Inflammatory Status Predicts Contact Lens Discomfort under Adverse Environmental Conditions.

Itziar Fernández, PhD;[1,2] Alberto López-Miguel, PhD;[2,3] Vicente Martín-Montañez, PhD;[2] Amalia Enríquez-de-Salamanca, PhD;[1,2] Margarita Calonge, PhD;[1,2] José M González-Méijome, PhD;[2,4] María J. González-García, PhD.[1,2]

Short title: Inflammatory status predicts contact lens symptoms.

[1]Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Valladolid, Spain.

[2]IOBA (Instituto de Oftalmobiología Aplicada), Universidad de Valladolid, Valladolid, Spain.

[3]Red Temática de Investigación Colaborativa en Oftalmología (OftaRed), Instituto de Salud Carlos III, Madrid, España.

[4]Clinical and Experimental Optometry Research Laboratory, Center of Physics, University of Minho, Braga, Portugal.

Corresponding author: Alberto López Miguel. IOBA, Universidad de Valladolid, Campus Universitario Miguel Delibes, Paseo de Belén 17, 47011, Valladolid, Spain. Telephone: +34983423274. Fax: +34983184723. Email: alopezm@ioba.med.uva.es

Conflict of interest: No conflicting relationship exists for any author.

Funding: Supported in part by the Spanish Ministry of Science, Innovation and Universities, (Carlos III National Institute of Health) through Research Projects

RETICS (RD16/008/0001 grant) (OftaRed); Biomedical Research Networking Center in Bioengineering; Biomaterials and Nanomedicine (CIBER-BBN); and SAF2016-77080-P grant from Agencia Estatal de Investigación (AEI) Ministry of Science, Innovation and Universities, Spain, and Fondo Europeo de Desarrollo Regional (FEDER), UE.

No funding organizations had a role in the design or conduct of this research.

ABSTRACT

Purpose: To characterize and predict the clinical and tear molecular response of contact lens (CL) wearers exposed to a controlled adverse desiccating environment (CADE).

Methods: Objective and subjective variables and tear cytokine levels of monthly silicone hydrogel CL wearers were evaluated pre- and post-90 min of CADE exposure. Unsupervised hierarchical agglomerative clustering based on relative change from baseline values was used to identify response profiles (clusters). A multiple logistic regression model was used to identify cluster membership predictors.

Results: Forty-seven CL wearers were divided into 3 clusters having similar age (mean: 27.7±7.7 years) and sex proportion. All of them showed a significant ($p \leq 0.05$) increase in limbal hyperemia and staining after CADE exposure. Additionally, Cluster-1 (n=22, 46.8%) membership was characterized by a significant ($p \leq 0.05$) higher worsening of corneal and limbal staining, increased CL wear symptoms, and reduced epidermal-growth-factor and increased interleukin (IL)-4 and IL-6 tear levels. Cluster-2 (n=22, 46.8%) showed no changes ($p > 0.05$) in symptoms after CADE; however, their IL-12p70, monocyte-chemoattractant-protein-1 and regulated-on-activation, normal-T-cell-expressed-and-secreted (RANTES) post-exposure tear levels significantly ($p \leq 0.05$) increased. Finally, Cluster-3 (n=3, 6.4%) mainly showed significant higher blink rate (78.1±21.7) during CADE. Corneal staining and tear IL-12p70 levels were identified as Cluster-1 membership predictors.

Conclusions: Most of silicone hydrogel CL wearers exposed to CADE showed a worsening of the ocular surface and an upregulated tear inflammatory status.

However, only half of them reported worsening of CL wear symptoms. These CL wearers were detected based on corneal integrity and tear inflammatory status. These findings can help reduce CL use discontinuation and drop out.

Keywords: Contact lens discomfort; contact lens symptoms; controlled adverse desiccating environment; tear cytokines; cluster; predictors.

