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CLINICAL TRIAL REPORT

Varenicline Solution Nasal Spray for the Treatment of Dry Eye Disease in Sjogren's Disease: A Pilot Study

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Purpose: We evaluated the efficacy of varenicline solution nasal spray (VNS) in treating dry eye disease (DED) associated with moderate to severe Sjogren's disease and analyzed tear film cytokine levels of patients with DED and Sjogren's disease before and after VNS use.

Methods: This was a pilot study involving a single-center, single-arm investigator-initiated trial. Patients with moderate to severe Sjogren's disease were given VNS 0.03 mg twice daily for 28 days. Patients were assessed on day 0 before VNS use, day 14 and day 28. Clinical exam findings, symptomatology as measured by the eye dryness score, and tear cytokines were assessed at baseline and day 28.

Results: Thirty-nine subjects were included. Between day 0 and day 28, there was a statistically significant improvement in the eye dryness score ($p = 0.01$), corneal staining ($p < 0.001$), and conjunctival staining ($p = 0.04$). There was a statistically significant increase in tear secretion by unanesthetized Schirmer's in subjects with a baseline Schirmer's ≤5 mm ($n = 35$ eyes, $p = 0.02$) and a non-statistically significant increase in tear secretion in subjects with a baseline Schirmer's of 6–10 mm ($n = 16$ eyes, $p = 0.79$). There was a statistically significant decrease in tear film cytokine concentration of IFNγ ($p = 0.0003$), IL-12p70 ($p < 0.0001$), IL-17a ($p = 0.004$), IL-1β ($p = 0.007$), IL-2 ($p < 0.0001$), IL-4 ($p = 0.01$), and TNF-α ($p = 0.02$), and no significant change in IL-6 ($p = 0.56$) and IL-10 ($p = 0.18$).

Conclusion: Our findings add to existing evidence that VNS improves subjective dry eye symptoms, corneal and conjunctival staining, and tear secretion in a subset of tear-deficient patients, while providing new evidence that VNS reduces concentration of pro-inflammatory cytokines in the tear film.

Clinical Trial Registration Number: NCT05700422.

Keywords: dry eye disease, Sjogren's disease, varenicline, tear film, cytokines

Introduction

Sjogren's disease (SjD) is a chronic inflammatory condition affecting the exocrine glands. Auto-antibody production and lymphocytic infiltration of the lacrimal and salivary glands results in the classic sicca complex characterized by dry eyes and dry mouth.[1] Various treatment modalities, focused on tear replacement, immunomodulation, and tear secretion preservation are used to help treat these patients with variable success.[2]

Varenicline solution nasal spray (VNS) 0.03 mg is an FDA approved treatment for the signs and symptoms of dry eye disease (DED);[3] it functions as a small-molecule nicotinic acetylcholine receptor (nAChR) that binds with high selectivity and affinity to multiple human nAChRs.[4,5] Tear secretion is regulated through neural reflex arcs, which includes the trigeminal parasympathetic pathway: activation of trigeminal afferent nerves in the nasal cavity leads to stimulation of trigeminal efferent parasympathetic nerves that innervate the lacrimal functional unit inclusive of the ocular surface, the lacrimal gland and the associated neural connections.[6,7] This trigeminal parasympathetic pathway is thought to be responsible for about one third of basal tear film production.[8] Research has shown that nAChRs can mediate afferent signals within the trigeminal nerve in response to nasal

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stimuli;[9,10] thus, the nAChR agonist varenicline, when used as a multi-dose, preservative-free nasal spray, may activate the trigeminal parasympathetic pathway, stimulating basal tear production and alleviating dry eye disease symptoms.

The pivotal ONSET-1 and ONSET-2, and supportive MYSTIC clinical trials have demonstrated the utility of VNS in improving both signs and symptoms of DED.[11–13] A post-hoc analysis from the ONSET-1 and ONSET-2 clinical trials demonstrated promising results that VNS improves both tear production and patient-reported symptoms in dry eye patients with an underlying autoimmune disease.[14] However, the specific utility of VNS remains unclear in patients with dry eye secondary to SjD.

Given the inflammatory nature of SjD, it is not surprising that elevated concentrations of inflammatory proteins and cytokines have been reported in tear fluid.[15] Upregulated cytokines including but not limited to IL-2, IL-4, IL-12p70, IL-17a and IFNγ in the tears and saliva of SjD patients have also been correlated significantly with reduced tear production, less stable tear film, and greater ocular surface damage.[16] In line with this observation, symptoms of SjD improve following treatment with anti-inflammatory agents like corticosteroids and cyclosporine A.[17]

The purpose of this pilot study was to evaluate the efficacy of VNS in subjects with DED associated with moderate to severe SjD. Additionally, an analysis was conducted to evaluate the pro-inflammatory cytokine levels within the tear film before and after the use of VNS in this patient population.

