale.**

**Fatigue**

- ☐ 0 = No problem
- ☐ 1 = Slight or mild problems; generally mild or intermittent
- ☐ 2 = Moderate; considerable problems; often present and/or at a moderate level
- ☐ 3 = Severe: pervasive, continuous, life disturbing problems

**Waking unrefreshed**

- ☐ 0 = No problem
- ☐ 1 = Slight or mild problems; generally mild or intermittent
- ☐ 2 = Moderate; considerable problems; often present and/or at a moderate level
- ☐ 3 = Severe: pervasive, continuous, life disturbing problems

**Cognitive symptoms**

- ☐ 0 = No problem
- ☐ 1 = Slight or mild problems; generally mild or intermittent
- ☐ 2 = Moderate; considerable problems; often present and/or at a moderate level
- ☐ 3 = Severe: pervasive, continuous, life disturbing problems

**Tally your score for Part 2a (not the number of checkmarks) and enter it here _____.**

# *Symptom Severity Score (SS score)- Part 2b*

**Check each of the following OTHER SYMPTOMS that you have experienced over the <u>past week?</u>**

- ☐ Muscle pain
- ☐ Irritable bowel syndrome
- ☐ Fatigue/tiredness
- ☐ Thinking or remembering problem
- ☐ Muscle Weakness
- ☐ Headache
- ☐ Pain/cramps in abdomen
- ☐ Numbness/tingling
- ☐ Dizziness
- ☐ Insomnia
- ☐ Depression
- ☐ Constipation
- ☐ Pain in upper abdomen
- ☐ Nausea
- ☐ Nervousness
- ☐ Chest pain
- ☐ Blurred vision
- ☐ Fever
- ☐ Diarrhea
- ☐ Dry mouth
- ☐ Itching
- ☐ Wheezing
- ☐ Raynauld's
- ☐ Hives/welts
- ☐ Ringing in ears
- ☐ Vomiting
- ☐ Heartburn
- ☐ Oral ulcers
- ☐ Loss/change in taste
- ☐ Seizures
- ☐ Dry eyes
- ☐ Shortness of breath
- ☐ Loss of appetite
- ☐ Rash
- ☐ Sun sensitivity
- ☐ Hearing difficulties
- ☐ Easy bruising
- ☐ Hair loss
- ☐ Frequent urination
- ☐ Painful urination
- ☐ Bladder spasms

Count up the number of symptoms checked above.
*If you tallied:

| | |
|---|---|
| 0 symptoms | Give yourself a score of 0 |
| 1 to 10 | Give yourself a score of 1 |
| 11 to 24 | Give yourself a score of 2 |
| 25 or more | Give yourself a score of 3 |

**Enter your score for Part 2b here _____.**

**Now add Part 2a <u>AND</u> 2b scores, and enter ____.**
This is your Symptom Severity Score (SS score), which can range from 0 to 12.

For information about Fibromyalgia Network, call our office Monday through Friday, 9:00 a.m. to 5:00 p.m. (PST) at (800) 853-2929 or visit us online at www.fmnetnews.com.

This survey is not meant to substitute for a diagnosis by a medical professional. Patients should not diagnose themselves. Patients should always consult their medical professional for advice and treatment. This survey is intended to give you insight into research on the diagnostic criteria and measurement of symptom severity for fibromyalgia.

**Modified Fatigue Impact Scale (MFIS)**

Fatigue is a feeling of physical tiredness and lack of energy that many people experience from time to time. But people who have medical conditions like MS experience stronger feelings of fatigue more often and with greater impact than others.

Following is a list of statements that describe the effects of fatigue. Please read each statement carefully, the circle the one number that best indicates how often fatigue has affected you in this way during the past 4 weeks. (If you need help in marking your responses, tell the interviewer the number of the best response.) Please answer every question. If you are not sure which answer to select choose the one answer that comes closest to describing you. Ask the interviewer to explain any words or phrases that you do not understand.

**Because of my fatigue during the past 4 weeks**

| | | Never | Rarely | Sometimes | Often | Almost Always |
|---|---|---|---|---|---|---|
| 1. | I have been less alert. | 0 | 1 | 2 | 3 | 4 |
| 2. | I have had difficulty paying attention for long periods of time. | 0 | 1 | 2 | 3 | 4 |
| 3. | I have been unable to think clearly. | 0 | 1 | 2 | 3 | 4 |
| 4. | I have been clumsy and uncoordinated. | 0 | 1 | 2 | 3 | 4 |
| 5. | I have been forgetful. | 0 | 1 | 2 | 3 | 4 |
| 6. | I have had to pace myself in my physical activities. | 0 | 1 | 2 | 3 | 4 |
| 7. | I have been less motivated to do anything that requires physical effort. | 0 | 1 | 2 | 3 | 4 |
| 8. | I have been less motivated to participate in social activities. | 0 | 1 | 2 | 3 | 4 |
| 9. | I have been limited in my ability to do things away from home. | 0 | 1 | 2 | 3 | 4 |
| 10. | I have trouble maintaining physical effort for long periods. | 0 | 1 | 2 | 3 | 4 |
| 11. | I have had difficulty making decisions. | 0 | 1 | 2 | 3 | 4 |
| 12. | I have been less motivated to do anything that requires thinking | 0 | 1 | 2 | 3 | 4 |
| 13. | My muscles have felt weak | 0 | 1 | 2 | 3 | 4 |
| 14. | I have been physically uncomfortable. | 0 | 1 | 2 | 3 | 4 |
| 15. | I have had trouble finishing tasks that require thinking. | 0 | 1 | 2 | 3 | 4 |
| 16. | I have had difficulty organizing my thoughts when doing things at home or at work. | 0 | 1 | 2 | 3 | 4 |
| 17. | I have been less able to complete tasks that require physical effort. | 0 | 1 | 2 | 3 | 4 |
| 18. | My thinking has been slowed down. | 0 | 1 | 2 | 3 | 4 |
| 19. | I have had trouble concentrating. | 0 | 1 | 2 | 3 | 4 |
| 20. | I have limited my physical activities. | 0 | 1 | 2 | 3 | 4 |
| 21. | I have needed to rest more often or for longer periods. | 0 | 1 | 2 | 3 | 4 |

# PTSD CheckList – Military Version (PCL-M)

***Instruction to patient:*** **Below is a list of problems and complaints that veterans sometimes have in response to stressful military experiences. Please read each one carefully, put an "X" in the box to indicate how much you have been bothered by that problem *in the last month.***

| | | Frequency: | | | | |
|---|---|---|---|---|---|---|
| **No.** | **Problem or Complaint:** | **Not at all (1)** | **A little bit (2)** | **Moderately (3)** | **Quite a bit (4)** | **Extremely (5)** |
| 1. | Repeated, disturbing *memories, thoughts,* or *images* of a stressful military experience? | | | | | |
| 2. | Repeated, disturbing *dreams* of a stressful military experience? | | | | | |
| 3. | Suddenly *acting* or *feeling* as if a stressful military experience *were happening again* (as if you were reliving it)? | | | | | |
| 4. | Feeling very upset when something reminded you of a stressful military experience? | | | | | |
| 5. | Having *physical reactions* (e.g., heart pounding, trouble breathing, or sweating) when *something reminded* you of a stressful military experience? | | | | | |
| 6. | Avoid *thinking about* or *talking* about a stressful military experience or avoid *having feelings* related to it? | | | | | |
| 7. | Avoid *activities* or *talking about* a stressful military experience or avoid *having feelings* related to it? | | | | | |
| 8. | Trouble *remembering important parts* of a stressful military experience? | | | | | |
| 9. | Loss of *interest* in things that you used to enjoy? | | | | | |
| 10. | Feeling *distant* or *cut off* from other people? | | | | | |
| 11. | Feeling *emotionally numb* or being unable to have loving feelings for those close to you? | | | | | |
| 12. | Feeling as if your *future* will somehow be *cut short*? | | | | | |
| 13. | Trouble *falling* or *staying* asleep? | | | | | |
| 14. | Feeling *irritable* or having *angry outbursts*? | | | | | |
| 15. | Having *difficulty* concentrating? | | | | | |
| 16. | Being "*super alert*" or watchful on guard? | | | | | |
| 17. | Feeling *jumpy* or easily startled? | | | | | |

**PCL-M for DSM-IV (11/1/94)**

**Weathers, F.W., Huska, J.A., Keane, T.M. PCL-M for DSM-IV. Boston; National Center for PTSD – Behavioral Science Division, 1991.**

# SF-12® Patient Questionnaire

**SF-12®:**
This information will help your doctors keep track of how you feel and how well you are able to do your usual activities. Answer every question by placing a check mark on the line in front of the appropriate answer. It is not specific for arthritis. If you are unsure about how to answer a question, please give the best answer you can and make a written comment beside your answer.

1. In general, would you say your health is:
   _____ Excellent (1)
   _____ Very Good (2)
   _____ Good (3)
   _____ Fair (4)
   _____ Poor (5)

The following two questions are about activities you might do during a typical day. Does YOUR HEALTH NOW LIMIT YOU in these activities? If so, how much?

2. MODERATE ACTIVITIES, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf:
   _____ Yes, Limited A Lot (1)
   _____ Yes, Limited A Little (2)
   _____ No, Not Limited At All (3)

3. Climbing SEVERAL flights of stairs:
   _____ Yes, Limited A Lot (1)
   _____ Yes, Limited A Little (2)
   _____ No, Not Limited At All (3)

During the PAST 4 WEEKS have you had any of the following problems with your work or other regular activities AS A RESULT OF YOUR PHYSICAL HEALTH?

4. ACCOMPLISHED LESS than you would like:
   _____ Yes (1)
   _____ No (2)

5. Were limited in the KIND of work or other activities:
   _____ Yes (1)
   _____ No (2)

During the PAST 4 WEEKS, were you limited in the kind of work you do or other regular activities AS A RESULT OF ANY EMOTIONAL PROBLEMS (such as feeling depressed or anxious)?

6. ACCOMPLISHED LESS than you would like:
   _____ Yes (1)
   _____ No (2)

7. Didn't do work or other activities as CAREFULLY as usual:
   _____ Yes (1)
   _____ No (2)

8. During the PAST 4 WEEKS, how much did PAIN interfere with your normal work (including both work outside the home and housework)?
   _____ Not At All (1)
   _____ A Little Bit (2)
   _____ Moderately (3)
   _____ Quite A Bit (4)
   _____ Extremely (5)

The next three questions are about how you feel and how things have been DURING THE PAST 4 WEEKS. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the PAST 4 WEEKS –

9. Have you felt calm and peaceful?

   _____ All of the Time (1)
   _____ Most of the Time (2)
   _____ A Good Bit of the Time (3)
   _____ Some of the Time (4)
   _____ A Little of the Time (5)
   _____ None of the Time (6)

10. Did you have a lot of energy?
    _____ All of the Time (1)
    _____ Most of the Time (2)
    _____ A Good Bit of the Time (3)
    _____ Some of the Time (4)
    _____ A Little of the Time (5)
    _____ None of the Time (6)

11. Have you felt downhearted and blue?
    _____ All of the Time (1)
    _____ Most of the Time (2)
    _____ A Good Bit of the Time (3)
    _____ Some of the Time (4)
    _____ A Little of the Time (5)
    _____ None of the Time (6)

12. During the PAST 4 WEEKS, how much of the time has your PHYSICAL HEALTH OR EMOTIONAL PROBLEMS interfered with your social activities (like visiting with friends, relatives, etc.)?
    _____ All of the Time (1)
    _____ Most of the Time (2)
    _____ A Good Bit of the Time (3)
    _____ Some of the Time (4)
    _____ A Little of the Time (5)
    _____ None of the Time (6)

# Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. **Please answer all questions.**

1. During the past month, what time have you usually gone to bed at night? _____
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night? _____
3. During the past month, what time have you usually gotten up in the morning? _____
4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.) _____

| 5. During the past month, how often have you had trouble sleeping because you… | Not during the past month | Less than once a week | Once or twice a week | Three or more times a week |
|---|---|---|---|---|
| a. Cannot get to sleep within 30 minutes | | | | |
| b. Wake up in the middle of the night or early morning | | | | |
| c. Have to get up to use the bathroom | | | | |
| d. Cannot breathe comfortably | | | | |
| e. Cough or snore loudly | | | | |
| f. Feel too cold | | | | |
| g. Feel too hot | | | | |
| h. Have bad dreams | | | | |
| i. Have pain | | | | |
| j. Other reason(s), please describe: | | | | |
| 6. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")? | | | | |
| 7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity? | | | | |
| | No problem at all | Only a very slight problem | Somewhat of a problem | A very big problem |
| 8. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done? | | | | |
| | Very good | Fairly good | Fairly bad | Very bad |
| 9. During the past month, how would you rate your sleep quality overall? | | | | |

| | No bed partner or room mate | Partner/room mate in other room | Partner in same room but not same bed | Partner in same bed |
|---|---|---|---|---|
| 10. Do you have a bed partner or room mate? | | | | |
| | Not during the past month | Less than once a week | Once or twice a week | Three or more times a week |
| If you have a room mate or bed partner, ask him/her how often in the past month you have had: | | | | |
| a. Loud snoring | | | | |
| b. Long pauses between breaths while asleep | | | | |
| c. Legs twitching or jerking while you sleep | | | | |
| d. Episodes of disorientation or confusion during sleep | | | | |
| e. Other restlessness while you sleep, please describe: | | | | |

**Based on the scale below, please rate the intensity of eye pain at this moment (scale 0 to 10, 10 being the worst).**

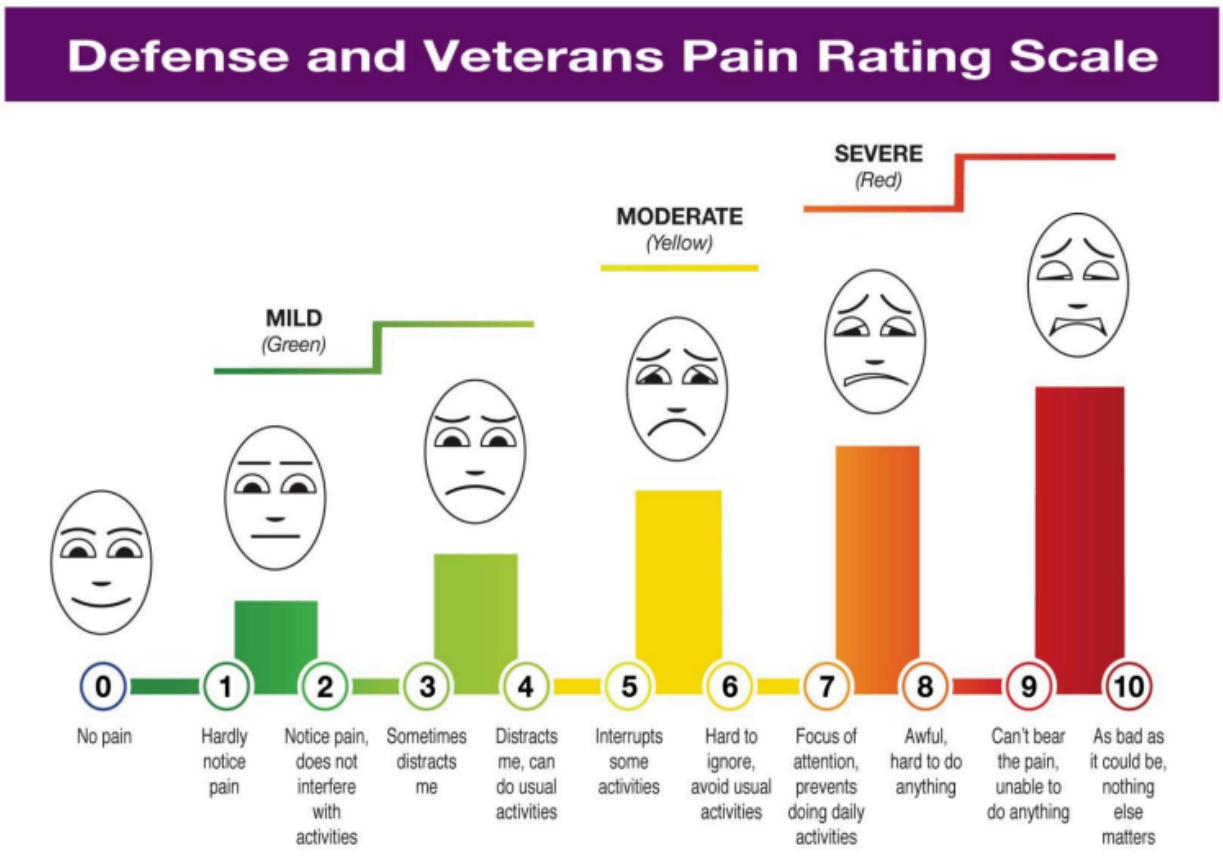
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Left Eye: | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Right Eye: | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

**Based on the scale below, please rate the intensity of eye pain on average over the last week (scale 0 to 10, 10 being the worst).**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Left Eye: | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Right Eye: | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

**Based on the scale below, please rate the intensity of eye pain at its worst over the last week (scale 0 to 10, 10 being the worst).**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Left Eye: | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Right Eye: | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |



# DEQ 5

1. Questions about **EYE DISCOMFORT**:
   a. During a typical day in the past month, **how often** did your eyes feel discomfort?

      0 Never
      1 Rarely
      2 Sometimes
      3 Frequently
      4 Constantly

   b. When your eyes felt discomfort, **how intense was this feeling of discomfort** at the end of the day, within two hours of going to bed?

| Never Have It | Not at all Intense | | | | Very Intense |
|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 5 |

2. Questions about **EYE DRYNESS**
   a. During a typical day in the past month, **how often** did your eyes feel dry?

      0 Never
      1 Rarely
      2 Sometimes
      3 Frequently
      4 Constantly

   b. When your eyes felt dry, **how intense was this feeling of dryness** at the end of the day, within two hours of going to bed?

| Never Have It | Not at all Intense | | | | Very Intense |
|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 4 | 5 |

3. Question about **WATERY EYES**:
   During a typical day in the past month, **how often** did your eyes look or feel excessively watery?

      0 Never
      1 Rarely
      2 Sometimes
      3 Frequently
      4 Constantly

Neuropathic Pain Symptom Inventory - Eye

We wish to know if you feel spontaneous eye pain, that is, pain without any stimulation. For each of the following questions, please select the number that best describes your *average spontaneous pain severity during the past 24 h*. Select the number 0 if you have not felt such pain (circle one number only).

Q1. Does your eye pain feel like burning?

No burning 0 1 2 3 4 5 6 7 8 9 10 Worst burning imaginable

Q2. Does your eye pain feel like squeezing?

No squeezing 0 1 2 3 4 5 6 7 8 9 10 Worst squeezing imaginable

Q3. Does your eye pain feel like pressure?

No pressure 0 1 2 3 4 5 6 7 8 9 10 Worst pressure imaginable

Q4. *During the past 24 h*, your spontaneous pain has been present:
Select *the response* that best describes your case

Permanently ____
Between 8 and 12 hours ____
Between 4 and 7 hours ____
Between 1 and 3 hours ____
Less than 1 hour ____

We wish to know if you have brief attacks of eye pain. For each of the following questions, please select the number that best describes the *average severity of your painful attacks during the past 24 h*. Select the number 0 if you have not felt such pain (circle one number only).

Q5. Does your eye pain feel like electric shocks?

No electric shocks 0 1 2 3 4 5 6 7 8 9 10 Worst electric shocks imaginable

Q6. Does your eye pain feel like stabbing?

No stabbing 0 1 2 3 4 5 6 7 8 9 10 Worst stabbing imaginable

Q7. *During the past 24 h*, how many of these pain attacks have you had?
Select *the response* that best describes your case

More than 20 ____
Between 11 and 20 ____
Between 6 and 10 ____
Between 1 and 5 ____

No pain attack ____

We wish to know if you feel eye pain provoked or increased by wind, light, or contact with cold/hot. For each of the following questions, please select the number that best describes the *average severity of your provoked pain during the past 24 h*. Select the number 0 if you have not felt such pain (circle one number only).

Q8. Is your eye pain provoked or increased by wind?

No pain 0 1 2 3 4 5 6 7 8 9 10 Worst pain imaginable

Q9. Is your eye pain provoked or increased by light?

No pain 0 1 2 3 4 5 6 7 8 9 10 Worst pain imaginable

Q10. Is your eye pain provoked or increased by *contact* with something cold or hot (air conditioned/warm weather)?

No pain 0 1 2 3 4 5 6 7 8 9 10 Worst pain imaginable

We wish to know if you feel abnormal eye sensations. For each of the following questions, please select the number that best describes the *average severity of your abnormal sensations during the past 24 h*. Select the number 0 if your have not felt such sensation (circle one number only).

Q11. Do you feel pins and needles?

No pins and needles 0 1 2 3 4 5 6 7 8 9 10 Worst pins and needles imaginable

Q12. Do you feel tingling?

No tingling 0 1 2 3 4 5 6 7 8 9 10 Worst tingling imaginable

# Ocular Surface Disease Index© (OSDI©)[2]

Ask your patient the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

**HAVE YOU EXPERIENCED ANY OF THE FOLLOWING *DURING THE LAST WEEK*:**

| | All of the time | Most of the time | Half of the time | Some of the time | None of the time |
|---|---|---|---|---|---|
| 1. Eyes that are sensitive to light? | 4 | 3 | 2 | 1 | 0 |
| 2. Eyes that feel gritty? | 4 | 3 | 2 | 1 | 0 |
| 3. Painful or sore eyes? | 4 | 3 | 2 | 1 | 0 |
| 4. Blurred vision? | 4 | 3 | 2 | 1 | 0 |
| 5. Poor vision? | 4 | 3 | 2 | 1 | 0 |

**Subtotal score for answers 1 to 5** (A)

**HAVE PROBLEMS WITH YOUR EYES LIMITED YOU IN PERFORMING ANY OF THE FOLLOWING *DURING THE LAST WEEK*:**

| | All of the time | Most of the time | Half of the time | Some of the time | None of the time | |
|---|---|---|---|---|---|---|
| 6. Reading? | 4 | 3 | 2 | 1 | 0 | N/A |
| 7. Driving at night? | 4 | 3 | 2 | 1 | 0 | N/A |
| 8. Working with a computer or bank machine (ATM)? | 4 | 3 | 2 | 1 | 0 | N/A |
| 9. Watching TV? | 4 | 3 | 2 | 1 | 0 | N/A |

**Subtotal score for answers 6 to 9** (B)

**HAVE YOUR EYES FELT UNCOMFORTABLE IN ANY OF THE FOLLOWING SITUATIONS *DURING THE LAST WEEK*:**

| | All of the time | Most of the time | Half of the time | Some of the time | None of the time | |
|---|---|---|---|---|---|---|
| 10. Windy conditions? | 4 | 3 | 2 | 1 | 0 | N/A |
| 11. Places or areas with low humidity (very dry)? | 4 | 3 | 2 | 1 | 0 | N/A |
| 12. Areas that are air conditioned? | 4 | 3 | 2 | 1 | 0 | N/A |

**Subtotal score for answers 10 to 12** (C)

**ADD SUBTOTALS A, B, AND C TO OBTAIN D (D = SUM OF SCORES FOR ALL QUESTIONS ANSWERED)** (D)

**TOTAL NUMBER OF QUESTIONS ANSWERED (DO NOT INCLUDE QUESTIONS ANSWERED N/A)** (E)

**Please turn over the questionnaire to calculate the patient's final OSDI© score.**

Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol. 2000;118:615-621

# Convergence Insufficiency Symptom Survey (CISS)

**Clinician/Assistant instructions**: Pose the following questions exactly as written. If the patient responds with "yes" - please qualify with frequency choices. Do not give examples.

**Patient instructions:** Please answer the following questions about how your eyes feel when reading or doing close work.

| Possible Subjective Symptoms | Frequency | | | | |
|---|---|---|---|---|---|
| | Never (0) | Infrequently/ not very often (1) | Sometimes (2) | Fairly often (3) | Always (4) |
| 1. Do your eyes feel tired when reading or doing close work? | | | | | |
| 2. Do your eyes feel uncomfortable when reading or doing close work? | | | | | |
| 3. Do you have headaches when reading or doing close work? | | | | | |
| 4. Do you feel sleepy when reading or doing close work? | | | | | |
| 5. Do you lose concentration when reading or doing close work? | | | | | |
| 6. Do you have trouble remembering what you have read? | | | | | |
| 7. Do you have double vision when reading or doing close work? | | | | | |
| 8. Do you see the words move, jump, swim or appear to float on the page when reading or doing close work? | | | | | |
| 9. Do you feel like you read slowly? | | | | | |
| 10. Do your eyes ever hurt when reading or doing close work? | | | | | |
| 11. Do your eyes ever feel sore when reading or doing close work? | | | | | |
| 12. Do you feel a "pulling" feeling around your eyes when reading or doing close work? | | | | | |
| 13. Do you notice the words blurring or coming in and out of focus when reading or doing close work? | | | | | |
| 14. Do you lose your place while reading or doing close work? | | | | | |
| **Total score** _____ | ___ x 0 | ___ x 1 | ___ x 2 | ___ x 3 | ___ x 4 |

**For Children** (< age 21) total score = **16 or higher** is suggestive of convergence insufficiency.
**For Adults** total score = **21 or higher** is suggestive of convergence insufficiency.

