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# Long-term activity and safety of a low-dose hydrocortisone tear substitute in patients with dry eye disease

**Running header:** Eye drops with low-dose hydrocortisone in DED

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## Declarations

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### *Conflicts of interest/Competing interests*

Maurizio Rolando and Stefano Barabino are consultants for Alfa Intes.

### *Authors' contributions*

MR contributed to writing the article, EV, LL, CL collected data from patients and impression cytology samples, PF provided insights for the projects, SB was responsible for the protocol. All Authors contributed to data collection and interpretation. All Authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

### *Ethics approval*

The study was conducted within the protocol approved by the ethics committee of Milano Area 1 (register number 39408/2019).

### *Consent to participate*

All the participants signed an informed consent form.

### *Consent for publication*

Not required as this manuscript does not include details, images, or videos related to the participants.

***Data availability statement***

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


## ABSTRACT

**Purpose:** A clinical trial was conducted to evaluate the activity of a new artificial tear containing hyaluronic acid (HA) and low-dose hydrocortisone to control dry-eye disease (DED) symptoms. **Methods:** a randomized, controlled, double-masked study was carried out at the Ocular Surface and Dry Eye Center, "Luigi Sacco" University Hospital (Milan, Italy), between June 2020 and June 2021. The study involved patients with DED for at least 6 months. After an initial 7-day treatment with corticosteroid, the treatment with the new artificial tear (four-times a day for 6 months) was compared with a control HA solution. **Results:** A total of 40 patients were considered. We observed a significant improvement in the frequency and intensity of DED symptoms in both groups. After corticosteroid discontinuation, the maintenance of the therapeutic advantage was observed only in the treatment group, which also showed a significant improvement of the tear film break-up time ($p \leq 0.05$) and infiltrated macrophages ($p < 0.05$). A significant reduction in fluorescein and Lissamine staining ($p < 0.05$) was observed in the treatment group, suggesting damage reduction at both corneal and conjunctival levels. Intraocular pressure did not change at the end of the treatment period and was maintained within the normal range, sustaining the product's safety. **Conclusions:** Our findings support the prolonged use of the new eye drop with low-dose hydrocortisone, also in

the DED initial stages, to prevent the degenerating towards a chronic condition (http://www.isrctn.com/ISRCTN16288419).

**Keywords**

Dry eye disease, hydrocortisone, hyaluronic acid, para-inflammation, ocular surface disease.

## Introduction

Dry-eye disease (DED) is a common ocular surface condition affecting millions of people worldwide.[1] It is a multifactorial disease caused by persistent tear film instability that leads to high discomfort and visual impairment, greatly impacting the patients' quality of life.[2–5]

In the context of DED, variable degrees of ocular surface epitheliopathy, excessive stimulation of the cold fiber sensors, overstimulation of the nociceptors, and tear hyperosmolarity induce a transitory adaptation response called para-inflammation, which is useful in restoring ocular surface homeostasis.[6–8] If the irritative stimulus is intense or long-lasting, para-inflammation becomes chronic and activates the ocular surface immune system, as Barabino and collaborators described.[9,10] The involved mechanisms of the innate immune response comprise the aberrant activation of Toll-like receptors and the increased expression of pro-inflammatory molecules. The expression of these molecules can trigger the activation of the antigen-presenting cells and the specific acquired immunity, with lymphocyte "homing" on the ocular surface, hyper-expression of pro-inflammatory cytokines, and the appearance of a chronic inflammatory condition.[1,9,10] Consequently, the prompt management of the subclinical para-inflammation plays a key role in the therapeutic approach to DED patients since its dysregulation represents one of the possible causes of the most frequent forms of DED;

moreover, the poor regulation of the inflammatory immune response seems to be responsible for the chronicity of this condition.[6,11]

Hyaluronic acid (HA) is a hydrophilic polymer with marked water retention and lubricant properties. HA is mainly a mucopolysaccharide of the connective tissues but is also highly contained in the vitreous and the aqueous humor.[12] Its physicochemical characteristics enable its use in different fields of medicine, such as ophthalmology.[13,14] In particular, regarding its potential for treating DED, HA has been shown to have protective properties against corneal epithelial damage and improved the optical quality of the retinal image.[15,16] Artificial HA-based tear drops have also been shown to improve ocular surface irregularity, stabilized precorneal tear film, and improved intensity of DED symptoms.[12,13,17]

