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# Efficacy and Safety of VisuEvo® and Cationorm® for the Treatment of Evaporative and Non-Evaporative Dry Eye Disease: A Multicenter, Double-Blind, Cross-Over, Randomized Clinical Trial

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**Purpose:** To compare the efficacy of the new lubricating product VisuEvo® (VSE) vs Cationorm® (CTN) in patients with dry eye disease (DED).

**Methods:** Seventy-two patients with evaporative (n=54) and non-evaporative DED (n=18) were included in a multicenter, double-blind, 12-week cross-over study to receive VSE (6 weeks) and CTN (6 weeks) in randomized sequence. After baseline, two visits were performed during each period (intermediate and final visit, respectively at 2 and 6 weeks from the beginning of each period). Primary (tear break-up time, TBUT) and secondary endpoints (Schirmer I, Ferning, blink rate, osmometry, cytokine and lipid expression, ocular surface staining, patient satisfaction, and OSDI score) were compared.

**Results:** Sixty-three patients were evaluated for efficacy and 68 patients for safety. The intergroup differences for mean TBUT values were not significant at any study visit (baseline 3.2 ±1.5 sec; intermediate visits 4.5 ± 1.9 and 4.5 ± 1.8 sec in VSE and CTN groups, respectively, p = 0.10; final visits 5.4 ± 2.4 and 6.0 ± 3.1, respectively, p=0.63). Also, the assessment of secondary endpoints showed no significant difference between the two groups. The two study treatments were equally effective in evaporative and non-evaporative DED. The safety profile was excellent for both ocular treatments; transient blurred vision was observed in 11 patients only during CTN, 10 patients only during VSE, and 16 during both treatments.

**Conclusion:** VSE was non-inferior to CTN in restoring tear film composition, increasing its stability and reducing ocular surface damage in evaporative and non-evaporative DED patients.

**Study Identifier:** NCT03833882.

**Keywords:** evaporative dry eye disease, tear break-up time, Ocular Surface Disease Index questionnaire, meibomian gland disturbance, glaucoma, ocular surface

## Introduction

Dry eye disease (DED) is

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.[1]

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Prevalence of DED ranges from approximately 5% to 50%;[2,3] incidence in a Caucasian population aged 48–91 years is about 2%, being more common in women (25%) than in men (17.3%).[2]

The most relevant risk factors for DED are increasing age, female sex and Asian ethnicity.[2] The endocrine system plays a significant role in the regulation of the ocular surface and adnexa. The decrease in serum androgen levels that occurs during menopause, pregnancy, lactation, or the use of estrogen-containing oral contraceptives may trigger tear deficiency. On the other hand, the breakdown of hormonal balance and induction of an androgen-deficient condition is associated with obstructive Meibomian gland disturbance (MGD).[4] Obstructive MGD is the most common cause of evaporative dry eye (EDE) and it is believed that MGD-dependent EDE is the most common form of DED overall.[5]

Other pathogenetic factors are abnormal immune response,[6,7] and ocular surface toxicity induced by the chronic use of medications and preservatives.[1] In glaucoma, the chronic use of topical medications is frequently associated with impairment of the corneal glycocalyx and of the mucins produced by conjunctival goblet cells,[8] which in turn can determine non-evaporative dry eye (NEDE).

Tear supplementation is considered a mainstay of DED therapy, in order to increase or stabilize the natural tear film. Two main categories of lubricating molecules are available: those increasing tear volume (polymers such as hyaluronic acid or cellulose derivates), and those improving tear stability (lipids). Despite eye drops targeted to aqueous supplementation are among the most commonly used, lipid-containing eye drops are growing in both availability and popularity, primarily due to the increased attention paid to hyper-evaporation in the pathogenesis of DED.[9]

Cationorm® (CTN)(Santen Ltd, Japan) is a clinically well-established lipid treatment for EDE. It is a preservative-free cationic nano-emulsion containing mineral oils, surfactants (cetalkonium chloride, tyloxapol, poloxamer), and glycerin. VisuEvo® (VSE)(Visufarma SpA, Italy) is a new multidose, non-preserved ophthalmic solution with antioxidant activity that uses a liposomal nano-dispersion associated with vegetable oil rich in Omega 3 (docosahexaenoic acid – DHA and eicosapentaenoic acid – EPA), Vitamin D3 and Vitamin A palmitate. Due to their compositions, both treatments are expected to be effective in EDE but also in non-Sjogren NEDE patients. The lipid structures are capable of effectively stabilizing impaired lipid layer (as already shown for CTN).[10] The presence of cetalkonium chloride in CTN would modulate inflammatory response,[11] as well as the presence of Omega 3 (DHA and EPA) and Vitamin D in VSE is able to modulate the immune and inflammatory responses in DED by means of resolving expression.[12,13] VSE also contains Vitamin A which may support goblet cells activity and epithelial integrity.[14]

In literature, there are numerous clinical trials on lubricating eye drops but, in most cases, they are cohort studies or uncontrolled studies; there is relative lack of prospective randomized head-to-head studies. The present study aimed at comparing the clinical performances of two lipid eye drops (VSE and CTN) by means of a prospective randomized study performed on both EDE and NEDE patients.

## Materials and Methods
### Patients and Study Design

This was a pre-market, multicenter, double-blind, randomized, prospective cross-over study. It was conducted at the Eye Clinic, ASST Santi Paolo Carlo, San Paolo Hospital, University of Milan, Milan, Italy and at the Department of Biomedical and Clinical Sciences, Fatebenefratelli-Sacco Hospital, Milan, Italy. It was registered at www.clinicaltrial.gov (identifier: NCT03833882) and approved by Comitato Etico Milano Area 1; it followed the Tenets of the Declaration of Helsinki; informed consent was obtained from participants.

72 consecutive patients with DED fulfilling the inclusion and exclusion criteria listed in Box 1 were included. Two groups of patients were studied: EDE (n = 54) and NEDE (n = 18). EDE patients had MGD or abnormal hormonal state or high tear film instability; NEDE were patients treated with BAK-preserved anti-glaucoma eyedrops for at least 2 years, showing mucous layer instability (Box 1).

