



# Short-Term Efficacy of Ophthalmic Cyclosporine: A 0.1% Cationic Emulsion in Dry Eye Patients Assessed Under Controlled Environment

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## ABSTRACT

**Introduction:** To evaluate the short-term efficacy of cyclosporine A (CsA)-0.1% cationic emulsion (CE) in patients with dry eye disease (DED) and mitigation of the inflammatory flares triggered by desiccating stress environments.

**Methods:** A single-center non-randomized clinical trial was performed at a tertiary care setting. Twenty patients with DED treated with CsA 0.1% CE were exposed to a normal controlled environment (NCE) (23 °C, 50% relative humidity) and an adverse controlled environment (ACE) (23 °C, 10% relative humidity, 0.43 m/s localized airflow) during baseline and

the 1- and 3-month visits. Patients underwent the following evaluations: conjunctival hyperemia and staining, corneal fluorescein staining (CFS) using the Oxford and Cornea and Contact Lens Research Unit (CCLRU) scale, meibomian gland (MG) secretion quality, Dry Eye Questionnaire-5, Symptom Assessment in Dry Eye (SANDE II), and Change in Dry Eye Symptoms Questionnaire. Multivariate models were adjusted for statistical analysis.

**Results:** Nineteen women and one man (mean age,  $58.9 \pm 12.3$  years) completed the study. All symptom questionnaires, CFS, conjunctival hyperemia and staining, and MG secretion quality improved ( $p \leq 0.003$ ) with 1 month of treatment; improvements were maintained after 3 months ( $p \leq 0.02$ ), except for SANDE II ( $p \geq 0.07$ ). The CFS worsening (total CCLRU) after baseline ACE exposure (from 8.6 to 10.1) was higher, although not significant ( $p = 0.64$ ), compared with 1 month (from 5.4 to 5.8) and 3 months (from 5.0 to 5.9) after treatment.

**Conclusion:** Topical CsA-0.1% CE improved DED signs and symptoms after 1 month of treatment under controlled environmental conditions. Future studies should confirm the benefit of CsA-0.1% CE in desiccating stress environments.

**Trial Registration:** ClinicalTrials.gov identifier, NCT04492878.

**Keywords:** Cyclosporine A; Dry eye; Ikervis; Controlled environment

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## Key Summary Points

### *Why carry out this study?*

The literature is scarce regarding the efficacy of topical treatment with cyclosporine (CsA) 0.1% cationic emulsion (CE) (Ikervis) for reducing signs and symptoms after 1 and 3 months of use in patients with dry eye disease with severe keratitis.

Patients with chronic dry eye disease (DED) suffer from episodic flares that are usually associated with exacerbation of DED signs and symptoms. These flares can be triggered by the exposition to adverse environmental conditions (e.g., air-conditioning office buildings).

### *What was learned from the study?*

Topical CsA 0.1% CE can improve signs and symptoms by 1 month of treatment in patients with moderate DED who previously did not benefit from tear substitute therapy. This improvement continues 3 months after starting the treatment.

Further studies are needed to show that topical CsA 0.1% CE could provide a prophylactic effect against aggravation of DED signs and symptoms when patients are exposed to desiccating stress environments.

Expositions to controlled environments can help to minimize potential confounding factors in DED clinical trials, which could be useful for assessing new treatments for DED more reliably.

## INTRODUCTION

Dry eye disease (DED) is one of the most prevalent ocular diseases and affects 5–50% of the population depending on the diagnosis

criteria [1]. This multifactorial inflammatory disorder is characterized by loss of homeostasis and instability of the tear film accompanied by ocular surface damage and symptoms [1].

The external environment is one of the most important triggers of DED. Adverse environmental conditions such as high temperature, airflow, or low relative humidity exacerbate DED signs and symptoms [2–5]. Environmental chambers allow re-creation of specific controlled environmental conditions in clinical studies and evaluation of individual responses to different stimuli in a standardized way [3, 6].

Artificial tears and lubricants ameliorate DED symptoms and signs only in the short term and do not address the pathogenic inflammatory mechanism of the disease [7]. Topical corticosteroids improve DED signs and symptoms by ameliorating the inflammatory process [8], but their known potential adverse effects such as intraocular pressure elevation or cataract development may prevent long-term use [9]. Long-term topical anti-inflammatory drugs for DED, such as cyclosporine A (CsA) and lifitegrast ophthalmic solution (Xiidra, Novartis, Basel, Switzerland), are also commercially available. Only topical CsA (multiple formulations), available in most countries, is safe for long-term use [10]. CsA is an anti-inflammatory and immunosuppressant drug that reduces the inflammatory status of the ocular surface [11–13] and improves signs and symptoms of DED [14, 15].