Recently, a novel HA-based artificial tear formulation has been introduced on the market. In addition to its diluent, protective and lubricating activities due to the presence of 0.2% HA, this new class III medical device has been enriched with a very low concentration of hydrocortisone sodium phosphate (0.001%) to control the para-inflammation and prevent or delay the risk of any recurrence of inflammation. Low levels of endogenous hydrocortisone are normally present in the aqueous humor to preserve the immune-privilege status of the anterior chamber, together with TGF-β2 and the a-melanocyte-stimulating hormone.[18] Notably, the low-dose hydrocortisone tear has not been shown to alter these endogenous hydrocortisone concentrations.[18]

This work presents the results of a long-term clinical trial conducted to evaluate the activity and safety of the new tear with low-dose hydrocortisone used to control the DED signs and symptoms, compared with the sole use of HA, after an initial combined treatment with a 7-day high-dose corticosteroid.

## Patients and methods

***Study design and setting***

This was a randomized, controlled, double-masked study carried out at the Ocular Surface and Dry Eye Center, "Luigi Sacco" University Hospital (Milan, Italy), between June 2020 and June 2021. The study involved patients with DED for at least 6 months. All patients underwent a screening examination that included ophthalmic history, assessment of the visual acuity and intraocular pressure (IOP), slit-lamp examination, corneal fluorescein staining and conjunctival Lissamine green staining, tear film evaluation, break-up time (T-BUT), Symptom Assessment in Dry Eye (SANDE) questionnaire and pregnancy test for women of childbearing age.

Inclusion criteria were a SANDE questionnaire score ≥30 (see study measure section for details) with concurrent positivity to at least one among corneal fluorescein staining score ≥3 (National Eye Institute [NEI] grading scale), conjunctival Lissamine green staining score ≥3 (NEI grading scale), T-BUT ≤10 seconds. Diagnosis of glaucoma was not an exclusion criteria. The ability to provide written informed consent and to follow study procedures was also required.

Patients on systemic and/or local therapy with other anti-inflammatory products, patients with other ocular surface diseases, patients undergoing surgery or para-surgical interventions in the study eye in the 3 months before the start of the treatment, patients with diabetes or other systemic diseases that may affect ocular surface health, pregnant or lactating women were excluded from the study. Tear substitutes were discontinued 7 days prior to the start of the study. Warm compresses were continued if used at the time of inclusion.

Enrolled patients were treated as follows:

- Treatment group: standard corticosteroid treatment (fluorometholone eye drop, twice daily) and tear with low-dose hydrocortisone (Idroflog®; Alfa Intes, Italy; twice daily) for 7 days, and then treated with the tear with low-dose hydrocortisone alone (four-times a day) for 6 months.

- Control group: standard corticosteroid treatment (fluorometholone eye drop, twice daily) and administration of a solution based on 0.2% HA (Zerodue®; Alfa Intes, Italy; twice daily) for 7 days, and then treated with the HA solution alone (four-times a day) for 6 months.

The standard corticosteroid treatment aimed to bring all patients to a comparable baseline condition (para-inflammation), from which the performance of the two study treatments could be better evaluated.

The study was conducted in accordance with the UNI EN ISO 14155 (Rev. January 2012) guidelines and MEDDEV 2.12/2 (Rev. January 2012) guidelines, and the Declaration of Helsinki. The Ethics Committee of Milan approved this study – Area 1 (Protocol number: 39408/2019). All participants provided written informed consent. The study is listed in the ISRCTN registry (http://www.isrctn.com/ISRCTN16288419).

***Study measures***

The study's primary aim was the assessment of DED symptoms by comparing the SANDE questionnaire score of the follow-up visits to baseline values. The SANDE questionnaire is composed of two questions about the frequency and severity of DED symptoms. At each visit, patients place a mark on two given lines of 1 cm each, based on the extent of their symptoms. The locations of the marks were measured in mm from left to right and recorded.[19]

Secondary outcomes were the regression of the corneal fluorescein staining (fluorescein sodium 2.0% eye drops) and the liquid conjunctival Lissamine green staining (Lissagreen, Polifarma, Italy). Corneal and conjunctival staining was graded according to the NEI grading scale. The T-BUT was assessed after the instillation of fluorescein strips according to the DEWS 2007 guidelines and using fluorescein sodium 2.0% eye drops.[20] Measurements were repeated three-times, and the mean value was used.