The flow of the study is described in Figure 1. The study lasted 12 weeks for each patient (two 6-week treatment cycles) and included six visits: Screening visit (V1,1 week prior to baseline), baseline visit (V2, randomization, dispensation of the first treatment to be started the following day), week 2 (V3, intermediate visit of the first cycle), week 6 (V4, final visit of first cycle, dispensation of the second treatment to be started the following day), week 8 (V5, intermediate visit of the second cycle), and week 12 (V6, final visit of second cycle and end of study visit). At screening visit patients stopped any eventual lubricating treatment;

**Box I** Inclusion Criteria and Exclusion Criteria

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Inclusion criteria were:

1. At least 18 years old
2. Schirmer I test >10 mm at 5 minutes
3. BUT< 7 seconds
4. OSDI score of 13 or more
5. Patients falling in one of the following groups:

   a. presence of active obstructive Meibomian gland disease, defined as at least one of the following:
      ○ Meibomian orifice plugging
      ○ Eyelid margin foaminess
      ○ Changes in orifice position with respect to the mucocutaneous junction
      ○ Abnormal Meibomian gland secretions (opaque and viscous-like form that is difficult to express)

   b. high tear film evaporation

   c. females in menopause, both using hormonal integration or not

   d. glaucomatous patients receiving one or more BAK preserved treatments for at least 2 years, showing an abnormal Ferning test (Types 3 or 4 according to Rolando et al[15])

The exclusion criteria were:

1. Diseases or conditions known affect corneal sensitivity: Diabetes, long-standing contact lens wearing, previous ocular herpes infections, previous refractive surgery
2. Coexisting corneal diseases
3. Autoimmune diseases
4. Past or active cicatricial conjunctivitis
5. Past ocular surface burns
6. Keratinization of the eyelid margin
7. Sjogren syndrome
8. History of corneal trauma
9. Any eye surgery performed 3 months before inclusion
10. Current use of contact lenses
11. Pregnant and lactating women
12. Inability to self-administer study medications
13. Known allergic sensitivity to any of the device ingredients or any other known allergy
14. Participation in a clinical trial during the 3 months prior to the beginning of the study

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at baseline untreated DED parameters were measured and patients were then randomized (centralized randomization by means of a list of random numbers) to receive either VSE or CTN with a 1:1 ratio for the following 6 weeks. After this time lapse, patients were switched to the opposite therapy for 6 additional weeks without wash-out period. From the day after baseline to the end of the study, patients self-administered one drop of the study devices into conjunctival sac three times daily.

## Study Procedures

At each visit, patients underwent the following tests in the following order: Ocular Surface Disease Index questionnaire (OSDI, Copyright 1995, Allergan Inc, Irvine, California, US),[16] measurement of blinking, osmometry, TBUT, fluorescein staining, lissamine staining, Schirmer I test, tear Ferning test, tear sampling and cytokine and lipid expression. Measurement of blinking, osmometry, tear sampling, Ferning test was performed on subgroups of patients. Test procedures are described in detail on Box 2.

## Outcome Assessments

The primary objective was the comparison of both ophthalmic treatments in improving tear film stability (TBUT increase in seconds). The secondary outcomes were improvements of symptoms (OSDI score), and the other ocular signs (Box 2). The safety profile of both medical devices was assessed by monitoring the occurrence of adverse events (AEs) during the study.

## Statistical Analysis

Both eyes of each patient, if eligible, were tested and treated. The analyses were then performed only on the worse eye per patient based on TBUT. In case of identical TBUT value the right eye was used.

### Sample Size

Sample size estimate was calculated setting the error to 5%, the worth-detecting difference was 1 second, delta of −0.5, standard deviation of 2.3 seconds, with a power of 80%. 64 patients were necessary. Taking into account a 10% drop-out rate, 72 patients were enrolled.

### Efficacy Analysis

This analysis was performed using intent-to-treat set. Cross-over data were analyzed by summing up the data of patients treated with the same eye drop during the first and the second 6-week period. A mixed-model analysis of variance was used to compare treatment groups and changes from baseline to each study visit, including the following effects: patients as random effect, drug, visits, drug by visit interaction, treatment sequence and baseline value as fixed effects. This model corrected the estimates for baseline value. The presence of a potential carry-over effect was evaluated using a likelihood ratio test between two mixed models including carryover effect or not. Study groups were also compared at each visit by means of $t$-test for paired data after Kolmogorov–Smirnov test was used to check data normality.

To test non-inferiority of VSE vs CTN, confidence intervals (CI) were estimated from mixed model and
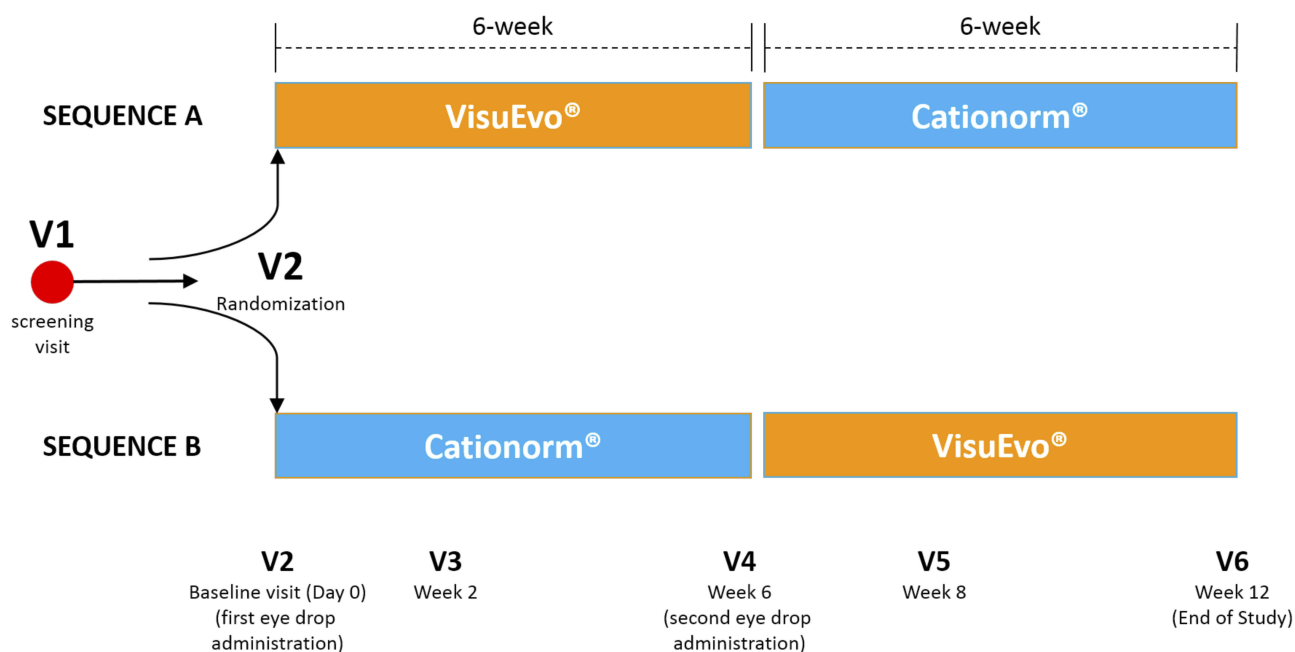
**Figure 1** Flow of the study design. After verifying eligibility at V1, patients received washout from lubricating treatments until V2. They then entered two 6-week periods. The first product was randomly assigned at V2, the second at V4. V3 and V5 were the intermediate visits of the first and second 6-week period, respectively. No washout occurred between V4 and V5.