Materials and Methods

This was a single center, single arm, investigator-initiated trial in human subjects (NCT05700422, 2023–01-25). All study participants were identified by reviewing medical records of patients seen at the Dry Eye and Ocular Surface Disease Center at the Scheie Eye Institute for a diagnosis of DED who also had biopsy and/or serology-confirmed moderate to severe SjD by a Rheumatologist. Exclusion criteria included previous ocular surgery in the past year inclusive of thermal pulsation or Intense Pulsed Light therapy in the past 3 months, use of topical ophthalmic corticosteroid therapy in the prior 4 weeks, a history of clinically significant ocular trauma, current active ocular inflammation inclusive of blepharitis or infection inclusive of herpes simplex or herpes zoster, eyelid abnormalities that significantly affect lid function, ocular surface abnormalities that may compromise corneal integrity, retinal pathology that may limit visual potential and refractive outcomes, systemic conditions or diseases not stabilized or judged by the investigator to be compatible with participation in the study, chronic or recurrent epistaxis or coagulation disorders, recent nasal or sinus surgery, or a female who is pregnant, nursing or planning a pregnancy. Subjects were recruited until a target sample size of 40 patients was reached, allowing for each patient to be evaluated thoroughly during the duration of the study period. Race and ethnicity were self-reported by patients. Patients were not required to stop topical ocular medications prior to onset of study, but were excluded if they utilized a topical steroid ocular medication within 4 weeks of study initiation. All participants provided informed written consent prior to being a part of the study.

All patients were given VNS 0.03 mg nasal spray to use twice daily for 28 days, the same length of time used in the initial ONSET-1 and ONSET-2 studies.[11,13] The primary endpoints for this trial were the eye dryness score and mean change in corneal and conjunctival staining. The secondary outcomes included best corrected visual acuity, nasal dryness score, dry mouth score, change in unanesthetized Schirmer's test strips (STS) results in subgroups including all study patients, study patients with moderate Sjogren's disease (STS 6–10 mm), and study patients with severe SjD (STS ≤ 5 mm), and tear-film concentration of pro-inflammatory cytokines for all study patients. Sub-analyses of tear film concentration of pro-inflammatory cytokines were performed for patients with STS ≤ 5 mm at baseline and patients who continued cyclosporine A during the study period. The study was reviewed and approved by the Institutional Review Board (IRB) at the University of Pennsylvania (IRB protocol 851655), was HIPAA compliant, and adhered to the tenets of the Declaration of Helsinki.

At baseline (day 0) before the use of VNS, subjective and objective measures of dry eye were obtained. Regarding subjective measures, subjects individually graded their eye, nose and mouth dryness using the eye dryness score, which is a visual analogue scale that has successfully been used in prior dry eye studies.[18] The visual analogue scale allows the subject to grade their symptoms of ocular comfort and dryness on a scale of 0–100, where 0 is no discomfort and 100 is maximum discomfort. Subjects also completed the Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR), a 16-question survey designed to screen for depression and measure changes in severity of symptoms. In terms of objective clinical exam findings, visual acuity was tested by the study coordinator in the same fashion for each patient, while corneal fluorescein staining was graded by the NEI scale, lissamine green conjunctival staining was graded by the Van Bijsterveld and Utrecht scale, and unanesthetized Schirmer's

test was performed all by the study lead M.M. in the consistent standard of care fashion for each patient. Adverse events were also recorded. Subjects had a virtual visit on day 14 to assess the subjective measures of eye, nose and mouth dryness score alone, and an in-person visit on day 28, where the same objective and subjective measures from day 0 were measured. Additionally, on day 0 and day 28, tear samples were collected from both eyes individually via STS by study investigator M.M., immediately placed into separate vials and stored in the −80 °C freezer until shipped to BioAgilytix where a validated bead-based multiplex immunoassay (Luminex®) was performed to quantify concentrations of pro-inflammatory cytokines in the tear film including Interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17a, interferon (IFN)γ, and tumor necrosis factor (TNF)-α.

Descriptive statistics of study participants were calculated for demographics, dry eye symptoms and signs using mean, standard deviation (SD), median, inter-quartile and range for the continuous measures and using count and percentage for the categorical measures. Comparison of DED signs between baseline and follow-up visits (either day 14 or day 28) was performed using generalized estimating equations to account for the repeated measures over time and to address inter-eye correlation. For the comparison of tear cytokines between baseline and day 28, the clustered Wilcoxon signed rank test for paired data (as implemented in R package "clusrank") was used, due to the skewness of the cytokine data.[19] All statistical analyses were performed using SAS version 9.4 or R, and two-sided $p < 0.05$ was considered statistically significant.

Results

A total of 39 subjects were included in the study. One patient withdrew due to adverse effects from the study medication, two withdrew due to personal reasons unrelated to the study, and one was lost to follow-up. Baseline characteristics of 39 participants are summarized in Table 1. The mean age of subjects was 59.5 years (SD 14.9), and 100% were female which is reflective of the

Table 1 Demographics and Baseline Characteristics of Study Participants

Baseline Characteristics	Total (n=39)
Length of SjD diagnosis (yrs)	
Mean (SD)	10 (5.79)
Age (yrs)	
Mean (SD)	59.5 (14.9)
Median (IQR)	63.0 (48.0, 72.0)
Range	26.0, 85.0
Gender n (%)	
Female	39 (100.0%)
Race n (%)	
White	32 (82.1%)
Black/African American	4 (10.3%)
Asian	1 (2.6%)
Indian	1 (2.6%)
Other	1 (2.6%)
Ethnicity n (%)	
Hispanic or Latino	3 (7.7%)
Non-Hispanic or Latino	35 (89.7%)
Unknown	1 (2.6%)

Abbreviation: SjD, Sjogren's Disease.