In addition, total immune (CD45+) cells and $CD14^+$ immune cell subpopulations were quantified on impression cytology specimens collected from the superior-temporal part of the bulbar conjunctiva by flow cytometry to study the involvement of the immune response.[21] Cytology filters were placed in a 15 ml tube containing RPMI supplemented with 10% fetal bovine serum and vigorously vortexed for 15 minutes at room temperature to release cells from the filter. Cells were harvested from the suspension by centrifugation and then resuspended in FACS buffer (PBS+1% BSA) and stained for 15 minutes at room temperature with the appropriate mouse fluorophore-labeled anti-human antibody (anti-CD45, BV421; anti-CD14, PE), with unstained sample serving as a negative control. Finally, cells were washed twice with FACS buffer and loaded on a FACS Celesta flow-cytometer, and data were analyzed by FlowJo software (both from Becton Dickinson, San Jose, CA, USA). All antibodies used in this study were purchased from Becton Dickinson.

The monitoring of the IOP was used to assess the safety of the treatments and was performed by applanation tonometry.

All the above-mentioned assessments were conducted at the baseline (V0) and repeated after the first week of treatment (V1). The evaluations were then repeated after 1 month (V2), after 3 months (V3), and after 6 months (V4) to assess the activity and safety of the study products, which were administered without the corticosteroid therapy.

The expression of inflammatory markers was evaluated on the cytological sampling from the baseline (V1), the 1-month treatment, and the 6-month treatment. At each follow-up patients were asked to report about the number of instillations of the eye drops and the mono-dose vials number was checked to assess compliance with the treatment.

***Statistical analysis***

Descriptive statistics were used to summarize relevant study information. ANOVA for repeated measures followed by *post-hoc* comparisons was applied to evaluate the experimental results. For the sample size calculation, the following assumptions have been applied:

1. Applicability of distributional assumptions on errors required by ANOVA.

2. Effect size: $\eta 2 = 0.039$ (corresponding to an $f = 0.2014515$). This value corresponds to the minimum threshold of effects of clinical interest.

3. $\alpha = 5\%$

4. $1-\beta = 80\%$ (minimum)

5. Number of groups (between): 2, Control group and experimental group of equal size and allocated to a single center.

6. Number of measures (within): 5, baseline, 1 week, 1 month, 3 and 6 months.

7. Correlation between repeated measures: 0.5

8. Non-sphericity correction: 1

With these assumptions, the need to enroll 32 total patients (16 for each of the groups) was identified. It was assumed that not all patients were evaluable and therefore increases this number by 20% (40 patients total considering the rounding to the nearest decade). The first and second type error thresholds were $\alpha=5\%$ and $\beta=20\%$ 121, respectively (i.e., implying a power of 80%).

**Results**

A total of 40 patients (87.5% female, n=35) were considered in the present study, 20 in the treatment group and 20 in the control group. The overall mean±SD age was 62±14 years, 64±15 years in the treatment group and 63±10 in the control group.

***Symptom's frequency and severity***

The mean SANDE scores for frequency and severity of DED symptoms were significantly reduced during the study period, compared with baseline values, in both study groups ($p<0.05$) (Figure 1).

Comparing the two study groups, no significant differences were observed between the mean SANDE scores in the frequency or severity of symptom.

***Secondary outcomes***

*Evaluation of corneal and conjunctival lesions*

The fluorescein and Lissamine green staining intensities were comparable between the two study groups in the first 7 days of combined treatment. In the subsequent period, a strong reduction was observed for both stainings in the treatment group, with a statistically significant difference ($p<0.05$) compared with the baseline from V1 (Lissamine green) and V2 (fluorescein) (Figure 2).

The control group showed an increase in both stainings at V2 up to comparable (Lissamine green) or higher (fluorescein) levels than baseline evaluations at V3 and V4 (Figure 2).

A significant reduction of the fluorescein and Lissamine green stainings was reported from V3 and at V4 in the treatment group ($p<0.05$) (Figure 2) compared with the control treatment.