In 2015, the European Medicines Agency approved CsA 0.1% cationic emulsion (CE) (Ikervis, Santen Oy, Tampere, Finland) to treat severe keratitis in patients with DED refractory to treatment with tear substitutes [16]. CsA 0.1% CE is safe and effective for reducing DED signs and symptoms after 6 and 12 months of use [17–20]. However, little evidence is available from high-quality clinical trials that assessed the safety and efficacy of CsA 0.1% CE during shorter follow-up periods [17, 18]. This information should be valuable for ophthalmologists because it could prevent patients from using other concomitant medications until the effectiveness of CsA has been determined. In addition, outcomes of DED clinical trials can be biased because of environment-related

conditions [5], especially in large sample phase III multicenter studies. Controlling the environmental conditions when evaluating patients can help to better assess the efficacy of any DED therapy. Therefore, the purpose of the current study was to evaluate the efficacy of CsA 0.1% CE on the signs and symptoms of DED after 1 and 3 months of therapy in patients with moderate to severe disease under controlled environmental conditions. The study also assessed the potential prophylactic benefits of CsA 0.1% CE to reduce inflammatory worsening in patients exposed to controlled adverse environmental conditions.

## METHODS

This was a single-center, open-label, non-controlled, phase IV clinical trial that explored the short-term efficacy of CsA 0.1% CE and its response after exposure to a controlled adverse indoor environment. The Ethics Committee of the Valladolid University Clinical Hospital (Valladolid, Spain) and the Spanish Drugs and Health Products Administration (<https://www.aemps.gob.es/>) (EudraCT number: 2019-000982-19) approved the trial. It was registered at the US National Institutes of Health (ClinicalTrials.gov, ID: NCT04492878). It was conducted at the Institute of Applied Ophthalmobiology, University of Valladolid, Valladolid, Spain. The study followed the tenets of the Declaration of Helsinki and the Good Clinical Practices.

### Study Design

Patients completed four visits (inclusion, baseline, and follow-up visits at 1 and 3 months). All visits except the inclusion visit were performed at the Controlled Environment Laboratory (CELab, Vision R&D, Valladolid, Spain). Participants were evaluated first after a 30-min exposure to a normal controlled environment (NCE) (23 °C temperature and 50% relative humidity). They then were evaluated after a 2-h exposure to an adverse controlled environment (ACE) (23 °C temperature, 10% relative humidity, and

localized 0.43 m/s airflow). Clinical evaluations were performed at each visit (Fig. 1).

During the inclusion visit, the nature of the study, associated potential risks and benefits, and visit schedule were explained and informed consent was obtained from the patients. Inclusion and exclusion criteria were checked, and if the criteria were met, the baseline visit was scheduled.

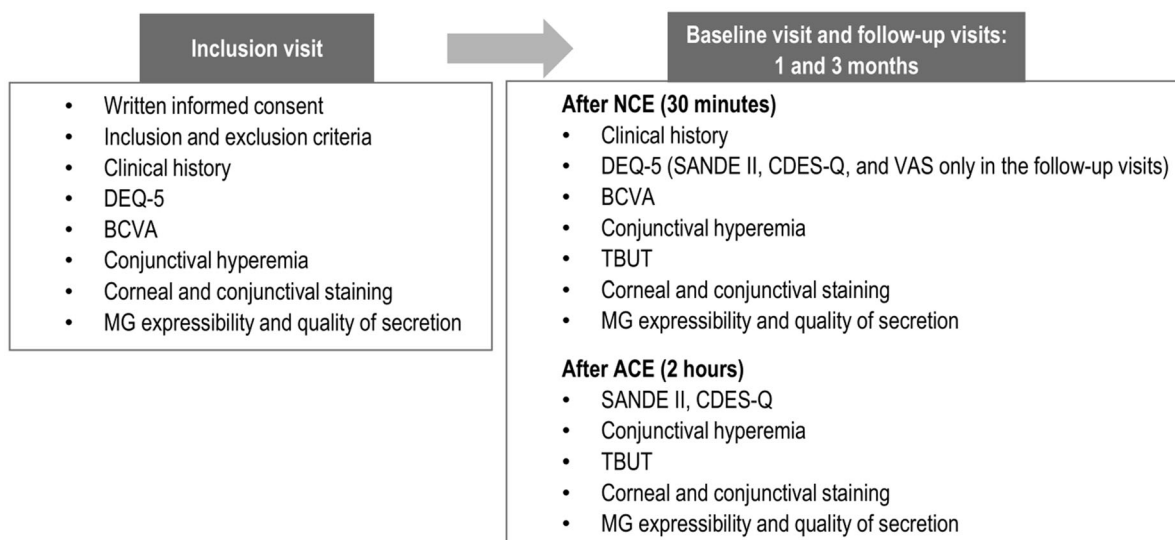
The baseline visit was performed  $7 \pm 1$  days from the inclusion visit. Baseline measurements were obtained in the NCE and ACE. The inclusion/exclusion criteria were re-evaluated, and subjects who did not meet the established criteria were excluded. The included patients received the study treatment, i.e., one drop of CsA 0.1% CE in each eye every day until the end of the study. The follow-up visits followed the same methodology as at the baseline visit.