Reference: Borsting EJ, Rouse MW, Mitchell GL, et al and the CITT group. Validity and reliability of the revised convergence insufficiency symptom survey in children. Optometry and Vision Science 2003; 80(12):832-838.

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# Ocular manifestations and biomarkers of Gulf War Illness in US veterans

Brandon S. Baksh[1,2], Kristen L. Zayan[1,2], Raquel Goldhardt[1,3], Elizabeth R. Felix[4,5], Nancy Klimas[4,6] & Anat Galor[1,3]✉

**Gulf War Illness (GWI) is a multisystem disease with variable presentations, making diagnosis difficult. Non-invasive biomarkers would aid in disease diagnosis. We hypothesized that the eye could serve as a biomarker for GWI. We performed a retrospective case–control study using a sample of 1246 patients seen during a 5-month period in an optometry clinic. We identified veterans who were active duty during the Gulf War Era and either had a questionnaire-based diagnosis of GWI (cases) or did not (controls). Medical records were reviewed for eye and medical co-morbidities, medication use, and retinal macular and nerve fiber layer (NFL) thicknesses based on optical coherence tomography (OCT) images. Compared to controls (n = 85), individuals with GWI (n = 60) had a higher frequency of dry eye symptoms (50% vs 32.9%, p = 0.039). Multivariable analysis revealed average retinal NFL thickness (odds ratio; OR = 0.95), cup-to-disc ratio (OR = 0.005), age (OR = 0.82), and PTSD (OR = 20.5) were predictors of a GWI diagnosis. We conclude that GWI is associated with dry eye symptoms and RNFL thinning may serve as a biomarker for disease.**

On return from the 1990 to 1991 Gulf War, about 200,000 veterans reported a wide range of symptoms that have been categorized as Gulf War Illness (GWI)[1]. GWI covers a wide range of symptoms including (1) fatigue (2) mood and cognition disorders and (3) musculoskeletal disorders. The pathophysiology of GWI is believed to involve central nervous system (CNS) dysfunction manifesting in multiple systems. Studies have examined CNS abnormalities in GWI. In a study of 96 veterans with GWI, functional magnetic resonance imaging (fMRI) showed significant decreases in the pre-frontal cortex and white-matter activity during high-demand working memory tasks compared to 44 matched controls[2]. These neurological changes have been linked to chemical exposure while in theater, including pesticides. A study of 7,971 United Kingdom Gulf War veterans (GWV) with GWI symptoms revealed a positive correlation between neurological symptoms and days handling pesticides, $r = 0.08$, $p < 0.001$[3]. Taken together, these data suggest GWI involves nervous system alterations in response to chemical exposures that have widespread biological effects.

Several age-related diseases have been found to be more common in GWI veterans compared to Gulf War Era (GWE) veterans not deployed to the Gulf War. These diseases include hypertension, coronary heart disease, and chronic obstructive pulmonary disease[4]. There is a paucity of data, however, on the frequency of age-related eye diseases in GWI, even though there is an increased frequency of blurry vision and photophobia in GWV compared to non-GWV[1]. Thus veterans with GWI may be at increased risk for age-related eye disease and this association should be explored. Furthermore, GWI may specifically be at risk for dry eye (DE) given the overlap in symptom profile between GWI, fibromyalgia, and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), the latter two of which have been associated with DE symptoms[5,6]. Given these data, we hypothesized that individuals with GWI have a higher frequency of age-related eye diseases, including DE symptoms, compared to GWE veterans that do not meet GWI diagnostic criteria.

Beyond the frequency of overt disease, there is a need to identify sub-clinical biomarkers of GWI as diagnosis is difficult given varied presentations. A potential modality to identify sub-clinical disease is OCT, which has been employed to diagnose and monitor disease progression in Multiple Sclerosis (MS), Parkinson's disease (PD), and Alzheimer's disease (AD)[7–10]. For example, OCT parameters, such as retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) thickness correlated with changes in clinical status[11,12], visual acuity, and disability in

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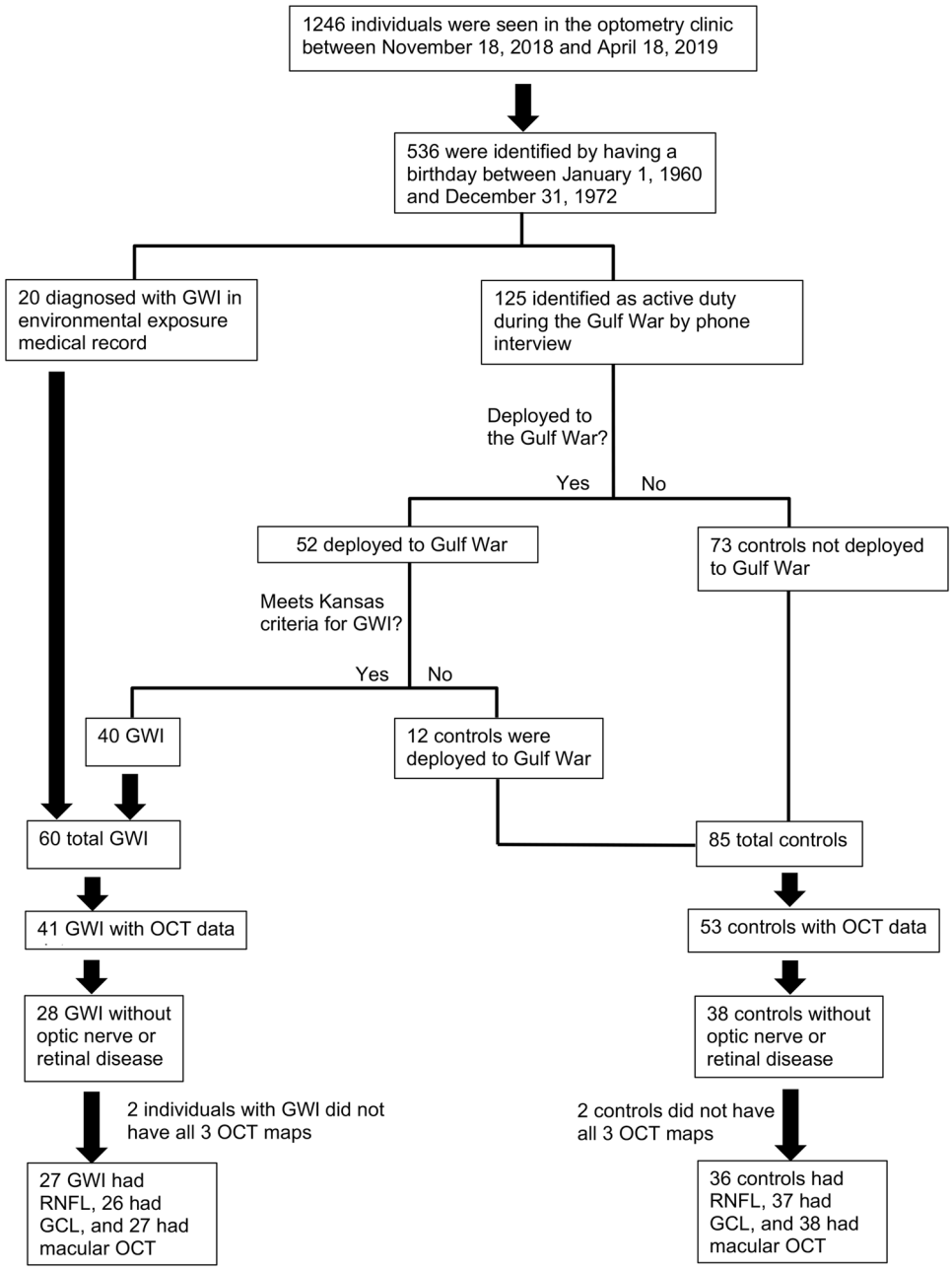


**Figure 1.** Flow chart of identification of veterans with Gulf War Illness. Of note, 2 of 28 individuals with GWI and 2 of 38 controls did not have all 3 OCT maps (RNFL, GCL-IPL, macula). RNFL images were available for 27 GWI and 36 controls, GCL for 26 GWI and 37 controls, and macula for 27 GWI and 38 controls. OCT = optical coherence tomography; GWI = Gulf War Illness. Figure was created using Microsoft Word for Mac (version 16.16.15, https://www.microsoft.com/en-us/microsoft-365/word).

MS[13–15]. Based on findings in other neurodegenerative diseases, we hypothesized that individuals with GWI, but without overt retinal and optic nerve pathology, would have differences in OCT measures compared to GWE veterans without a GWI diagnosis. To evaluate this hypothesis, we performed a retrospective case–control study.

## Materials and methods

**Study population and Gulf War Illness diagnosis.** The study population consisted of 1246 patients who were seen between November 18, 2018, and April 18, 2019, in the optometry clinic at the Miami Veterans Affairs Hospital (VA). Individuals were split into two groups: those with a diagnosis of GWI and those who served during the GWE who did not meet the criteria for GWI (controls) (Fig. 1). To identify all potential GWE veterans, we contacted 536 individuals seen in the optometry clinic during the relevant date range with a birthday between January 1, 1960, and December 31, 1972. Patients were diagnosed with GWI if they were

deployed to the Gulf War and met the Kansas criteria via clinic or phone interview[1]. The Kansas criteria requires: symptoms started during or after deployment and were present in the year prior to assessment, and one severe or two moderate symptoms in at least three of six domains, including (1) fatigue, (2) pain, (3) neurologic and mood, (4) gastrointestinal, (5) respiratory, and (6) skin[1]. Veterans were included in the control group if they were active duty and deployed to the Gulf War, but did not meet Kansas criteria, or were active duty, but not deployed to the Gulf War.

Individuals with GWI were further sub-typed based on reported symptoms. Specific symptom clusters have been found to be useful when grouping GWI veterans[16]. In our study, we identified individuals with "severely impaired cognition" syndrome if they had at least 5 out of 6 of the following symptoms: problems with memory, feelings of irritability/angry outbursts, headaches, depression, difficulty concentrating, and trouble finding words when speaking.

The study was first approved by the Miami VA Institutional Review Board (IRB) as a quality assurance study. Approval was then obtained to link the questionnaire to clinical records. Informed consent was waived by the Miami VA IRB. The study was conducted in accordance with the principles of the Declaration of Helsinki and complied with the requirements of the United States Health Insurance Portability and Accountability Act.

**Data collected.** Patient charts were retrospectively reviewed for demographics, co-morbidities, medications, and diagnoses of eye diseases including glaucoma, age-related macular degeneration, diabetic retinopathy, and DE symptoms or signs. DE symptoms were considered present if terms such as "dryness", "irritation", or "foreign body sensation" were listed as complaints in the clinical records. DE signs were considered present if any of the criteria were documented: fast tear break-up time (TBUT), positive fluorescein corneal staining, low tear lake, or Schirmer's test < 5 mm wetting at 5 min.

**Imaging.** Of 145 veterans identified via phone survey or medical record review as having served during the GWE, 94 individuals underwent OCT imaging (RNFL, GCL-inner plexiform layer (IPL), and macular maps) during their next routine clinic visit using a Cirrus HD-OCT (Carl Zeiss Meditec Inc, Dublin, California, USA). Of note, the 94 individuals with imaging were slightly older than the 51 who did not have OCT imaging available (51 ± 4.1 vs 52 ± 4.2, p = 0.013) but the remaining demographics were similar between the groups. OCT data from 28 individuals were subsequently excluded from the final analysis given overt retinal or optic nerve disease, including glaucoma, ocular hypertension retinopathy, retinal hemorrhage, diabetic retinopathy, or dry age-related macular degeneration (ARMD). Thus, 66 individuals with no diagnosis of retinal or optic nerve pathology and were included in the imaging analysis. Of note, 2 of 28 individuals with GWI and 2 of 38 controls did not have all 3 OCT maps (RNFL, GCL-IPL, macula). RNFL images were available for 27 GWI and 36 controls, GCL for 26 GWI and 37 controls, and macula for 27 GWI and 38 controls. For all analyses, the thinner RNFL, GCL, and macular value from either eye was used.

**Data analysis.** Statistical analyses were performed using SPSS 24.0 (IBM Corp, Armonk, NY) statistical package. Descriptive statistics were used to summarize patient demographic and clinical information. Normality of the data was assessed using the Kolmogorov–Smirnov test. Differences in continuous variables between two groups were analyzed using the Student's t-test or Mann–Whitney U test, as appropriate. Differences in continuous variables between more than two groups were analyzed using the Kruskal–Wallis H test. Differences in categorical data were compared using Chi-square or Fisher's exact test, as appropriate[17]. Predictors of GWI were analyzed using forward stepwise binary logistic regression. All reported p-values are two-tailed and $p < 0.05$ was considered statistically significant. In this paper, we opted to give information on all variables being compared as opposed to correcting the p-value (e.g. Bonferroni) since the latter methodology has its own limitations[18].

## Results

**Study population.** During the above timeframe, 1246 veterans were seen in the optometry clinic. Of those, 145 served during the GWE, 60 met the criteria for GWI, and 85 served as controls. Twenty-eight GWI veterans were identified as having "severely impaired cognition." Demographics were comparable between GWI veterans and controls (Table 1). Veterans with GWI had significantly higher frequencies of post-traumatic stress disorder (PTSD) (45% vs 20%, p = 0.001), chronic fatigue syndrome (13% vs 1%, p = 0.004), and fibromyalgia (18% vs 2%, p = 0.001) compared to controls. Of note, non-steroidal anti-inflammatory drug (NSAID) and naltrexone use were significantly more common in GWI vs controls (60% vs 42%, p = 0.036 and 15% vs. 0%, p < 0.001, respectively).

**Frequency of eye diseases in the populations.** Overall, individuals with GWI had a similar frequency of any eye disease, 73% vs 61%, p = 0.13. DE symptoms were significantly more common in GWI compared to controls, 50% vs 33%, p = 0.04. The GWI group tended to have higher frequencies of diabetic retinopathy (7% vs 4%, p = 0.45), and dry ARMD (3% vs 0%, p = 0.17), compared to controls, but the results were not significant with low frequencies in both groups. Compared to controls, GWI veterans with "severely impaired cognition" had significantly higher frequencies of both DE symptoms (61% vs 33%, p = 0.009) and signs (39% vs 19%, p = 0.028).

**Optical coherence tomography as a potential biomarker of Gulf War Illness.** Of the 94 individuals with available OCT images, 66 veterans (28 GWI and 38 controls) had no known optic nerve or retinal disease. Although not significant, almost all mean RNFL measurements were thinner in GWI compared to controls, with the largest difference seen in the inferior RNFL (109.33 μm ± 26.20 vs 117.00 μm ± 24.29,

| | GWI (n = 60) | Control (n = 85) | P-value |
|---|---|---|---|
| **Demographics** | | | |
| Age (years) | 52.1 ± 4.78 (45–71) | 52.7 ± 3.84 (46–60) | 0.39 |
| Male gender | 85% (51) | 82% (70) | 0.67 |
| White race | 41% (25) | 37% (32) | 0.81[a] |
| Hispanic ethnicity | 26%(16) | 20% (17) | 0.42 |
| **Non-ocular comorbidities** | | | |
| Diabetes | 33% (20) | 24% (21) | 0.26 |
| Hypertension | 43% (26) | 50% (43) | 0.39 |
| Hypercholesterolemia | 51%(31) | 49% (42) | 0.92[a] |
| PTSD | 45% (27) | 20% (17) | 0.001* |
| Depression | 38% (23) | 37% (32) | 0.93 |
| Arthritis | 20% (12) | 8% (7) | 0.039* |
| Sleep apnea | 56% (34) | 47% (40) | 0.25 |
| Chronic fatigue syndrome | 13% (8) | 1% (1) | 0.004[a]* |
| Fibromyalgia | 18% (11) | 2% (2) | 0.001* |
| **Ocular comorbidities** | | | |
| Dry eye** | 50% (30) | 34% (29) | 0.06 |
| Symptoms | 50% (30) | 33% (28) | 0.039* |
| Signs | 23% (14) | 18% (16) | 0.51 |
| Ocular hypertension | 5% (3) | 10% (9) | 0.36[a] |
| Glaucoma | 15% (9) | 17% (15) | 0.67 |
| Cataract | 11% (7) | 7% (6) | 0.34 |
| Diabetic retinopathy | 7% (4) | 4% (3) | 0.45[a] |
| Dry ARMD | 3% (2) | 0% (0) | 0.17[a] |
| Wet ARMD | 0% (0) | 0% (0) | |
| Retinal hemorrhage | 1% (1) | 1% (1) | 1.00[a] |
| Vitreous degeneration | 3% (2) | 4% (4) | 1.00[a] |
| Keratoconus | 5% (3) | 2% (2) | 0.65[a] |
| Any eye disease | 73% (44) | 61% (52) | 0.13 |

**Table 1.** Demographic and comorbidities of the study population. Continuous variables are expressed as mean ± standard deviation (minimum–maximum). Categorical variables are expressed as percent (n). Mann–Whitney U test was used for all continuous variables. Pearson Chi Square was used for all categorical variables unless otherwise noted. *GWI* Gulf War Illness, *Control* Individuals who served in 1990–91 who do not meet criteria for GWI, *ARMD* age-related macular degeneration, *SD* standard deviation, *n* number in group, *PTSD* post-traumatic stress disorder. *Statistically significant difference at a p-value < 0.05 between GWI and control. **Symptoms or signs. [a]Fisher's Exact Test.

p = 0.13, a 6.6% decrease) (Supplementary Table S1 and Fig. 2). Similarly, all mean macular OCT measurements were thinner in veterans with GWI vs controls, with the largest decrease in the superior outer segment of the macula (271.00 μm ± 14.03 vs 277.45 μm ± 14.20, p = 0.12, a 2.32% decrease). Interestingly, almost all mean GCL parameters were thicker in the GWI group, with the largest increase in the inferotemporal GCL segment (78.65 μm ± 9.03 vs 77.29 μm ± 11.03, p = 0.245, a 1.75% increase). All other OCT measurements for GWI and controls are shown in Supplementary Table S1.

Further sub-grouping the population by severity of cognitive deficit, OCT data were available for 20 GWI veterans with "severely impaired cognition," 7 GWI veterans without "severely impaired cognition," and 38 controls. Of note, one individual with GWI was not included in this sub-analysis as questionnaire sub-score data was not available. Since OCT data for these groups were non-parametric, the Kruskal–Wallis H test was used to assess statistical differences between groups. GWI veterans with "severely impaired cognition" had significantly thinner inferior GCL (72.5 μm ± 12.0 vs 82.66 μm ± 2.74, p = 0.004, a 12% decrease) and inferotemporal GCL (76.8 μm ± 9.4 vs 85.0 μm ± 3.22, p = 0.011, a 9.7% decrease) compared to GWI veterans without the syndrome. The findings were similarly pronounced when GWI veterans with "severely impaired cognition" syndrome were compared to controls without GWI. Interestingly, GWI veterans without "severely impaired cognition" had significantly thicker values in inferior (82.67 μm ± 2.73 vs 76.24 μm ± 9.66, p = 0.015, a 8.42% increase) and inferotemporal GCL (85.00 μm ± 3.22 vs 77.30 μm ± 11.03, p = 0.006, a 9.97% increase) compared to controls without GWI.

**Predictors of Gulf War Illness.** To determine if specific demographics and OCT parameters could predict a diagnosis of GWI, we used all veterans with available OCT data to perform forward stepwise binary logistic regression with GWI (yes/no) as the dependent variable. Beyond OCT measures mentioned in the methods sec-

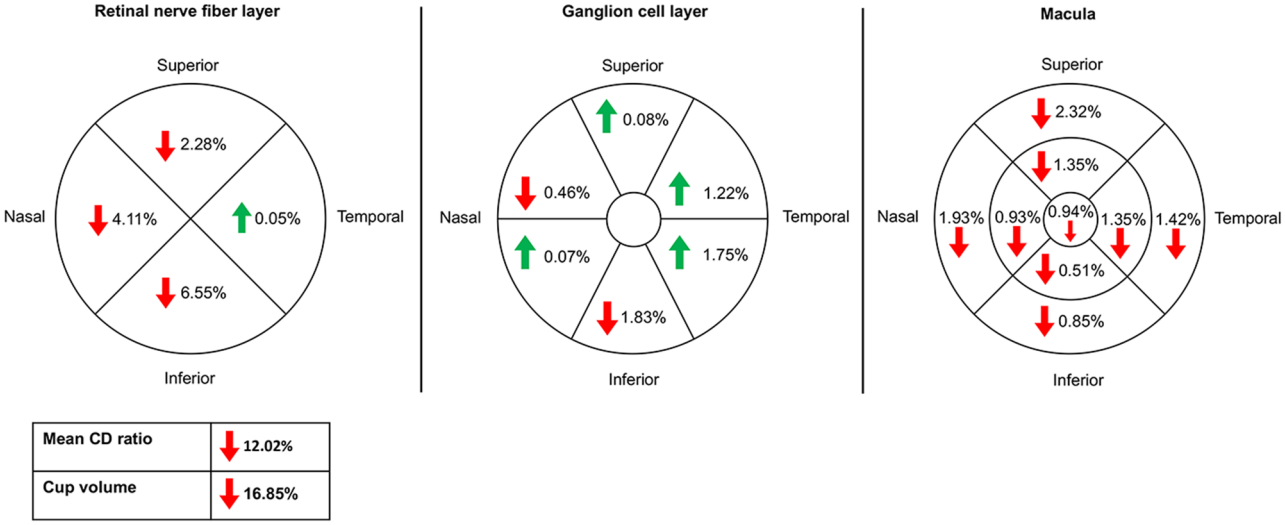


**Figure 2.** Percent change in optical coherence tomography measurements in Gulf War Illness. Percent change in optical coherence tomography (OCT) measurements Gulf War Illness (GWI) compared to controls. RNFL: n = 27 GWI and 36 controls. GCL: n = 26 GWI and 37 controls. Macula: n = 27 GWI and 38 controls. indicates a decreased percent change for GWI compared to controls. indicates an increased percent change. CD = cup-to-disc; OCT = optical coherence tomography. Figure was created using Microsoft PowerPoint for Mac (version 16.16.15, https://www.microsoft.com/en-us/microsoft-365/powerpoint).

| Predictor | β | S.E | Wald statistic | P value | OR | OR 95% CI |
|---|---|---|---|---|---|---|
| Age | − 0.20 | 0.08 | 6.03 | 0.014 | 0.82 | 0.70–0.96 |
| PTSD | 3.02 | 0.81 | 13.87 | < 0.001 | 20.51 | 4.2–100.5 |
| Mean RNFL thickness | − 0.05 | 0.03 | 4.03 | 0.045 | 0.95 | 0.90–0.999 |
| Mean CD ratio | − 5.22 | 1.85 | 7.97 | 0.005 | 0.005 | 0.0–0.20 |

**Table 2.** Results of forward stepwise binary logistic regression analysis for predictors of Gulf War Illness. *PTSD* post-traumatic stress disorder, *RNFL* retinal nerve fiber layer, *CD* cup-to-disc, *OR* odds ratio, *CI* confidence interval, *S.E.* standard error for β.

tion, other metrics included as independent variables were demographics (age, gender, race, ethnicity), co-morbidities (PTSD and arthritis), NSAID use, and eye diseases (DE signs or symptoms, dry ARMD, diabetic retinopathy, ocular hypertension, retinal hemorrhage, and glaucoma). Fibromyalgia and chronic fatigue syndrome were not included in the model since their symptoms overlap with GWI. Naltrexone use was also excluded as it perfectly separated GWI from controls. Of note, population based differences in the 94 individuals included in the prediction analysis mirrored that of the entire population (n = 145), with individuals with GWI having a lower mean age, a higher frequency of PTSD and dry eye symptoms, and a trend toward thinner RNFL and macular thicknesses on OCT compared to controls. After confirming non-collinearity between predictors, the final model (Table 2) included age (odds ratio; OR = 0.82, 95% confidence interval; CI 0.70–0.96), PTSD (OR = 20.5, CI 95% 4.2–100.5), average RNFL thickness (OR = 0.95, CI 0.90–0.999), and average CD ratio (OR = 0.005, CI: 0.0–0.20). ROC analysis demonstrated an area under the curve (AUC) of 0.80 (95% CI 0.71–0.90, p < 0.001; Fig. 3) for this model in predicting a GWI diagnosis. The best cut-off value for the prediction model, as determined by Youden's index (top left point on the ROC curve), was associated with a sensitivity of 76% and 60%. When excluding average RNFL thickness from the model, its predictive ability decreased (AUC = 0.68, 95% CI 0.59–0.77, p < 0.001).