*Tear film break-up time*

The two study groups revealed a different trend regarding the mean T-BUT. After an initial improvement reported at T1 for both treatment regimens and the discontinuation of the steroid

therapy, the improvement in the mean T-BUT was maintained only in the treatment group, with a significant improvement at V3 and V4 compared with the control group ($p \leq 0.05$; Figure 3). In the control group, a worsening trend of the T-BUT was reported at V3 and V4 (Figure 3).

*Involvement of the immune response*

During the treatment period, the number of infiltrating macrophages ($CD14^+$) compared with the number of total leukocytes ($CD45^+$; reported as $CD14^+/CD45^+$ ratio) was constantly lower for the treatment group, reaching a statistically significant difference from the control group at V2 ($p<0.05$; Figure 4).

***Safety evaluation***

IOP did not change at the end of the treatment period in either the treatment group (baseline, $14.2 \pm 2.1$; V4, $14.4 \pm 1.4$ mmHg) or in the control group (baseline, $14.5 \pm 1.9$; V4, $13.9 \pm 1.8$ mmHg). No difference between the two groups was observed. The recorded IOP values were within the normal range of variability for both treatment groups (Figure 5).

No adverse events were reported during the study period.

## Discussion

In the present study, the clinical activity of a new tear drops containing 0.2% HA and a low concentration of hydrocortisone was compared with the control eye drop use (0.2% HA) for the long-term management of DED signs and symptoms after an initial 7-day high-steroid combination treatment.

Study results showed a significant improvement in the frequency and intensity of DED symptoms in both groups. This effect was particularly evident in 1 week (V1), thanks to the

protective effect of standard corticosteroid treatment. Of note, after the discontinuation of the corticosteroid, the maintenance of the therapeutic advantage was observed only in the treatment group and persisted for the entire duration of the follow-up period (6 months). The symptom improvement in the treatment group was accompanied by the improvement of T-BUT, which was significantly reduced compared with the use in the control group.

A significant increase in the infiltrated macrophages/total leukocytes ratio was demonstrated in treated subjects compared with controls, suggesting that DED causes an immune response activation leading to the chronicity of the pathology.[10] In this study, a reduction of infiltrated macrophages was observed during the use of the new tear. Of note, this effect, not observed during treatment with HA alone, was further supported by results of both vital stainings, where the significant reduction in fluorescein intensity and Lissamine green suggested the reduction of damage at both corneal and conjunctiva levels.

Therefore, the low concentration of hydrocortisone seemed to control the macrophage infiltration better than the control treatment, prolonging the control of the immune system involvement and through the para-inflammation, leading to an easier recovery of the homeostasis conditions.[6]

The product's safety was sustained by maintaining the IOP values within the normal range. Therefore, this new artificial tear containing a very low dose of hydrocortisone can be used for a long time without any risk of increased IOP, in line with pre-clinical and clinical data that showed the absence of 0.001% hydrocortisone penetration in the anterior chamber, and thus its accumulation.[18,20] In addition, during the first week of treatment, there was no additive effect between the traditional steroid treatment and the low-dose hydrocortisone, sustaining the feasibility of the combined use of the two products.

In conclusion, understanding the role of parainflammation within the inflammatory process that leads to the progression and chronicity of DED is fundamental to implementing adequate

therapeutic measures to control the inflammatory status from an early stage.[6] According to this evidence, our findings support the use of the new eye drop with low-dose hydrocortisone, even in the initial stages of DED, to achieve a faster rebalance of the ocular surface alterations, preventing the degenerating toward a pathological condition that could become chronic. Furthermore, the product's safety has been observed even after continuous use, allowing a prolonged treatment.