Subjects were instructed not to use any eye drops in the 2 h before the visits. Patients were asked about adherence to the protocol before beginning the follow-up visits; the vials used until that time point were collected and counted.

### Patient Selection

The inclusion criteria included age of least 18 years; moderate or worse DED defined as an Oxford scale corneal fluorescein staining (CFS)  $\geq 2$  bilaterally and a dry eye questionnaire (DEQ)-5 score exceeding six points; use of artificial tears four or more times daily; and when concomitant medication was used that could affect the ocular surface, patients had to have been using it at least 3 months before the inclusion visit and keep using it according to the same schedule for the study duration.

Patients were excluded if they had any ocular disease except DED, a history of eye surgery or trauma that could affect corneal sensitivity or tear film distribution within 6 months before inclusion in the study, used any ocular medication for anomalies other than DED, and used any ocular medication for DED other than artificial tears during the previous month such as steroids or during the previous 3 months such as cyclosporine or tacrolimus. Patients also



**Fig. 1** Sequence of clinical evaluations. *ACE* adverse controlled environment, *BCVA* best corrected visual acuity, *CDES-Q* Change in Dry Eye Symptoms Questionnaire, *DEQ* Dry Eye Questionnaire, *MG* Meibomian

gland, *NCE* normal controlled environment, *SANDE* Symptom Assessment in Dry Eye, *TBUT* tear film break-up time, *VAS* visual analog scale

were excluded if they had any uncontrolled systemic disease that may affect the eyes, used any systemic treatment that could affect the DED during the last 3 months before the inclusion visit, underwent occlusion of the lacrimal punctum within the month before the study, wore contact lens during the month before the study or during its duration, or were pregnant or breastfeeding.

### Clinical Evaluation

Both eyes of all patients were clinically evaluated. The following parameters were evaluated following the schedule shown in Fig. 1.

#### Ocular Symptoms

The presence of DED symptoms was assessed using the DEQ-5. The questionnaire score ranges from 0 to 22, and the cut-off value to detect DED is a score of 6 or higher [21].

The Symptom Assessment in Dry Eye (SANDE) II questionnaire was used to compare symptoms among study visits and between both environmental conditions. This instrument

records the frequency and intensity of dry/irritated eyes, and the score ranges from – 50 to 50 [22].

Change in Dry Eye Symptoms Questionnaire (CDES-Q), which ranges from – 100 to 100 [23], also was used to assess changes.

For the SANDE II and CDES-Q, negative values corresponded to a worsening of symptoms and positive values to an improvement.

Finally, the visual analog scale (VAS) ranging from 0 to 100 evaluated satisfaction with the study treatment [24].

#### Visual Acuity

The best-corrected visual acuity (BCVA) was measured monocularly (Topcon Corp. Ltd., Tokyo, Japan) at 4 m using a logarithm of the minimum angle of resolution (logMAR) scale with 100% contrast.

#### Ocular Surface Evaluation (I)

A slit-lamp (SL-D7, Topcon Corp.) examination was performed to assess the ocular surface. Bulbar and tarsal hyperemia was evaluated

based on the Cornea and Contact Lens Research Unit (CCLRU) scale [25].

### ***Tear Film Break-up Time (TBUT)***

The TBUT was measured after the instillation of 5  $\mu$ l of 2% sodium fluorescein into the inferior fornix. A cobalt blue filter (Topcon Corp.) and a yellow Wratten no. 12 filter (Eastman Kodak, Rochester, NY, USA) were used. Three measurements were taken, and the average was recorded.

### ***CFS***

After 2 min of fluorescein instillation, the CFS was evaluated using the Oxford (0–5) and CCLRU (0–4 extent score for each of the 5 corneal areas) scales [25, 26]. The total CCLRU (extent) score was calculated as the sum of each score obtained in the five corneal areas.