Abbreviations: **AUC** = area under the receiver operation characteristic curve; **CI** = confidence interval; **CADE** = controlled adverse desiccating environment; **EGF** = Epidermal growth factor; **FC** = fold change; **IFN-g** = interferon - gamma; **IL**= Interleukin; **IP-10** = interferon- gamma– Induced Protein-10; **LOOCV** = leave-one-out-cross-validation; **MCP-1** = matrix metalloproteinase-9; **MMP-9** = matrix metalloproteinase-9; **PC** = principal component; **PCA** = principal component analysis; **RANTES** = Regulated on Activation, Normal T cell Expressed and Secreted; **ROC** = receiver operation characteristic; **SANDE** = symptom assessment in dry eye.

1. INTRODUCTION

It is estimated that up to 50% of contact lens (CL) wearers are daily struggling with their habitual CL because of a diverse range of symptoms. This condition, previously known as CL related-dry eye or CL-induced dry eye, is currently named CL discomfort (CLD) [1]. CLD usually leads to CL wear discontinuation, which result in a final drop out in around 20% of total wearers every year [2]. Consequently, CLD has become a major concern not only for CL wearers and clinicians, but also for industry.

Quality of life is positively affected by CL use in comparison with spectacles in both adults and teenagers [3,4]. Thus, CL users are willing to continue using CL comfortably. Clinicians and researchers have focused in developing adequate strategies for better diagnosing and treating CLD [5]. In addition, industry is continuously making efforts to delivery new high-quality biocompatible materials [6]. And even, the current FDA review process for the approval of these medical devices, aims to contribute also to the comfort of CL wearers [7]. However, CLD is still the first unmet need for several millions of CL users worldwide.

The understanding of CLD etiology is currently limited. However, there are several contributing elements that can be CL-related like CL material, design or care; patient-related (e.g. age, sex, diseases, drugs, etc); or environment-related (e.g. ocular or external) [8]. Besides, once CL care solutions are not necessary, the decrease in comfort can be driven by ocular factors [9]. In fact, it has been demonstrated that the decrease in comfort during CL wear occurs when CL users are exposed to daily life adverse environmental conditions [10-12]. The appearance or even worsening of CLD can result in high rates of CL wear discontinuation, thus, predicting what CL users are going to develop CL

symptoms during adverse indoor environments is essential. Several studies have attempted to determine the clinical and CL factors that predict CLD, or even CL drop out, in current and neophyte soft CL users [13-18]. However, none of these studies considered the environmental conditions that CL users were exposed to. And, it is well-known that environmental conditions can negatively affect not only the CL dehydration [19], but also the ocular surface [20] even in normal individuals [21].

Recently, it has been hypothesized that inflammation is involved in the sensations of discomfort so that CLD may be a form of subacute inflammation [22]. In fact, several studies have pointed out that the presence of inflammatory mediators in tears are associated to CLD [23-27]. Considering the increasing importance of tear biomarkers not only in ocular surface anomalies [28-31], but also in CL wearers [32], it is worth to study the ability of tear inflammatory mediators to predict worsening of symptoms in CL wearers. Consequently, we aimed to assess what clinical and tear biochemical variables could characterize those CL wearers that might suffer objective and subjective ocular surface worsening when exposed to indoor adverse conditions, and specially, what clinical and tear biomarkers could predict that worsening.

2. METHODS

2.1. Participants and study design

This prospective cross-sectional study adhered to the tenets of the Declaration of Helsinki. The University of Valladolid Ethics Committee approved the study protocol. Informed consent was obtained from all CL wearers after explanation of the nature and possible consequences of the study.

Recruited CL wearers should have worn CLs for at least the last 6 months before the screening visit. Inclusion criteria were: age between 18 and 45 years, myopic spherical equivalent to ≥-1.00 and ≤-5.00 diopters (D), astigmatism error ≤0.75 D and logMAR VA ≤0.00. Exclusion criteria were being under systemic or ocular medication (artificial tears for CLD were allowed), presence of ocular abnormalities, and having a history of ophthalmic disease or surgery (including refractive surgery). Inclusion and exclusion criteria were checked during the screening visit, in addition, the following clinical tests were performed: fluorescein corneal and conjunctival staining (Oxford scheme) and Schirmer I test.