## Discussion

In this study, we did not detect significant differences in the overall frequency of age-related eye diseases in individuals with GWI. However, dry eye symptoms were significantly more common in GWI compared to controls, which aligns with other diseases that have similar symptomology, such as fibromyalgia and chronic fatigue syndrome[5,6]. There are many potential contributors to the noted association, including systemic inflammatory processes that lead to ocular surface inflammation and peripheral and/or central nerve abnormalities that lead to persistent symptoms of dryness[19,20].

Given the need for GWI biomarkers, we also compared retinal and optic nerve measures with OCT imaging. We found trends for macular OCT and RNFL thinning but GCL thickening in cases compared to controls. These differences became significant when GWI individuals with "severely impaired cognition" were compared

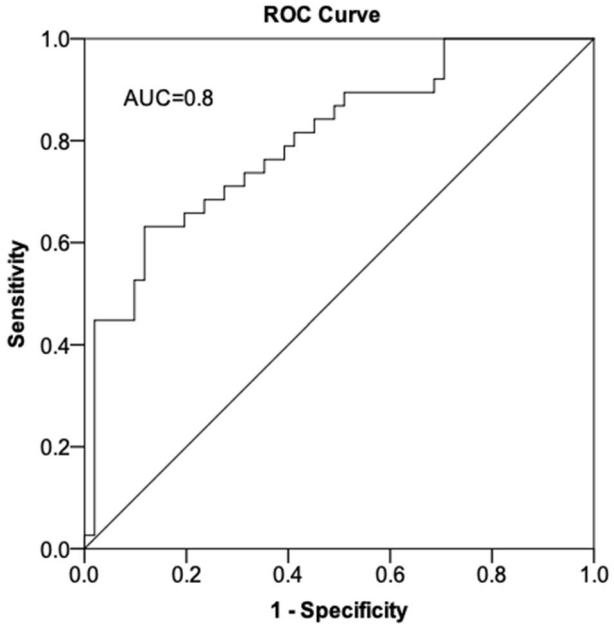


**Figure 3.** Receiver operating characteristic curve for predictors of Gulf War Illness. The curve is for a model using age, diagnosis of post-traumatic stress disorder (PTSD), overall retinal nerve fiber layer (RNFL) thickness, and cup-to-disc ratio as predictors. Forward stepwise binary logistic regression was used to develop the model. Receiver operating characteristic was used to test the ability of the model to predict a GWI diagnosis (yes/no). ROC = receiver operating characteristic. Figure was created using SPSS (version 24.0, https://www.ibm.com/products/spss-statistics).

to both individuals with GWI but without "severely impaired cognition" and controls. These data highlight the heterogeneous nature of GWI and suggest that different disease processes likely drive the clinical heterogeneity. Diagnostics tests are thus needed to detect disease but also to sub-type based on underlying pathophysiological mechanisms.

Similar to GWI, RNFL thinning has also been described in individuals with PD as compared to controls, with the temporal and nasal regions most affected[21]. In fact, the magnitude of inferior RNFL reduction in our GWI cohort (6.6%) is in the range of what has been reported in PD (6.2–15%[21]). This becomes relevant as studies have found other similarities between GWI and PD. In an MRI study of 293 Gulf War veterans compared to healthy controls, individuals with GWI had significantly more PD-like symptoms and reduced basal ganglia volumes (a common radiological feature of PD[22])[23]. RNFL thinning has also been described in AD, with an overall mean reduction of 6.8–40% as compared to controls. Interestingly, the superior and temporal regions were most significantly affected in AD[21], as compared to the nasal and inferior regions in GWI. OCT findings have also been reported in MS, with overall mean reductions in RNFL thickness of 7.2% and temporal reductions of 23% as compared to controls[24]. While the magnitude of overall RNFL reduction in GWI is smaller as compared to studies in PD, AD, and MS, these data highlight RNFL thinning as a marker of neurodegeneration with GWI showing similar trends.

Interestingly, in contrast to RNFL thinning, GCL values were thicker in GWI as compared to controls. This is the opposite of what has been described in other neurodegenerative diseases[21,25]. We hypothesize that the discrepancy between GCL findings in GWI as compared to other neurodegenerative disorders are driven by competing mechanisms in GWI. While RNFL thinning can be an indicator of neurodegeneration, GCL thickening may indicate inflammation, with secondary edema, glial cell infiltration, and vascular changes[26]. Both processes have been implicated in GWI. A prospective MRI study found significant reductions in brainstem, cerebellar, and thalamus volumes in 17 GWI veterans compared to 23 controls, aligning with a neurodegenerative process[27]. On the other hand, neuroinflammation has also been described in GWI in the form of autoantibodies to neural and glial cell tissue, including calmodulin kinase II (CaMKII) and neurofilament triplet proteins (NFP), which are also found on retinal ganglion cells (RGC)[28–30]. Linking neurodegeneration and inflammation in GWI, one study found that increased serum concentrations of the inflammatory marker, soluble receptor II for tumor necrosis factor was significantly associated with reduced hippocampal volume in GWI veterans[31]. Thus, it is possible that individuals with diffuse inflammation on top of neurodegeneration may have RNFL thinning but GCL thickening while those with a more prominent neurodegeneration component have thinning in both layers[32,33]. However, longitudinal studies are needed to evaluate our hypotheses.

In our final analysis, we explored whether specific OCT parameters could help discriminate between GWI and controls. We found that RNFL thickness, in conjunction with other parameters, predicted 80% of the variability in GWI risk. Similar regression analysis using minimum RNFL thickness and age predicted brain atrophy in patients with MS[34]. Other OCT parameters were also predictive of a GWI diagnosis including decreased average

cup-to-disc (CD) ratio. This finding may be driven by mechanisms related to GWI, such as toxic or inflammatory changes, or may be due to an unrelated confounder, such as a higher frequency of individuals with physiologic cupping in the non-GWI group. Younger age also remained in the model as a predictor of GWI. This may simply reflect that of the population of individuals who were in service in 1990–1991, younger individuals were more likely to be deployed (a requirement for receiving a GWI diagnosis) than older individuals. Alternatively, younger age may be an unexplained contributor to GWI risk. Nevertheless, our data suggest that OCT has the potential to detect GWI and perhaps monitor disease progression. This is needed as GWI is a disease significant with morbidity and no disease-modifying therapies. Methods are thus needed to detect GWI early, identify GWI sub-types, and monitor for disease progression. Similar approaches have been investigated in other neuroinflammatory diseases, such as MS. In one study, OCT detected MS in the early stages of disease[35], leading to early treatment which improved disease severity and morbidity[36].

Our findings must be considered in light of the study limitations which included a retrospective design in a defined study population with a fixed sample size. As such, assessment of eye diseases was not performed in a standard method by individual clinicians. Furthermore, we used both phone and clinic-based interviews to define GWI. However, we used one of the two questionnaires (Kansas criteria) recommended by the Institute of Medicine and the United States Department of Defense in both settings[37]. Balancing the limitations are the strengths of this study which include the only study to our knowledge to evaluate ocular manifestations and non-invasive ocular biomarkers of GWI. Additional studies are thus needed in independent cohorts to replicate our findings and examine change over time, as has been done for other neurodegenerative diseases[38]. In fact, our plan is to further explore and validate the predictive markers discussed in this manuscript in a novel population. Despite these limitations, our findings open the possibility of studying OCT as biomarkers of GWI, which is greatly needed as GWI is a disease with high morbidity but with no therapeutic interventions.

## Data availability

The datasets generated during and/or analyzed during the current study are not publicly available due to lack of permission from Veterans Health Administration to share data.

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## Author contributions

All authors substantially contributed to this manuscript. Data collection: B.S.B., K.Z., and A.G. Data analysis: B.S.B., A.G., and R.G. Drafting manuscript: B.S.B., K.Z., A.G., and R.G. Critical revision of manuscript: B.S.B., K.Z., R.G., E.F., N.K. and A.G.

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## Competing interests

The authors declare no competing interests.

## Additional information

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REVIEW

# Exploring the Link Between Dry Eye and Migraine: From Eye to Brain

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*Eye and Brain*

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**Abstract:** Dry eye and migraine are common diseases with large societal and economic burdens that have recently been associated in the literature. This review outlines the link between dry eye and migraine, which may have implications for reducing their respective burdens. We highlight possible shared pathophysiology, including peripheral and central sensitization, as the potential link between dry eye and migraine. Finally, therapies targeting similar pathophysiological mechanisms between dry eye and migraine are discussed.

**Keywords:** dry eye, migraine, brain, sensitization

## Introduction

Awareness of dry eye has increased in recent years including its association with specific diseases, such as migraine headaches. However, our understanding of the link between dry eye and migraine is contingent on what is currently known about them as separate diseases. Specifically, dry eye and migraine are both highly prevalent in the population. The prevalence of dry eye ranges from 5% to 50% in the worldwide population, depending on disease definition and population studied, with an overall estimated societal economic burden of $55.4 billion in the United States.[1] As with dry eye, the prevalence of migraine headache is also high. In western countries, the lifetime prevalence of migraine is up to 9.5% in males and 25% in females.[2] The societal economic burden of migraine in the United States is estimated at $36 billion.[3] Thus, migraine headaches and dry eye are important health concerns, and their association warrants further exploration. Understanding shared connections between the two diseases may provide insight into shared pathophysiology and treatments, with a potential decrease in disease morbidity.

To understand the link between dry eye and migraine, we must first define them as separate diseases. Dry eye is defined by the Tear Film and Ocular Surface Society Dry Eye Workshop II as

> a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.[4]

The symptoms of dry eye are variable and can include sensations of “dryness”, “grittiness”, “burning” and “stinging”, to name a few.[5] Individuals may also report that these sensations are spontaneous and/or evoked by wind or light.[5] Others complain of visual phenomena, such as blurry or fluctuating vision.[6] Dry eye

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symptoms are commonly assessed in the clinic with specific questionnaires, such as Dry Eye Questionnaire-5 (DEQ-5; range 0–22)[7] and Ocular Surface Disease Index (OSDI; range, 0–100),[8] which incorporate many of the above complaints. The DEQ-5 focuses on intensity and frequency of dryness and discomfort, along with tearing, while the OSDI considers spontaneous and evoked pain, visual complaints, and impact on daily activities. These questionnaires lump responses and generate severity scores, with DEQ-5 scores ≥6 considered indicative of any dry eye symptoms[7] and scores ≥12 considered severe symptoms. OSDI scores are interpreted as normal=0–12, mild=12–32, and severe=33–100.[9] Of note, these severity scales incorporate a number of different aspects of dry eye to reach a final score, including pain, visual complaints, tearing, and activity limitations. Given that specific symptoms may be driven by different contributors, other questionnaires have been developed to specifically assess for ocular pain complaints, including the Ocular Pain Assessment Survey (OPAS), a 28-question survey, that focuses on intensity of eye pain, non-eye related pain, and aggravating factors,[10] and the Neuropathic Pain Symptom Inventory-Eye (NPSI-Eye; range, 0–100), which focuses on neuropathic pain features, inquiring about descriptors such as burning pain and evoked pain to wind and light.[11]

In addition to symptoms, clinical signs are also included under the purview of dry eye. The tear film is composed of 2 layers, a thicker muco-aqueous layer that interacts with the corneal epithelium, and a thinner lipid layer that sits on top of the muco-aqueous layer and inhibits its evaporation.[12] Broadly speaking, dry eye is sub-grouped into categories by dysfunction in these two layers, that is aqueous tear deficient and evaporative dry eye.[13] Signs of aqueous deficiency include decreased tear volume, assessed by examining the tear meniscus under the slit lamp examination or with the Phenol Red Thread (PRT) test, and reduced tear production, assessed with Schirmer strips (strips of paper placed in the corner of the eye and left in place for 5 minutes, mm of wetting recorded). The main sign of evaporative deficiency is a rapid tear break up time (TBUT, measured in seconds until a black spot appears in the tear film), which can occur with a dysfunctional lipid layer. However, any tear abnormality, including aqueous deficiency, can result in a rapid TBUT. Furthermore, the sub-types co-exist and individuals may present with both aqueous and evaporative deficiency. Punctate epithelial erosions, which are small disruptions in the corneal epithelium visualized with vital dyes such as sodium fluorescein, rose bengal, or lissamine green, can be seen in both dry eye sub-types and with other ocular surface abnormalities (eg anatomic abnormalities of the eyelid, conjunctivae, or cornea).

The lipid layer is produced by the Meibomian glands (MG) in the upper and lower eyelids. Eyelid abnormalities such as plugging of the MG orifices, MG atrophy, and production of a thicker than normal lipid product (eg abnormal meibum quality) can accompany signs of tear dysfunction.[14] Point of care tests have also been developed to assess tear composition and inflammation and can specifically evaluate tear osmolarity (TearLab, San Diego)[15] and ocular surface inflammation (matrix metalloproteinase-9, Inflammadry, Quidel Corporation, San Diego)[16] in the clinical setting. Some individuals with clinical tear film abnormalities will have high or unstable tear osmolarity levels and/or detectable inflammation on their ocular surface.

A challenge in evaluating dry eye is that the symptoms and signs of disease are often disparate.[17,18] The presenting symptoms of dry eye can vary even in the same individual and are frequently discordant from the clinical signs and their severity, which can make the diagnosis and management of dry eye difficult. For example, a systematic review of 33 studies assessing associations between dry eye symptoms and signs found that out of 175 individual symptom-sign analyses, only 42 (24%) were significantly correlated with one another. This study also found that the majority (129/148; 87%) of individual analyses reporting correlation coefficients were in the low-to-moderate range (−0.4 to 0.4).[17] In addition, the lack of a single objective test with which to evaluate dry eye signs and the low repeatability of tests (eg Schirmer) contributes to the complexity of the disorder.

One of the reasons it is important to screen for dry eye is that dry eye symptoms have a negative impact on individuals' lives as they decrease the ability to work and carry out activities of daily living.[19] For example, a study recruited 56 individuals with a dry eye diagnosis (International Classification of Diseases, Ninth Revision, ICD-9, codes) for assessment of ophthalmic and quality of life parameters. This study found that individuals with severe dry eye disease (composite score of symptoms [9-level subjective facial expression scale] and signs [Schirmer and corneal surface staining]) had quality of life scores (measured by the time trade-off method) in the range of severe (class III/IV) angina (mean utility

score, range 0 to 1, lower values indicate worse quality of life: 0.72 for severe dry eye disease and 0.71 for class III/IV angina).[20] In addition, dry eye symptoms have a negative impact on mental health and several studies have linked depression and anxiety to dry eye.[21,22] Finally, individuals with dry eye have sleep abnormalities. For example, a meta-analysis of 17 studies found that individuals with dry eye symptoms or disease (diagnosed using varying criteria across studies) or primary Sjogren's syndrome had worse sleep quality scores (using Pittsburgh Sleep Quality Index) compared to controls (weighted mean difference=1.69, 95% confidence interval (CI): 0.82–2.56).[23] Taken together, dry eye is a debilitating disease with profound impacts on social functioning and perception of life quality.

Similar to dry eye, migraine is a prevalent condition in the general population.[2] The International Headache Society (IHS) defines migraine as a "recurrent headache disorder manifesting in attacks lasting 4–72 hours".[24] Migraine headaches are characterized by unilateral location and pulsating quality and can include nausea, photophobia, and/or phonophobia. Migraine attacks are classified into those with or without aura. Migraine with aura involves reversible prodromal symptoms, such as visual, sensory, or other central nervous system disturbance lasting a few minutes.[24] Migraine can also be separated into chronic and episodic. Chronic migraine is characterized as occurring ≥15 days per month for three months, which, on at least eight days per month, has features of migraine, while episodic migraine occurs less than 15 days per month.[24]

As with dry eye, migraine symptoms can be debilitating and decrease quality of life.[25] An observational study of 102 individuals with migraine found disability and health-related quality of life scores were significantly lower than the general population.[26] Similarly, a retrospective cross-sectional survey study of 80,600 European patients found lower health-related quality of life and decreased work productivity among those with ≥4 monthly migraine headaches compared to non-migraine controls.[27] Interestingly, lower quality of life scores among those with migraine closely associate with dry eye symptoms. In a cross-sectional survey-based study of 62 individuals with migraine, visual function (measured via visual functioning questionnaire-25) and overall quality of life (measured via headache impact test-6) correlated with dry eye symptoms (measured via OSDI score).[28] Together, these data show that both dry eye, migraine, and perhaps their interaction, have significant negative impacts on patient quality of life. Thus, in this review, we explore the association between dry eye and migraine with the goal of illuminating overlapping pathophysiology and potential therapies. To do so, we reviewed recent studies that investigated the relationship between dry eye and migraine.

## Methods

A PubMed search was conducted using the terms "dry eye" AND "migraine". All published scientific articles were considered including original research, meta-analyses, and systematic reviews. All searches were limited to the English language. Eligible articles were reviewed and summarized.

## Clinical Associations Between Dry Eye and Migraine

### Epidemiology of Dry Eye, Migraine, and Their Co-Existence

Dry eye and migraine are co-morbid. Using survey data from a Korean population-based cross-sectional study of 14,329 participants, the prevalence of migraine and dry eye diagnosis was found to be similar among participants: 24.2% reported migraine headaches (positive answer to "Do you have, or have you ever experienced migraine [pulsatile pain unilaterally in your head]?"), 22.6% reported a dry eye diagnosis (positive answer to "Have you ever been diagnosed with dry eye by an ophthalmologist?"), and 37.1% reported dry eye symptoms (positive answer to "Do your eyes tend to be dry, with a foreign body sensation including itching and burning or sandy feeling lately?").[29] Furthermore, the frequency of dry eye diagnosis was found to be higher in those with migraine. Of those with migraine, 14.4% reported a dry eye diagnosis compared to 8.2% without migraine, $p<0.0001$. Similarly, of those with migraine, 22% reported dry eye symptoms compared to 15.1% without migraine, $p<0.0001$.[29] While limitations of this study included the use of data assessed via questionnaires, other studies have reported similar results. In a hospital-based case–control study of 72,969 individuals from University of North Carolina-affiliated hospitals, individuals with migraine and dry eye were identified using International Classification of Diseases, Ninth and Tenth Revision (ICD-9 and ICD-10) codes. The prevalence of a migraine or dry eye diagnosis was 7.3% and 13.2%, respectively. Again, individuals with migraine had a higher frequency of a co-morbid dry eye

diagnosis. Of those with migraine, 19.6% had a dry eye diagnosis compared to 12.7% without migraine.[30]

Looking at the data as odds ratios (OR), in the population-based questionnaire study of 14,329 Korean individuals, after adjusting for confounders, the presence of migraine was found to increase the odds of a dry eye diagnosis 1.58 fold (95% confidence interval (CI) 1.34–1.86) and the odds of dry eye symptoms 1.3 fold (95% CI, 1.15–1.50).[29] In the study of 72,969 individuals from North Carolina, the presence of migraine increased the odds of a dry eye diagnosis 1.42 fold (95% CI, 1.20–1.68). The association was strongest among women ≥65 years old (OR, 2.47; 95% CI, 1.75–3.47).[30]

Other studies have investigated the reverse relationship, that is the odds of migraine in individuals with dry eye. In a large Taiwanese study using ICD-9 codes (n=48,028), the presence of a dry eye diagnosis increased the odds of a migraine diagnosis 1.76 fold (95% CI, 1.57–1.98), after adjusting for co-morbidities.[31] While these studies suggest a reciprocal relationship between dry eye and migraine, they are limited by their retrospective or cross-sectional nature and reliance on ICD coding and questionnaires for migraine and dry eye diagnosis. This is especially relevant as dry eye is a heterogeneous disease and it is unclear which combination of symptoms and/or signs led to the coded diagnosis. Overall, these studies suggest that dry eye and migraine are common conditions and that individuals with migraine are more likely to have dry eye symptoms and carry a dry eye diagnosis compared to those without. However, a limitation of the studies is that they did not look at dry eye signs and as such, it is difficult to understand what component of dry eye is most closely related to migraine.

## Dry Eye Characteristics Among Individuals with Migraine

To further explore relationships between dry eye and migraine, several smaller studies investigated associations between migraine and dry eye symptoms and signs. In a cross-sectional study of South Florida veterans seen in a dry eye clinic, 31 individuals with migraine (defined via the American Migraine Study/American Migraine Prevalence and Prevention (AMS/AMPP) migraine diagnostic module)[32] were compared to 219 individuals without migraine. Migraineurs had significantly higher dry eye symptom scores (via OSDI) but similar tear metrics (TBUT, corneal staining, tear production) compared to controls.[33] Interestingly, NSPI-Eye scores, assessing for neuropathic features of eye pain, were also higher among individuals with migraine compared to controls. These data suggest dry eye symptoms, but not dry eye signs, are related to migraine. This conclusion is supported by other studies, as well. One observational study of Turkish individuals seen in a dry eye clinic compared 33 individuals with migraine to 33 controls. Migraine was diagnosed by different neurologists. Dry eye symptoms were assessed using OSDI and dry eye signs using TBUT, corneal staining, and Schirmer test. Migraineurs had significantly higher dry eye symptoms, lower TBUT, and Schirmer scores, and higher corneal staining compared to controls.[34] However, Schirmer scores were within normal limits in both groups (mean >10 mm/5 min) and thus the clinical relevance of the differences in values is unclear. Similar findings were reported in another study of 46 Turkish patients with migraine and 50 controls that were assessed for Sjogren's Syndrome, dry eye symptoms (via OSDI), and dry eye signs (TBUT, Schirmer) in a rheumatology clinic.[35] Migraine was diagnosed by the referring neurologist. In this study, individuals with migraine had significantly higher dry eye symptoms and lower TBUT and Schirmer scores compared to controls, however again, Schirmer results were still within normal limits (mean >10 mm/5 min). Another case–control study performed in a United States ophthalmology clinic assessed dry eye symptoms and signs and corneal nerve parameters in 19 individuals with chronic migraine. This study used 30 controls from a normative dataset for corneal nerve comparisons, but no control data were included for dry eye parameters. Chronic migraine was defined by the International Headache Society guidelines. Dry eye symptoms via measured DEQ-5 were abnormal in all subjects (DEQ-5 >6), but tear parameters were within normal limits among all individuals with chronic migraine (data not reported).[36] Interestingly, corneal nerve fiber density was significantly lower in individuals with migraine compared to controls (48 ± 23 vs 71 ± 15 fibers/mm$^2$). However, given the lack of standard nomograms for corneal nerve fiber density, the interpretation of this finding is uncertain. Together, these studies point to dry eye symptoms being more closely related to migraine than dry eye signs.

## Migraine Characteristics Among Individuals with Dry Eye

As above, while some studies evaluated dry eye characteristics in individuals with migraine, other studies evaluated

whether specific migraine characteristics were more closely associated with dry eye. A Turkish study that evaluated 58 individuals with migraine reported that the odds of having dry eye (defined if 2 of 3 criteria met: OSDI >33, TBUT <10 seconds or Schirmer <10 mm/5 min) were 5.03 times higher in those with migraine and aura compared to those without aura (95% CI, 1.42–17.83).[37] These data suggest that migraine with aura is more closely associated with aspects of dry eye than migraine without aura.

In addition to aura, the lifetime duration of migraine has also been explored in its relationship to dry eye. In the above Turkish study, individuals with a dry eye diagnosis had a longer median lifetime duration of migraine compared to those without a diagnosis (10 vs 6 years, p=0.01).[37] Similarly, another Turkish study of 46 individuals with migraine (diagnosed by a neurologist) found that migraine lifetime duration correlated with both dry eye symptom severity (OSDI score) (r=0.3, p=0.01), tear stability (TBUT: r= −0.23, p=0.05), and tear production (Schirmer: r= −0.28, p=0.01). Of note, the negative correlations imply that longer duration of migraine associated with faster break-up time and lower tear production.[35] Taken together, these studies suggest that migraine with aura and longer disease duration are associated with aspects of dry eye. However, it is important to note that definitions of dry eye were not uniform among studies, and migraine criteria were not always clearly outlined.