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higher prevalence of Sjogren's syndrome in women. The mean length of diagnosis of SjD was 10 years (SD = 5.79). Five patients had a history of an autoimmune disease: three with systemic lupus erythematous and two with rheumatoid arthritis. In terms of ocular medications, fourteen patients were on and continued the regular use of topical cyclosporine A at the start of the study and throughout the study period, and no patients concurrently administered topical steroids during the study period.

On follow-up at the day 14 virtual visit, there was no statistically significant change in eye, mouth, or nasal dryness scores (Table 2). On follow-up at day 28, there was a statistically significant improvement in the eye dryness score (56.08 vs 41.57, p = 0.01), corneal staining (3.65 vs 1.87, p < 0.001), and conjunctival staining (2.33 vs 1.87, p = 0.04) (Table 3); there was no significant change in best corrected visual acuity, nose dryness, mouth dryness, or depressive symptoms as measured by QIDS-SR (Table 3); there was a statistically significant increase in tear secretion as measured by STS in subjects with baseline STS ≤ 5 mm (n = 35 eyes, 3.71 vs 5.37, p=0.02), and a non-statistically significant increase in tear secretion in subjects with baseline STS of 6–10 mm (n = 16 eyes, 7.88 vs 8.19, p = 0.79) (Table 3); there was a non-statistically significant decrease in tear secretion as measured by STS inclusive of all subjects (n = 70 eyes, 10.37 vs 9.34, p = 0.30) (Table 3).

Table 2 Dryness Scores from Baseline to Day 14

Outcome Measures	Baseline	Day 14	Change (Day 14 – Baseline)	P-value[a]
	Mean (SE)	Mean (SE)	Mean (95% CI)	
Primary Outcomes				
Eye dryness score	56.08 (3.47)	51.25 (3.83)	−4.83 (−14.94, 5.29)	0.35
Secondary Outcomes				
Nasal dryness score	36.59 (3.98)	37.03 (4.39)	−0.44 (−11.16, 12.05)	0.94
Dry mouth score	58.33 (3.75)	48.75 (4.14)	−9.58 (−20.52, 1.35)	0.09

Notes: [a]Comparison of DED signs between baseline and follow-up visits (either day 14 or day 28) were performed using generalized estimating equations to account for the repeated measures over time and inter-eye correlation.

Table 3 Change in Subjective and Objective Measures of Dry Eye Disease at Day 28

Outcome Measures	Baseline	Day 28	Change (Day 28 – Baseline)	P-value[a]
	Mean (SE)	Mean (SE)	Mean (95% CI)	
Primary Outcomes				
Eye dryness score	56.08 (3.89)	41.57 (4.11)	−14.51 (−25.60, −3.41)	0.01
Corneal stain score	3.65 (0.21)	1.87 (0.16)	−1.78 (−2.09, −1.47)	<0.0001
Conjunctival stain score	2.33 (0.23)	1.87 (0.25)	−0.46 (−0.91, −0.01)	0.04
Secondary Outcomes				
Best-corrected visual acuity (logMAR)	0.049 (0.016)	0.047 (0.016)	−0.002 (−0.031, 0.027)	0.89
Nasal dryness score	36.59 (4.31)	31.29 (4.55)	−5.30 (−17.59, 6.98)	0.40
Dry Mouth Score	58.33 (4.24)	46.97 (4.48)	−11.36 (−23.46, 0.73)	0.07
QIDS-SR	5.54 (0.63)	4.37 (0.66)	−1.17 (−2.96, 0.63)	0.20
Schirmer test (n=70 eyes)	10.37 (1.40)	9.34 (1.40)	−1.03 (−2.99, 0.93)	0.30
Schirmer test ≤ 5mm at baseline (n = 35 eyes)	3.71 (0.20)	5.37 (0.78)	1.66 (0.33, 2.99)	0.02
Schirmer test 6–10mm at baseline (n = 16 eyes)	7.88 (0.33)	8.19 (1.22)	0.31 (−1.96, 2.57)	0.79

Notes: [a]Comparison of DED signs between baseline and follow-up visits (either day 14 or day 28) were performed using generalized estimating equations to account for the repeated measures over time and inter-eye correlation.

Tear film concentration of inflammatory cytokines was measured in 70 eyes. We found a statistically significant decrease in the median tear film cytokine concentration from baseline to day 28 for the following cytokines: IFNγ (0.704 x 10^{-3} vs 0.000×10^{-3} pg/μL, p = 0.0003), IL-12p70 (4.575 x 10^{-3} vs 0.811×10^{-3} pg/μL, p < 0.0001), IL-17a (0.675 x 10^{-3} vs 0.000×10^{-3} pg/μL, p = 0.004), IL-1β (0.645 x 10^{-3} vs 0.000×10^{-3} pg/μL, p = 0.007), IL-2 (1.550 x 10^{-3} vs 0.000 × 10^{-3} pg/μL, p < 0.0001), IL-4 (0.147 x 10^{-3} vs 0.000 × 10^{-3} pg/μL, p = 0.011), and TNF-α (1.233 x 10^{-3} vs 0.000 × 10^{-3} pg/μL, p = 0.017) (Table 4). There was no statistically significant change in IL-10 (0.000 x 10^{-3} vs 0.000 × 10^{-3} pg/μL, p = 0.18) and IL-6 (0.812 x 10^{-3} vs 0.000 × 10^{-3} pg/μL, p = 0.56) (Figure 1). Overall, >50% of all patients had a decrease in tear film cytokine levels for all cytokines except IL-10 between baseline and day 28 (Table 5).