### ***Lissamine Green Conjunctival Staining***

Staining was evaluated with the Oxford scale using lissamine green strips (GreenGlo, HUB Pharmaceuticals LLC, Rancho Cucamonga, CA, USA) wet with 25  $\mu$ l sodium chloride and applied to the inferior fornix [26].

### ***Meibomian Glands (MGs) Assessment***

The expressibility and quality of secretion of the MG were measured on a 0–3 scale [27].

## **Outcome Measures**

The primary outcome measure was a significant reduction in CFS after 1 and/or 3 months of treatment. The secondary outcome measures were significant differences in the worsening of clinical signs and/or symptoms after exposure to ACE at the 1- and 3-month follow-up visits compared with baseline.

Safety outcome measures were assessed by recording adverse events, BCVA, and slit-lamp findings.

## **Sample Size and Statistical Analysis**

The calculation indicated that 25 patients were needed to detect a minimum change of 1 point

in the CFS score (Oxford scale) considering a 20% loss ( $\alpha = 0.05$ ;  $p = 0.90$ ).

A PhD licensed statistician (I.F.) performed the statistical analysis using the R statistical package version 4.0.4 (R Core Team. Foundation for Statistical Computing, Vienna, Austria (available at <https://www.R-project.org/>). To evaluate the effect of CsA 0.1% CE treatment and exposure to ACE and their interaction in quantitative variables, linear mixed effects models were adjusted. In addition, scores from both eyes also were considered for analysis. The required model assumptions (normality, homoelasticity, linearity, and lack of outliers) were checked. Post-hoc Tukey's method for multiple comparisons adjustment was performed. Cumulative link mixed models were used to assess the effect of CsA 0.1% CE treatment and exposure to ACE (and their interaction) in non-parametric variables. Odds ratio (OR) calculations were obtained to characterize the changes observed in non-parametric variables after treatment or ACE exposure.

Parametric variables are expressed as the mean  $\pm$  standard deviation and non-parametric variables as the median and interquartile range (IQR).  $P \leq 0.05$  was considered statistically significant.

## **RESULTS**

### **Baseline Characteristics**

Twenty-six patients with DED were recruited, three did not meet the inclusion criteria, two dropped out of the study because of lack of tolerability to treatment, and one left because of scheduling constraints. In addition, nine patients could not complete the 1-month follow-up visit because of the COVID-19 pandemic lockdown, but they were contacted by phone to complete the DED questionnaires.

Twenty patients with DED (19 women, 1 man) (age  $58.9 \pm 12.3$ ; range 35–77 years) completed the study. Four had Sjögren syndrome-related DED, and the others had no extraocular involvement. At the inclusion visit, the mean BCVA was  $0.10 \pm 0.21$  (range – 0.20/0.85) logMAR in the right eye and  $0.04 \pm 0.11$

(range – 0.12/0.28) in the left eye. The mean DEQ-5 score was  $14.8 \pm 3.3$  points (range 7.0–19.0), and the median CFS (Oxford scale) was 3 (IQR, 1) bilaterally.

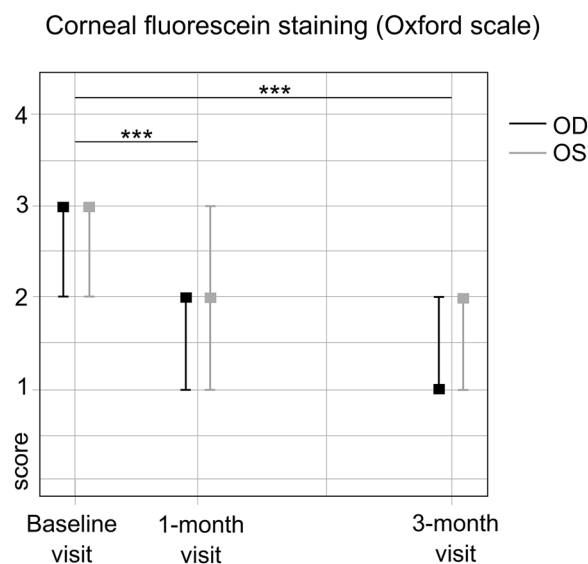
### Efficacy Analysis

The primary endpoint was achieved as the CFS (Oxford scale) decreased significantly ( $p < 0.001$ ) after 1 and 3 months of CsA 0.1% CE treatment compared to baseline in the NCE (Fig. 2). No significant ( $p = 0.69$ ) differences were seen between both eyes. After 1 and 3 months of treatment, the cumulative link mixed model estimated that the ORs for a 1-point increase in the Oxford scale were 0.02 (95% confidence interval [CI], 0.006–0.04) and 0.06 (95% CI 0.03–0.13), respectively.