## References


1. Reyes JL, Vannan DT, Eksteen B, Avelar IJ, Rodríguez T, González MI, Mendoza AV. Innate and adaptive cell populations driving inflammation in dry eye disease. Mediators Inflamm. 2018;2018:2532314. doi: 10.1155/2018/2532314
2. Tsubota K, Pflugfelder SC, Liu Z, Baudouin C, Kim HM, Messmer EM, Kruse F, Liang L, Carreno-Galeano JT, Rolando M, Yokoi N, Kinoshita S, Dana R. Defining dry eye from a clinical perspective. Int J Mol Sci. 2020;21(23):9271. doi: 10.3390/ijms21239271.
3. Guo OD LW, Akpek E. The negative effects of dry eye disease on quality of life and visual function. *Turk J Med Sci* 2020;50:1611–1615.
4. Craig JP, Nelson JD, Azar DT, Belmonte C, Bron AJ, Chauhan SK, de Paiva CS, Gomes JAP, Hammitt KM, Jones L, Nichols JJ, Nichols KK, Novack GD, Stapleton FJ, Willcox MDP, Wolffsohn JS, Sullivan DA. TFOS DEWS II report executive summary. Ocul Surf. 2017;15(4):802-812. doi: 10.1016/j.jtos.2017.08.003.
5. Barabino S, Aragona P, di Zazzo A, Rolando M; with the contribution of selected ocular surface experts from the Società Italiana di Dacriologia e Superficie

Oculare. Updated definition and classification of dry eye disease: Renewed proposals using the nominal group and Delphi techniques. Eur J Ophthalmol. 2021;31(1):42-48.

6. Rolando M, Barabino S. The subtle role of para-inflammation in modulating the progression of dry eye disease. Ocul Immunol Inflamm. 2021;29(4):811-816. doi: 10.1080/09273948.2021.1906908
7. Perez VL, Stern ME, Pflugfelder SC. Inflammatory basis for dry eye disease flares. Exp Eye Res 2020;201:108294.
8. Rolando M, Barabino S, Mingari C, Moretti S, Giuffrida S, Calabria G. Distribution of conjunctival HLA-DR expression and the pathogenesis of damage in early dry eyes. Cornea. 2005;24(8):951-4. Doi: 10.1097/01.ico.0000157421.93522.00.
9. Barabino S, Chen Y, Chauhan S, Dana R. Ocular surface immunity: homeostatic mechanisms and their disruption in dry eye disease. Prog Retin Eye Res. 2012;31(3):271-85. Doi: 10.1016/j.preteyeres.2012.02.003
10. Barabino S, Montaldo E, Solignani F, Valente C, Mingari MC, Rolando M. Immune response in the conjunctival epithelium of patients with dry eye. Exp Eye Res. 2010;91(4):524-9. doi: 10.1016/j.exer.2010.07.008.
11. Knop N, Knop E. Regulation of the inflammatory component in chronic dry eye disease by the eye-associated lymphoid tissue (EALT). Dev Ophthalmol 2010;45:23–39.
12. You IC, Li Y, Jin R, Ahn M, Choi W, Yoon KC. Comparison of 0.1%, 0.18%, and 0.3% hyaluronic acid eye drops in the treatment of experimental dry eye. J Ocul Pharmacol Ther. 2018;34(8):557-564. doi:10.1089/jop.2018.0032

13. Huynh A, Priefer R. Hyaluronic acid applications in ophthalmology, rheumatology, and dermatology. Carbohydr Res. 2020;489:107950. doi: 10.1016/j.carres.2020.107950.

14. Mateo Orobia AJ, Saa J, Ollero Lorenzo A, Herreras JM. Combination of hyaluronic acid, carmellose, and osmoprotectants for the treatment of dry eye disease. Clin Ophthalmol. 2018;12:453-461. doi:10.2147/OPTH.S157853

15. Johnson ME, Murphy PJ, Boulton M. Effectiveness of sodium hyaluronate eyedrops in the treatment of dry eye. Graefes Arch Clin Exp Ophthalmol. 2006;244(1):109-12. doi: 10.1007/s00417-005-0028-1.

16. Montés-Micó R, Cerviño A, Ferrer-Blasco T, García-Lázaro S, Ortí-Navarro S. Optical quality after instillation of eyedrops in dry-eye syndrome. J Cataract Refract Surg. 2010;36(6):935-40. doi: 10.1016/j.jcrs.2009.12.044.

17. Ang BCH, Sng JJ, Wang PXH, Htoon HM, Tong LHT. Sodium hyaluronate in the treatment of dry eye syndrome: A systematic review and meta-analysis. Sci Rep. 2017;7(1):9013. doi: 10.1038/s41598-017-08534-5.

18. Cagini C, Muzi A, Castellucci G, Ragna G, Lupidi M, Alabed HBR, Pellegrino RM. Kinetics of hydrocortisone sodium phosphate penetration into the human aqueous humor after topical application. Int J Clin Pract. 2021;75(12):e14987. doi: 10.1111/ijcp.14987.