In the sub-group of patients with baseline STS ≤ 5 mm (n = 35 eyes), we found a statistically significant decrease in the median tear film cytokine concentration from baseline to day 28 for the following cytokines: IFNγ, IL-12p70, IL-17a and IL-2; there was a non-statistically significant decrease in IL-1β, IL-4, IL-6, and TNF-α, and no change in IL-10 (Table 6). Overall, there was a greater percent of patients who had decreased tear film cytokine levels in the baseline STS > 5 mm group compared to the baseline STS ≤ 5 mm group for all cytokines except IL-10, though this was only statistically significant for IFNγ and IL-4 (Table 7). In the sub-group of patients who continued cyclosporine A (n= 28 eyes) throughout the study period, we found a statistically significant decrease in the median tear film cytokine concentrations from baseline to day 28 for the following cytokines: IL-12p70, IL-2 and IL-4; there was a non-statistically significant decrease in IFNγ, IL-17a, IL-1β and TNF-α, a non-statistically significant increase in IL-6, and no change in IL-10 (Table 8). Overall, there was a greater percent of patients who had decreased tear film cytokine levels in the no cyclosporine A use group compared to the cyclosporine A use group for all cytokines except IL-10, though this was only statistically significant for IL-6 (Table 9).

The most common adverse events reported were sneezing (56%), cough (5%), throat irritation (1%), and instillation site irritation (1%). VNS was considered well tolerated by study participants.

Discussion

Varenicline solution nasal spray (VNS) is a novel first-in-class drug therapy and is a potential DED treatment for patients with SjD. In this pilot study, we found that treatment with VNS use two times per day for 28 days was associated with a significant improvement in DED signs and symptoms as measured by the eye dryness, corneal stain and conjunctival staining scores. This is congruent with the findings in ONSET-1 whereby patients taking VNS 0.03 mg BID for 28 days

Table 4 Comparison of Median Tear Film Cytokine Levels Between Baseline and Day 28: All Subjects

Tear Cytokine	Baseline (pg/μL)	Day 28 (pg/μL)	P-value[a]
	Median (n = X x 10^{-3} (Q1, Q3))	Median (n = X x 10^{-3} (Q1, Q3))	
IFNγ	0.704 (0.000, 2.601)	0.000 (0.000, 0.160)	0.0003
IL-10	0.000 (0.000, 0.000)	0.000 (0.000, 0.000)	0.18
IL-12p70	4.575 (1.538, 8.185)	0.811 (0.000, 2.453)	<0.0001
IL-17a	0.675 (0.000, 2.307)	0.000 (0.000, 0.489)	0.004
IL-1β	0.645 (0.000, 1.603)	0.000 (0.000, 0.132)	0.007
IL-2	1.550 (0.000, 3.454)	0.000 (0.000, 0.000)	<0.0001
IL-4	0.147 (0.000, 1.127)	0.000 (0.000, 0.000)	0.01
IL-6	0.812 (0.000, 2.953)	0.000 (0.000, 2.269)	0.56
TNF-α	1.233 (0.000, 2.546)	0.000 (0.000, 0.957)	0.02

Notes: [a]The clustered Wilcoxon signed rank test for paired data (as implemented in R package "clusrank") was used to compare cytokine data at baseline and day 28, due to the skewness of the cytokine data.