## Photophobia is a Feature of Both Dry Eye and Migraine

Thus far, we have discussed associations between dry eye and migraine. However, the diseases also share a common feature, that is, the presence of photophobia. Although photophobia is variably defined in the literature, in this review, photophobia refers to light-induced neurological symptoms, which usually emerge in the form of (i) increased sensitivity to light or glare, (ii) intensification of headache and (iii) ocular pain or discomfort.[38] With regards to dry eye, our group reported that 75% of 236 veterans with dry eye symptoms (DEQ-5 score ≥6) reported pain sensitivity to light (defined as score ≥1 on a 0–10 numerical rating scale (NRS)).[39] In another study, we found that of 102 South Florida veterans, individuals with persistent dry eye symptoms (DEQ-5 score ≥6 over a 2-year period) were more likely to report photophobia compared to those without persistent symptoms (OR, 15.6; 95% CI, 2.0 to 123, p=0.009).[40] Our data suggest that photophobia is a common feature in individuals with dry eye symptoms, and in fact, presence and severity of photophobia is the first question on the OSDI.

Photophobia is also a common feature in migraine. In a cross-sectional survey of 6045 respondents in the Migraine in America: Symptoms and Treatment Study, 49.1% reported photophobia as the 'most bothersome symptom.[41] In a retrospective cross-sectional study of 117 individuals with chronic migraine (≥15 headache days/month), 80% rated their photophobia (via 0–10 NRS) as severe (a score of ≥7/10; mean 7.91 ± 2.05).[42] Together, the data demonstrate that photophobia is a feature of both dry eye and migraine. The presence of photophobia in both diseases has implications for shared pathophysiology and treatments as discussed later in the review.

## Neural Pathways Mediating Photophobia

Studies have explored the neural circuitry underlying photophobia, both in the context of dry eye[43] and migraine.[44] One pathway involves light-evoked signals in rod and cone cells that are transmitted to retinal ganglion cells (RGC) via amacrine and bipolar cells. Some signals in RGCs are transmitted to the olivary pretectal nucleus (OPN), then to the superior salivatory nucleus, and subsequently to the sphenopalatine ganglion, which stimulates parasympathetic-mediated vasodilation of ocular[45] and dural[38] vessels that are innervated by trigeminal afferents. Trigeminal signals subsequently travel to the trigeminal nucleus caudalis, posterior thalamus, and cortical structures (Figure 1).[38] Evidence for this pathway comes from immunocytochemistry experiments in rats that demonstrated light-evoked neuronal activity in the trigeminal brainstem, which was reduced after intravitreal injection of norepinephrine. These data suggest that constriction of ocular blood vessels by norepinephrine plays a role in light-evoked neuronal activity, thus implicating ocular vasculature in the trigeminal brainstem pathway of photophobia.[45] A mouse study similarly found a trend for reduced blue-light aversion behavior (measured by amount of time mice spent in the illuminated portion of a box) after intravitreal injection of norepinephrine, but the reduction did not reach statistical significance.[46]

A second neural pathway involves light-sensitive neurons in the posterior thalamus, specifically the lateral

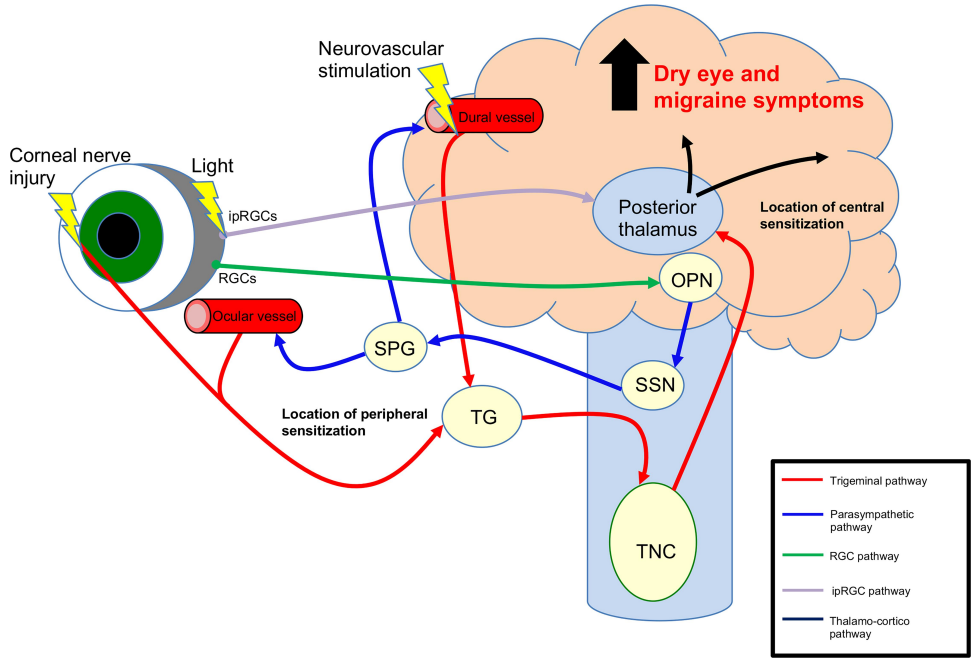


**Figure 1** Selected photophobia neural pathways in dry eye and migraine. Light evokes signals from rod and cone cells that are transmitted via amacrine and bipolar cells (not shown) to retinal ganglion cells (RGC), which project to the olivary pretectal nucleus (OPN, green line). Blue line: parasympathetic signals travel from the OPN to the superior salivatory nucleus (SSN), then to the sphenopalatine ganglion (SPG), and ocular and dural vessels to mediate vasodilation. Red line: afferent trigeminal signals from cornea (stimulated by corneal disruptions), ocular vessels, and dural vessels (stimulated by vasodilation) travel to the trigeminal ganglion (TG) then to the trigeminal nucleus caudalis (TNC) and finally the posterior thalamus. Alternatively, light-evoked signals from intrinsically photosensitive RGCs (ipRGC) travel directly to the posterior thalamus (purple line). Black line: signals from the posterior thalamus travel to somatosensory and visual cortices to mediate dry eye and migraine symptoms. Note other pathways of photophobia that involve the hypothalamus and retinal rod and cone cells are not depicted.

posterior (LP) and posterior nuclei (PO),[38] which receives input from both intrinsically photosensitive RGCs (ipRGC) and dural trigeminal afferents, and subsequently send signals to somatosensory and visual cortices (Figure 1).[38,47] Evidence for this pathway comes from a rat study using electrophysiologic and histopathologic techniques which demonstrated that cell bodies and dendrites of dura- and light-sensitive neurons in the posterior thalamus were in close apposition to axons originating from ipRGCs.[48] Other studies have further connected the posterior thalamic nuclei to photophobia. A mouse model found that stimulation of posterior thalamic nuclei (LP and PO nuclei) by optogenetics or injection of calcitonin gene-related peptide (CGRP)[49] triggered light aversive behavior.[50] Beyond these two pathways, other postulated, but less well studied pathways in photophobia involve the hypothalamus, retinal rod and cone cells, and the iris.[47,51,52]

## Dry Eye and Migraine Share Underlying Pathophysiology

The clinical overlap between dry eye symptoms and migraine, including the presence of photophobia, suggests pathophysiological links between them. One unifying theory is that dry eye symptoms and migraine involve abnormal peripheral trigeminal nerve activation with subsequent peripheral and central sensitization. Peripheral sensitization is defined as "increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields"[53] and below we focus on corneal peripheral nerve abnormalities that have been described in dry eye and migraine. Central sensitization is defined as "increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input"[53] and below we focus on changes in secondary and tertiary nerves that connect corneal afferents to higher cortical areas.

## Tests Used to Evaluate Nerve Abnormalities in Dry Eye and Migraine in Animal Models and Humans

In animals, peripheral nerve function is often evaluated via electrophysiological recordings of polymodal (~70%), mechano- (10–15%), and cold thermoreceptors (10–15%) within corneal nerves or via recordings in ciliary nerves.[54–56] Polymodal nociceptors respond to mechanical force, heat, chemical irritants and inflammatory mediators,[57] mechanoreceptors to mechanical forces, and cold thermoreceptors to

temperature drop and changes in tear osmolarity.[58] Electrophysical recordings are also used to evaluate central nerve function along trigeminal pathways, such as in the trigeminal nucleus caudalis.[59] Corneal sensitivity is also tested in animals with von Frey filaments, where increasing force is used to contact the central cornea until a blink-response is elicited.[55]

In humans, electrophysiological recordings of corneal and central nerves are not feasible. As such, clinicians have developed several tests that evaluate corneal nerve pathway function. In the clinic, corneal sensitivity is typically qualitatively checked with a cotton tip or dental floss (rated as 0=absent, 1=reduced, 2=normal, 3, increased). In the research arena, corneal sensitivity can be quantitatively assessed using a Cochet-Bonnet esthesiometer where a nylon monofilament 6 cm in length is used to contact the ocular surface and then retracted in 0.5-cm increments until corneal sensation is felt. For this test, a higher result corresponds to a higher corneal sensitivity.[60] Alternatively, a Belmonte esthesiometer utilizes a non-contact air jet to provide the stimulus, which can either be mechanical (variable airflow), thermal (hot or cold pulses), or chemical (varying CO2 concentrations). In contrast to Cochet-Bonnet, lower values with the Belmonte esthesiometer correspond to increasing sensitivity. The presence of hypo- or hyper-sensitivity suggests abnormalities in the corneal nerve pathway, although it is not possible to determine if the abnormality is in peripheral and/or central nerves. Overall, a wide range of corneal sensitivity values has been found in individuals with and without dry eye.[61] One study of 403 individuals with dry eye symptoms (DEQ5 ≥6) found a mean corneal mechanical detection threshold (using Belmonte esthesiometer) of 87 ± 46 mL/min, with a 10th percentile of 40 mL/min and a 90th percentile of 145 mL/min. Twenty-four percent of individuals had values that fell at or outside this range, 13% (n=51) were hypersensitivity (≤40mL/min) and 11% (n=46) hyposensitive (≥145mL/min).[61]

Peripheral nerve structure can be assessed using in vivo confocal microscopy (IVCM). IVCM images can be used to examine corneal subbasal nerves for density, branching, beading, tortuosity, and abrupt termination with swelling (termed microneuroma).[62] IVCM, however, has limitations in that it lacks built-in software to analyze nerve parameters, there are no normative databases with which to compare values across populations, it provides a small field of view, and it is difficult to scan the exact same location over time.[63]

In humans, certain symptom profiles suggest central abnormalities including the presence of allodynia (pain due to a stimulus that does not normally provoke pain,[53] such as with light), hyperalgesia (increased pain from a stimulus that normally provokes pain,[53] such as with wind) and expansion of the receptive fields (such as pain to light touch of the periocular skin).[43,64] The proparacaine challenge is another clinical test used to detect central abnormalities. Individuals are first asked to rate their ocular pain intensity (typically on a 0–10 scale) immediately prior to placement of topical anesthetic. After one drop is instilled in each eye and 30 seconds to 2 minutes have passed (different investigators use different time periods), ocular pain is reassessed. Elimination of pain suggests nociceptive or peripheral contributors to pain while persistence of pain suggests central or non-ocular contributors. A limitation of this test is that it is not informative if no pain is present at the start of testing. In the research arena, brain functional studies[65] and quantitative sensory testing have been used to identify central abnormalities in trigeminal pathways.[66]

## Abnormalities in Peripheral Nerves Have Been Detected in Dry Eye and Migraine

The literature suggests that both dry eye symptoms and migraine pain are driven in part by peripheral sensitization.[67,68] In dry eye, peripheral injury and activation may result from a number of sources including chronic epithelial disruptions, high tear osmolarity, ocular surface inflammation, and/or surgically induced nerve injury (eg refractive surgery).[67] On the other hand, initiators of peripheral nerve injury in migraine remain controversial.[69]

Electrophysiology studies have detected corneal nerve abnormalities in dry eye. In a guinea-pig model of aqueous tear deficiency using lacrimal gland excision, changes in peripheral nerve function were detected in mechanoreceptor spontaneous activity at 1 week post-surgery (0.30 ± 0.22 vs 0.02 ± 0.02 impulses/second, $p<0.05$) and cold-thermoreceptor spontaneous activity at 4 weeks post-surgery (13.22 ± 1.00 vs 10.27 ± 0.78 impulses/second, $p<0.05$) compared to sham controls. Furthermore, a change in cold-thermoreceptor thresholds was observed 4 weeks post-surgery (32.42 ± 0.14 vs 29.87 ± 0.35 °C, respectively, $p<0.05$) compared to controls, indicating increased sensitivity to cooling.[56] In a mouse model, lacrimal gland excision resulted in an

increase in spontaneous ciliary nerve activity compared to sham controls (86.8 ± 7.6 vs 43.4 ± 4.9 impulses/sec, p<0.001). Concomitantly, corneal mechanical thresholds decreased (implying increased sensitivity) compared to sham controls (0.012 ± 0.001 vs 0.028 ± 0.002 g, p<0.0001).[70] Together, the studies demonstrate that the initiation of aqueous tear deficiency causes a change in corneal nerve function, manifesting as hypersensitivity. Unfortunately, corneal nerve electrophysiology studies in migraine animal models are lacking.

Alterations in corneal nerves structure and function have also been reported in various dry eye populations compared to controls. Overall, most studies have reported decreased corneal nerve density and sensitivity in individuals with aqueous tear deficiency and Sjögren's syndrome but not in individuals with evaporative dry eye.[62] For example, an Italian study examined corneal nerves in 39 individuals with symptomatic aqueous tear deficiency (low TBUT, corneal staining, low Schirmer) compared to 30 controls. They found significantly lower corneal nerve fiber density and length, but higher width in the dry eye vs control group (respectively, 20.5 ± 8.7 vs 25 ± 6.7 n/mm$^2$, p=0.008; 12.6 ± 4.4 vs 14.5 ± 2.9 mm/mm$^2$, p=0.02; 0.021 ± 0.001 vs 0.019 ± 0.001 mm/mm$^2$, p<0.001).[71] For corneal sensitivity, an American study of 33 individuals with symptomatic aqueous tear deficiency (OSDI >20, TBUT ≤7 seconds, tear meniscus height <220μM) found decreased sensitivity (measured via Cochet-Bonnet) compared to 10 healthy controls (3.6 ± 1.6 vs 5.5 ± 0.83 cm, p<0.05). Similar to density, individuals with other dry eye sub-types (Meibomian gland dysfunction and conjunctivochalasis) did not have differences in corneal sensitivity compared to controls.[72] Together, the above studies suggest that individuals with aqueous tear deficiency have lower nerve densities and sensitivity than controls, but that these differences are not as robust in other dry eye sub-types.

Corneal nerve alternations have also been documented in migraine. A Chinese study examined corneal nerves in 10 individuals with episodic migraine and 10 controls. Corneal nerve branching and tortuosity were significantly increased in individuals with migraine compared to controls (91 ± 13.8 vs 75 ± 14.2 branches/mm$^2$, p=0.03 and 2.3 ± 4.6 vs 1.6 ± 0.5, p=0.01, respectively).[73] Photophobia has also been linked to peripheral corneal nerve abnormalities. In a prospective Indian study, individuals with chronic migraine and photophobia (n=36) had significantly lower subbasal nerve parameters, including corneal nerve fiber length (14.8 ± 4.0 vs 18.1 ± 3.3 mm/mm$^2$, p=0.007), compared to those with migraine but no photophobia (n=24).[74]

Individuals with migraine have also been found to have increased corneal sensitivity compared to controls. One Turkish study compared 58 individuals with chronic migraine to 30 controls. Corneal sensitivity (measured by Cochet-Bonnet) in the nasal region was higher (increased sensitivity) in the migraine vs control group [median (IQR); 5.5 (5.25–6.0) vs 5.37 (5.0–5.75) cm, p=0.02]. Interestingly, in individuals with unilateral migraine, corneal sensitivity was higher in the affected vs unaffected side (median (IQR); 5.4 (5.0–5.7) vs 5.3 (5.0–56.5), p=0.049).[75] The data on sensitivity, however, are limited in that the Cochet-Bonnet can only measure sensitivity up to 6 cm and most healthy individuals can detect the filament when fully extended. No studies have evaluated corneal sensitivity in migraine with Belmonte esthesiometry which has a wider testing range. Overall, while not as robust as for dry eye, studies demonstrate that individuals with migraine have changes in their corneal nerve structure and function compared to controls.

## Abnormalities in Central Nerves Have Been Detected in Dry Eye and Migraine

The literature suggests that both dry eye symptoms and migraine pain are driven in part by central sensitization. Given that corneal nerve fibers project to the trigeminal brainstem region, studies have used this region to investigate central nerve changes in dry eye.[70] In a lacrimal gland excision mouse model, an increase in spontaneous firing rate of trigeminal subnucleus interpolaris/caudalis (Vi/Vc) neurons was noted compared to sham controls (6.4 ± 1.9 vs 2.9 ± 1.4 Hz, p<0.05). Additionally, periocular cutaneous receptive field areas of Vi/Vc and Vc/C1 units were significantly enlarged compared to sham controls.[59] These data suggest that aqueous tear deficiency can lead to central nerve abnormalities.

As with dry eye, central nerve abnormalities have been demonstrated in migraine. In a rat model of migraine using dural stimulation with an "inflammatory soup" (i.e histamine, serotonin, bradykinin), electrophysiologic recordings from trigeminovascular neurons in the posterior thalamus showed an increased firing rate and increased magnitude of responses to pressure, pinch, cephalic and extracephalic brush after dural stimulation compared to baseline. In contrast, control animals (dura stimulated

with fluid) showed no change in responsiveness after stimulation compared to baseline.[76]

Central abnormalities have also been noted in humans with dry eye and migraine. With regards to dry eye, a cross-sectional study of 224 South Florida veterans with dry eye symptoms (DEQ-5 ≥6) found that 18 (41%) had persistent ocular pain (0–10 NRS) after topical anesthesia placement. Individuals with persistent ocular pain also had worse dry eye symptoms (DEQ-5, 14.6 ± 3.7 vs 12.7 ± 3.3, p=0.001) and photophobia intensity (5.6 ± 3.1 vs 3.2 ± 3.2, p<0.0005, 0–10 NRS scale) compared to individuals without pain after topical anesthesia.[77] These data highlight multiple clinical features suggestive of central abnormalities in individuals with dry eye symptoms. However, brain imaging studies would provide stronger evidence of central nerve abnormalities. While lacking for aqueous tear deficiency, a case report of functional magnetic resonance imaging (fMRI) in an individual with contact lens overuse (one contributor to dry eye)[78] and photophobia reported activation at the level of the trigeminal ganglion, trigeminal nucleus caudalis, and thalamus when presented with 6-second blocks of light.[65] The strength of this report is that it links corneal epithelial cell disruption to photophobia to activation of central trigeminal pathways. However, more imaging studies in a variety of dry eye sub-types are needed to supplement these findings. Quantitative sensory testing has also been applied to the study of dry eye, with higher dry eye symptoms associated with enhanced temporal summation and the presence of after-sensations, both of which suggest central contributions to symptoms.[66]

Similar to dry eye, central abnormalities have been found in individuals with migraine pain.[79] In a Chinese study of 16 individuals with chronic migraine, 18 with episodic migraine, and 18 controls, individuals with chronic migraine demonstrated increased resting-state functional connectivity between bilateral amygdala and several brain regions compared to those with episodic migraine on fMRI. Compared to controls, those with chronic migraine had decreased functional connectivity between the right amygdala and several brain regions, whereas those with episodic migraine had increased functional connectivity in the left amygdala.[80] In a Korean study, 19 individuals with chronic migraine had increased resting-state functional connectivity between pain processing areas and the dorsal raphe nucleus compared to 45 individuals with episodic migraine on fMRI.[81] Together, these studies demonstrate central abnormities in animal models and humans with migraine, with greater abnormalities noted in individuals with chronic vs episodic migraine.

Inflammation is an important contributor to peripheral and central nerve abnormalities in dry eye and migraine.

Inflammatory mediators likely contribute to the development of peripheral and central sensitization in individuals with dry eye and migraine. For example, CGRP, a neuropeptide involved in neurogenic inflammation, as well as cardiovascular, gastrointestinal and endocrine processes,[43] has been associated with changes in nerve function in dry eye and migraine. In a rat model of corneal abrasion using heptanol, CGRP increased in peripheral corneal nerves at one week (measurement at 24 hours was limited by the abrasion) and in the trigeminal ganglion at 24 hours compared to controls. Concomitantly, rats displayed corneal hyperalgesia (increased eye wipes after corneal application of menthol) at 24 hours compared to controls. Both CGRP levels and hyperalgesia decreased to baseline at 1 week. These results suggest an association between CGRP and peripheral nerve function.[82]

Inflammatory mediators have also been found to increase in the central nervous system in dry eye. In a mouse model of lacrimal gland excision, increased mRNA levels of pro-inflammatory markers were noted in the trigeminal ganglion and brainstem compared to sham controls 21 days post-surgery. Similar to the rat model, these mice also exhibited corneal hypersensitivity after injury. Additionally, increased spontaneous electrical activity in their ciliary nerve was noted compared to controls. Centrally, increased synaptic plasticity in the trigeminal brainstem complex (measured using immunofluorescence of presynaptic zone components) was observed at 21 days.[70] This study demonstrates an association between aqueous tear deficiency, inflammation in central trigeminal pathways, and peripheral and central nerve abnormalities.

Human studies also support the link between inflammation and corneal nerve abnormalities. A Turkish study of 37 individuals with dry eye symptoms and signs (TBUT<7 seconds, corneal staining, Schirmer<10 mm) measured corneal sensitivity (via Cochet-Bonnet) before and after topical cyclosporine 0.05% (an anti-inflammatory agent). Corneal sensitivity increased post vs pre cyclosporine therapy (58.8 ± 2.1 vs 52.1 ± 5.5 mm, p<0.001).[83] These data suggest that inflammation impacts corneal nerve sensitivity in dry eye.

Inflammation, specifically CGRP, has also been linked to nerve abnormalities in migraine.[49] For example, a rat model

of migraine (recurrent administration of nitroglycerin) found that CGRP-immunoreactive fibers significantly increased in the trigeminal nucleus caudalis compared to controls. This was clinically accompanied by thermal hyperalgesia (withdrawal latency after infrared radiation on hind paw). Furthermore, hyperalgesia was ameliorated by knocking down CGRP with short hairpin RNA.[84] In a rat model of migraine (glass micropipette inserted into the visual cortex), a propagating wave of depolarization was induced with a resultant increase in the firing rate of spinal trigeminal nucleus neurons.[85] The increased firing rate was blocked when rats were pretreated with a CGRP-blocking antibody.[86] These data demonstrate that CGRP impacts nerve sensitivity in migraine.

CGRP has also been linked to migraine in humans. In a placebo-controlled, cross-over study of 13 individuals with migraine, intravenous CGRP induced migraine-like attacks in 10 individuals compared to 0 after placebo (isotonic saline), p=0.002. Median peak headache intensity score (NRS scale 0 to 10) was 5 (5–9) after CGRP compared to 2 (0–4) after placebo (p=0.004).[87] The effectiveness of anti-CGRP antibodies in treating migraine provides further support for the role of CGRP in migraine pathophysiology.[88] Together, the above studies support the interaction between CGRP and nerve function in migraine.

## CGRP is Also Related to Light Sensitivity, Independent of Dry Eye and Migraine

CGRP can induce light sensitivity. In wild-type mice, peripheral (intraperitoneal) and central (intracerebroventricular) injection of CGRP induced light-aversive behavior (time spent in illuminated portion of a light/dark box). Furthermore, an anti-CGRP monoclonal antibody attenuated light aversion after the peripheral injection of CGRP.[89] In transgenic mice that overexpressed the CGRP receptor, central, but not peripheral, CGRP administration induced light aversion. In another mouse model, peripheral injection of CGRP produced spontaneous pain (measured by a squint assay) both in complete darkness and in bright light.[90] Together, these studies support the role of CGRP in pain and photophobia via multiple mechanisms.

## Light Can Trigger Corneal Inflammation and Nerve Abnormalities

In a mouse model, blue light, but not yellow light, increased corneal sensitivity (via von Frey hair test) 3 hours post vs pre exposure. Exposure to blue light also led to observable changes on in-vivo confocal microscopy including activation of the superficial corneal epithelium (defined as the appearance of hyperreflective nuclei), increased numbers of dendritic (inflammatory) cells in the sub-basal plexus, and increased numbers of keratocytes in the stroma.[91,92] Additionally, blue-light increased inflammation in both the trigeminal ganglia and spinal trigeminal nucleus, as measured by mRNA expression of cFOS and ATF3.[46] These data suggest that the pathophysiology of dry eye and migraine is complex with multiple potential entry points (light, aqueous tear deficiency, corneal epithelial damage, cortical disruptions) that lead to inflammation and nerve abnormalities in multiple compartments (peripheral and central).