lower boundary of the CI was compared to the non-inferiority margin (equal to −0.5) in order to test the non-inferiority. The null hypothesis was TBUT difference between two treatments of 0.5 sec or less. If it was rejected, non-inferiority of the study treatment was accepted.

## Results

Overall, 72 subjects were enrolled in the study; age was 65 ±17 years; 73% were female. In EDE patients age was 63 ± 17 years; 82% were females. In NEDE patients age was 71 ± 12 years; 43% were females. Nine patients were excluded from efficacy analysis (n=63); four patients were excluded from safety analysis (n=68) because they did not self-administer the eye drop correctly.

### Efficacy Data, Whole Population

TBUT increased from baseline to intermediate visits by +1.3 seconds in both treatment groups; afterwards, a further increase was shown: +0.9 for VSE, and +1.5 seconds for CTN ($p < 0.001$ vs baseline, Table 1). Differences between the two treatment groups were not significant at any visit ($p > 0.10$). The estimated effect of treatment products was −0.57 (CI 95% −1.19 to 0.05), and non-inferiority of VSE over CTN was achieved. The

similar effect of the two products is also confirmed by Blandt–Altmann plot between baseline and final visits (Figure 2).

OSDI scores significantly decreased from baseline (37 ± 12) to the following visits (23 ± 15 in both groups at intermediate visit, and 19 ± 13 in VSE group and 19 ± 14 in CTN group at final visits; $p < 0.001$ for both treatments compared with baseline, Table 1, Figure 3), with no inter-treatment differences at any visit ($p > 0.43$).

Osmometry was 314 ± 23 mOsm/L at baseline and 305 ± 16 at intermediate visits in both groups. At final visits, it was 305 ±15 in VSE groups and 307 ± 20 in CTN group ($p < 0.05$ compared with baseline for all visits and both treatments). No significant differences between treatments were shown ($p > 0.46$).

Blinking analysis was performed excluding 4 outliers due to side effects (data not shown). The trend of blinks during the study was similar in CTN and VSE: the number of complete blinks was overall stable, whereas a significant, progressive reduction of incomplete blinks was shown throughout the study ($p < 0.001$); this phenomenon also caused a significant reduction of the number of total blinks ($p < 0.001$; Table 1).

In both treatment groups, fluorescein staining showed an initial improvement by 15% between baseline and the intermediate visit, then remaining constant until the end of

**Box 2** Diagnostic Procedures of the Study

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**OSDI**

OSDI questionnaire was administered to the patient by a nurse who helped in filling in the answers without interfering on patient's judgement. OSDI score ranges from 0 to 100 (0–12, normal; 13–22, mild DED; 23–32, moderate DE; 33 or more, severe DED).[16] OSDI was measured on the whole population at all visits.

**Blinking**

Measurement of the number of complete and incomplete blinking was performed by recording a 1-minute video under normal room temperature (20–26 °C) and room humidity (rH up to 50per cent) in a room with medium lighting.

This test was performed at all visits in patients of Site 2 (N=36).

**Osmometry**

Tear osmometry was measured by means of i-Pen (I-MED Pharma Inc., Dollard-des-Ormeaux, QB, Canada).

The sample was taken from just above the lower eyelid tear meniscus, with the patient fixating upwards. Care was paid to avoid contact between the probe and the globe, and to avoid eyelid dislocation from the eye.[17,18] Tear osmolarity was measured at least one hour after the latest eye drops instillation. Osmometry was measured to both eyes on a random sample of 20 patients at visits between V2 and V6.

**TBUT**

TBUT was measured by determining the lapse between end of blinking and tear break-up. TBUT was performed after instillation of 2 μL of 2% preservative-free sodium fluorescein solution into the inferior conjunctival cul-de-sac of each eye. To thoroughly mix the fluorescein with the tear film, the patient was instructed to blink several times. In order to achieve maximum fluorescence, the examiner waited approximately 30 seconds from instillation before evaluating TBUT. With the aid of a slit lamp at 10X magnification using cobalt blue illumination, the examiner monitored the integrity of the tear film, noting the time it took to form lacunae (black spaces in the fluorescent tear film) from the time that the eye was opened after the last blink. TBUT was measured twice during the first minute after the instillation of the fluorescein. If the 2 readings differ by more than 2 seconds, then a third reading was taken. The TBUT value was the average of the 2 or 3 measurements.[17]

TBUT was measured on the whole population at all visits.

**Corneal and Conjunctival Fluorescein Staining**

Corneal fluorescein staining was assessed immediately after TBUT. Reading was performed between 1 and 4 minutes after fluorescein instillation, to ensure that the dye did not diffuse into stroma, blurring the discrete margin of any staining defects. The eye was examined at the slit lamp (16X magnification) using a yellow barrier filter and cobalt blue illumination.

Staining using fluorescein was graded using the Oxford scale for cornea and conjunctiva separately.[17]

Lissamine was instilled after fluorescein staining had been evaluated by a moistened and saturated filter paper strip. A red filter (567–634 nm) to enhance contrast against the sclera was used to enhance staining visibility, and van Bijsterveld grading system was used.[17]

Corneal and conjunctival staining were evaluated on the whole population at all visits.