Regarding CFS as measured with the CCLRU scale (extent score), the linear mixed effects model showed that CsA 0.1% CE treatment had a significant ( $p < 0.0001$ ) positive effect on the CFS. The estimated marginal means for the total CCLRU (extent score) provided by the statistical model for baseline, and the 1- and 3-month visits were 9.25 (95% CI 8.05–10.45), 6.20 (95% CI 4.90–7.50), and 5.85 (95% CI 4.65–7.10), respectively (range 0–20). The differences in the marginal means of the total CCLRU scale between baseline and the 1-month (3.05, 95% CI 2.05–4.05) and 3-month (3.40, 95% CI 2.60–4.15) follow-up visits were both significant ( $p < 0.0001$ ).

### Secondary Outcome Measures

Regarding ocular symptoms, the linear mixed effects model showed that values obtained for all questionnaires (DEQ-5, SANDE II, and CDES-Q) significantly ( $p \leq 0.003$ ) improved after 1 month of CsA 0.1% CE treatment (Table 1). In addition, the DEQ-5 values (baseline vs. 3-month visit) improved significantly ( $p < 0.0001$ ) after the 3 months of treatment (Table 1). The CDES-Q scores showed improvement ( $p = 0.02$ ) 3 months after treatment compared with the 1-month visit. The SANDE II also showed a marked tendency for improvement in the frequency and intensity items ( $p = 0.08$  and



**Fig. 2** Corneal fluorescein staining (Oxford scale) under a normal controlled environment at baseline and after 1 and 3 months of topical cyclosporine 0.1% cationic emulsion. Both eyes were considered for statistical analysis. The squares represent medians, and vertical bars represent the 25th and 75th percentiles. OD, right eye; OS, left eye. \*\*\* $p < 0.0001$

$p = 0.07$ , respectively). After ACE exposure, the linear mixed effects model showed that DED symptoms significantly ( $p < 0.0001$ ) worsened as measured with the SANDE II (frequency and intensity) and CDES-Q (Table 1). Finally, patients reported a high level of satisfaction with the treatment based on the VAS score (mean  $\geq 75$  units), which was similar for both follow-up visits (Table 1).

The outcomes of the remaining clinical signs evaluated are shown in Table 2. A significant ( $p \leq 0.03$ ) positive effect of CsA 0.1% CE treatment was seen in conjunctival bulbar and tarsal hyperemia, corneal (Oxford and CCLRU scales) and conjunctival staining, and quality of MG secretion. In contrast, exposure to the ACE had a significant ( $p \leq 0.02$ ) negative effect on bulbar hyperemia and CFS (Oxford and CCLRU scales). Specifically, the cumulative link mixed model showed that the ORs for an increase of 1 point in the bulbar hyperemia score after ACE exposure during the 1- and 3-month visits were 0.74 (95% CI 0.03–0.15) and 0.19 (95% CI 0.09–0.38), respectively. Regarding CFS, the OR

**Table 1** Values obtained for the dry eye disease questionnaires at baseline and after 1 and 3 months of topical cyclosporine A 0.1% cationic emulsion during normal and after adverse environmental conditions

Instrument	Normal controlled environment (NCE)			Adverse controlled environment (ACE)			<i>p</i> value (statistical model) NCE vs ACE
	BL visit	1-month visit	3-month visit	BL visit	1-month visit	3-month visit	
DEQ-5 (0–22)	14.0 ± 3.1	9.7 ± 4.3	8.2 ± 5.0	–	–	–	–
SANDE II (frequency) (– 50/50)	–	23.0 ± 15.6	15.2 ± 24.2	– 8.8 ± 23.8	– 2.2 ± 9.8	– 5.6 ± 23.9	0.74
SANDE II† (intensity) (– 50/50)	–	23.2 ± 16.1	14.9 ± 23.4	– 9.5 ± 23.3	– 3.9 ± 11.4	– 5.6 ± 23.9	0.77
CDES-Q† (– 100/100)	–	57.3 ± 26.6	38.1 ± 43.0	– 12.5 ± 53.9	6.4 ± 35.8	– 10.2 ± 47.4	0.77
VAS (satisfaction) (0–100)	–	75.0 ± 18.2	75.5 ± 21.1	–	–	–	–