# Practical Implications for Diagnosing Dry Eye and Migraine

The overlap between dry eye and migraine has potential implications in the evaluation and treatment of individuals with these two diseases as illustrated in Figure 2. First, eye care providers should ask individuals with dry eye about comorbid headache and primary care doctors and neurologists should ask individuals with migraine about symptoms of dry eye. If present, appropriate referrals can be made.

Second, given shared pathophysiology involving nerve dysfunction, eye care providers should think about nerve status when evaluating an individual with dry eye symptoms. This includes assessing for ocular pain via standardized questionnaires (eg NRS, Neuropathic Pain Symptom Inventory-Eye [NPSI-Eye]) and evaluating nerve structure and function clinically. The presence of cutaneous allodynia can be assessed by evaluating for pain to touch around the eyes. In addition, corneal sensitivity can be qualitatively checked with a cotton tip or dental floss (generally rated as 0=absent, 1=reduced, 2=normal, 3, increased). The proparacaine test can help differentiate between nociceptive pain ("pain that arises from actual or threatened damage to non-neural tissue and is due to activation of nociceptors")[53] or peripheral neuropathic pain vs centrally mediated or non-ocular pain.[77]

Corneal nerves can be imaged with IVCM and certain nerve findings have been reported to suggest the presence of peripheral neuropathic pain. Specifically, one retrospective study found that in individuals with clinical suspected neuropathic pain, nerves in the subbasal layer abruptly terminated with hyperreflective enlargements.[93] This finding was termed microneuroma based on similar findings in

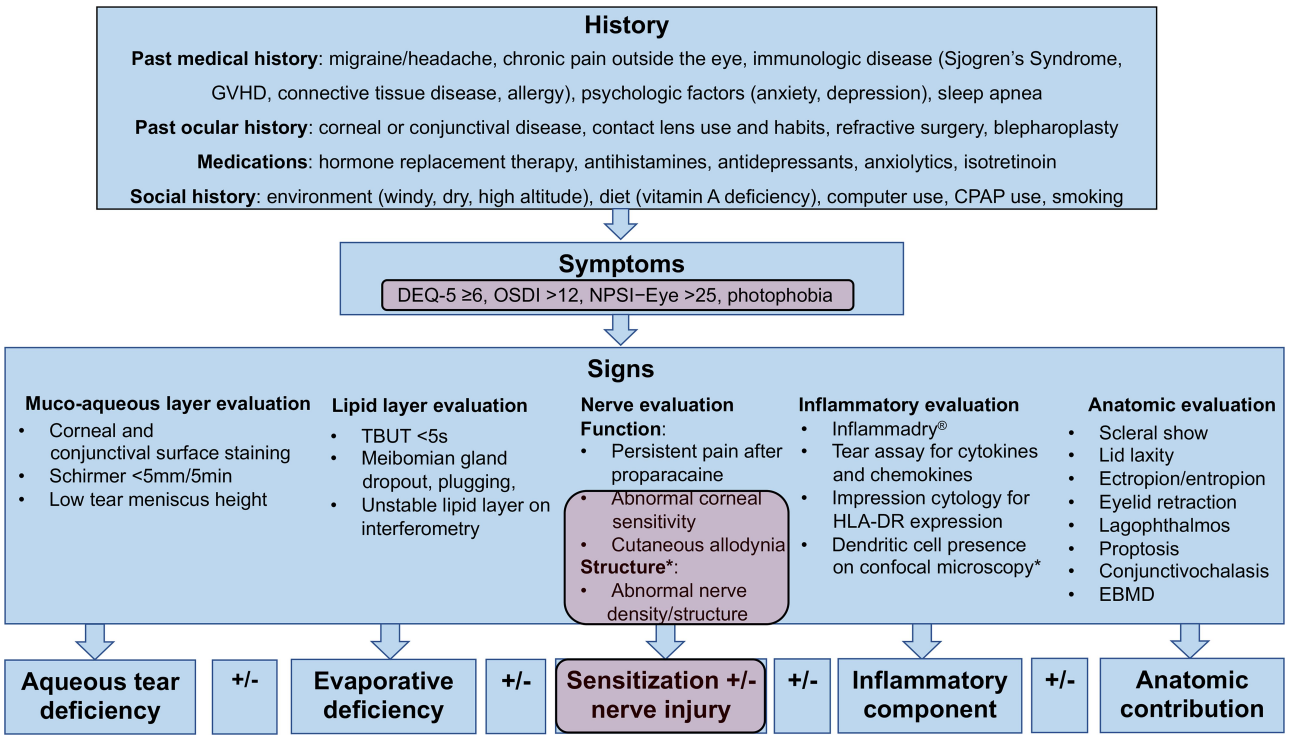


**Figure 2** Clinical assessment of patients with dry eye. Purple boxes indicate phenotypes that overlap with migraine. *Nerve structure findings using in-vivo confocal microscopy.

**Abbreviations:** GVHD, graft versus host disease; DEQ-5, Dry Eye Questionnaire-5; OSDI, Ocular Surface Disease Index; NRS, numerical rating scale; NPSI-Eye, Neuropathic Pain Symptom Inventory-Eye; TBUT, tear break up time; EBMD, epithelial basement membrane dystrophy.

animal models.[94] Microneuromas were observed in all subjects with ocular pain (n=30), but were not present in any subjects without pain (n=30).[93] Other studies, however, failed to replicate these findings in other dry eye populations.[95]

Understanding nerve status in an individual patient may help explain their clinical presentation as different sensitivity profiles have been described in different dry eye populations (eg hyposensitivity in aqueous tear deficiency, hypersensitivity in individual with presumed neuropathic ocular pain[96] and/or migraine).[75] This heterogeneity may explain the disconnect often seen between dry eye symptoms and signs, as nerve function drives sensation, and thus symptomatic interpretation, of dry eye signs (decreased tear volume, rapid tear evaporation). Understanding nerve status can also help tailor an individualized treatment plan.

## An Updated Paradigm for the Treatment of Dry Eye Based on Data in Migraine

The current paradigm for managing dry eye is to target tear dysfunction. This new paradigm suggests that when this approach does not sufficiently relieve dry eye symptoms, therapies targeting nerve dysfunction should be considered. Given similarities between dry eye and migraine, therapies that are of benefit in migraine may be beneficial in dry eye.

### Anti-Inflammatory Therapy

Anti-inflammatory medications are a first-line treatment in dry eye and migraine.[69,97] Specifically, in dry eye, short-term topical corticosteroids, and long-term cyclosporine and lifitegrast are first-line agents.[67] Decreasing ocular surface inflammation may improve tear composition and dry eye symptoms.[98] However, similar to migraine,[99] not all patients with dry eye respond to anti-inflammatory therapy.[100] Interestingly, baseline nerve status may predict who responds to anti-inflammatory therapy. In an American study, 60 individuals with dry eye (OSDI>22, corneal staining, meibomian gland dysfunction) were grouped by subbasal corneal nerve length (<16.84 (n=9) vs ≥16.84 mm/mm$^2$, n=11). Symptoms and signs in individuals with higher baseline SNFL improved 4 weeks after starting loteprednol (Symptom Assessment in Dry Eye, SANDE: 60.1 ± 17.4 vs 50.0 ± 22.7, p=0.04 and corneal

staining: 6.7 ± 3.2 vs 4.6 ± 2.9, p=0.01) while those with low baseline nerve length showed no improvement.[100] In patients who fail anti-inflammatory therapies, other therapies need to be considered.

## Oral Nerve Modulators

In individuals with features suggestive of centrally mediated pain (peri-ocular allodynia to light touch, photophobia, persistent pain after anesthesia), systemic nerve modulators should be considered. Oral nerve modulators have been effective for migraine prevention including, serotonin and norepinephrine reuptake inhibitors and tricyclic antidepressants (TCAs),[25] and for aborting acute migraine attacks, such as triptans.[101] Given similar pathophysiology to migraine, patients with dry eye may also benefit from oral nerve modulators. Indeed, gabapentin and pregabalin, both alpha 2 delta (α2γ) ligands, have been examined in dry eye. These agents are thought to exert their effect by reducing voltage-gated calcium channel currents in the central nervous system leading to decreased excitatory neurotransmission.[102] A case series evaluated the efficacy of α2γ ligands in 8 individuals with ocular pain unresponsive to topical therapies. Gabapentin was escalated to a dose of 600–900 mg three times daily and pregabalin to 150 mg twice in the study. Two individuals reported complete pain relief after adding a α2γ ligand to their multi-modal regimen while 3 individuals reported significant relief.[102] Interestingly, the 2 individuals with complete pain relief were also on concomitant duloxetine. This study demonstrates that α2γ ligands may alleviate ocular pain in dry eye as part of a multi-modal regimen. However, additional studies are needed.

As with migraine, groups have studied the impact of TCAs in nerve-related ocular pain. TCAs inhibit central and peripheral serotonin and norepinephrine reuptake as well as cholinergic, histaminergic, and sodium channels.[98] In a retrospective cohort study of 30 patients who failed other therapies and had persistent pain after anesthesia, nortriptyline (at least 4 weeks of use, started at 10 mg and increased up to 100 mg based on response and tolerability) improved ocular pain in the last 24-hours (measured via NRS) from 5.7 ± 2.1 to 3.6 ± 2.1 after 10.5 ± 9.1 months (p<0.0001) of use. In addition, quality of life score (via an OPAS sub-score) improved from 6.0 ± 2.5 to 4.3 ± 2.4 (p=0.019).[103] Taken together, the above studies suggest that in individuals with dry eye symptoms and clinical features suggestive of central nerve abnormalities, oral nerve modulators may improve ocular pain symptoms. However, in patients with either dry eye or migraine who show no or partial response to oral therapies, adjuvant approaches may be considered.

## Adjuvant Approaches

Adjuvant therapies are often employed in migraine and may also be beneficial in the treatment of dry eye. For example, botulinum toxin is an approved medication in migraine[104] and has been explored in dry eye. Botulinum toxin is thought to target pain responses by reducing facial muscle contraction and thus decreasing trigeminal afferent signaling as well as by reducing synaptic release of CGRP.[42] In migraine, a Cochrane meta-analysis of 26 double-blind randomized controlled trials found that botulinum toxin treatment reduced the frequency of migraine (mean difference= −2.39 migraine days/month; 95% CI, −4.02 to −0.76) and migraine severity (measured by NRS 0–10; mean difference= −3.30; 95% CI, −4.16 to −2.45) compared to placebo in those with episodic or chronic migraine.[105] In dry eye, a retrospective study of 117 South Florida veterans with chronic migraine (≥15 headaches or headache days/month) found that botulinum toxin A (mean units injected: 114.4 ± 24.5) improved migraine pain (mean change= −3.43; 95% CI, −3.95 to −2.92; p<0.001), photophobia (mean difference= −2.64; 95% CI, −3.18 to −2.11; p<0.001), and dry eye symptoms (mean difference= −0.716; 95% CI, −1.18 to −0.249; p=0.003) (all measured via NRS 0–10) compared to pre-injection scores.[106] This effect was found to be independent of tear volume,[42] suggesting that mechanisms beyond tear dysfunction drive eye symptoms. In 4 individuals with dry eye symptoms without migraine, a modified botulinum toxin A protocol (35 units in 7 sites) improved photophobia and dry eye symptoms 1 month post vs pre injection.[107] Together, these data suggest that botulinum toxin A may improve photophobia and dry eye symptoms in individuals with and without migraine.

Another adjuvant treatment with success in migraine is device neuromodulation, and this entity has also been studied in dry eye. Specifically, transcutaneous electrical nerve stimulation (TENS) uses pulsed low voltage electrical currents across the intact surface of the skin to stimulate peripheral nerves.[108] TENS has been postulated to improve pain by stimulating deep sensory afferents that secondarily inhibit nociceptive input via gate control theory.[108] As applied to ocular pain, TENS may stimulate deep Aβ fibers in the V1 and V2 distribution and block nociceptive input from unmyelinated C fibers. In terms of migraine, one meta-analysis of four studies using different

TENS devices, Cefaly (company, location), LH202H Han Electrostimulator (company, location), GammaCore® (company, location), HANS-200A machine (company, location), with varying protocols (five times weekly, daily, three times daily) found that TENS significantly reduced monthly headache days (standard mean difference= −0.48; 95% CI, −0.73 to −0.23; p<0.001) and analgesic intake (standard mean difference= −0.78; 95% CI, −1.14 to −0.42; p<0.001) compared to sham TENS (TENS device was applied with far less electrical stimulation or none at all).[108] Similar to migraine, TENS has also shown promise in dry eye. In a retrospective study of 10 individuals with ocular pain, some of which had dry eye signs, an RS4i (RS medical, Vancouver) was used at varying intervals (range 3–21 times weekly) for a median of 6.5 months (range: 3–14 months). Overall, pain scores (one-week recall measured via NRS 0–10) decreased by 27.4% (p=0.02) post- vs pre-treatment.[109] Together, these data suggest that TENS may be incorporated as an adjuvant treatment in individuals with dry eye and migraine.

Another modality less frequently used in migraine is the blockage of peripheral nerve afferents with local anesthetic.[102] In migraine, a meta-analysis of 33 articles showed that blockade of the greater occipital nerve was associated with a significant decrease in the number of headache days (pooled mean difference in headache days= −3.6; 95% CI, −1.39 to −5.81) and headache severity (pooled mean difference in pain scores= −2.2; 95% CI, −1.56 to −2.84).[110] This approach may also benefit patients with ocular pain when applied to trigeminal nerve afferents. A retrospective series of eleven individuals who failed conservative therapy for dry eye and ocular pain reported outcomes after periocular nerve block with 4 mL of 0.5% bupivacaine mixed with 1 mL of 80 mg/mL methylprednisolone acetate targeting the supraorbital, supratrochlear, infratrochlear, and infraorbital nerves. Seven of 11 individuals experienced pain relief after nerve block lasting hours to months and five individuals underwent repeat nerve blocks.[102] Of note, four of the seven individuals who responded to nerve blocks had ocular surgery as the pain trigger, whereas this was the case for one of the four non-responders. The above studies suggest that nerve blocks may benefit some patients with refractory ocular pain. However, these data are limited by their observational nature and limited number of subjects.

In addition to trigeminal afferent blockade, other nerve block sites have shown promise for treatment of migraine and dry eye, such as sphenopalatine ganglion (SPG) blocks.[111,112] In fact, some ocular pain is thought to be mediated by parasympathetic fibers, whose presence has been documented on the cornea.[113] Although biologic plausibility exists, studies are needed to evaluate the effects of SPG blocks in individuals with dry eye symptoms and ocular pain. Overall, the data presented in this section support the use of nerve blocks in appropriate individuals, especially those with surgically induced chronic ocular pain.

## Conclusions

To conclude, this review discusses potential links between dry eye and migraine, prompted by an association between the two diseases in the literature. This information can be used to better understand pathophysiological mechanisms and develop targeted treatments by applying therapies successful in reducing migraine pain to dry eye. Neuronal injury leading to peripheral and central sensitization through trigeminal pathways are important mechanisms in some individuals with dry eye symptoms. Clinically, these individuals may manifest as hyperalgesia (evoked pain with wind), photophobia, and expansions of the receptive field (pain to light touch of the skin around the eye). These data highlight the need to test for nerve function in individuals with dry eye and consider the use of therapies that target nerve abnormalities in appropriate individuals.

## Disclosure

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52 Abstract

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**Recent Findings:** Subjects with DED have a heterogeneous symptom and sign profile along with variability in nerve structure and function. Most studies have reported lower nerve density and sensitivity in aqueous tear deficiency, while findings are more inconsistent for other DED subtypes. Examining nerve status, along with profiling symptoms and signs of disease, can help categorize subjects into disease phenotypes (structural and functional patterns) that exist under the umbrella of DED. This, in turn, can guide therapeutic decision-making.

**Summary:** Due to the heterogeneity in symptoms and signs of DED, corneal nerve evaluations can be valuable for categorizing individuals into disease sub-types and for guiding clinical decision-making.



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# How Should Corneal Nerves Be Incorporated Into the Diagnosis and Management of Dry Eye?

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**Summary** Due to the heterogeneity in symptoms and signs of DED, corneal nerve evaluations can be valuable for categorizing individuals into disease sub-types and for guiding clinical decision-making.

**Keywords** Corneal nerves · Confocal microscopy · Aethesiometry · Dry eye disease · Phenotype

## Introduction

The corneal nerve system derives innervation from the trigeminal nerve and functions in ocular healing and processing of sensory stimuli. Studies examining this system have provided insight into its structure and function in health and disease states. Structural and functional nerve abnormalities have been reported in the setting of several disorders including dry eye disease (DED), a common cause of morbidity in the general population. This review will summarize this data and discusses how the evaluation of nerve status may be better incorporated into the clinical examination of DED.

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## Corneal Nerves

### Structure of Corneal Nerves

By combining findings of light and electron microscopy studies with later studies using vivo confocal microscopy (IVCM), the morphology of corneal nerves has been studied in detail [1–3]. Today, IVCM is the most popular method of studying nerve structure, with images providing information for diagnosis and measurement of treatment response for several disorders [4, 5]. The benefit of IVCM includes non-invasive imaging at high resolution (1–2 μm laterally, 5–10 μm axially, magnification ×600) [6]. Cons also exist, including a small field of view, a need for trained operators, and a lack of built-in quantification software [7, 8]. It is also difficult to scan the same area repeatedly, which must be considered when reviewing studies that evaluated nerve changes over time.

Many IVCM microscopes exist, including slit-scanning (e.g., ConfoScan 4; Nidek), tandem-scanning (e.g., tandem scanning; Reston), and laser-scanning (e.g., Heidelberg Retina Tomograph; Heidelberg) instruments. Tandem-scanning instruments have a small aperture (30 μm), narrow depth of field (7–11 μm), and limited magnification, creating difficulty in telling <5-μm objects apart, making them better suited for the anterior stroma rather than nerves. Slit-scanning microscopes have wider apertures (e.g., 300 μm) and a larger depth of field (10–26 μm), allowing for improved resolution. Laser-scanning microscopes offer the highest contrast and resolution for nerves given the pinhole aperture (1 μm) and small depth of field (4–7 μm).

Several nerve parameters have been examined via IVCM, including nerve density (nerves within a defined area [μm/mm$^2$ or mm/mm$^2$], nerve length (density of nerves in a frame [mm/mm$^2$], often used as a proxy for density), nerve count (fibers within a frame), reflectivity (graded 0–4), and tortuosity (twisting, graded on a Likert scale or by tortuosity coefficient) [9]. IVCM also allows for morphologic examination of various cell types (epithelial, endothelial, dendritiform cells (DCs)). Dendritiform cells are thought to be antigen-presenting cells that are found within the cornea. In non-inflamed states, they are mostly located in the peripheral cornea. In the setting of systemic immune disorders (Sjögren's syndrome, graft-versus-host disease (GVHD), and local inflammation (keratitis), these cells increase in number and size and migraine into the central cornea [10]. DCs are further categorized by maturity or "activation" state. Based on studies in animals [11–13], when DCs become "activated," they enlarge, and the number and length of their dendrites increase. A limitation of IVCM is that it is often challenging to compare findings across studies due to resolution differences between microscopes and variability in the reporting of outcome measures [14, 15].

## Function of Corneal Nerves

Corneal nerves are sensory, and several types of sensory fibers exist in the system [16]. The majority of fibers are polymodal nociceptors, which process mechanical, heat, and chemical stimuli. A smaller number of fibers are mechanonociceptors, which identify mechanical stimuli. Finally, a minority of nerves are cold-sensitive receptors, which respond to a decrease in temperature [17]. Sensitivity evaluation is the most common method for assessing nerve function—this can be done qualitatively in clinic with the use of a cotton tip or floss, or quantitatively using an aesthesiometer. The Cochet-Bonnet (CB) aesthesiometer has a nylon filament that contacts the eye; the filament is then retracted until the individual detects the stimulus, providing measurement of a mechanical detection threshold. A lower reading corresponds to a *lower* sensitivity. The Belmonte instead propels air jets (with or without $CO_2$, at varied intensities and temperatures), allowing testing of mechanical, chemical, thermal, and pain thresholds [18]. A lower reading (lower threshold) corresponds to a *higher* sensitivity.

Both instruments have limitations. Both devices require that the eye is open during testing. For CB, the operating technician must be weary of proper filament placement and pressure in order to take reproducible measurements. Furthermore, the CB requires sterilization between patients and its filament can be aged by humidity and temperature. Also, the CB has a narrow testing range and many healthy individuals can detect the filament when fully extended. Thus, it is difficult to use this instrument to examine for corneal hypersensitivity [19]. The Belmonte is not commercially available, has a bulky exterior, and requires more time for sensitivity readings. Both instruments have been found to have higher reproducibility in the central cornea compared to the conjunctiva [20, 21]. Finally, various studies have used different protocols (different distances from the cornea, air temperature, locations of testing) make comparisons across the literature, even with the same instrument, challenging.

## Nerves in Healthy Individuals

A handful of studies have described nerve attributes in healthy individuals. One tandem-scanning study of 65 healthy individuals (mean age 46±19 years, range 15–79) reported a mean nerve density of 8,404±2,012 μm/mm$^2$ (range 4,735–14,018 μm/mm$^2$) [22]. In comparison, a slit-scanning study of healthy individuals (n=60 (age <35 or >50 years)) reported a nerve density of 14,731±6,056 μm/mm$^2$ [23]. Finally, two laser-scanning studies of healthy subjects (n=85 (mean age 38±16 years, range 18–87) and n=106 (mean age 50, range 15–88)) estimated nerve densities at 20,300±6,500 μm/mm$^2$ (range 5,000–35,000 μm/mm$^2$) and 19,000±4,500 μm/mm$^2$ (range 13,400 to 23,400 μm/mm$^2$), respectively [24, 25]. It is important to note the overlap in reported nerve values across studies when examining healthy individuals. Such overlap is expected between different IVCM instruments. However, the overlap remains even when comparing values obtained with one instrument. While this may be due to operator technique or the section of cornea sampled, it may also indicate a heterogeneity in density values in healthy and diseased eyes.

Fewer studies have examined sensitivity in healthy individuals. In an Australian study of 18 healthy subjects (mean age 34.40±8.09 years), detection thresholds on CB and Belmonte were 5.50±0.80 cm and 64.40±29.40 mL/min, respectively. However, 56% of the measurements were beyond the maximum threshold of the CB (individuals felt the stimulus when the thread was at its full length of 6 cm), while all values were within the testing range of the Belmonte [18]. Near identical results were reported in a British study of 17 subjects (mean

age = 20.30 years, range = 19–26) where 61% of subjects felt the stimulus at 6 cm, while no threshold reached the max Belmonte stimulus intensity [26].

## Nerves in DED

DED is defined as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles" [27]. Its pathogenesis is complex, involving a cyclical process of tear osmolarity changes, inflammatory damage, and tear film instability, among others [28]. There exists heterogeneity in DED in terms of phenotypes and underlying mechanisms. DED is often subtyped into aqueous tear deficiency (ATD), evaporative DED, and a mixed sub-type depending on diagnostic findings. Also, DED can occur as an isolated phenomenon or secondary to a systemic disorder, such as Sjögren's syndrome or GVHD [14, 29]. Nerve attributes have also been evaluated in DED, with the additional challenge that different studies used different definitions for the disease [30]. Overall, similar to what was seen in healthy individuals, there was a wide range of nerve parameters with overlapping values between DED and healthy controls. This further demonstrates the heterogeneity of nerve status in individuals with and without eye disease.