**Schirmer Test**

Schirmer test was performed without anesthesia, 15 minutes after corneal fluorescein test, in a dimly lit room. While the patient looked upwards, the lower lid was drawn gently downwards and temporally. The rounded bent end of a sterile strip was inserted into the lower conjunctival sac over the temporal one-third of the lower eyelid margin. Care was paid not to directly touch the strip with the fingers to avoid contamination of skin oils. After 5 minutes the strip was removed and the length of the tear absorption was measured.[17]

Schirmer Test was measured on the whole population at all visits.

**Tear Ferning Test**

A tear sample (1–2μL) was pipetted onto a clean glass microscope slide and allowed to dry for 7–10 minutes under normal room temperature (20–26°C) and room humidity (rH up to 50%). The slide then will be observed under a light microscope at high magnifications and classified according to Rolando's classification (Type 1: uniform large arborization; Type 2: abundant Ferning of smaller size than type 1; Type 3: partially incomplete Ferning; Type 4: no Ferning. Type 1 and 2 are reported to be normal).[15,19]

Ferning test was performed to study eye on 18 NEDE patients at V1, V2, V4, V6.

**Cytokine and Lipid Analysis**

Tear collection of at least 100 μL was performed on study eye by means of capillary tubes. Tear samples were then split in two and analyzed.

Tear samples were analyzed for pro-inflammatory cytokines IFN, IL-6, IL-8, by means of ELISA using a Luminex multiplex basis (which enables the quantification of the three proteins simultaneously in the same sample).[20] Lipid expression was evaluated by mass spectrometry. Four molecules were studied: ceramides, sphingomieline, sphingosine and sphingosine 1 phosphate.[21]

These tests were performed on a subgroup of 20 patients at Site 2 at V2, V4, V6.

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**Table 1** Results of Primary and Secondary Endpoints on the Whole Study Population (N=63)

| Endpoints | Total | Mean±SD | | P§ |
|---|---|---|---|---|
| | | VSE | CTN | |
| **TBUT (sec)** | | | | |
| Baseline | 3.2±1.5 | 3.2±1.5 | | |
| Intermediate visit | 4.5±1.9 | 4.5±1.9** | 4.5±1.9** | 0.10 |
| Final visit | 5.7±2.7 | 5.4±2.4** | 6.1±3.0** | 0.63 |
| Significant ANOVA? No, p=0.073 | | Significant carryover effect?No ($X^2$ = 2.7)# | | |
| **OSDI questionnaire score** | | | | |
| Baseline | 37±12 | 37±12 | | |
| Intermediate visit | 23±15 | 23±15** | 23±15** | 0.43 |
| Final visit | 19±13 | 19±13** | 19±14** | 0.44 |
| Significant ANOVA? No, p=0.19 | | Significant carryover effect?Yes ($X^2$ = 7.1)# | | |
| **Schirmer I test (mm/5 min)** | | | | |
| Baseline | 16±5 | 16±5 | | |
| Intermediate visit | 15±8 | 15±8 | 15±8 | 0.37 |
| Final visit | 15±9 | 15±9 | 15±9 | 0.31 |
| Significant ANOVA? No, p=0.16 | | Significant carryover effect?Yes ($X^2$ = 6.4)# | | |
| **Osmolarity (mOsm/L)** | | | | |
| Baseline | 314±23 | 314±23 | | |
| Intermediate visit | 305±16 | 305±16 | 305±16 | 0.46 |
| Final visit | 306±17 | 305±15* | 307±20* | 0.60 |
| Significant ANOVA? No, P=0.36 | | Significant carryover effect? Yes ($X^2$ = 7.9)# | | |
| **Complete blinkings (N/min)** | | | | |
| Baseline | 14±12 | 14±12 | | |
| Intermediate visit | 17±10 | 17±10 | 17±10 | 0.42 |
| Final visit | 16±14 | 15±13 | 17±15 | 0.79 |
| Significant ANOVA? Yes, p=0.017 | | Significant carryover effect?Yes ($X^2$ =11.3)# | | |
| **Incomplete blinkings (N/min)** | | | | |
| Baseline | 7±5 | 7±5 | | |
| Intermediate visit | 5±5 | 5±5* | 5±5* | 0.35 |
| Final visit | 3±3 | 4±4* | 2±2** | 0.0004 |
| Significant ANOVA? Yes, p=0.005 | | Significant carryover effect?No ($X^2$ = 3.6)# | | |
| **Total blinkings (N/min)** | | | | |
| Baseline | 21±12 | 21±12 | | |
| Intermediate visit | 22±13 | 22±13** | 22±13** | 0.45 |
| Final visit | 19±14** | 19±12** | 19±15** | 0.48 |
| Significant ANOVA? Yes, p=0.02 | | Significant carryover effect?Yes ($X^2$ = 12.0)# | | |