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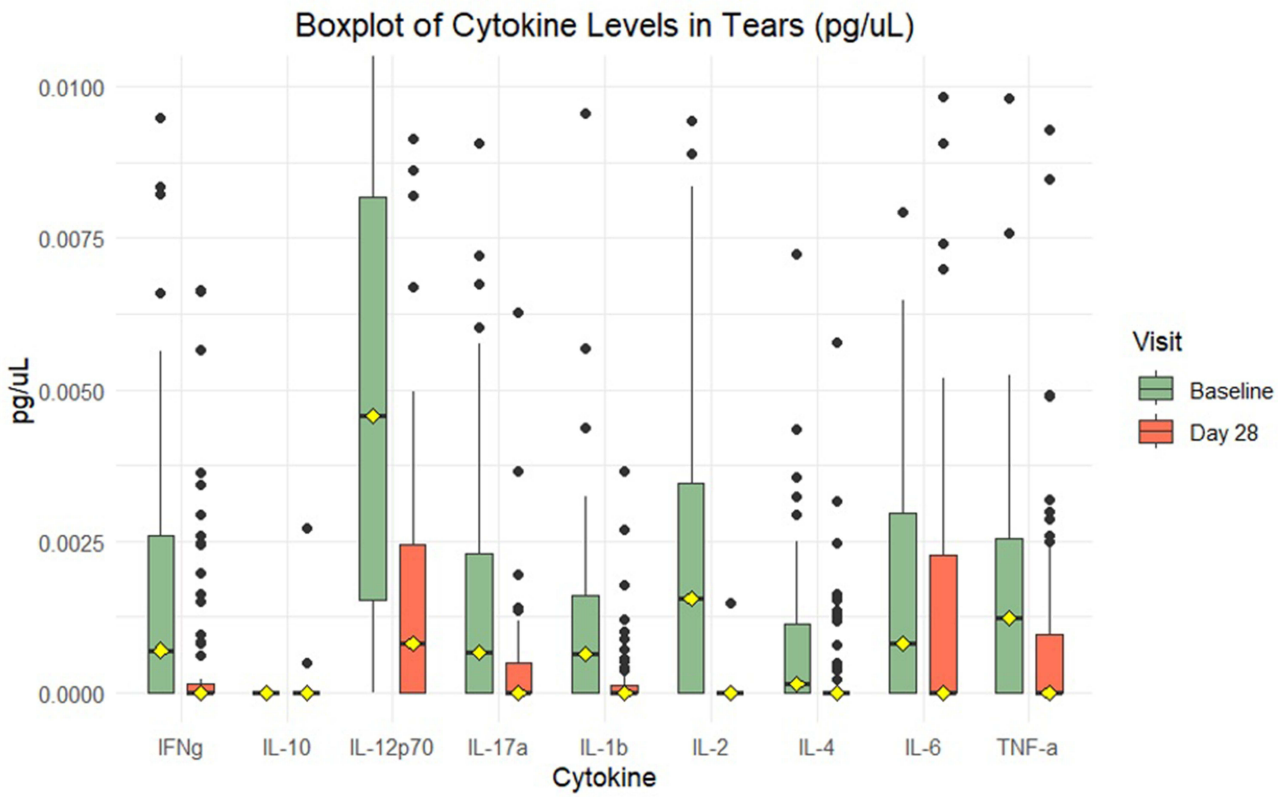

Figure 1 Tear cytokine levels from baseline to day 28. [a] The clustered Wilcoxon signed rank test for paired data (as implemented in R package "clusrank") was used to compare cytokine data at baseline and day 28, due to the skewness of the cytokine data.

had a statistically significant mean reduction in eye dryness score and statistically significant improvement in tear film production by anesthetized STS compared to controls. This is also in line with findings in ONSET-2, whereby a statistically significant improvement in STS and eye dryness score at week 4 in patients taking VNS BID compared

Table 5 Number of Patients with Median Tear Film Cytokine Level Change Between Baseline and Day 28: All Subjects (n = 70)

	N=70		
	Decrease	**Increase**	**No Change**
IFNγ	45 (64.3%)	10 (14.3%)	15 (21.4%)
IL-10	0 (0.0%)	2 (2.9%)	68 (97.1%)
IL-12p70	55 (78.6%)	14 (20.0%)	1 (1.4%)
IL-17a	41 (58.6%)	12 (17.1%)	17 (24.3%)
IL-1β	38 (54.3%)	17 (24.3%)	15 (21.4%)
IL-2	43 (61.4%)	1 (1.4%)	26 (37.1%)
IL-4	34 (48.6%)	12 (17.1%)	24 (34.3%)
IL-6	33 (47.1%)	21 (30.0%)	16 (22.9%)
TNF-α	40 (57.1%)	15 (21.4%)	15 (21.4%)

Table 6 Comparison of Median Tear Film Cytokine Levels Between Baseline and Day 28: Baseline STS ≤ 5 mm (n = 35)

Tear Cytokine	Baseline (pg/μL)	Day 28 (pg/μL)	P-value[a]
	Median (n = X x 10^{-3} (Q1, Q3))	Median (n = X x 10^{-3} (Q1, Q3))	
IFNγ	2.236 (0.277, 3.448)	0.000 (0.000, 2.219)	0.01
IL-10	0.000 (0.000, 0.000)	0.000 (0.000, 0.000)	0.18
IL-12p70	5.071 (2.082, 9.307)	1.166 (0.000, 3.599)	0.002
IL-17a	0.503 (0.000, 2.360)	0.000 (0.000, 0.602)	0.005
IL-1β	0.432 (0.000, 1.464)	0.000 (0.000, 0.263)	0.16
IL-2	1.554 (0.000, 4.506)	0.000 (0.000, 0.000)	0.0004
IL-4	0.000 (0.000, 1.067)	0.000 (0.000, 0.470)	0.08
IL-6	0.000 (0.000, 2.951)	0.000 (0.000, 2.524)	0.79
TNF-α	1.371 (0.000, 3.533)	0.000 (0.000, 1.577)	0.08

Notes: [a]The clustered Wilcoxon signed rank test for paired data (as implemented in R package "clusrank") was used to compare cytokine data at baseline and day 28, due to the skewness of the cytokine data.