### Structural Anomalies in DED

Many studies have examined nerve structure in individuals with DED. Most studies focused on individuals with ATD while a minority examined individuals with evaporative DED. Overall, most studies (using laser-scanning IVCM) found that individuals with ATD (variably defined) had lower nerve densities compared to controls. For example, a French study examined 12 subjects with ATD (irritation, tear instability, staining ≥2, Schirmer ≤10 mm) and 10 controls and found a lower nerve density in the ATD group (9,426±2,640 vs. 15,956±2,431 μm/mm$^2$, p<0.0001) [31]. Similar findings were described in a Korean study that examined 40 individuals with ATD (symptoms, tear film break-up time (TBUT) <5 s, Schirmer <10 mm) and 18 controls and reported lower density (9,884±2,548 vs. 12,030±2,203 μm/mm$^2$, p<0.005) and higher tortuosity (3.70±0.50 vs. 1.60±0.60, p<0.001) in the ATD group [32]. Other studies have used nerve counts as a surrogate for nerve density and reported similar results—a Chinese study evaluated 43 subjects with ATD (symptoms, TBUT <10 s, Schirmer <5 mm) and 14 controls and noted a lower nerve count (34.91±8.08 vs. 45.87±4.21 nerves/mm$^2$ [frame = 400×400 μm], p<0.001) and higher tortuosity (3.01 ±0.49 vs. 1.94±0.46, p<0.001) in the ATD group [33]. Similarly, an Italian study that examined 15 subjects with ATD (definition not provided) and 15 controls reported lower nerve counts in ATD (3.90±0.50 vs. 5.80±1.30 nerves/frame, p<0.001) [34]. Contrasting from these findings, a Chinese study that used slit-scanning IVCM on 30 subjects with ATD (symptoms, staining, Schirmer ≤8 mm) reported higher nerve density in the ATD group, but differences between groups were not significant (1,424±610 vs. 1,316±665 μm/frame [frame = 340×255 μm], p=0.50) [35].

Fewer studies have examined evaporative DED, but overall, no significant differences in density were found compared to controls using laser-scanning IVCM. An Indian study examined 52 subjects with evaporative DED (high Ocular Surface Disease Index (OSDI) score, low TBUT, normal Schirmer; further specifications not provided) and 43 controls and found no difference in nerve density between the groups (27.20±0.60 vs. 28.60±0.80 nerves/mm$^2$ [frame of 400×400 μm], p>0.05) [36]. Another Indian study that examined 47 subjects with evaporative DED (symptoms, TBUT <10s, Schirmer >10 mm) and 33 controls also reported no difference in nerve density (~30.00 nerves/mm$^2$ both groups, p>0.05) [37].

It is not known why individuals with ATD seem to have more nerve abnormalities than individuals with evaporative DED. Hypotheses include distinct pathophysiologic mechanisms for the two diseases that differentially affect corneal nerves, including the close association between inflammation and ATD and the finding that many individuals with ATD (especially in the setting of Sjögren's) have a component of neurotrophic keratitis [29]. Inflammation has been closely linked to corneal nerve dysfunction in other studies—in particular, one study that utilized a mouse model found that exposure of an "inflammatory soup" (bradykinin, histamine, prostaglandin E2, serotonin, and ATP) to mouse corneas leads to alterations in firing pattern (paroxysmal discharges and silent periods with stimulation) and waveform morphology (flatter, with lower peak amplitude) of recorded impulses from actively firing cold and polymodal nociceptors [38].

### Functional Anomalies in DED

Similar to nerve density, corneal sensitivity has been frequently reported to be decreased in ATD. However, studies in mixed DED populations have not replicated these findings. Specifically, the French study above used CB to examine 12 subjects with ATD and 10 controls and reported a lower sensitivity in the ATD group (5.00±0.83 vs. 5.89±0.22 cm, p=0.01) [31]. Similarly, an American study that used CB on 10 subjects with ATD (OSDI >20, TBUT ≤6 s, tear meniscus height <220 μm on optical coherence tomography) and 10 healthy controls reported lower sensitivity in the ATD group (3.60±1.65 vs. 5.45±0.83 cm, p<0.05) [39]. Hypoesthesia has also been found in studies using Belmonte. A Spanish study

that used Belmonte on 10 subjects with ATD (symptoms, Schirmer <10 mm) and 10 controls found hypoesthesia (higher mechanical threshold) in the ATD cohort (134.0 ±24.0 vs. 78.0±12.0 mL/min, p=0.02) [40]. Another Spanish study that used Belmonte on 44 subjects with DED (symptoms, staining, TBUT ≤6 s; n=14 with Sjögren's) and 42 controls found hypoesthesia in the DED group (mechanical (152.60±33.80 vs. 109.0±23.30 mL/min, p<0.001), chemical (23.90±4.30 vs. 16.40±3.10 %$CO_2$, p<0.001), heat (+0.34 ±0.13 vs. +0.26±0.05 °C, p<0.001), cold (−0.14±0.15 vs. −0.05±0.04 °C, p<0.001)) [41]. Diverging from these findings, other studies have reported hypersensitivity in DED. Specifically, an American study that used Belmonte on 20 subjects with DED (symptoms, TBUT ≤5 s, staining ≥2) and 20 controls noted hyperesthesia (lower mechanical threshold) vs. the control group (34.60±21.09 vs. 61.50±20.07 mL/min, p<0.05) [42].

## Animal Models of AT

Animal studies support findings of lower density and sensitivity in ATD. One study exposed mice to environmental stress (fan) for 5 h/day for 3 days and found lower nerve density after the stressor (2,813±762 to 1,898±286 pixels/frame, p=0.01) while tortuosity (0.81±0.33 to 0.96±0.40, p=0.31) and reflectivity (0.83±0.37 to 0.78±0.43, p=0.76) did not change with stress [43]. Another study exposed mice to scopolamine (with a subsequent reduction in tear production) and noted hypoesthesia on CB at 2 weeks compared to controls (2.72±0.30 vs. 3.50±0.40 cm, p<0.0001) [44]. Lower sensitivity has also been described in a breed of dogs that spontaneously develop ATD—in a study of West Highland White Terriers, sensitivity was lower in dogs with ATD (discharge, conjunctival hyperemia/chemosis, chronic keratitis, Schirmer <15 mm) vs. controls of the same breed via corneal touch threshold (1.40 (range 0.60–2.30) vs. 2.20 (range 1.20–10.30) g/$mm^2$, p=0.07) [45]. Unfortunately, animal models of evaporative DED have yet to be examined, making comparisons to human findings difficult.

## Relationship Between Structure and Function

A number of studies have evaluated relationships between structure and function in DED, with the majority reporting positive relationships, e.g., lower nerve density via laser-scanning IVCM correlating with lower sensitivity. The Chinese study that examined 43 subjects with ATD and 14 controls reported a positive association between density and CB sensitivity (r = 0.38, p=0.04) [33]. Similarly, the French study that examined 12 subjects with ATD and 10 controls also reported positive associations between CB sensitivity and density (r = 0.64, p=0.05) and nerve count (r = 0.65, p=0.04) [31]. Finally, the Spanish study that examined 10 subjects with ATD and 10 controls found associations between nerve density with mechanical (r = −0.79, p<0.001), chemical (r = −0.80, p<0.001), and thermal cold thresholds on Belmonte (r = −0.63, p<0.001) [40]. This indicates a positive association between density and sensitivity.

## Relationships With Symptoms

A common symptom of DED is pain, for which several symptom measures exist. The Ocular Surface Disease Index (pain, vision, triggers, and quality of life [46]) and Dry Eye Questionnaire (DEQ5; dryness, discomfort, and tearing [47]) include a variety of questions regarding painful and non-painful symptoms. The Neuropathic Pain Symptom Inventory modified for the Eye (NPSI-E; pain metrics specific to neuropathic pain [48••]) and Ocular Pain Assessment Survey (OPAS [49••]) was developed specifically to assess ocular pain. Importantly, some studies correlated nerve metrics with pain-specific questions (e.g., OSDI-discomfort, NPSI-E) while others examined relationships with total questionnaire scores, of which pain is only one component.

Studies in ATD have demonstrated negative relationships between symptoms and nerve parameters, in which higher symptoms in individuals are associated with lower nerve density (via laser-scanning IVCM). For example, an American study examined 22 individuals with Sjögren's DED, 12 with non-Sjögren's DED (symptoms, TBUT <10s, corneal staining), and 7 healthy controls and found a strong negative correlation between density and OSDI (r = −0.91, p<0.001) [50]. The Chinese study that examined 43 subjects with ATD and 14 controls also found a negative relationship between nerve length and OSDI, albeit at a lower magnitude (r = −0.27, p=0.02) [33]. However, this finding was not reproduced in evaporative DED—the Indian study that examined 52 subjects with evaporative DED and 43 controls did not find relationships between nerve density (r=0.10, p=0.33) or length (r=0.15, p=0.13) with OSDI discomfort [36].

Inconsistent relationships have been reported for sensitivity and DED symptoms. A French study examined 30 subjects with post-keratectomy DED (defining specifications not provided), reporting a negative association between CB sensitivity and OSDI (r = −0.65, p<0.01) [51]. Conversely, an American study that examined 129 subjects with DED symptoms (DEQ5 score ≥6) noted a significant, but weak, negative association between Belmonte thresholds and pain (OSDI: r = −0.18, p=0.04 and r = −0.20, p=0.03; NPSI-E: r = −0.23, p=0.01 and r = −0.21, p=0.02). This translates into a positive relationship between sensitivity and pain as lower thresholds on Belmonte indicate higher sensitivity [52]. It is important to note however that the latter study excluded individuals with Sjögren's, GVHD, and a history of refractive surgery; thus, study populations were not similar across studies. Other studies have reported both positive and negative weak associations

between CB sensitivity and OSDI in ATD (e.g., r = 0.13 [33], r = 0.20 [39], r = −0.14 [53]; p<0.05 for each).

Overall, lower nerve density has been associated with higher symptoms in individuals with ATD, while inconsistencies have been found across the literature with regard to the relationship between corneal sensitivity and symptoms.

### Relationships With Signs

Studies have also examined relationships between nerve parameters and DED signs. Overall, most studies reported negative relationships between nerve metrics (via laser-scanning IVCM) and corneal staining, while inconsistent relationships were found for TBUT and Schirmer.

For example, the Chinese study that examined 43 subjects with ATD and 14 controls found that nerve density (r = −0.49, p=0.01), nerve length (r = −0.31, p=0.04), nerve count (r = −0.36, p=0.02), nerve reflectivity (r = −0.34, p=0.03), and corneal sensitivity (r = −0.30, p=0.04) negatively correlated with corneal staining, while none of these measures related to TBUT or Schirmer [33]. Similarly, that American study of 10 subjects with ATD found that CB sensitivity negatively correlated with corneal staining (r = −0.46, p<0.01), but not TBUT (r = 0.25, p>0.05) [39]. Further supporting these findings, the Spanish study that examined 44 individuals with DED and 42 controls found a positive association between mechanical and chemical Belmonte thresholds and corneal staining (coefficients not provided, p<0.05), but no associations with TBUT and Schirmer [41]. Finally, an American study that examined 403 subjects with DED symptoms (DEQ5≥6; Sjögren's, GVHD, post-refractive patients excluded) found higher staining scores in individuals with corneal hyposensitivity (n=46; defined as Belmonte mechanical threshold ≥145 mL/min) compared to those with normal sensation (n=306) or hypersensitivity (n=50; defined as threshold ≤ 40 mL/min (2.40 ±2.90 vs. 2.10±2.50 vs. 1.40±1.90 respectively, p<0.05 for each)), while no differences were noted in TBUT or Schirmer scores between the 3 groups [54]. Other studies, however, reported relationships between nerves and TBUT—an American study that examined corneal nerve density in 4 regions (nasal, temporal, superior, and inferior quadrants) in 46 subjects with DED (defining specifications not provided) found that density correlated with staining (r = −0.42, r = −0.39, r = −0.36, r = −0.47; p<0.001 each) and TBUT (r = 0.57, r = 0.40, r = 0.50, r = 0.58; p<0.05 each) in all four regions, but not to Schirmer [55].

Overall, individuals with ATD have lower nerve density and sensitivity that relate to a higher degree of corneal staining. In comparison, there are inconsistencies across the literature regarding relationships between nerve parameters and TBUT or Schirmer.

## Nerve Evaluations in the Diagnosis and Treatment of DED

DED may be understood as an umbrella term, characterized by multiple phenotypes with different symptoms, signs, and nerve findings [56]. This is exemplified by the lack of consistent relationships between symptoms and signs of disease, the lack of a "gold standard" disease definition, and the overlap of nerve parameters in DED and healthy individuals [54, 57]. Given this variability, nerve evaluations should be incorporated into the workup of individuals with DED because they can help define and categorize DED phenotypes. This is especially the case for sensitivity, which can help identify contributors to symptoms and signs and provide information on the origin of symptoms.

### Nerve Definitions as They Relate to DED Phenotypes

When describing nerve parameters as they relate to DED, several terminologies arise that must be first defined.

1) *Nociceptive pain*: defined as "pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors" [58]. When applied to the ocular surface, nociceptive pain occurs due to any noxious stimuli that triggers a nociceptor response and causes a painful sensation. Tear film abnormalities (e.g., decreased tear production, high or unstable tear osmolarity, presence of inflammatory mediators), environmental factors (e.g., air pollution), abnormal ocular anatomy (e.g., pterygium), or toxicity (e.g., topical glaucoma medications) are common sources of ocular surface nociceptive pain.

2) *Neuropathic pain*: defined as "pain caused by a lesion or disease of the somatosensory nervous system" [58]. As such, neuropathic pain stems from an abnormality in the nerves themselves. This can occur due to an abnormality in peripheral sensory neurons (e.g., peripheral neuropathic pain), central neurons (e.g., central neuropathic pain), or both. Hyperalgesia and allodynia are features often seen in individuals with neuropathic pain [58, 59]. Hyperalgesia is defined as "increased or augmented pain response from a stimulus that normally **does** provoke pain," while allodynia is defined as "pain due to a stimulus that normally **does not** provoke pain," for example pain evoked by light touch to the skin (e.g., cutaneous allodynia). Secondary hyperalgesia is defined as an increase in pain sensitivity when a noxious stimulus is delivered to a region surrounding, but not including the zone of injury (increased pain sensitivity outside of the area of

injury or inflammation), and its presence suggests a central component to pain [60].

3) *Sensitization*: defined as "increased responsiveness of nociceptive neurons to their normal input, and/or recruitment of a response to normally subthreshold inputs" [58]. Sensitization is used to describe the changes in nerve function (peripheral or central) that underlie neuropathic pain.
4) *Neurotrophic keratitis* (*NK*): a phenotype that describes decreased sensitivity and corneal epithelial abnormalities (grade 1: corneal staining; grade 2: epithelial defect; grade 3: ulceration or perforation) that may or may not be accompanied by pain [29, 61].

It is important to remember that the corneal nerve pathway is dynamic and that individuals may have more than one pain type. For example, ongoing nociceptive pain (e.g., inflammatory mediators) may lead to peripheral nerve sensitization, and ongoing peripheral nerve input may lead to centralization of pain [60]. Overall, nerve hypersensitivity tends to manifest as chronic pain (i.e., neuropathic pain) while hyposensitivity often manifests with epithelial changes (i.e., neurotrophic keratitis). However, individuals can have both neurotrophic keratitis and neuropathic pain, as is seen outside the eye in individuals with painful diabetic neuropathy [62].

## Diagnostic Tests That Can Be Incorporated Into the DED Evaluation to Evaluate Nerve Structure and Function

1) *Analysis of ocular and non-ocular symptom profiles:* The DED examination begins with symptom assessment, as certain characteristics (e.g., burning, tingling, electricity-like pains, and sensitivity to light and wind) are suggestive of neuropathic etiology [63]. This can be gleaned by examining responses to specific questions within the OSDI (e.g., Q1—eyes that are sensitive to light? Q3—eyes that feel painful or sore? Q10—eyes that are uncomfortable during windy conditions? [46]) or by using an ocular pain-specific questionnaire like the NPSI-E [48••]. Also, it is important to query for the presence of systemic pain conditions like migraine or fibromyalgia, as pain often travels together [64]. Demonstrating this, an American study on 154 subjects with DED symptoms (DEQ5≥6) found that subjects with multiple comorbid pain syndromes (n=97; mean = 6.2 disorders, 3.8 pain locations) reported more severe ocular symptoms than subjects with fewer syndromes (n=57; mean = 2.5 disorders, 1.1 pain locations) using multiple scales (NPSI-E: 29.0±23.0 vs. 19.0±19.0, p=0.006; OSDI: 44.0±25.0 vs. 29.0±22.0, p<0.0005; DEQ5 13.60±3.70 vs. 11.70±3.90, p=0.004), while tear parameters were similar (TBUT: 8.90±3.80 vs. 9.40±3.60 s, p=0.39; corneal staining: 2.20±2.80 vs. 2.20±2.30, p=0.85; Schirmer 13.80±6.60 vs. 14.00±6.20 mm, p=0.87) [65•]. Similarly, an American study of 250 subjects with DED symptoms (DEQ5≥6) found that subjects with comorbid migraine (n=31) had higher NPSI-E scores (39.39±23.33 vs. 21.86±20.17, p=0.0001), light sensitivity (5.77±3.59 vs. 3.45±3.17, p=0.0001), and wind sensitivity (5.19±3.49 vs. 2.88±3.07, p=0.0001) than those without migraine, but again had similar tear parameters (TBUT: 8.35±3.59 vs. 9.61±5.02 s, p=0.39; corneal staining: 1.69±1.93 vs. 2.14±2.56, p=0.53; Schirmer: 14.15±9.04 vs. 12.93±7.32 mm, p=0.56) [66•]. This suggests that neuropathic mechanisms may contribute to painful DED symptoms in individuals with systemic pain co-morbidities.
2) *Corneal sensitivity:* Corneal sensitivity is often qualitatively assessed in the clinical setting with a cotton tip applicator or dental floss, with sensation graded on a 0–3 scale (absent, decreased, normal, increased). Corneal sensation can be evaluated centrally or in various quadrants. Increased or decreased sensitivity suggests an abnormality in the sensory pathway, but cannot determine its origin (i.e., peripheral, central, or both).
3) *Persistent pain after anesthesia:* This test assesses pain before and after topical anesthetic placement, such as proparacaine. The test often requires reworking the clinic flow as the provider must assess the patient prior to placement of topical anesthesia. The patient is first asked to grade ocular pain intensity in each eye (range 0–10), after which a drop of topical anesthetic is placed in each eye. Pain intensity (range 0–10) is re-assessed after ~30 s (some investigators wait 1–2 min). If pain persists after anesthesia, this suggests a central neuropathic or non-ocular component to pain, as peripheral nociceptors should be quieted by the anesthetic. If pain is eliminated with anesthesia, this suggests a nociceptive or peripheral neuropathic origin to the pain [67, 68]. The test is not informative if the patient does not have pain prior to anesthesia.
4) *Nerve architecture via IVCM:* IVCM provides high-resolution images of nerves (Fig. 1). However, there is no built-in software to quantify nerves, so providers must rely on qualitative assessments. As such, IVCM provides a general feel on nerve density (e.g., reduced vs. normal) and morphology (e.g., no, mild, severe tortuosity). Reduced density and tortuosity have been consistently reported in ATD [29, 69•]. One group reported that in individuals with clinically diagnosed peripheral neuropathic pain, some nerves were hyperreflective and abruptly terminated with a swelling at the nerve ending. They termed this finding microneuroma (MN) based on similar findings in animal studies [70], and found it to be a specific marker for peripheral neuropathic pain (Fig. 2) [71••]. On the other hand, other studies cited that

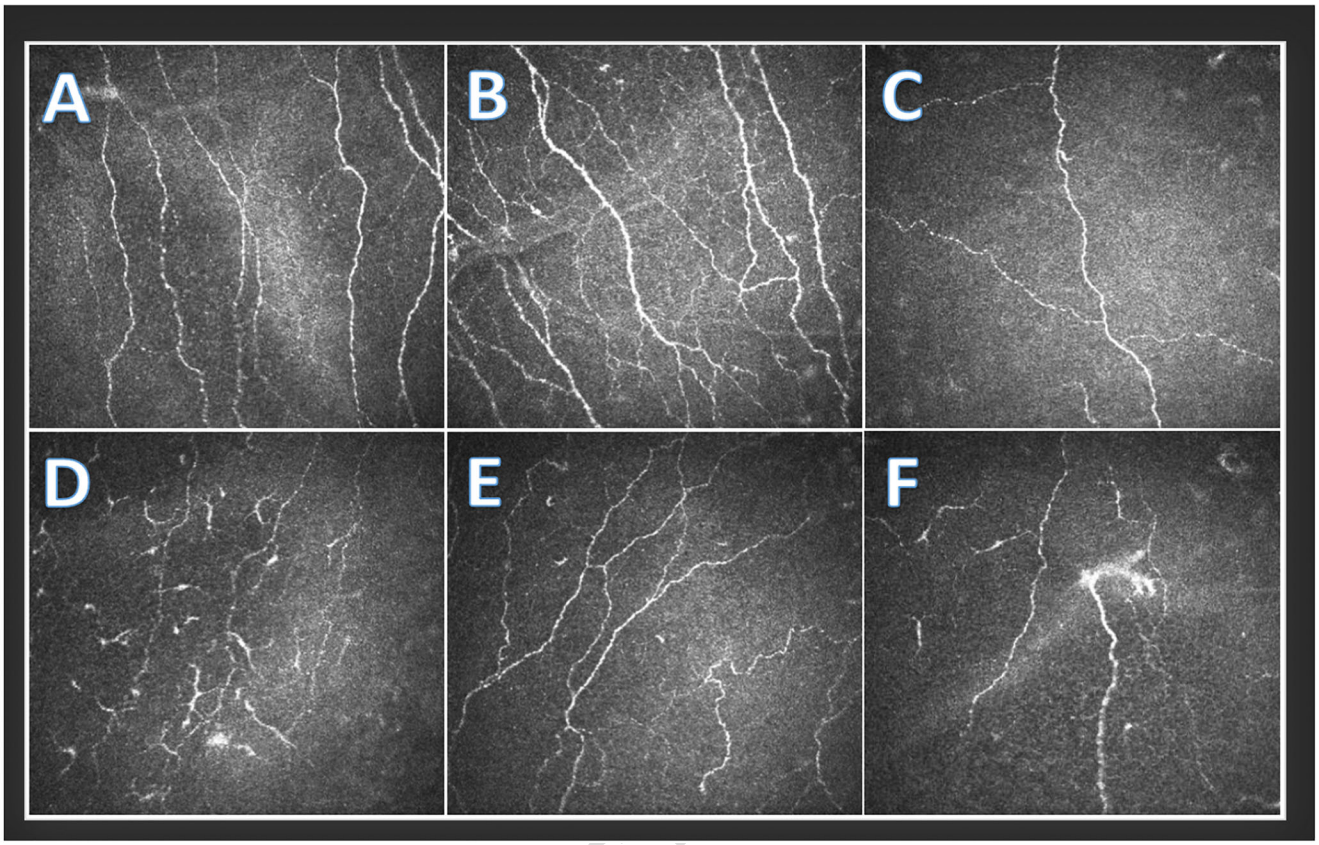


**Fig. 1** In vivo confocal microscopy of sub-basal nerves (Heidel Retina Tomograph/Cornea Rostock Module by Heidelberg Engineering, Heidelberg, Germany) depicting **A** a normal nerve pattern with no dendritic cells, **B** increased nerve branching, **C** decreased nerve density, **D** decreased nerve density and many activated dendritic cells, **E** increased nerve tortuosity, and **F** decreased nerve density, a probable microneuroma, and a few activated dendritic cells



inconsistencies in definitions for terms and use of language, limitations in IVCM imaging, and lack of standardized sampling and reporting may have led to inaccurate classification of physiological nerve characteristics as pathological microneuromas [72]. Supporting this, some researchers have described that nerves near the stroma bend 90° when entering the sub-basal nerve layer and that depending on the cut, this bend may look similar to a MN [73•]. Our group evaluated for this confocal feature in 153 subjects with DED symptoms (DEQ5≥6) and did not find correlations between abrupt termination and nerve swelling and other metrics suggestive of neuropathic pain [69•]. As such, more research is needed to determine how IVCM images can best be incorporated into the nerve evaluation.

5) *Presence of cutaneous allodynia* (*e.g.*, *pain to light touch*): The presence of cutaneous allodynia can be easily assessed by lightly touching the periocular skin surrounding the eyes and assessing for a pain response.
6) In addition to evaluating nerves, ocular surface status (TBUT, corneal staining, tear volume/production) and ocular surface anatomy should be examined. Abnormalities in these compartments often manifest as nociceptive sources of pain.

## Sub-categorizing DED Phenotypes Can Aid in Guiding Treatment Algorithms

The treatment ladder for DED begins by treating any nociceptive sources of pain (rapid TBUT, corneal staining, low tear production) using artificial tears, topical anti-inflammatories, and/or addressing underlying anatomical abnormalities (e.g., Meibomian gland dysfunction [MGD], conjunctivochalasis, pterygium) [29]. If pain persists despite these approaches, or if certain symptoms (e.g., burning, wind and light sensitivity) or comorbidities (migraine, fibromyalgia, post-surgical pain) are present, neuropathic pain should be considered (Fig. 2) [74]. Suspected neuropathic pain based on symptoms should be fully examined with the above-discussed diagnostic processes.