**Notes:** §Paired *T*-test to compare two treatment group, adjusting by baseline value. #Cut-off value being 3.84. *T*-test vs baseline, p<0.05. **T*-test vs baseline, p<0.001.
**Abbreviations:** CTN, Cationorm; VSE, VisuEvo.
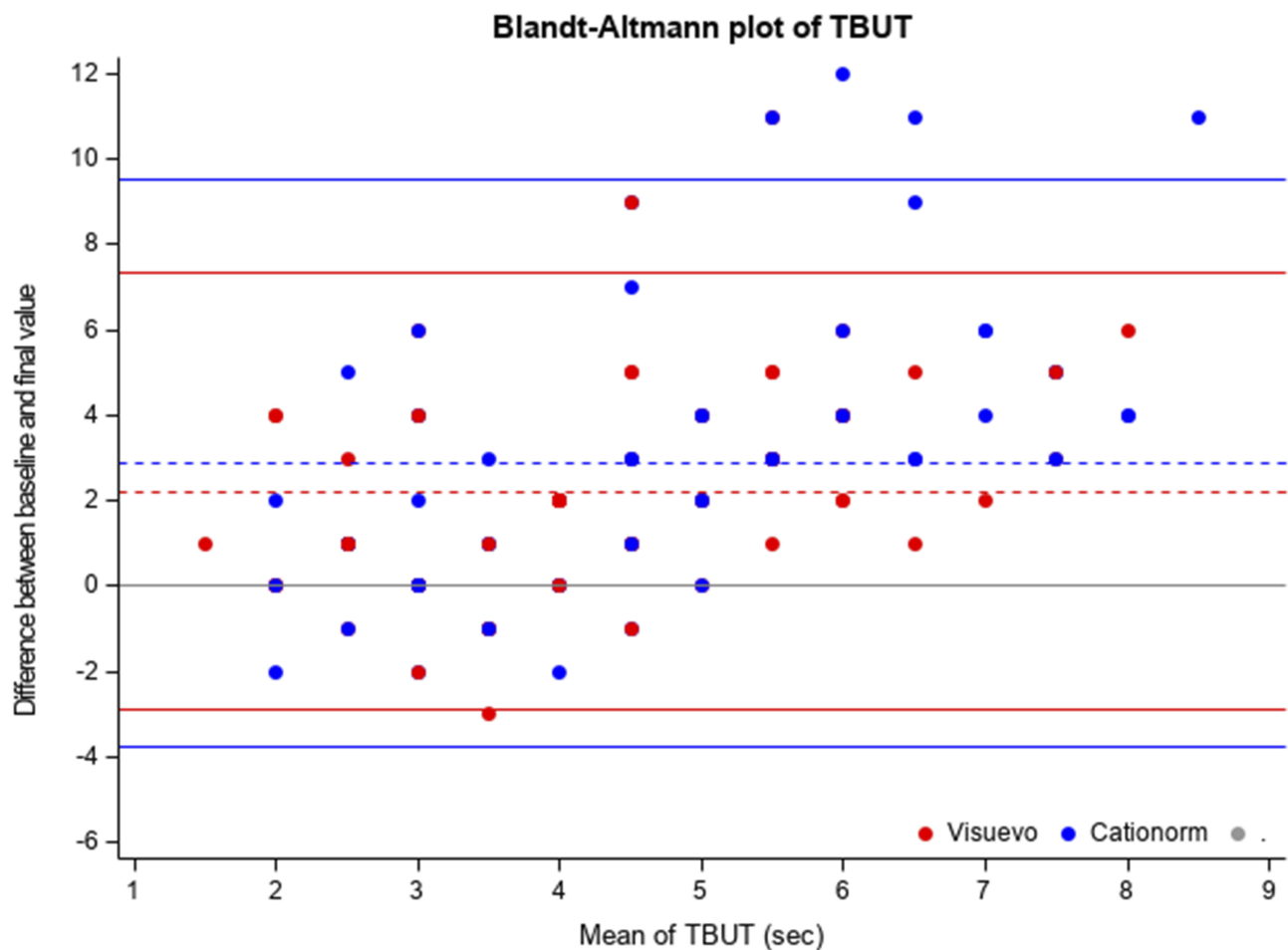
**Figure 2** Blandt–Altmann plot for comparison of baseline and final TBUT values between study treatments.

the study (Figure 4), with negligible inter-treatment differences (p > 0.60).

The expression of cytokine IL-6, IL-8, and IFN and lipids is reported in Table 2. All these parameters reduced during the study; the difference was statistically significant for IFN, sphingosine and sphingosine 1 phosphate both in the whole population ($0.06 < p < 0.0014$); no inter-treatment differences were shown.

## Efficacy Data, EDE and NEDE Patients

The main findings for these patients are shown in Table 3. The significant improvements of TBUT shown in the global data with both treatments were confirmed in both EDE and NEDE patients, even though NEDE had worse TBUT at baseline and a minor response throughout the study. The effects of treatments on OSDI improvements were similar for EDE and NEDE patients. Of note, NEDE were less symptomatic at baseline ($39 \pm 13$ EDE vs $32 \pm 8$ NEDE). Relevant differences were shown at

osmometry. EDE had similar findings with both treatments (p=0.26), whereas VSE showed better performances than CTN in NEDE patients ($299 \pm 18$ vs $318 \pm 26$ mOsm/L) although this difference was not statistically significant (p = 0.10).

Blinking patterns were also very different in the two groups. At baseline, EDE patients had a slightly higher number of total blinks ($23 \pm 12$ EDE, $20 \pm 13$ NEDE), a significantly higher number of complete blinks ($16 \pm 13$ EDE, $9 \pm 5$ NEDE, p = 0.02), and a significantly lower number of incomplete blinks ($7 \pm 6$ EDE, $11 \pm 9$ NEDE, p = 0.04). At the end of the study, the scenario between EDE and NEDE was completely changed. In EDE patients, no statistically significant changes were found: total blinks had slightly increased to $25 \pm 17$ and complete blinks to $22 \pm 18$; incomplete blinks halved to $3 \pm 4$. In NEDE patients, total blinks nearly halved ($11 \pm 6$, p < 0.001), complete blinks remained stable, and an important decrease of incomplete blinks was shown ($3 \pm 4$, p < 0.001).