19. Amparo F, Schaumberg DA, Dana R. Comparison of two questionnaires for dry eye symptom assessment: The Ocular Surface Disease Index and the symptom assessment in dry eye. *Ophthalmology* 2015;122:1498–1503.

20. Bucolo C, Fidilio A, Fresta CG, et al. Ocular pharmacological profile of hydrocortisone in dry eye disease. Front Pharmacol. 2019;10:1240. 10.3389/fphar.2019.01240

21. Rolando M, Barabino S. Are there clinical ways to assess inflammation in dry eye disease? *Ocul Immunol Inflamm*. 2021;29:1183-1189. doi: 10.1080/09273948.2021.1916540

**Figure 1. Mean±SD SANDE scores related to (A) symptoms frequency and (B) symptoms severity in treatment (blue line) and control (grey line) groups.** *p<0.05, compared with baseline values.

**Figure 2. Mean±SD (A) fluorescein and (B) Lissamine green signals evaluated on treatment (blue line) and control (grey line) cytological samples.** *p<0.05, compared with baseline values. #p<0.05 between the two study groups.

**Figure 3. Mean±SD break-up time observed in the control group (grey line) and treatment group (blue line).** #p≤0.05 between the two study groups.

**Figure 4. Mean±SD $CD14^+/CD45^+$ macrophages ratio observed in treated (blue line) and control (grey line) conjunctival samples.** #p<0.05 between the two study groups.

**Figure 5. Mean±SD intraocular pressure (IOP) values reported by treated (blue line) and control (grey line) patients.** The dotted line and the shadow area refer to the mean and the range of normal IOP values, respectively.

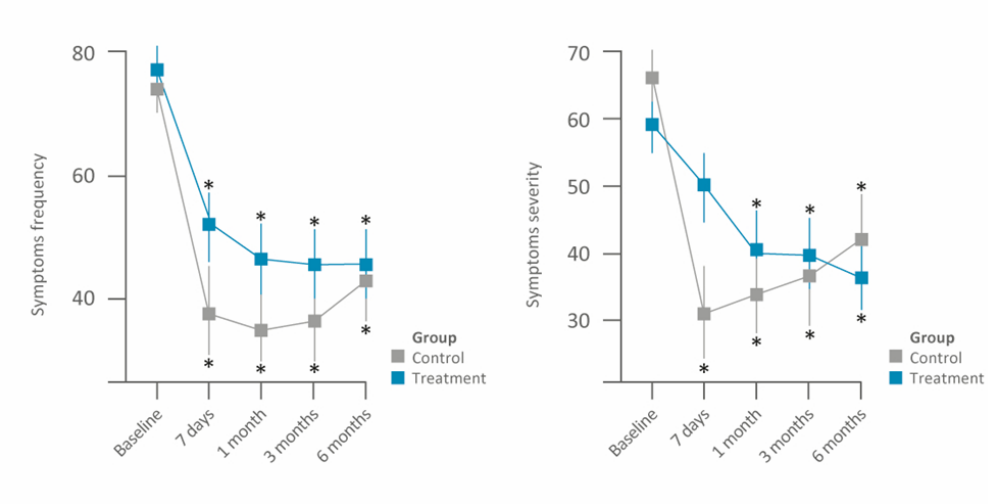


Figure 1.