Table 7 Number of Patients with Median Tear Film Cytokine Level Change Between Baseline and Day 28: Baseline STS ≤ 5 mm (n = 35) Vs Baseline STS > 5 mm (n = 35)

	Schirmer's ≤ 5mm			Schirmer's >5mm			P-value[a]
	N = 35			N = 35			
	Decrease	Increase	No Change	Decrease	Increase	No Change	
IFNγ	21 (60.0%)	9 (25.7%)	5 (14.3%)	24 (68.6%)	1 (2.9%)	10 (28.6%)	0.016
IL-10	0 (0.0%)	2 (5.7%)	33 (94.3%)	0 (0.0%)	0 (0.0%)	35 (100.0%)	0.15
IL-12p70	25 (71.4%)	9 (25.7%)	1 (2.9%)	30 (85.7%)	5 (14.3%)	0 (0.0%)	0.27
IL-17a	18 (51.4%)	8 (22.9%)	9 (25.7%)	23 (65.7%)	4 (11.4%)	8 (22.9%)	0.37
IL-1β	15 (42.9%)	10 (28.6%)	10 (28.6%)	23 (65.7%)	7 (20.0%)	5 (14.3%)	0.14
IL-2	19 (54.3%)	0 (0.0%)	16 (45.7%)	24 (68.6%)	1 (2.9%)	10 (28.6%)	0.23
IL-4	12 (34.3%)	9 (25.7%)	14 (40.0%)	22 (62.9%)	3 (8.6%)	10 (28.6%)	0.037
IL-6	13 (37.1%)	13 (37.1%)	9 (25.7%)	20 (57.1%)	8 (22.9%)	7 (20.0%)	0.23
TNF-α	19 (54.3%)	7 (20.0%)	9 (25.7%)	21 (60.0%)	8 (22.9%)	6 (17.1%)	0.68

Note: [a]Chi-square or Fisher's Exact Test (if counts < 5).

to controls were reported as secondary outcomes. Interestingly, although VNS improved signs and symptoms of DED in many patients with SjD in our study, VNS was most efficacious in increasing tear secretion in patients with STS ≤ 5 mm. This is congruent with the prior ONSET-2 and MYSTIC trials, where the average baseline STS was approximately 5 mm.[12,13] The efficacy of VNS in patients with DED and SjD in this trial suggests that these patients retain some functional gland structures. There have also been reports describing SjD patients to have anti-muscarinic acetylcholine receptor antibodies, and blocking of receptors may impair nerve impulses to the lacrimal gland;[20,21] it is possible that VNS use overcomes this antibody-associated inhibition, leading to increased tear secretion in this patient population.

Table 8 Comparison of Median Tear Film Cytokine Levels Between Baseline and Day 28: Cyclosporine A Use (n = 28)

Tear Cytokine	Baseline (pg/μL)	Day 28 (pg/μL)	P-value[a]
	Median (n = X x 10^{-3} (Q1, Q3))	Median (n = X x 10^{-3} (Q1, Q3))	
IFNγ	1.021 (0.000, 2.484)	0.000 (0.000, 0.995)	0.06
IL-10	0.000 (0.000, 0.000)	0.000 (0.000, 0.000)	0.32
IL-12p70	5.169 (2.166, 11.270)	1.291 (0.000, 3.403)	0.008
IL-17a	0.606 (0.000, 2.344)	0.000 (0.000, 0.648)	0.08
IL-1β	0.821 (0.000, 1.794)	0.000 (0.000, 0.792)	0.44
IL-2	1.746 (0.000, 3.913)	0.000 (0.000, 0.000)	0.006
IL-4	0.578 (0.000, 2.164)	0.000 (0.000, 0.090)	0.029
IL-6	0.000 (0.000, 3.360)	1.622 (0.000, 4.710)	0.27
TNF-α	1.554 (0.000, 2.787)	0.000 (0.000, 1.853)	0.40

Notes: [a]The clustered Wilcoxon signed rank test for paired data (as implemented in R package "clusrank") was used to compare cytokine data at baseline and day 28, due to the skewness of the cytokine data.

Table 9 Number of Patients with Median Tear Film Cytokine Level Change Between Baseline and Day 28: Cyclosporine A Use (n = 28) vs No Cyclosporine A Use (n = 42)

	Cyclosporine A use			No Cyclosporine A use			Pp-value[a]
	N = 28			N = 42			
	Decrease	Increase	No Change	Decrease	Increase	No Change	
IFNγ	17 (60.7%)	6 (21.4%)	5 (17.9%)	28 (66.7%)	4 (9.5%)	10 (23.8%)	0.36
IL-10	0 (0.0%)	1 (3.6%)	27 (96.4%)	0 (0.0%)	1 (2.4%)	41 (97.6%)	0.77
IL-12p70	22 (78.6%)	5 (17.9%)	1 (3.6%)	33 (78.6%)	9 (21.4%)	0 (0.0%)	0.45
IL-17a	15 (53.6%)	5 (17.9%)	8 (28.6%)	26 (61.9%)	7 (16.7%)	9 (21.4%)	0.75
IL-1β	15 (53.6%)	8 (28.6%)	5 (17.9%)	23 (54.8%)	9 (21.4%)	10 (23.8%)	0.73
IL-2	17 (60.7%)	0 (0.0%)	11 (39.3%)	26 (61.9%)	1 (2.4%)	15 (35.7%)	0.70
IL-4	15 (53.6%)	4 (14.3%)	9 (32.1%)	19 (45.2%)	8 (19.0%)	15 (35.7%)	0.77
IL-6	8 (28.6%)	12 (42.9%)	8 (28.6%)	25 (59.5%)	9 (21.4%)	8 (19.0%)	0.04
TNF-α	15 (53.6%)	7 (25.0%)	6 (21.4%)	25 (59.5%)	8 (19.0%)	9 (21.4%)	0.83

Note: [a]Chi-square or Fisher's Exact Test (if counts < 5).