Data are expressed as the mean ± standard deviation

BL baseline, CDES-Q Change in Dry Eye Symptoms Questionnaire, DEQ Dry Eye Questionnaire, N/A not applicable, SANDE Symptom Assessment in Dry Eye, VAS Visual Analog Scale

†For NCE, comparison with baseline; for ACE, comparison before and after exposure. Positive values indicate improved dry eye symptoms and negative values worsening of dry eye symptoms

**Table 2** Values obtained for clinical signs at baseline and after 1 and 3 months of topical cyclosporine A 0.1% cationic emulsion

	Normal controlled environment (NCE)			<i>p</i> value (statistical model)			Adverse controlled environment (ACE)			<i>p</i> value (statistical model) NCE vs ACE
	Baseline visit	1-month visit	3-month visit	Among visits	BL vs 1-month	BL vs 3-month	BL visit	1-month visit	3-month visit	
Bulbar hyperemia	2.5 (1)	2 (0)	2 (2)	< 0.0001	< 0.0001	< 0.0001	3 (1)	2 (1)	2 (1.25)	0.02
Tarsal hyperemia	2 (1)	2 (1)	1 (1.25)	< 0.0001	< 0.0001	< 0.0001	2 (1)	2 (1)	1.5 (1)	0.09
TBUT	2.3 ± 0.9	3.1 ± 1.3	2.8 ± 2.0	0.27	–	–	2.4 ± 1.0	2.9 ± 1.7	2.9 ± 2.7	0.68
CFS (Oxford scale)	3 (1)	2 (1)	1 (1)	< 0.0001	< 0.0001	< 0.0001	3 (1.25)	2 (2)	2 (2)	0.003
CFS (CCLRU scale)	8.6 ± 3.6	5.4 ± 2.3	5.0 ± 3.2	< 0.0001	< 0.0001	< 0.0001	10.1 ± 4.0	5.8 ± 2.6	5.9 ± 3.4	0.001
Conjunctival staining	1 (1.5)	1 (1)	1 (1.25)	0.03	0.03	0.81	1 (1.25)	2 (2)	1 (1)	0.71
MG expressibility	0 (1)	1 (1)	0 (0)	0.15	–	–	0 (1)	0 (1)	0 (0.25)	0.51
MG secretion quality	1 (0.25)	1 (0.5)	1 (1)	< 0.0001	< 0.0001	0.001	1 (0)	1 (0.5)	1 (1)	0.39

Quantitative variables are expressed as the mean ± standard deviation and qualitative variables as median (interquartile range). *P* values obtained from the cumulative link mixed models performed and linear mixed effects models for TBUT and CCLRU scales

BL baseline, CFS corneal fluorescein staining, CCLRU Cornea and Contact Lens Research Unit, MG meibomian gland, TBUT tear film break-up time



of a 1-point increase in the Oxford scale score after ACE exposure was 2.46 (95% CI 1.35–4.47). Regarding the total CCLRU scale (extent score), the linear mixed effects model showed that exposure to ACE increased the estimated marginal means by 1.0 point (95% CI 0.4–1.5). Regarding the Oxford and CCLRU scales, the interactions between CsA 0.1% CE treatment and environmental exposures were not significant ( $p = 0.26$  and  $p = 0.64$ , respectively). No significant ( $p \geq 0.15$ ) differences were seen in the TBUT or MG expressibility among study visits or between environmental conditions. No significant ( $p \geq 0.06$ ) differences were seen between both eyes for any clinical sign.

### Safety Analysis

No significant differences were seen in the BCVA among the study visits or between both exposures. Sixteen of the 20 study patients reported mild adverse reactions after treatment instillation, i.e., ocular discomfort, itching, or stinging, with the last the most frequent. These symptoms resolved within a few minutes after instillation.

## DISCUSSION

Treatment strategies for DED should follow staged management depending on the disease severity [28, 29]. Anti-inflammatory medications are included in a stepwise approach. They are also very useful in chronic DED to ameliorate flares triggered by desiccating stress environments [30]. CsA is an immunomodulatory drug with anti-inflammatory benefits, and its topical administration is recommended for DED management [29]. The efficacy of topical CsA has been demonstrated without causing severe side effects [31]; however, the tolerability during instillation should be fully addressed. Recently, a new CsA formulation, CsA 0.1% CE (Ikervis), has resulted in improved DED signs and symptoms 6 and 12 months after treatment [17–20]. However, data from clinical trials assessing the safety and efficacy of CsA 0.1% CE after 1 month of treatment are scarce. In

addition, the prophylactic effect of CsA 0.1% CE against flares due to environmental stress, which are very common in chronic DED [32], has not been tested. The present study provides not only such relevant information but also data obtained under controlled environmental conditions. Thus, reliability of the outcomes is higher because this methodology reduces bias from weather-related conditions [5, 33], in contrast to previous CsA 0.1% CE clinical trials in which environmental conditions were not controlled.