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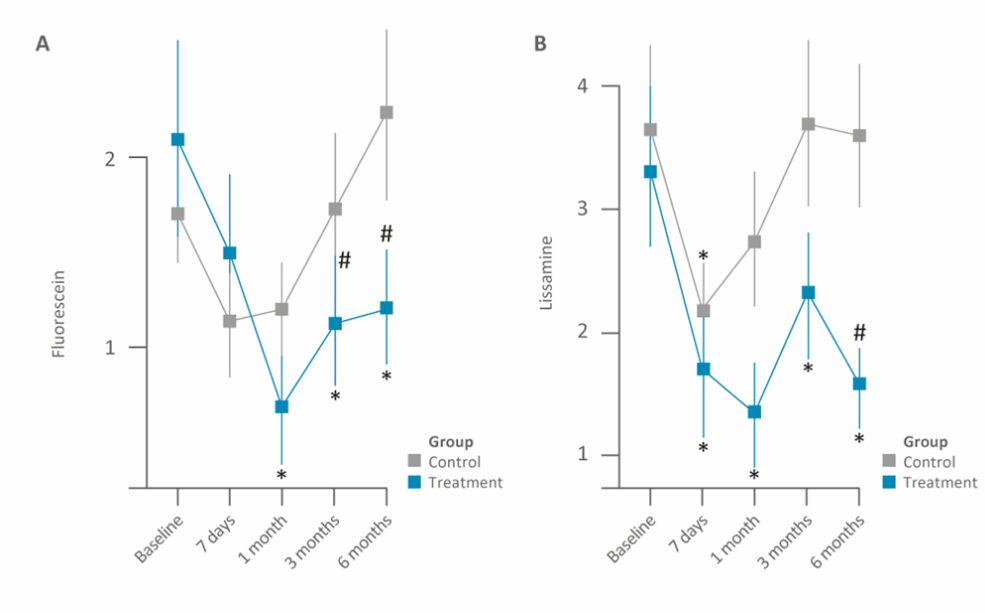


Figure 2.

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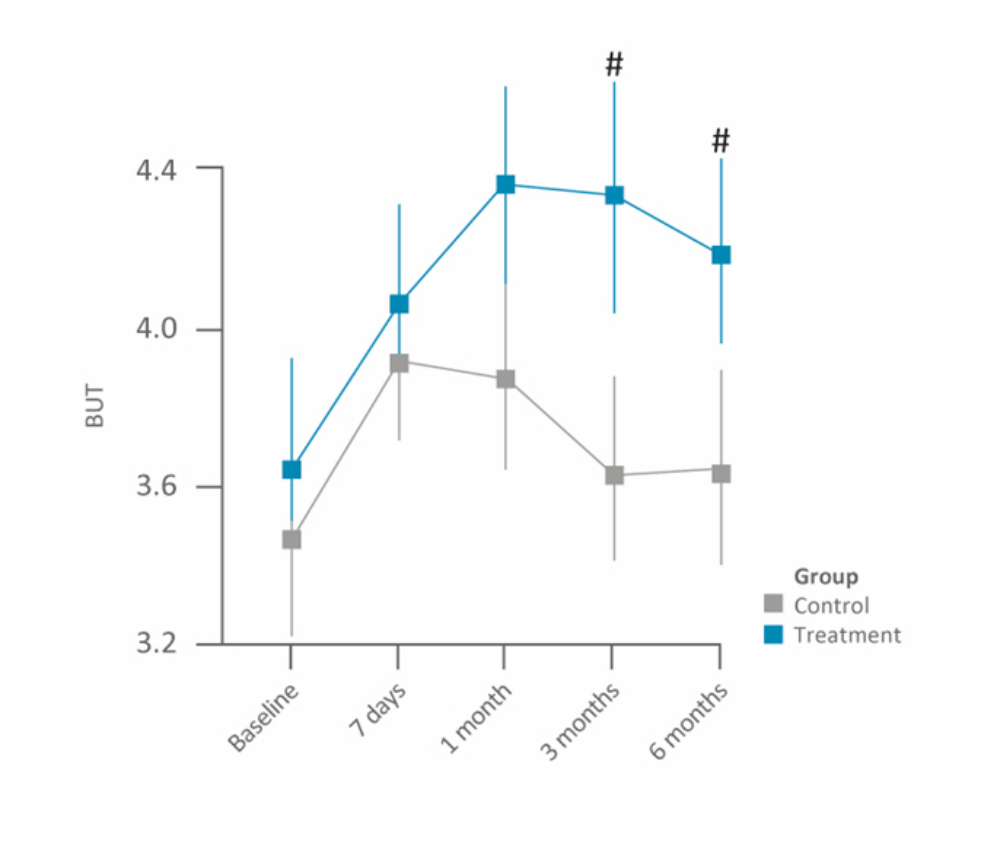


Figure 3.