## Blandt-Altmann plot of OSDI Questionnaire
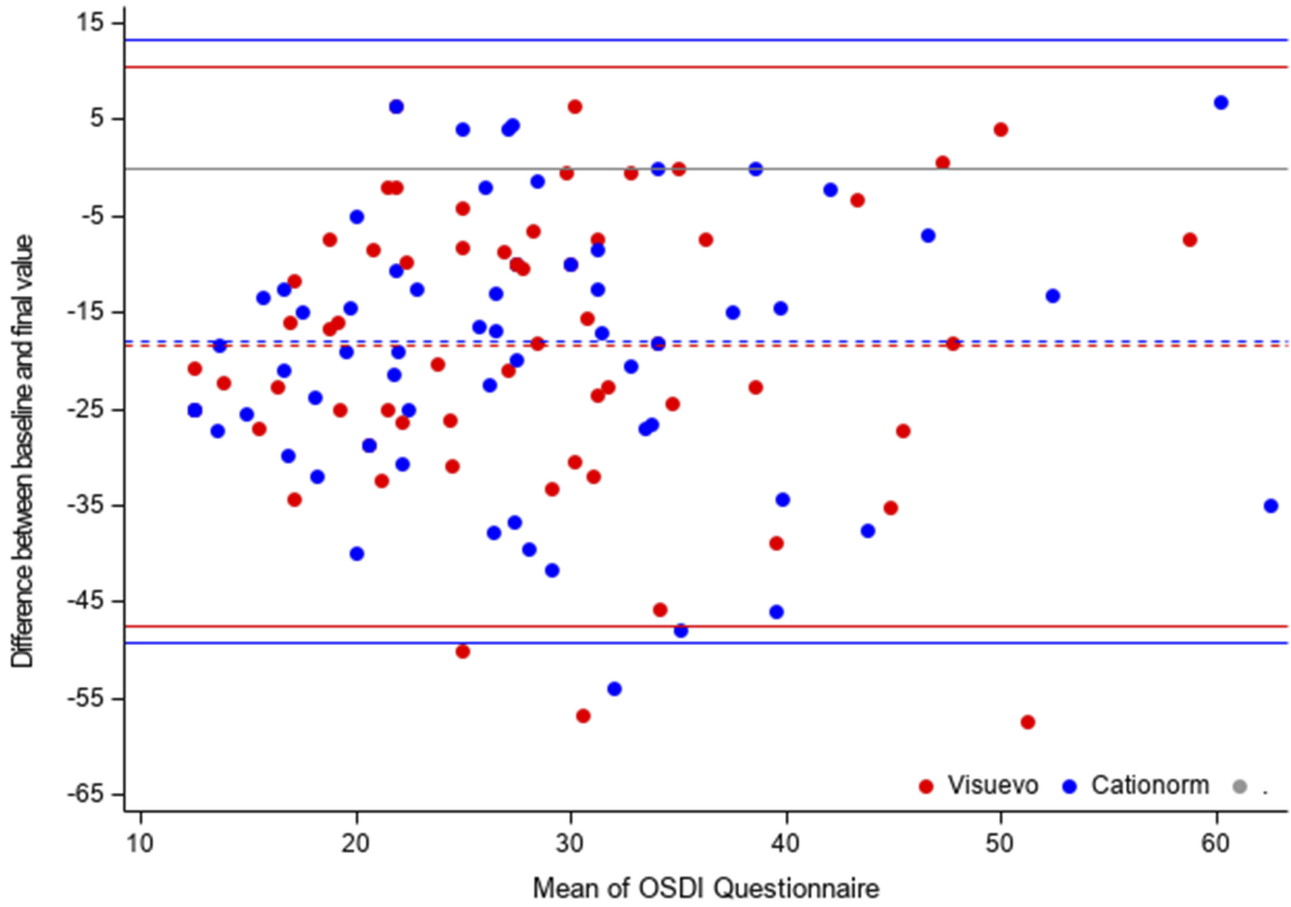


**Figure 3** Blandt–Altmann plot for comparison of baseline and final OSDI scores between study treatments.

## Staining grade with fluorescein
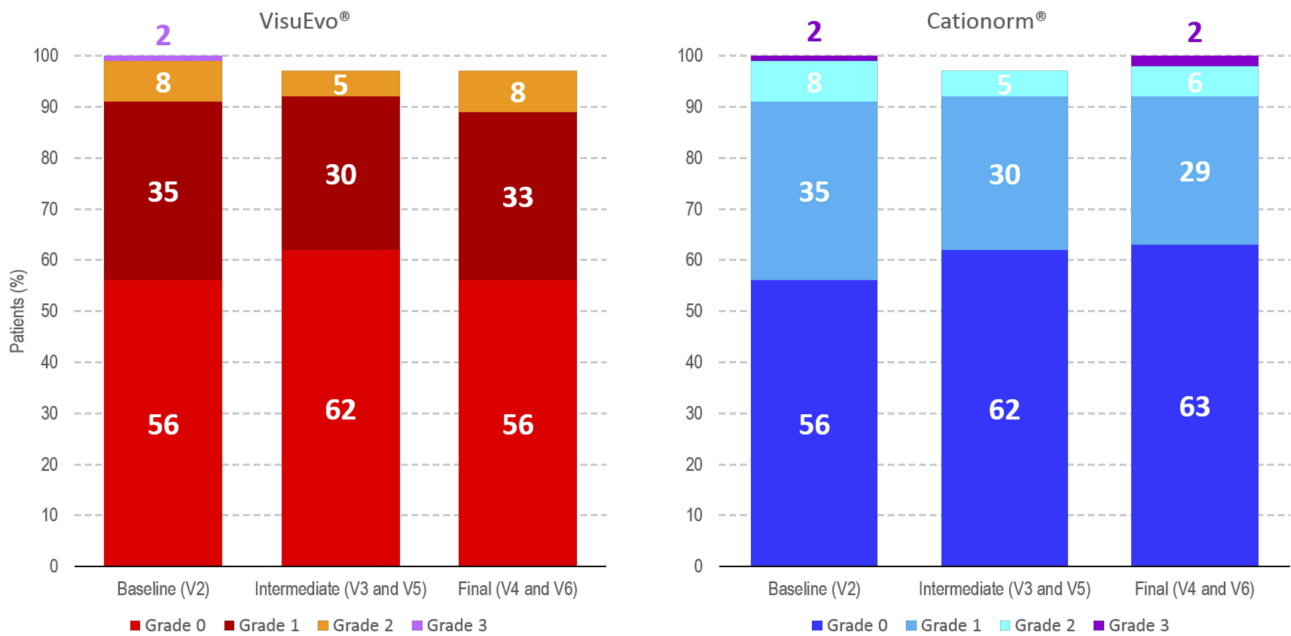


**Figure 4** Changes of proportion (%) of patients with staining grade with fluorescein during the study visits with both ophthalmic solutions (VisuEvo® and Cationorm®).

**Table 2** Tear Expression of Cytokines and Lipids

| | TOTAL | EDE | NEDE |
|---|---|---|---|
| **IL-6 (pg/mL)** | | | |
| Baseline | 12.62 ± 14.67 | 15.45±16.80 | 6.48±5.38 |
| Final visit | 8.15 ± 11.26 | 9.98±12.96 | 3.82±2.74 |
| **IL-8 (pg/mL)** | | | |
| Baseline | 623.7 ± 719.6 | 738.6±853.6 | 374.7±242.7 |
| Final visit | 502.5 ± 1266.0 | 619.8±1499.3 | 225.4±173.3 |
| **IFN (pg/mL)** | | | |
| Baseline | 2.81 ± 2.09 | 3.11±2.42 | 2.16±1.17 |
| Final visit | 1.69 ± 1.97* | 1.89±2.22* | 1.24±1.14 |
| **Ceramides (pmol/mg)** | | | |
| Baseline | 181 ± 248 | 202 ± 295 | 137 ± 93 |
| Final visit | 100 ± 106 | 106 ± 125 | 86 ± 33 |
| **Sphingomieline (pmol/mg)** | | | |
| Baseline | 596 ± 653 | 648 ± 733 | 484 ± 393 |
| Final visit | 379 ± 378 | 405 ± 443 | 319 ± 139 |
| **Sphingosine (pmol/mg)** | | | |
| Baseline | 537 ± 435 | 581 ± 502 | 441 ± 193 |
| Final visit | 179 ± 135* | 155 ± 120* | 236 ± 158 |
| **Sphingosine 1 phosphate (pmol/mg)** | | | |
| Baseline | 4 ± 2 | 4 ± 2 | 4 ± 3 |
| Final visit | 2 ± 1* | 2 ± 1* | 2 ± 1 |

**Notes:** *T-test to compare intermediate visit and final visit with baseline n the totality of subjects. p-value < 0.05.