Inflammation is recognized as a contributing factor to the pathophysiology of DED in patients with SjD. Tear cytokines have previously been found to be elevated in patients with SjD and correlate to tear production, tear film stability, and damage to the ocular surface.[15,16] We found that VNS use two times daily for 28 days in patients with DED and SjD led to a statistically significant decrease in tear film concentration of pro-inflammatory cytokines, including IFNγ, IL-12p70, IL-17a, IL-2, IL-1β, IL-4, and TNF-α, and >50% of patients realized a decrease in tear film cytokine levels for all cytokines except IL-10 between baseline and day 28. These cytokines have been implicated in the pathogenesis of SjD. For example, Th1 cells, which are classically associated with autoimmunity, produce IL-2, IFNγ, and TNF-α,[22] which were found to be reduced in the tear film after treatment with VNS. IL-2 may represent a biomarker

of disease gravity in DED, and IFN-γ and TNF-α may also be secreted by macrophages and have been implicated in the perpetuation of chronic inflammation in autoimmune diseases such as SjD.[23] IL-12p70, which was also reduced by VNS treatment, is mainly produced by macrophages and dendritic cells, and can promote the development of Th1 response; IL-12p70 levels have been found to be elevated in patients with SjD-related DED compared to patients with DED without SjD.[24] IL-17a is produced by Th17 cells and is one of the main effector cytokines of Th17 cells; Th17 cells have been hypothesized to contribute to autoimmune disease progression by supporting autoreactive B cell responses,[25] and IL-17a has also been found to be elevated in SjD.[26,27] IL-4 is a pro-inflammatory cytokine that is classically associated with allergy but has been found to be overexpressed on the ocular surface of patients with DED and SjD,[28,29] and can be secreted by a variety of cell types. Finally, IL-1β-secreting circulating lymphocytes are significantly upregulated in SjD patients compared to healthy controls and non-SjD sicca patients; its level has been shown to correlate with disease duration and rheumatoid factor levels and interestingly, immunohistochemical staining of salivary glands of SjD patients showed expression of IL-1β whereas biopsies from controls did not.[30–32]

VNS treatment did not have a significant effect on IL-10 in our study. IL-10 is a pleomorphic cytokine which is primarily considered anti-inflammatory.[33] Upregulation of IL-10 has been associated with improved ocular surface disease in a rabbit model.[34] The concentrations measured in our study were too low to glean any insight regarding the effect of VNS treatment on this particular cytokine; this may be the result of the chronic nature of the underlying SjD state leading to overall low levels. The other cytokine that was not significantly affected by VNS was IL-6, a pro-inflammatory cytokine that has been implicated in SjD and/or DED and can be secreted by a variety of cell types.[28,29] This cytokine was reduced but not significantly after VNS treatment, which may indicate some effect vs noise; further investigation into this cytokine concentration in relation to VNS use is warranted.

It is unclear why VNS treatment only affected the production of certain cytokines in the tear film in our study. Both the lacrimal gland and conjunctival cells can produce cytokines that are secreted in tears.[35] Thus, it is unclear if VNS administration affects the cytokine secretion from the lacrimal gland directly, or if this is a secondary effect from increased production of basal tears that either restores ocular surface homeostasis and thus reduction of cytokine secretion from conjunctival cells or leads to a dilution effect due to the increase in basal tears alone. Further studies are needed to investigate the pathophysiology and mechanism behind the anti-inflammatory effects of VNS.

We performed a sub-group analysis on tear film cytokine level changes in patients with baseline STS ≤ 5 mm, which found a statistically significant decrease in median tear film cytokine concentrations for IFNγ, IL-12p70, IL-17a and IL-2 and a non-statistically significant decrease in IL-1β, IL-4, IL-6, and TNF-α. Interestingly, there was a greater percent of patients who had decreased tear film cytokine levels in the baseline STS > 5 mm group compared to the baseline STS ≤ 5 mm group for all cytokines except IL-10, though this was only statistically significant for IFNγ and IL-4. We postulate that all patients experience some dilution effect due to increase in basal tears alone from use of VNS, but a greater dilution effect may be had by those with SjD with baseline STS > 5 mm, as they may retain more natural functional gland structures compared to those patients with SjD with baseline STS ≤ 5 mm. Further, the patients in the baseline STS > 5 mm group may require less of an increase in basal tear production to restore ocular surface homeostasis compared to the patients with baseline STS ≤ 5 mm, which may also lead to decreased inflammatory cytokine release in the tear film.