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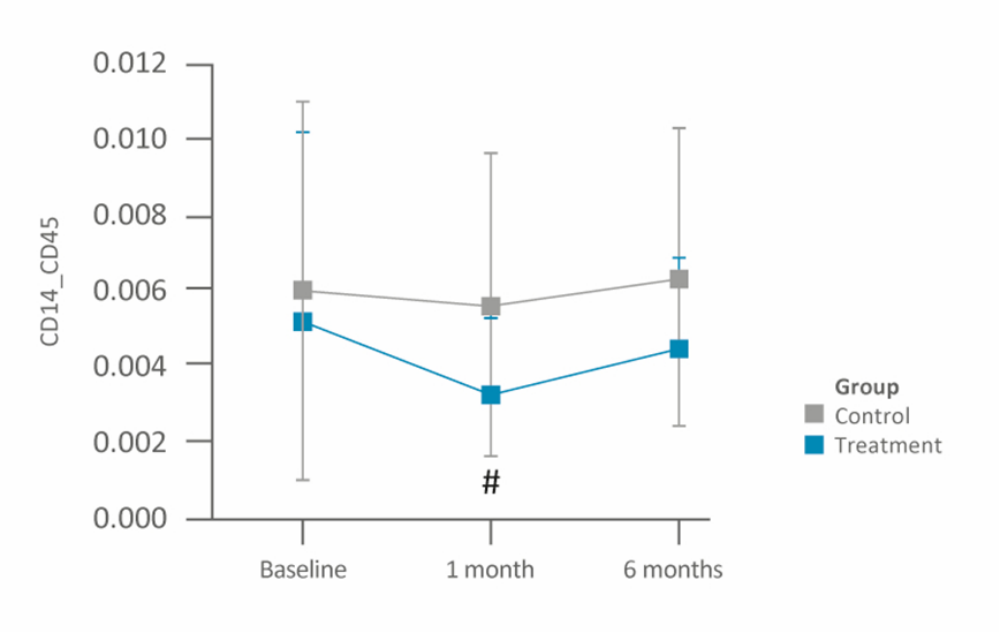


Figure 4.

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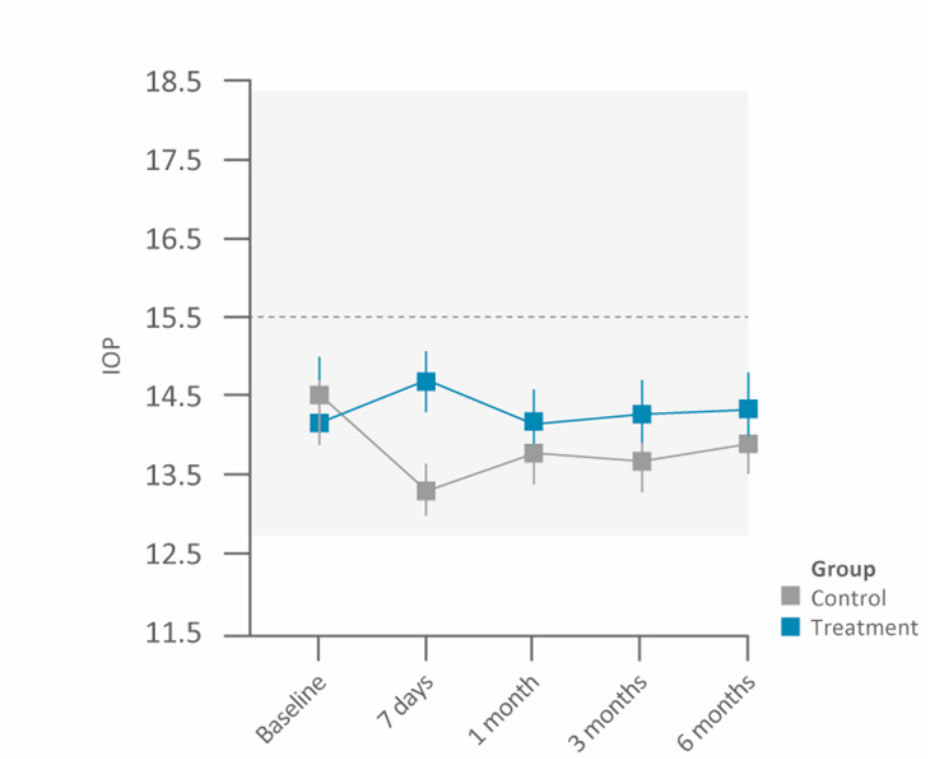


Figure 5.

132x113mm (150 x 150 DPI)