Relevant differences in the two groups were also shown at fluorescein staining. EDE patients had no staining in 58% of cases at baseline, and this percentage progressively increased at 66% at final visits. On the other hand, NEDE patients had no staining in just 35% of cases at baseline. After a temporary improvement at intermediate visits, this percentage returned stable at final visits. The effects of the two treatments were similar in the two DED populations.

On EDE patients, biochemical analysis reflected the data on the whole population, with a statistically significant reduction of IFN, sphingosine and sphingosine 1 phosphate in the course of the study. No differences were shown for NEDE patients.

Ferning test was assessed in 17 NEDE patients who had type 3–4 at screening visit. The two treatments were effective in progressively improving the proportion of

normal (type 1–2) Ferning tests: at intermediate visit, they were 21% in both groups; at the final visit, 57% with VSE and 50% with CTN (p = 0.70, Fisher exact test) (Figure 5).

## Safety Data
The two treatments had high safety profiles, with mild or unrelated to treatment AEs. VSE caused less blurred vision than CTN, both for number of days with blurring (median of 6 days for CTN and 3 days for VSE, p = 0.37) and the minutes with blurred vision after instillation (13 vs 11 min, respectively, p = 0.96).

## Discussion
This randomized prospective study compared the efficacy of a new lipid eye drop (VSE) with one of the most commonly prescribed lipid eye drops (CTN) and it showed that the two treatments are equally effective in reducing signs and symptoms on a population with DED.

VSE was as effective as CTN in providing a significant and progressive TBUT amelioration throughout the study, reaching +2.5 sec. This beneficial effect was confirmed by a reduction of symptoms (mean OSDI was 39 at baseline, ie severe symptoms, and 19 at the end of the study, ie mild symptoms). A 15% amelioration of ocular surface staining was also found with both treatments. The agreement of TBUT, OSDI and ocular surface staining has been shown in other studies,[21,22] and it is probably, together with ease of use, the main reason of the diffusion of these tests. Schirmer test did not change over the course of the study, and this is an expected finding in a group of non-Sjogren patients with normal function of the main lacrimal gland.

Also "second-level" tests showed consistent results. Osmometry showed a progressive, similar reduction in the two populations, from a mean of 314 mOsm/L at baseline (corresponding to mild DED) to 305–307 mOsm/L at the end of the study (corresponding to normality according to Sullivan et al[4]). Another sign of enhanced tear film stability was the progressive reduction of incomplete blinks found with both treatments.[23,24] Also, proinflammatory cytokine and lipids reduced in the course of study in both treatment arms.

The effects of the two drops used in this study are close to those observed for CTN using TBUT on mild to moderate DED,[10] and OSDI and ocular surface staining on moderate to severe DED.[25] The limited number of clinical studies comparing polymers and lipids suggests
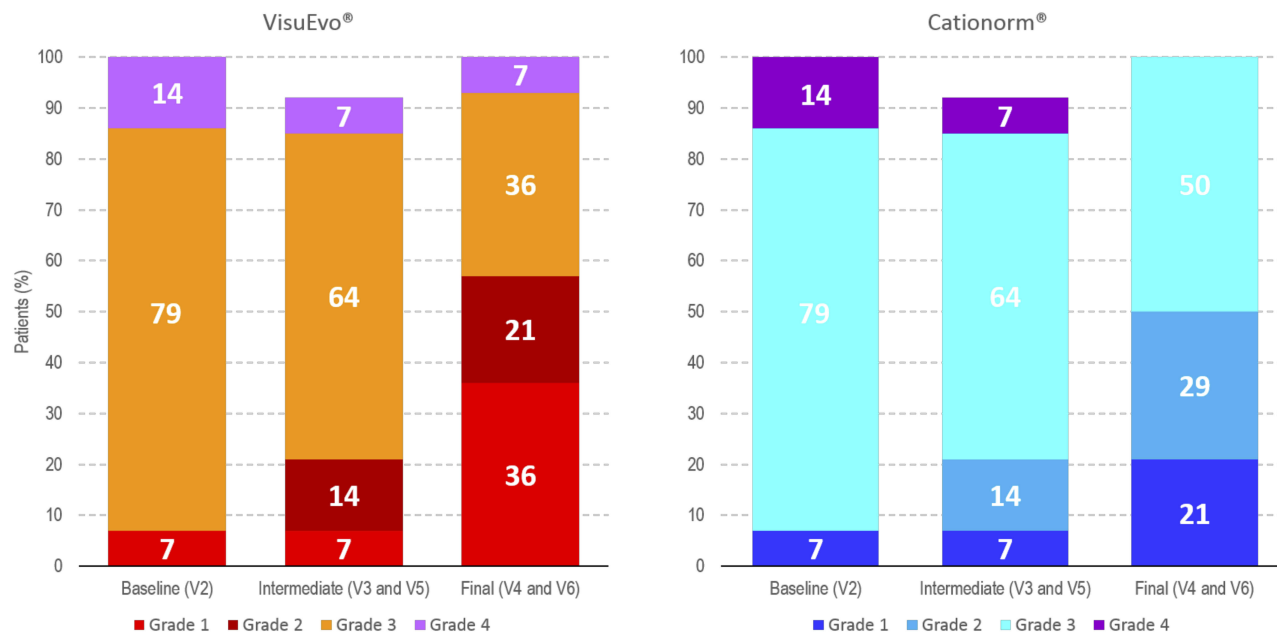
**Figure 5** Distribution of proportion (%) of patients during study visits, according to Ferning test grading scale, between the two treatment groups.

a superiority of lipids,[10,25–27] particularly on evaporative DED.[24] In the absence of a gold standard for DED treatment, our data may have been modified using a different comparator, as well as a population with different types and severity of DED.