Regarding the primary outcome measure of this clinical trial, CFS significantly improved by 1 point (median values: 3 [1] vs. 2 [1]) as measured with the Oxford scale (0–5) after 1 month of CsA 0.1% CE use, and the effect was maintained out to the 3-months visit (Fig. 1). The OR showed that the likelihood of a 1-point increase in the CFS score (Oxford scale) was 50.0 (1/0.02) and 16.6 (1/0.06) times lower after 1 and 3 months of treatment, respectively. The CFS was evaluated using the CCLRU scale (extent score), in which the five corneal areas were assessed individually and could provide more precise measures of corneal integrity. We also wanted to characterize these possible changes in CFS using a more detailed scale instead of only using the Oxford scale that assesses the cornea. In the present study, assessment of the CFS using the total CCLRU scale also showed improved corneal integrity after CsA 0.1% CE. Regarding DED symptoms, the DEQ-5 scores improved 1 and 3 months after treatment compared with baseline. SANDE II (frequency and intensity) and CDES-Q scores improved after 1 month of treatment; however, further symptom relief was not reported during the 3-months visit compared with 1 month (Table 1, NCE).

Several questionnaires were used to assess the conventional efficacy of the treatment to ameliorate DED over 3 months and the possible symptom changes after ACE exposure. The DEQ-5 is a questionnaire recommended by TFOS DEWS II diagnostic methodology report [34]. However, because this questionnaire asks for the symptoms during the last month, it could not be used to assess changes before and after the ACE exposure. Instead, the SANDE II

and CDES-Q were designed specifically to evaluate the changes in DED symptoms [22, 23].

The SANSIKA study [17], a phase III clinical trial that assessed the effect of CsA 0.1% CE in severe DED compared with vehicle, also reported significant CFS improvements using a modified Oxford scale (0–5) after 3 and 6 months of treatment. However, the reduction in CFS after 1-month treatment was not significant from a statistical viewpoint [17]. In the SANSIKA study, the severity of the DED in patients recruited regarding the CFS score was higher than in the present study. It is possible that keratitis can take longer to improve in the presence of more severe DED, and patients might obtain only mild benefits from the protective effect of the vehicle and need more time for the DED signs to improve until the effect of CsA 0.1% showed clinical advancements. Previous authors have reported improved signs and symptoms in mild and moderate DED as soon as 2 weeks after treatment using a compound similar to the CsA 0.1% CE vehicle [35]. Thus, this might be a reason for the slightly different findings between the SANSIKA study and the present study. In fact, Geerling et al. [36] reported significant improvements in the CFS after 1 month of treatment with CsA 0.1% CE in less severe DED (Mean Oxford scale, baseline, 2.56 vs. 1 month, 1.77) in a prospective multicenter study from routine clinical practice. Another important reason to observe CFS improvement after 1 month of treatment in the present study is that the patients were always evaluated under the same environmental conditions. Thus, more consistent results should be expected during the visits when assessing ocular surface parameters, because the environmental conditions might not have biased the outcomes as occurs in other studies. The SICCANOVE study was another phase III clinical trial that assessed the efficacy and safety of CsA 0.1% CE in DED [18]. The authors used a modified Oxford scale (0–7) to assess the CFS and reported that the treatment group had lower CFS score (– 0.77, CsA 0.1% CE) than the vehicle group (– 0.52, CsA 0.1% CE) during the 1-month follow-up visit. Differences in the CFS between the CsA 0.1% CE and the vehicle (CE-CKC) were clinically negligible. The reason

might be that the vehicle can act alone as a tear substitute as previously observed [17], and patients with non-severe DED are even more likely to obtain benefits from the drug (CsA 0.1%) and the vehicle (CE).

Regarding the safety outcomes, no changes in BCVA or marked slit-lamp findings were observed. However, most patients experienced discomfort immediately after CsA instillation, which resolved within a few minutes, except for two patients whose tolerability was much lower and caused them to leave the study. In fact, the most commonly reported ocular adverse event in the literature for CsA treatments was mild instillation site pain or eye irritation [17–20]. This drug-related ocular adverse event is usually well tolerated in most patients with DED; thus, study discontinuation due to this secondary effect was observed in only 9.3% of the recruited patients in the previous CsA 0.1% CE clinical trials [37]. In the present study, two patients recruited discontinued CsA 0.1% CE treatment, which is a similar proportion to that in previous clinical trials [38]. Geerling et al. [36] also assessed the tolerability of CsA 0.1% CE in routine clinical practice and reported that only 6.6% of the participants discontinued the treatment due to poor local tolerance.