One common phenotype is neurotrophic keratitis (NK), which presents with decreased sensitivity and signs of corneal

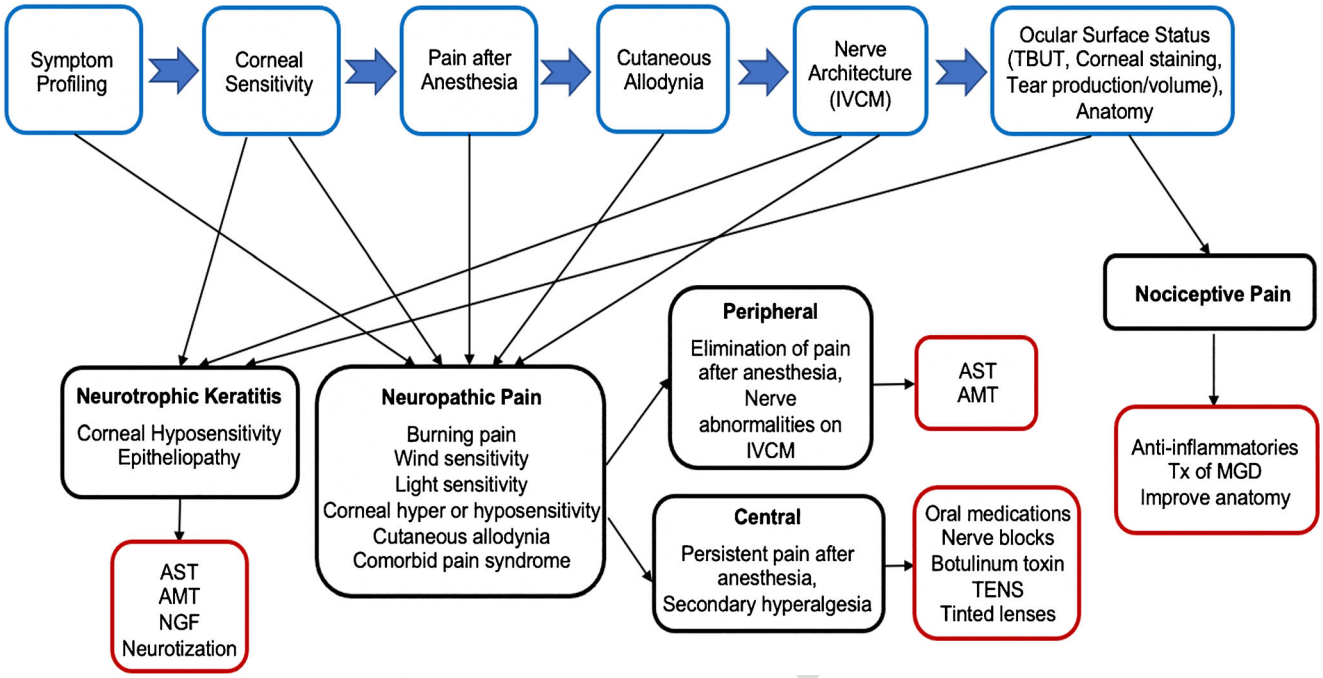


**Fig. 2** Phenotyping algorithm based on signs, symptoms, and nerve evaluation and how this can aid in clinical decision-making; with demonstrated examples for neurotrophic, neuropathic, and nociceptive profiles. IVCM, in vivo confocal microscopy; TBUT, tear break-up time; AST, autologous serum tears; AMT, amniotic membrane transplant; NGF, nerve growth factor; TENS, transcutaneous electrical nerve stimulation; Tx, treatment; MGD, Meibomian gland dysfunction

epitheliopathy (e.g., increased corneal staining). NK is often observed in the setting of diabetes, viral infection, anesthetic abuse, and after neurosurgical procedures [29, 75]. Autologous serum tears (ASTs) are helpful in treating NK [75]. For example, in a Japanese study of 11 subjects with NK treated with 20% topical AST drops 5–10 times daily, epithelial defects resolved in all eyes within 6–32 days (mean 17.10±8.0 days) and sensitivity improved on CB compared to baseline (3.00±2.29 cm vs. 1.18±1.16 cm, p<0.05) [76]. Similarly, in an American study of 6 subjects with NK, subjects treated with autologous plasma for a mean of 4.7 months (range 3–6 months) showed decreased symptoms on OSDI (39.5±11.2 to 16.8±6.0, p=0.003), increased sensitivity on CB (0.90±1.24 to 4.20±1.40 cm, p<0.0001), increased nerve count on laser-scanning IVCM (0.75±0.81 to 4.81±1.98, p=0.0004), and decreased corneal staining (values not provided, p=0.0003) at mean 5 month follow-up (range 3–6 months) [77]. Besides AST, amniotic membrane transplantation (AMT) has been used to treat individuals with NK [78]. More recently, recombinant human nerve growth factor (NGF) (Oxervate, Cenegermin, Dompe) was approved for the treatment of NK [79•, 80•]. In recalcitrant NK, neurotization is a surgical procedure that can be performed to increase sensation and improve epitheliopathy [81, 82].

Several treatment options have been studied in individuals with presumed peripheral (corneal) neuropathic pain. Like in NK, ASTs have been studied in individuals with peripheral neuropathic pain. In 16 individuals with light sensitivity with a presumed neuropathic component (decreased nerve length/count, normal slit-lamp exam), treatment with AST (mean 3.80±0.50 months, range 1–8) decreased pain severity (9.10 ±0.20 to 3.10±0.30, p<0.0001) and increased nerve count (10.50±1.40 to 15.10±1.60 nerves/frame, p<0.0001) [83]. Besides AST, AMT has also been studied in individuals with peripheral pain—an American study of 9 patients who received AMT (mean retention time 6.4 ± 1.1 days) reported reduced pain scores (6.3 ± 0.8 to 1.9 ± 0.6, p = 0.0003) and increased nerve density on laser-scanning IVCM (17,700.9 ± 1315.7 to 21,891.3 ± 2040.5 μm/mm$^2$, p = 0.05) [84]. While there is interest, no data are available on the use of recombinant NGF for the treatment of peripheral neuropathic pain. Finally, several agents have been evaluated in animal models and are now making their way to the clinical realm. For example, TRPV1 antagonists mitigated capsaicin (50 μL 0.02%) induced ocular pain in animal models [85]. A clinical trial in humans with post-refractive pain is underway.

In individuals with a suspected central component to pain (persistent pain after anesthesia, cutaneous allodynia, comorbid fibromyalgia or migraine), oral medications including α2γ ligands (gabapentin or pregabalin), selective serotonin-norepinephrine reuptake inhibitors (e.g., duloxetine), and/or tricyclic antidepressants (e.g., nortriptyline) can be considered [29]. In a case series of 8 subjects with presumed neuropathic ocular pain (pain out of proportion, poor response to topical therapies), gabapentin (starting 300 mg daily, escalation to 600–900 TID) and pregabalin (starting 75 mg daily, escalation

to 150 mg BID) led to complete pain relief in 2 subjects (NRS = 0 on a 0–10 scale), marked improvement in 3 subjects (NRS ≤ 2), and slight improvement in 1 subject (NRS = 10 to 7), while 2 subjects had no improvement in pain. The 2 subjects who noted complete pain relief were also on concomitant duloxetine (starting 20 mg, escalation to 60 mg daily) [86]. In a British study, 25 subjects with clinically diagnosed peripheral neuropathic pain were treated with nortriptyline (10–25 mg starting dose, escalation to 100 mg daily) which led to lower pain at 4 weeks post-treatment (via NRS; 3.80±2.39 vs. 6.36±2.18, p<0.0001). Overall, 84% of subjects (n = 21) reported pain improvement (28% with >50% improvement (n = 7), 40% with 25–50% improvement (n = 10), and 32% with <25% improvement (n = 8)) [87].

In individuals with cutaneous allodynia who fail or are intolerant to oral medications, nerve blocks may be utilized. This modality entails long-term reversible blockade of depolarizing sodium channels, which prevents generation of action potentials involved in propagating the pain signal, combined with a long-acting corticosteroid to strengthen the effects [88]. A case series reported on outcomes of 11 subjects with presumed neuropathic ocular pain after periocular (supraorbital, supratrochlear, infratrochlear, and infraorbital) nerve blocks (4 mL of 0.5% bupivacaine with 1 mL of 80 mg/mL methylprednisolone acetate). In total, 7 subjects experienced pain relief, lasting from hours to months [86].

In individuals with comorbid headache and light sensitivity, migraine treatments can be initiated, such as botulinum toxin A (BoNT-A) or transcutaneous electrical nerve stimulation (TENS) [89, 90]. In a study of 76 individuals with chronic migraine who received BoNT-A (100–150 units every 3 months), improvements in photophobia scores were noted following BoNT-A (via Visual Light Sensitivity Questionnaire-8 (VLSQ-8); 3.37 vs. 4.89, p<0.001). Furthermore, dry eye symptoms significantly improved but only in the subset of patients with severe DED symptoms at baseline (DEQ5 score ≥12; n=38) (via DEQ5; 13.80±4.02 vs. 15.40±2.47, p=0.03) [91]. Similarly, a study evaluating the efficacy of TENS found that an in-office 30-minsession improved ocular pain in an open-label fashion in 14 individuals with chronic ocular pain. Overall, mean pain intensity was reduced 5 min post- vs. pre-treatment (0–10 NRS: right eye 4.54±3.20 to 1.92±2.50, p=0.01; left eye 4.46±3.36 to 2.00±2.38, p=0.01) [92]. Tinted lens spectacles that block out specific wavelengths of light (~480 nm) are also helpful in managing individuals with photophobia [93], including those with comorbid migraine [94].

Importantly, individuals with ocular pain are often found to have an emotional component to their symptoms [95]. Clinicians must pair with other members of the care team to address the emotional consequences of chronic pain. Cognitive behavioral therapy (CBT) [96, 97], medications, and acupuncture [98] have all been used to target chronic pain–related depression and anxiety for pain conditions outside the eye and thus may be helpful in individual with ocular pain. Finally, all of the therapies listed above can be used concomitantly with traditional DE medications.

## Conclusion

DED is an umbrella term applied to individuals with a wide range of symptoms and signs. The various phenotypic presentations of DED are due, in part, to individual differences in nerve function. Tests that can be used to evaluate nerve status, including structure and function, should be incorporated into the clinical examination of individuals with DED as this can aid in disease sub-typing. This in turn can guide therapeutic decision-making, especially in individuals who do not respond to first-line treatment modalities.

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## Compliance with Ethical Standards

**Human and Animal Rights** All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

**Conflict of Interest** The authors declare no competing interests.

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- • Of importance
- •• Of major importance

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# AUTHOR QUERIES

**AUTHOR PLEASE ANSWER ALL QUERIES.**

Q1. Please check if the author names and affiliations are captured and presented correctly.
Q2. Please check if the sentence “Finally, an American study that examined...” is presented correctly.
Q3. Please check if section headings are assigned to appropriate levels.
Q4. Please check if the Acknowledgements statement is presented correctly.
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American Journal of Ophthalmology

# Dry eye symptoms and signs in US veterans with Gulf War Illness

--Manuscript Draft--

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## Abstract

**Purpose**: To examine dry eye (DE) symptoms and signs in individuals with versus without Gulf War Illness (GWI).

**Methods**: We performed a prospective, cross-sectional study of South Florida veterans who were active duty during the Gulf War Era (GWE; 1990-91) and seen at an eye clinic between October 1, 2020, and March 13, 2021. Veterans were split into two groups: those who met Kansas criteria for GWI (cases, N=30) and those who did not (controls, N=41). DE symptoms were assessed via standardized questionnaires while DE signs were assessed using a series of ocular surface parameters. Differences between groups were assessed via Mann-Whitney U test. Linear regressions analyses were used to examine which GWI symptoms most closely aligned with DE symptoms.

**Results**: Veterans with GWI had higher DE symptoms scores compared to controls (Ocular Surface Disease Index (OSDI) scores: mean 41.20±22.92 vs 27.99±24.03, p=0.01). In addition, veterans with GWI had higher eye pain scores compared to controls (average eye pain over past week: 2.63±2.72 vs 1.22±1.50, p=0.03), including on neuropathic ocular pain questionnaires (Neuropathic Pain Symptom Inventory- modified for the Eye (NPSI-E): 17.33±17.20 vs 9.63±12.64, p=0.03). DE signs were mostly similar between the groups. GWI symptoms “nausea or upset stomach” (β=14.58. SE=3.02, p<0.001) and “headache” (β=7.90, SE=2.91, p=0.011) correlated with higher OSDI scores.

**Conclusion**: Individuals with GWI have more severe DE symptoms and ocular pain scores but similar tear and ocular surface parameters compared to controls without GWI.

This finding suggests that mechanisms beyond tear dysfunction drive eye symptoms in GWI.

July 15, 2021

Richard Parrish II, MD
Editor-in-Chief
American Journal of Ophthalmology

Dear Dr. Parrish II,

Thank you for the opportunity to submit a manuscript entitled, "*Dry eye symptoms and signs in US veterans with Gulf War Illness*" for consideration of publication in *American Journal of Ophthalmology*.

Our study is the first prospective study to examine dry eye (DE) symptoms and signs in veterans with Gulf War Illness (GWI). The results have the potential to elucidate the pathophysiology of DE symptoms in those affected by Gulf War Illness and to inform their treatment in clinical settings. In addition, and of particular interest to the ophthalmologist, our study adds to the growing list of disorders (e.g. migraine, fibromyalgia) associated with DE symptoms but not signs. The pathophysiology of these disorders shed light onto the possible contribution of nerve abnormalities to DE symptoms in these individuals.

The study was funded by the Department of Defense, but the authors do not have any individuals funding sources or conflicts of interest to report.

All authors have participated in either the study concept and design, analysis and interpretation of data, or drafting of the manuscript. All authors have approved the manuscript as submitted. The manuscript is being submitted only to the *American Journal of Ophthalmology* for publication. It has not been previously submitted or published elsewhere.

Thank you for your consideration of our work.

Sincerely

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**Title**: Dry eye symptoms and signs in US veterans with Gulf War Illness

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**Introduction:**

Upon their return from 1991 Gulf War (GW), many veterans began suffering from a wide range of health symptoms of unknown etiology. These symptoms, which include fatigue, headaches, cognitive dysfunction, musculoskeletal pain, and gastrointestinal and respiratory complaints have come to be categorized as Gulf War Illness (GWI). GWI is a multisystem disease estimated to affect 25% of Gulf War Era veterans.[1] The etiology of GWI is unknown, however, chemical exposures have been postulated as potential causes of GWI.[2,3] For example, one study of 304 Gulf War veterans found an independent association between veterans who reported use of uniforms with pesticides (OR: 2.91, $p<0.05$, 95% CI: 1.41, 6.01) and pyridostigmine bromide (PB) pills (prescribed to protect against acute effects of nerve agents during wartime) (OR: 2.88, $p<0.05$, 95% CI: 1.68, 4.94) and GWI.[4] Chemical exposure can affect the central nervous system (CNS), as demonstrated by a study of 80 Gulf War (GW) veterans that found significantly reduced total gray matter and hippocampal volumes in individuals exposed vs unexposed to sarin and cyclosarin gas. Combining these findings, one hypothesis is that symptoms of GWI are driven by CNS abnormalities that occurred secondary to exposures during the the Gulf War.[5]

GWI symptoms encompass 6 major domains which include cognitive and sleep, pain, neurologic and cognitive, respiratory, gastrointestinal, and skin. Encompassed within these domains, several organ systems can be involved in GWI including the brain, musculoskeletal system, and gastrointestinal tract.[6] Only a few studies have examined eye involvement in GWI. One study of 1,844 Gulf War veterans found an increased

likelihood of photophobia (OR: 2.62, 95% CI: 1.84, 3.74) and blurred or double vision (OR: 2.49, 95% CI: 1.55, 4.00) in GW-era veterans who were deployed in the Persian Gulf War compared to controls (undeployed GW-era veterans).[6] In a previous retrospective study of 145 GW veterans, we found that dry eye (DE) symptoms were significantly more frequent in individuals diagnosed with GWI compared to controls (GW veterans without GWI) (50% vs. 33%, p=0.04). DE signs, however, were similar between the groups. Individuals with impaired cognition, however, had a higher frequency of DE symptoms and signs compared to controls. This is not entirely surprising as DE symptoms and signs have been associated with other diseases that share similarities to GWI such as fibromyalgia and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS).[7] However, a major limitation of our previous study was its retrospective nature and non-standardized assessments of DE symptoms and signs.

To build on our previous observation, we prospectively examined Gulf War veterans and comprehensively profiled them for symptoms and signs of DE. We hypothesized that veterans with GWI would have more severe DE symptoms, but similar DE signs, compared to GW veterans without GWI. We also hypothesized that GWI sub-types with “severe cognitive impairment” would most closely associate with DE symptoms and signs. Characterizing the relationship between DE and GWI can serve to elucidate the pathophysiology of GWI and potentially improve treatment algorithms for the disease.

## Materials and Methods

### Study population and Gulf War Illness Diagnosis

The study population included 71 prospectively enrolled Gulf War veterans seen at the Miami Veterans Affair Hospital GWI Clinic between October 1, 2020 and May 30, 2021. Participants were identified based on birthday and a history of being active duty during the 1990-91 Gulf War. Study exclusions included the use of topical medications (e.g. glaucoma medications), devices (e.g. contact lens use), and anatomical abnormalities (e.g. pterygium) that could confound DE. Participants were enrolled in the study after informed consent was signed. The diagnosis of GWI was made using the Kansas criteria which requires: symptoms that started during or after deployment and were present in the year prior to assessment, and one severe or two moderate symptoms in at least three of six domains, including (1) fatigue, (2) pain, (3) neurologic and mood, (4) gastrointestinal, (5) respiratory, and (6) skin.[6] Veterans who met this criteria and were deployed during the 1991 Gulf War were placed in the GWI group. Veterans who were active duty but not deployed to the Gulf War or were deployed but did not meet the Kansas Criteria were included in the control group.

Individuals with GWI were further sub-typed based on reported symptoms. In our study, we identified individuals with “severely impaired cognition” syndrome if they had at least 5 out of 6 of the following symptoms: problems with memory, feelings of irritability/angry outbursts, headaches, depression, difficulty concentrating, and trouble finding words when speaking. We also identified individuals with “musculoskeletal symptoms” based on the validated Widespread Pain Index (WPI)/Symptom Severity

Scale (SS).[8] Veterans who met the criteria based on GWI and subsequently scored 7 or greater on the WPI and 5 or greater on the SS or 3-6 on the WPI and 9 or greater on the SS were subtyped as having musculoskeletal symptoms. The study was approved by the Miami VA Institutional Review Board (IRB). The study was conducted in accordance with the principles of the Declaration of Helsinki and complied with the requirements of the United States Health Insurance Portability and Accountability Act.

**Data collected**

DE symptoms and signs were assessed on the same clinic visit after individuals signed informed consent. DE symptoms were assessed using the Ocular Surface Disease Index (OSDI, range 0-100)[9] and 5-Item Dry Eye Questionnaire (DEQ-5, range 0-22).[10] Ocular pain intensity was graded using a numerical rating scale (NRS, range 0-10) and using the Neuropathic Pain Symptom Inventory modified for the Eye (NPSI-E, total score: range 0-100; sub-score range 0-10).[11] NRS scores were acquired for pain felt “right now,” “averaged over the last week,” and “worst over the last week.” Convergence insufficiency was assessed using the Convergence Insufficiency Symptoms Survey (CISS, 0-60).[12]

DE signs included, in the order assessed, Inflammadry (Quidel, San Diego), tear break-up time (TBUT), fluorescein corneal staining, pain intensity rating pre and post anesthetic placement with proparacaine hydrochloride 0.5%, anesthetized Schirmer’s test at 5 min, and eyelid and Meibomian gland. InflammaDry is a point of care test that measures MMP-9 presence on the ocular surface.[13] The intensity of the pink stripe was qualitatively graded as none, mild, moderate, or severe. TBUT was measured three

times in each eye after instilling 5 µl of fluorescein dye and values averaged. For corneal staining, the cornea was divided into five areas and staining was graded in each area on a scale of 0=none to 3=severe, and the scores summed based on the National Eye Institute scale.[14] For pain pre and post anesthesia, subjective eye pain was assessed using a 10-point NRS before prior to and 30 seconds after application of 10 µL of proparacaine. Schirmer's test was performed with anesthesia and measured at 5 minutes. Eyelid vascularity was graded on a scale of 0 to 3 (0 none; 1 mild engorgement; 2 moderate engorgement; 3 severe engorgement) and meibum quality on a scale of 0 to 4 (0 = clear; 1 = cloudy; 2 = granular; 3 = toothpaste; 4 = no meibum extracted). Inferior Meibomian gland dropout was graded to the Meiboscale based on Lipiscan (Johnson & Johnson, New Brunswick, NJ) images.[15] DE signs were assessed by a provider that was masked to the clinical symptoms for each patient.

**Data analysis**

Statistical analysis was performed using SPSS 24.0 (IBM Corp, Armonk, NYU) statistical package. Descriptive statistics were used to summarize patient demographic and clinical information. Normality of the data was assessed using the Kolmogorov-Smirnov test. Given that some values did not fit a normal distribution, Mann Whitney U tests was used to calculate differences in continuous variables. The more severe value from each eye was used when examining DE signs. Chi square or Fischer's exact test were used, as appropriate, for categorical variables. After examining residuals, we examined which GWI symptoms associated with DE symptom scores (DEQ-5 and OSDI)

through linear regression analyses. Reported p-values were two-tailed and $p<0.05$ was considered significant.

**Results:**

**Study population**

Our population included 71 individuals who were active duty during the 1990-1991 Gulf War Era. The mean age for the population was 54.24 $\pm$ 4.30 years and 91.5% self-identified as male gender and 59.2% as White. When separated by a GWI diagnosis, 30 individuals were grouped as GWI and 41 as controls. Demographics were similar between the groups with the exception of male gender whose frequency was higher among controls (Table 1).

**DE symptoms and ocular pain in the GWI and control groups**

Ocular symptoms were assessed in all participants (Table 2). The GWI group had higher DE symptoms scores compared to controls, including significantly higher OSDI scores and marginally higher DEQ-5 scores. GWI veterans also reported higher ocular pain scores, both at the time of survey and in the prior week, as compared to controls. Individuals with GWI also had higher total NPSI-E scores, as well as “burning” and “evoked” pain sub-scores, compared to controls.

**Dry eye signs in the GWI and control groups**

DE signs were overall similar between the GWI and control groups (Table 3). The exception was meibomian gland drop-out graded on Lipiscan (Johnson & Johnson, New

Brunswick, NJ) images which was significantly higher in the GWI cohort compared to controls (2.27 ± 1.26 vs 1.66 ± 1.30, p=0.048).

**GWI subtypes and DE signs and symptoms**

The GWI group was subclassified to participants with symptoms of “severely impaired cognition” in their GWI presentation. Of the 30 participants in the GWI cohort, 25 met criteria for “severely impaired cognition” while 5 did not. DE symptoms and signs and were compared across groups. DE symptoms, via the OSDI, were higher in GWI veterans with “severely impaired cognition” compared to those without (45.57 ± 20.76 vs 19.30 ± 21.67, p=0.03) and to controls (27.99 ± 24.04, p=0.002). DEQ-5 scores were similar between the two GWI groups (10.24 ± 4.26 vs 8.25 ± 6.95, p=0.74). Neuropathic ocular pain scores, via the NPSI-E, also tended to be higher in individuals with GWI and “severely impaired cognition” compared to those without (19.84 ± 17.50 vs 4.80 ± 8.56, p=0.07) and significantly higher than controls (9.63 ± 12.64, p=0.002). However, DE signs were again similar between the 3 groups.

In a similar manner of 30 individuals who met GWI criteria, 19 met criteria for “musculoskeletal symptoms” while 11 did not. Veterans in the “musculoskeletal” group had higher DEQ-5 (10.74 ± 4.23 vs 7.83 ± 4.57, p=0.02) and OSDI (47.24 ± 20.10 vs 28.12 ± 24.33, p=0.002) scores compared to controls but only slightly higher scores compared to the GWI “no musculoskeletal” group (DEQ-5: 10.74 ± 4.23 vs 8.50 ± 5.15, p=0.29, OSDI: 47.24 ± 20.10 vs 30.74 ± 24.34, p=0.10). However, the GWI “musculoskeletal” cohort had higher NPSI-E total scores compared to the “no musculoskeletal” group (22.16 ± 17.62 vs 9.0 ± 13.36, p=0.02) and to controls (22.16 ±

17.62 vs 9.57 ± 12.79, p=0.002). The GWI "musculoskeletal" group also had higher CISS scores compared to both the GWI "no musculoskeletal" group (30.0 ± 11.25 vs 14.80 ± 12.75, p=0.006) and to controls (30.0 ± 11.25 vs 19.08 ± 12.85, p=0.003). Again, DE signs were similar across the 3 groups.