The analysis performed on EDE and NEDE patients clearly shows how DED may be a very different condition between different types of patients. Yet, lipids were highly effective in both subgroups. At baseline NEDE patients had more severe TBUT and ocular surface staining than EDE, though symptoms at OSDI were lower. During the study, NEDE patients had a lower TBUT increase than EDE; nevertheless, the clinical amelioration was sufficient to reduce corneal staining by about 10% and increase the mucous characteristics of tear (Ferning test), with VSE patients performing slightly better than CTN (57% vs 50%). NEDE also showed peculiar blinking patterns: during study period, the number of complete blinks remained stable (though largely less than EDE: $9 \pm 5$ vs $16 \pm 13$ at baseline, $8 \pm 5$ vs $22 \pm 18$ at the end of the study), whereas incomplete blink rate reduces from $11 \pm 9$ at baseline to $3 \pm 4$ at final visits; as a consequence total blinks nearly halved in the course of the study ($20 \pm 13$ vs $11 \pm 6$). Also, in these subgroups of patients VSE proved at least non-inferior to CTN, with better final osmometry

in NEDE patients ($299 \pm 18$ vs $318 \pm 26$ mOsm/L, $p = 0.10$).

Lipids have been introduced in clinical practice as ideal treatments in EDE, but in this paper, we showed a clinically usefulness also on glaucoma patients. This could be due to several factors: the advantage of tear film stabilization also in NEDE, the anti-inflammatory activity[11,12] of lipids and, for VSE, the role by Vitamin D[14] and the trophic effect on tear mucous layer[13] by vitamin A. In NEDE patients, VSE showed better Ferning tests and osmometry than CTN. This may be due to a more favorable composition of VSE over CTN on patients with associated mucous deficits, a fact which require further investigation. Also, future studies may explore the efficacy of VSE and, more generally of lipids, on patients with combined DED (hyposecretive and hyperevaporative).

The strength of the study is randomization and crossover design, which allowed all patients to receive both treatments and therefore provided a true head-to-head comparison. Yet it also has some limitations, the most important being the drop-out rate which may limit our results; luckily the small inferiority to the desired number apparently did not affect study power, which – using a post hoc analysis – was 0.93. When present, carry-over effect in this study likely reflects the progressive amelioration of the ocular surface thanks to chronic treatments rather than

**Table 3** Results of Primary and Secondary Endpoints for Evaporative (EDE) and Non-Evaporative Dry Eye (NEDE) Groups

| Endpoints | EDE Group (N=54) | | | | NEDE Group (N=18) | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | Mean±SD | | P§ | Total | Mean±SD | | P§ |
| | | VSE | CTN | | | VSE | CTN | |
| **TBUT (sec)** | | | | | | | | |
| Baseline | 3.3±1.6 | 3.3±1.6 | | | 2.9±1.1 | 2.9±1.2 | | |
| Intermediate visit | 4.6±1.8 | 4.6±1.8** | 4.6±1.8** | 0.08 | 4.1±2.4 | 4.1±2.4* | 4.1±2.4* | 0.29 |
| Final visit | 6.0±2.8 | 5.6±2.4** | 6.5±2.8** | 0.74 | 4.6±2.4 | 4.6±2.3* | 4.6±2.6* | 0.30 |
| **OSDI questionnaire score** | | | | | | | | |
| Baseline | 39±13 | 39±13 | | | 32±8 | 32±8 | | |
| Intermediate visit | 24±15 | 24±15** | 24±15** | 0.44 | 20±15 | 20±15* | 20±15* | 0.46 |
| Final visit | 20±13 | 20±15** | 20±15** | 0.38 | 18±12 | 18±11** | 18±14* | 0.49 |
| **Schirmer I test (mm/5 min)** | | | | | | | | |
| Baseline | 16±5 | 16±5 | | | 16±6 | 16±6 | | |
| Intermediate visit | 16±8 | 16±8 | 16±8 | 0.38 | 14±9 | 14±9 | 14±9 | 0.44 |
| Final visit | 14±9 | 15±9 | 14±9 | 0.34 | 16±10 | 17±11 | 16±10 | 0.40 |
| **Complete blinkings (N/min)** | | | | | | | | |
| Baseline | 16±13 | 16±13 | | | 9±5 | 9±5 | | |
| Intermediate visit | 19±10 | 19±10 | 19±10 | 0.43 | 11±8 | 11±8 | 11±8 | 0.46 |
| Final visit | 22±18 | 20±15 | 25±21 | 0.81 | 8±5 | 7±4 | 9±6 | 0.77 |
| **Incomplete blinkings (N/min)** | | | | | | | | |
| Baseline | 7±6 | 7±6 | | | 11±9 | 11±9 | | |
| Intermediate visit | 5±5 | 5±5 | 5±5 | 0.37 | 3±3 | 3±3* | 3±3* | 0.40 |
| Final visit | 3±4 | 4±5 | 2±2** | 0.005 | 3±4** | 5±4* | 1±2 | 0.04 |
| **Total blinkings (N/min)** | | | | | | | | |
| Baseline | 23±12 | 23±12 | | | 20±13 | 20±13 | | |
| Intermediate visit | 24±13 | 24±13 | 24±13 | 0.45 | 13±7 | 13±7 | 13±7 | 0.45 |
| Final visit | 25±17 | 24±15 | 27±20 | 0.68 | 11±6** | 13±6* | 10±6** | 0.22 |
| **Osmolarity (mOsm/L)** | | | | | | | | |
| Baseline | 313±20 | 313±20 | | | 318±30 | 318±30 | | |
| Intermediate visit | 309±15 | 309±15 | 309±15 | 0.47 | 296±17 | 296±17 | 296±17 | 0.48 |
| Final visit | 305±15 | 307±14 | 303±17 | 0.26 | 308±23 | 299±18 | 318±26 | 0.85 |

**Notes:** §Paired *T*-test to compare two treatment group, adjusting by baseline value. *T-test vs baseline, p<0.05. **T-test vs baseline, p<0.001.
**Abbreviations:** CTN, Cationorm; VSE, VisuEvo.

a study bias. Finally, the results on secondary results and NEDE group should be considered with caution because of the low number of patients.

## Conclusion

In conclusion, we were able to prove that the new ophthalmic solution (VSE) was as effective and safe as the reference cationic emulsion (CTN) in restoring proper tear film composition, increasing tear film stability and reducing ocular surface damage and symptoms in patients with different subclasses of DED.

## Data Sharing Statement

The datasets during and/or analyzed during the current study are available from the corresponding author on request.

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