Regarding the secondary outcome measures of the present study, we assessed the DED signs and symptoms after ACE exposure because most of the population are exposed daily to adverse outdoor and indoor environmental conditions (i.e., auto climate control cars, airplane cabins, office buildings) [38]. It is well known that patients with chronic DED have episodic flares that are usually associated with exacerbation of DED signs and symptoms [32]. The flares can be triggered using environmental chambers to produce desiccating stress environments [38]. However, they also can be limited if patients were previously treated using drugs having a prophylactic effect [30, 39]. In the present study, the worsening of CFS after ACE exposure during baseline was higher (CCLRU scale from 8.6 to 10.1) than the one observed after CsA 0.1% CE treatment during the 1-month (from 5.4 to 5.8) and 3-month (from 5.0 to 5.9) visits (Table 2). However, the interaction between treatment and environmental exposures was

not significant ( $p = 0.64$ ). A larger sample size could have helped to observe significant differences in CFS before and after treatment; however the sample size was calculated based on the primary outcome measure. Regarding the DED symptoms, although questionnaire scores significantly improved 1 and 3 months after treatment ( $p \leq 0.002$ ), the worsening reported by patients after ACE was not significant for any visit ( $p \geq 0.74$ ). Thus, further larger studies should confirm that topical CsA 0.1% CE also might prevent symptom worsening provoked by adverse environments.

The main limitation of this clinical trial was the lack of a vehicle-treated control group. However, the effects of the vehicle in DED have been reported widely during previous large-sample clinical trials, the SANSIKA and SICCA-NOVE studies [17–20]. The aims of the present study were to provide new more reliable data describing the early short-term efficacy of CsA 0.1% CE controlling the environmental conditions to reduce bias and to assess the ability of CsA 0.1% CE to ameliorate flares when patients with DED are exposed to adverse conditions. Thus, there was no need to include a control group using the CsA 0.1% CE vehicle because an adequate statistical analysis can be performed using multivariate models that consider the clinical parameters assessed during all visits and during both environmental conditions. Another limitation was that the study followed an open-label unmasked design. It was decided this way because it was already proven that CsA 0.1% CE was safe and effective after 6 months of use [17–20], and the goal of the present study was different from a pivotal phase III clinical trial aiming to achieve regulatory agency approval. Besides, the investigator who performed the qualitative assessments was masked to the data corresponding to previous visits; consequently, bias associated with the design was likely to be low. Finally, another limitation is that the study started before the COVID-19 pandemic lockdown, and several participants could not complete the 1-month visit. Nonetheless, data from the rest of the participants was collected and properly analyzed from a statistical viewpoint.

## CONCLUSION

In conclusion, the present study showed that topical CsA 0.1% CE can improve signs and symptoms by 1 month of treatment in patients with moderate DED who previously did not benefit from tear substitute therapy. This improvement in the signs and symptoms of ocular surface damage continued 3 months after starting treatment. Larger studies should corroborate that topical CsA 0.1% CE can protect patients from exacerbation of DED signs and symptoms when exposed to desiccating stress environments.

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**Data Availability.** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Conflict of Interest.** No conflicting relationship exists for any author. Disclosures of Dr. Margarita Calonge are the following: Research/clinical trials contracts, consultancies, advisory boards and/or lectures for Novartis, Santen Pharmaceutical, Johnson & Johnson, Horus Pharma, Fidia Farmaceutica, UrsaPharm GmbH, and Thea Laboratories. Alberto Lopez Miguel is an Editorial Board member of *Ophthalmology and Therapy*. Alberto Lopez Miguel was not involved in the selection of peer reviewers for the manuscript nor any of the subsequent editorial decisions. The remaining authors have no relationship to disclose.

**Ethical Approval.** The Ethics Committee of the Valladolid University Clinical Hospital (Valladolid, Spain) and the Spanish Drugs and Health Products Administration (<https://www.aemps.gob.es/>) (EudraCT number: 2019-000982-19) approved the trial. It was registered at the US National Institutes of Health (ClinicalTrials.gov, ID: NCT04492878). It was conducted at the Institute of Applied Ophthalmobiology, University of Valladolid, Valladolid, Spain. The study followed the tenets of the Declaration of Helsinki and the Good Clinical Practices.

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