We performed a sub-group analysis on tear film cytokine level changes in eyes who continued cyclosporine A use that found a statistically significant decrease in median tear film cytokine concentrations for IL-12p70, IL-2 and IL-4, a non-statistically significant decrease in IFNγ, IL-17a, IL-1βand TNF-α, a non-statistically significant increase in IL-6, and no change in IL-10. There were a greater percent of patients who had decreased tear film cytokine levels in the no cyclosporine A use group compared to the cyclosporine A use group for all cytokines except IL-10, though only statistically significant for IL-6. This is in line with the current literature whereby cyclosporine A use twice daily for two weeks has shown decreased tear film cytokine levels of IL-1β, TNF-α, and IL-6,[36] and also suggests that VNS use allows for decreased cytokine concentrations in the tear film above and beyond the levels of cyclosporine A alone and may be synergistic in treatment of DED in patients with SjD.

In congruence with prior studies, VNS was well tolerated with no serious adverse events or ocular adverse events noted.[37,38] Many patients, however, did report nasal-cavity related symptoms, such as sneezing and cough. Patients who have presumably low to no nerve function to stimulate the lacrimal gland (secondary to trauma, infection, toxicity,

tumors, etc.) would likely not realize marked results with VNS use, as VNS requires nerves to be at least semi-functional and the gland to retain some function as well. However, given it is difficult to know if all nerves are non-functional, and since VNS therapy is relatively low risk, it may be worth trying despite the question of nerve or gland functionality.

There are several potential advantages to the use of VNS to treat DED in Sjogren's patients. Varenicline solution nasal spray (VNS) 0.03 mg is an FDA-approved treatment for the signs and symptoms of DED that functions as a cholinergic agonist to activate basal tear secretion.[3] Neurostimulation via the cholinergic agonist harnesses the ability of the lacrimal functional unit to secrete a complex milieu of endogenous aqueous solution, which includes antibodies, growth factors, and cytokines. Neurostimulation has also been found to stimulate mucin and meibum secretion,[39] and the tears produced are similar to those produced naturally,[40] providing a distinct advantage over the use of artificial tears. Utilizing these patient's residual gland functionality with VNS offers a unique treatment option that should not disrupt the tear film balance in the same manner as artificial tears. Other studies have hypothesized how VNS may function in patients with autoimmune-associated dry eye.[14] For example, neural stimulation of the lacrimal gland may help maintain lacrimal gland weight and morphology, although this is likely a delayed response.[41] Neurostimulation by means of VNS also may be a useful tool for those who cannot self-administer eye drops easily. In addition, in comparison to other treatment modalities, such as anti-inflammatory agents, VNS has been shown to have a rapid effect in improving patients' symptoms, supported by our results whereby a cohort of patients with DED and SjD had improvement in dry eye symptoms at 28 days post-treatment initiation.

This is the first pilot study to evaluate the effect of VNS treatment on patients with DED and SjD. Overall, there is a paucity of research on VNS treatment in dry eye given the medication's more recent FDA approval. We hope this pilot study will provide a launchpoint from where more studies on patients with complex and difficult to treat DED, such as those with Sjogren's, can occur, and ultimately with the goal of providing this difficult to treat population with more options to manage their symptoms and improve their quality of life. However, there were several limitations to our study including lack of a control group and small sample size. However, this was pilot study so further larger studies that include a control group would be helpful. Additionally, the 28-day study duration may not be sufficient to fully capture long-term effects, as chronic inflammatory remodeling may require 3–6 months for significant changes; future studies looking at extended follow-up could provide insight into the potential for either more substantial or less pronounced effects of VNS on the tear film in this patient population over time, given the chronic nature of Sjögren's disease. Further, we did not require cessation of current dry-eye regimen for our cohort of patients; while other dry eye medication use could confound the results of VNS use during the study period, the baseline measurements while on their current dry eye regimens should help to account for this potential confounder. Additionally, our sub-group analysis demonstrates the majority of patients had a decrease in tear film cytokine levels across all cytokines except IL-10 in patients who were taking cyclosporine A as well as those who were not. Future studies may consider a washout period of all topical ocular medications prior to administration of VNS during the study period. Finally, permanent damage to the lacrimal glands in Sjögren's disease could limit the efficacy of treatment in some patients, as irreversible gland destruction might explain why some individuals do not experience meaningful improvements. Despite the limitations, the results are promising, and we hope will form the basis for future larger studies that can establish the effect of VNS use on the ocular surface in this patient population.

Conclusion

In this pilot study, we found that VNS is a promising treatment modality for the treatment of DED in the setting of SjD and can be considered in DED treatment in this difficult to treat population by clinicians today. This trial adds to the existing evidence that VNS use in patients with DED provides relief of dry eye symptoms, improves corneal and conjunctival staining, improves tear secretion in a subset of severely tear-deficient patients. We also found that VNS reduces tear film concentrations of pro-inflammatory cytokines which points to one potential mechanism for how VNS may be effective in treating DED.

Abbreviations

VNS, Varenicline Nasal Spray; DED, Dry eye disease; STS, Schirmer's test strips; QIDS-SR, Quick Inventory of Depressive Symptomatology Self-Report; IL, Interleukin; SD, Standard deviation.

Data Sharing Statement

IPD sharing listed as no in submission. Authors do not intend to share individual deidentified participant data from this pilot study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Vatinee Y. Bunya is a consultant for Kowa and an unpaid board member of the Sjogren's Foundation. Mina Massaro is a consultant for Tarsus, Claris Bio, Oyster Point/Viatris, Alcon, Stellular Bio and Dompe. The other authors report no conflicts of interest in this work.

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