**Predicting DE symptoms based on GWI presentation**

To study which GWI symptoms most closely aligned with DE symptoms, we performed multiple linear regression analysis using only veterans in the GWI group with DE symptom scores (OSDI or DEQ-5) as the dependent variable and GWI symptoms on the Kansas questionnaire as the independent variables. GWI symptoms that predicted a higher OSDI score were "Nausea or upset stomach" and "headaches" (n=29; $R^2$=0.55 for model, Table 4). Symptoms that remained in the final model for DEQ-5 were "eyes very sensitive," "moderate or multiple neurological symptoms," and "symptomatic response to chemicals, odors" (n=28, $R^2$=0.53 for model, Table 4).

## Discussion

In summary, we found that veterans with GWI reported more severe DE symptoms, including neuropathic like symptoms, but similar DE signs compared to controls. Notably, only one sign, meibomian dropout was significantly higher in the GWI group compared to controls. These findings are consistent with our previous work that found a similar pattern, albeit with a weaker study design.[16] When examining only individuals with GWI, we found that among the myriad of GWI symptoms, it was the neurologic symptoms that most closely related to DE symptoms. Our findings suggest that mechanisms beyond tear dysfunction, such as neurological abnormalities, drive DE symptoms in individuals with GWI.

In fact, neurological abnormalities have been suggested to be a major contributing mechanism in GWI. First, several symptoms suggestive of underlying neuro-abnormalities are included in the Kansas criteria[6], such as memory deficits and headaches. Second, magnetic resonance imaging (MRI) has detected neuro-abnormalities in individuals with vs without GWI. For example, one study examined 293 GW veterans and found that veterans diagnosed with GWI had lower intracranial volume-adjusted (ICV-adjusted) basal ganglia volumes compared to healthy controls ($2.54 \times 10^8$ vs $3.16 \times 10^8$, $p<0.001$).[17] Interestingly, the basal ganglia is one structure implicated in modulating nociceptive information.[18] Another study focused on pain pathway abnormalities in GWI using functional MRI (fMRI). In this study, 55 GW veterans were exposed to a noxious heat stimuli applied to the ventral inner forearm of the right arm. Individuals with GWI (both with mild cognitive impairment (n=11) and severe confusion-ataxia (n=17)) exhibited hyper-activation in regions involved in pain

processing (bilateral S1, S2, insula, inferior parietal lobule, supplementary motor area) as compared to controls.[19] A clinical correlate of an increased response to non-noxious and noxious stimuli was seen in our population, with individuals with GWI reporting more evoked pain to light and wind compared to controls.

Animal models have also been used to study the link between neuro-abnormalities and GWI. One group exposed rodents to pyridostigmine bromide (PB), N,N-diethy-m-toluamide (DEET), and permethrin – chemicals believed to contribute to the development of GWI and then put the animals in a stressful situation (i.e. a restraint).[3] Rodents exposed to both chemicals and stress showed greater neuronal apoptosis on H&E stain in the cingulate cortex ($p<0.001$), dentate gyrus ($p<0.001$), lateral dorsal nucleus of the thalamus ($p<0.001$), and dorsomedial nucleus of the hypothalamus ($p<0.001$) as compared to rodents exposed to chemical alone, stress alone, or controls.[20] Again, this experiment links GWI to pain as the cingulate cortex[21] and thalamus[22] regions are both associated with pain processing. Extended to DE symptoms and eye pain, these same abnormalities in various CNS locations may explain differences in spontaneous and evoked eye symptoms (e.g. dryness and pain) in our GWI population (Figure).

One facet that has been proposed to drive neuro-degeneration in GWI is neuroinflammation. One study applied Positron Emission Tomography (PET) to the study of neuro-inflammation in GWI. This study used a radioligand, [$^{11}$C]PBR28, which binds to 18-kDA translocator protein (TSPO), a neuroinflammatory marker that is upregulated in activated microglia/macrophages and astrocytes. Veterans with GWI (n=15) showed elevations in [$^{11}$C]PBR28 standardized volume uptake (SUVR) in the

precuneus, prefrontal, and primary motor and somatosensory cortices as compared to healthy controls (n=33). Notably, serum analysis of inflammatory cytokines including TNF-α, IL-6 and IL-1β were similar between the GWI and control groups[23], suggesting that central vs peripheral inflammatory mechanisms drive disease manifestations. Further supporting these findings, another study assayed neuro-inflammation markers in the serum of 20 veterans with GWI and 10 non-veteran controls. Veterans with GWI had elevated autoantibodies to neural specific proteins including a 6.60 fold increase in GFAP ($p<0.001$), 9.27 fold increase in CaMKII ($p<0.001$), 2.45 fold increase in NFP ($p=0.02$) when analyzed via Western blot.[24] Again, neuroinflammation, leading to central neuronal abnormalities, may explain the more intense DE symptoms, eye pain, hyperalgesia and allodynia seen in our GWI groups, but the similar levels of ocular surface inflammation (measured via MMP-9) (Figure).

As with all studies, our findings should be considered in light of the study limitations, which includes a geographically restricted sample size and the self-reported nature of both DE and GWI symptoms. However, a strength of the study is its prospective nature, with a detailed assessment of both DE and GWI symptoms and DE signs. The novelty of the study is in examining the characteristics of DE in our patient population and determining that the disease in individuals with GWI is defined more by symptoms than by ocular surface abnormalities. This has implications for treatment as improving tear parameters with traditional means (artificial tears, topical anti-inflammatories) may not benefit this population. A better understanding of contributors to DE symptoms is needed to improve treatment algorithms. For example, given potentially central mechanisms, therapies that impact central nerves, such as α2γ

ligands, tricyclic antidepressants (TCAs), and anticonvulsants may be more appropriate treatments.[25-27] Anti-inflammatory therapeutics targeting CNS pathways may be another avenue to reduce both ocular and non-ocular symptoms in GWI.[28] Future work is needed to examine serum and CNS markers of inflammation in our population and examine the effects of oral medications targeting these mechanisms on eye symptoms in our population.

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## Figure Legend

**Figure:** This schematic model links dry eye (DE) symptoms to potential Gulf War Illness (GWI) contributors. Inciting factors in GWI, such as pyridostigmine bromide (PB) exposure, can lead to neuro-inflammation, as evidenced by elevated levels of microglia/astrocyte markers in the precuneus, prefrontal, and primary motor and somatosensory cortices and neural-specific antibodies in serum. This neuro-inflammation can result in a number of abnormalities including neuronal apoptosis († areas include cingulate cortex, dentate gyrus, thalamus, hypothalamus), decreased basal ganglia volume, and abnormal responses to stimuli in brain regions associated with pain processing (*bilateral S1, S2, insula, inferior parietal lobule, supplementary motor area). These neuronal abnormalities may result in abnormal nociceptive responses within the corneal pain pathway (trigeminal nerve (V1), trigeminal ganglia (TG), trigeminal nucleus caudalis (TNC), thalamus (red lines), S1, insula, etc.) leading to spontaneous pain (i.e. sensations of dryness, burning) and evoked pain to stimuli such as wind and light, in the absence of ocular surface pathology.

**Table 1. Demographic information for the GWI and control groups**

| | GWI (n = 30) | Control (n= 41) | P-value |
|---|---|---|---|
| **Age (mean years ± SD)** | 54.10 ± 4.14 | 54.34 ± 4.46 | 0.79 |
| **Male gender, n (%)** | 83% | 98% | 0.03* |
| **White, n (%)** | 60% | 59% | 0.47 |
| **Hispanic ethnicity, n (%)** | 40% | 54% | 0.19 |

*GWI* Gulf War Illness, *Control* Individuals who served in 1990-91 who do not meet the criteria for GWI, *SD* standard deviation, *n* number in group. *Statistically significant difference at p-value < 0.05.

**Table 2. Dry eye (DE) questionnaire scores in the GWI and control group**

| | GWI (n = 30) | Control (n= 41) | P-value |
|---|---|---|---|
| **Dry Eye Symptoms (mean ± SD)** | | | |
| OSDI | 41.20 ± 22.92 | 27.99 ± 24.03 | 0.01* |
| DEQ-5 | 9.97 ± 4.60 | 7.90 ± 4.54 | 0.06 |
| **Ocular Pain (mean ± SD)** | | | |
| NRS (Right Now) | 2.40 ± 2.93 | 0.73 ± 1.21 | 0.02* |
| NRS (Last Week Average) | 2.63 ± 2.72 | 1.22 ± 1.50 | 0.03* |
| NRS (Last Week Worst) | 2.97 ± 3.17 | 1.61 ± 1.99 | 0.09 |
| NPSI-E Total | 17.33 ± 17.20 | 9.63 ± 12.64 | 0.03* |
| NPSI-E Burning | 1.90 ± 2.60 | 1.61 ± 2.70 | 0.45 |
| NPSI-E Pressing | 2.07 ± 2.30 | 0.78 ± 1.51 | 0.008* |
| NPSI-E Paroxysmal | 1.20 ± 1.85 | 0.83 ± 1.75 | 0.09 |
| NPSI-E Evoked | 2.31 ± 2.32 | 1.18 ± 1.66 | 0.02* |
| NPSI-E Paresthesia/Dysesthesia | 0.98 ± 1.70 | 0.63 ± 1.30 | 0.10 |
| **Light sensitivity n (%)** | | | |
| NPSI-EQ9 Provoked by light | 70% | 44% | 0.03* |
| **Binocular function (mean ± SD)** | | | |
| Convergence Insufficiency | 24.76 ± 13.7 | 19.33 ± 12.78 | 0.10 |

*GWI* Gulf War Illness, *OSDI* Ocular Surface Disease Index *DEQ-5* Dry Eye Questionnaire 5 *NRS* Normal Rating Scale *NPSI-E* Neuropathic Pain Symptom Inventory-Eye, *SD*

standard deviation, *n* number in group. *Statistically significant difference at p-value < 0.05.

**Table 3. Dry eye (DE) signs in the GWI and control groups**

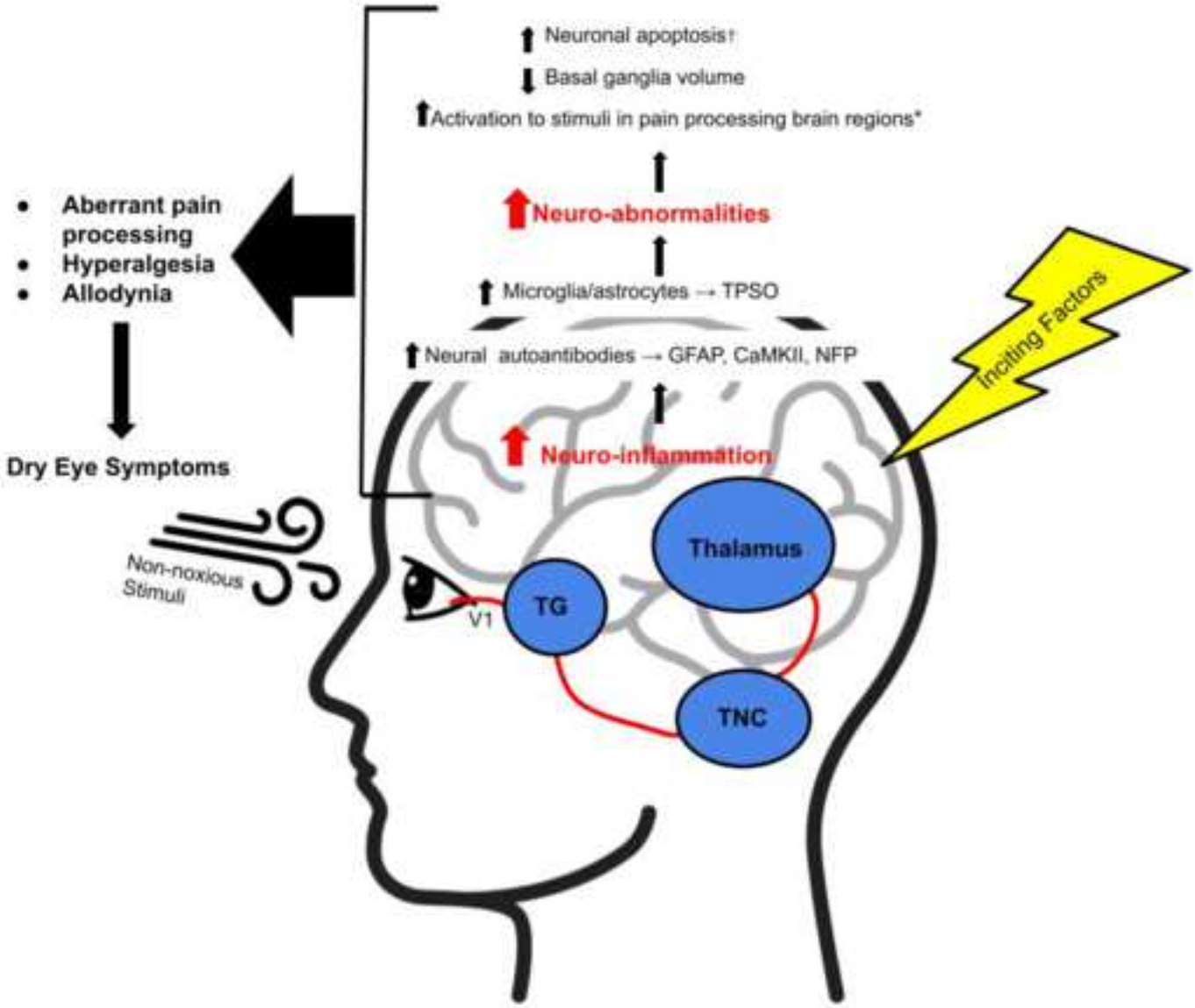
| DE signs | GWI (n = 30) | Control (n= 41) | P-value |
|---|---|---|---|
| **MMP9, n (%)** | 73% | 73% | 0.99 |
| **MMP, mean ± SD** | 1.13 ± 0.97 | 1.10 ± 0.89 | 0.99 |
| **TBUT**, seconds | 8.48 ± 3.92 | 8.88 ± 4.65 | 0.75 |
| **Corneal staining** | 0.73 ± 1.11 | 1.24 ± 2.44 | 0.98 |
| **Schirmer's test**, mm wetting | 13.37 ± 9.25 | 14.78 ± 8.25 | 0.42 |
| **Any eye pain prior to anesthesia, n (%)** | 53% | 41% | 0.98 |
| **Any persistent pain after anesthesia, n (%)** | 33% | 22% | 0.29 |
| **Eyelid telangiectasias** | 0.67 ± 0.92 | 0.51 ± 0.71 | 0.65 |
| **Meibum quality** | 1.23 ± 0.94 | 1.0 ± 0.81 | 0.29 |
| **Meibomian gland drop-out** | 2.27 ± 1.26 | 1.66 ± 1.30 | 0.048* |

*GWI* Gulf War Illness, *Control* Individuals who served in 1990-91 who do not meet the criteria for GWI, *TBUT* Tear break-up time, *MMP-9* ocular surface matrix metalloproteinase 9, *SD* standard deviation, *n* number in group *Statistically significant difference at p-value < 0.05.

**Table 4. GWI symptoms as predictors of DE Questionnaire scores**

| Predictor | **β** | **SE** | **P-Value** |
|---|---|---|---|
| **OSDI** | | | |
| Nausea or upset stomach | 14.58 | 3.02 | 0.000 |
| Headaches | 7.90 | 2.91 | 0.011 |
| **DEQ5** | | | |
| Eyes very sensitive | 2.84 | 0.74 | 0.001 |
| Moderate or multiple neurological symptoms | 3.02 | 0.86 | 0.002 |
| Symptomatic response to chemicals, odors | -2.11 | 0.83 | 0.02 |

*GWI* Gulf War Illness *OSDI* Ocular Surface Disease Index *DEQ-5* Dry Eye Questionnaire 5, β Beta coefficient for dependent variables, *SE* standard error for β Statistically significant difference at p-value < 0.05.



## Highlights

- Veterans with Gulf War Illness (GWI) have more severe dry eye (DE) symptoms, including neuropathic eye pain questionnaire scores, compared to controls who served during the Gulf War but did not meet criteria for GWI.
- Dry eye signs were mostly similar between the groups.
- Certain GWI symptoms (neurological, gastrointestinal) correlated with more severe DE symptoms.

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| **Time frame: Since the initial planning of the work** | | | |
| 1 | All support for the present manuscript (e.g., funding, provision of study materials, medical writing, article processing charges, etc.) **No time limit for this item.** | __x__ None | |
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| **Time frame: past 36 months** | | | |
| 2 | Grants or contracts from any entity (if not indicated in item #1 above). | __x__ None | |
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| 3 | Royalties or licenses | __x__ None | |
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| 4 | Consulting fees | __x__ None | |
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| 5 | Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events | __x__ None | |
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| 6 | Payment for expert testimony | __x__ None | |
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| 7 | Support for attending meetings and/or travel | __x__ None | |
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| 8 | Patents planned, issued or pending | _x___ None | |
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| 9 | Participation on a Data Safety Monitoring Board or Advisory Board | __x__ None | |
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| 10 | Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid | __x__ None | |
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| 11 | Stock or stock options | __x__ None | |
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| | | | |
| 12 | Receipt of equipment, materials, drugs, medical writing, gifts or other services | __x__ None | |
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| | | | |
| 13 | Other financial or non-financial interests | __x__ None | |
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**Please place an "X" next to the following statement to indicate your agreement:**

**_x__ I certify that I have answered every question and have not altered the wording of any of the questions on this form.**

# ICMJE DISCLOSURE FORM

**Date:**___7/16/2021_____
**Your Name:**_ Kimberly Cabrera_____
**Manuscript Title:**____ Dry eye symptoms and signs in US veterans with Gulf War Illness _____
**Manuscript number (if known):**_____

**In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.**

**The following questions apply to the author's relationships/activities/interests as they relate to the current manuscript only.**

**The author's relationships/activities/interests should be defined broadly. For example, if your manuscript pertains to the epidemiology of hypertension, you should declare all relationships with manufacturers of antihypertensive medication, even if that medication is not mentioned in the manuscript.**

**In item #1 below, report all support for the work reported in this manuscript without time limit. For all other items, the time frame for disclosure is the past 36 months.**

| | | Name all entities with whom you have this relationship or indicate none (add rows as needed) | Specifications/Comments (e.g., if payments were made to you or to your institution) |
|---|---|---|---|
| **Time frame: Since the initial planning of the work** | | | |
| 1 | All support for the present manuscript (e.g., funding, provision of study materials, medical writing, article processing charges, etc.) **No time limit for this item.** | ____ None<br>Department of Defense | Provides funding for the project from which this data is gathered |
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| **Time frame: past 36 months** | | | |
| 2 | Grants or contracts from any entity (if not indicated in item #1 above). | ____ None | |
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| 3 | Royalties or licenses | _X_ None | |
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| 4 | Consulting fees | X_ None | |
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| 5 | Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events | __X__ None | |
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| 6 | Payment for expert testimony | __X__ None | |
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| 7 | Support for attending meetings and/or travel | ___X_ None | |
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| 8 | Patents planned, issued or pending | __X__ None | |
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| 9 | Participation on a Data Safety Monitoring Board or Advisory Board | __X__ None | |
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| 10 | Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid | _X___ None | |
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| 11 | Stock or stock options | _X___ None | |
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| 12 | Receipt of equipment, materials, drugs, medical writing, gifts or other services | __X__ None | |
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| 13 | Other financial or non-financial interests | ___X_ None | |
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**Please place an "X" next to the following statement to indicate your agreement:**

**_X__ I certify that I have answered every question and have not altered the wording of any of the questions on this form.**

# ICMJE DISCLOSURE FORM

**Date:** July 17, 2021
**Your Name:** Katherine Jensen, OD, FAAO
**Manuscript Title:** Dry eye symptoms and signs in US veterans with Gulf War Illness
**Manuscript number (if known):**

**In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.**

**The following questions apply to the author's relationships/activities/interests as they relate to the current manuscript only.**

**The author's relationships/activities/interests should be defined broadly. For example, if your manuscript pertains to the epidemiology of hypertension, you should declare all relationships with manufacturers of antihypertensive medication, even if that medication is not mentioned in the manuscript.**

**In item #1 below, report all support for the work reported in this manuscript without time limit. For all other items, the time frame for disclosure is the past 36 months.**

| | | Name all entities with whom you have this relationship or indicate none (add rows as needed) | Specifications/Comments (e.g., if payments were made to you or to your institution) |
|---|---|---|---|
| **Time frame: Since the initial planning of the work** | | | |
| 1 | All support for the present manuscript (e.g., funding, provision of study materials, medical writing, article processing charges, etc.) **No time limit for this item.** | ____ None<br>Department of Defense | Payment to supply study items/materials, payment received for examining patients in study/collecting data |
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| **Time frame: past 36 months** | | | |
| 2 | Grants or contracts from any entity (if not indicated in item #1 above). | _X_ None | |
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| 3 | Royalties or licenses | _X_ None | |
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| 4 | Consulting fees | _X_ None | |
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| | | | |
| 5 | Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events | _X_ None | |
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| 6 | Payment for expert testimony | _X_ None | |
| | | | |
| | | | |
| 7 | Support for attending meetings and/or travel | _X_ None | |
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| 8 | Patents planned, issued or pending | X_ None | |
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| 9 | Participation on a Data Safety Monitoring Board or Advisory Board | X_ None | |
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| 10 | Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid | X_ None | |
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| 11 | Stock or stock options | X_ None | |
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| | | | |
| 12 | Receipt of equipment, materials, drugs, medical writing, gifts or other services | X_ None | |
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| 13 | Other financial or non-financial interests | X_ None | |
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**Please place an "X" next to the following statement to indicate your agreement:**

**_X I certify that I have answered every question and have not altered the wording of any of the questions on this form.**

# ICMJE DISCLOSURE FORM

**Date:**___7/16/2021_____
**Your Name:**_ Nancy Klimas_____
**Manuscript Title:**____ Dry eye symptoms and signs in US veterans with Gulf War Illness _____
**Manuscript number (if known):**_____

**In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.**

**The following questions apply to the author's relationships/activities/interests as they relate to the current manuscript only.**

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| **Time frame: past 36 months** | | | |
| 2 | Grants or contracts from any entity (if not indicated in item #1 above). | __X__ None | |
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| 3 | Royalties or licenses | _X_ None | |
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| 4 | Consulting fees | X_ None | |
| | | | |
| | | | |
| 5 | Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events | __X__ None | |
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| | | | |
| 6 | Payment for expert testimony | __X__ None | |
| | | | |
| | | | |
| 7 | Support for attending meetings and/or travel | ___X_ None | |
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| 8 | Patents planned, issued or pending | __X__ None | |
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| 9 | Participation on a Data Safety Monitoring Board or Advisory Board | __X__ None | |
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| 10 | Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid | _X___ None | |
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| 11 | Stock or stock options | _X___ None | |
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| | | | |
| 12 | Receipt of equipment, materials, drugs, medical writing, gifts or other services | __X__ None | |
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| | | | |
| 13 | Other financial or non-financial interests | ___X_ None | |
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**_X__ I certify that I have answered every question and have not altered the wording of any of the questions on this form.**

# ICMJE DISCLOSURE FORM

**Date:_____07/18/2021_____**

**Your Name:_____Victor Sanchez_____**

**Manuscript Title:____** Dry eye symptoms and signs in US veterans with Gulf War Illness **_____**

**Manuscript number (if known):_____**

**In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.**

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| **Time frame: past 36 months** | | | |
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| 3 | Royalties or licenses | __x__ None | |
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| 4 | Consulting fees | __x__ None | |
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| 6 | Payment for expert testimony | __x__ None | |
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| 7 | Support for attending meetings and/or travel | __x__ None | |
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| 8 | Patents planned, issued or pending | _x___ None | |
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| 9 | Participation on a Data Safety Monitoring Board or Advisory Board | __x__ None | |
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| 10 | Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid | __x__ None | |
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| 11 | Stock or stock options | __x__ None | |
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| 12 | Receipt of equipment, materials, drugs, medical writing, gifts or other services | __x__ None | |
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| | | | |
| 13 | Other financial or non-financial interests | __x__ None | |
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