CL wearers were provided with a new silicone-hydrogel CL (Comfilcon A; Biofinity; Coopervision, Fairport, NY) and assessed prior to and after a 90-minutes adverse exposure within the controlled environment laboratory (CELab) as previously described [33]. The environmental conditions selected were 5% relative humidity, a temperature of 23°C, and localized airflow (mean velocity: 0.43 m/s). These conditions are referred to as CADE (controlled adverse desiccating environment). Participants were watching a documentary on a conventional light-emitting diode television monitor during CADE exposure, thus, all subjects performed the same visual task.

2.2. Clinical tests

Objective and subjective ocular clinical examinations were performed. Before CL insertion, the following tests were performed: (i) Corneal fluorescein staining using a cobalt-blue filter over the light source of the slit-lamp biomicroscope (SL-8Z; Topcon Corp, Tokyo, Japan) and a yellow Wratten no.12 filter (Eastman Kodak, Rochester, New York, USA), 2 minutes after instillation of 5 µL of 2% sodium fluorescein. The Oxford, and the CCLRU grading scale [35] were used to

evaluate the extent of the staining (0-4; 0.5-unit steps) within each of five corneal areas (superior, inferior, nasal, temporal, and central) and their total score adding up the five zone scores. (ii) Limbal fluorescein staining divided into four zones (superior, inferior, nasal, and temporal) and their total sum using the Efron scheme (0–4; 0.5-unit steps) was also recorded. After performing these tests, the ocular surface was rinsed with saline solution to eliminate the presence of fluorescein, and then CL was inserted after 15 minutes.

The objective measures with the CL on before and after CADE exposure were: (i) Tear osmolarity measured with a TearLab Osmolarity System (TearLab Corporation, San Diego, California, USA). (ii) Pre-CL tear BUT. This was evaluated with the CL placed on the eye, using the Tearscope Plus instrument. (Keeler Instruments, Berkshire, UK). The mean of three consecutive measurements was calculated. (iii) Limbal and bulbar conjunctival hyperemia, that was graded for nasal, temporal, superior, and inferior areas. The Efron grading scale [34] was used (0–4; 0.5-unit steps) and the total sum of the four locations was also recorded. (iv) Phenol red thread test (Menicon Company Ltd, Nagoya, Japan) was used to evaluate tear production. When the CL was removed after CADE exposure, corneal and limbal fluorescein staining were performed as abovementioned.

CL dehydration was also measured calculating the CL mass loss. CL was weighed before insertion (prior to CADE) and immediately after CL removal after CADE exposure, as previously detailed [36]. Finally, average blink rate per minute was also recorded using a video camera in primary gaze conditions at four time intervals (5–10, 25–30, 55–60, and 85–90 min) during CADE exposure.

Regarding CL wear symptoms, participants were evaluated before and after CADE exposure using a slightly modified symptom assessment in dry eye (SANDE) questionnaire [37]. Thus, CL wearers should indicate the severity of dryness, comfort and blurred vision placing a mark on a 10-cm horizontal visual analog scale prior to and after CADE exposure (SANDE version 1). In addition, to easily compare the level of CL symptoms before and after CADE exposure, CL users were administered SANDE version 2. In this case, there is an anchor in the middle of the line, and CL wearers should place a mark to the left (less symptoms) or to the right (more symptoms), according to how much of a change they perceived.

2.3. Tear sample collection

A glass capillary tube (Drummond Scientific, Broomall, PA, USA) was used to collect 2-µL of basal unstimulated tear sample of the right eye of all participants. The samples were diluted 1/10 in ice-cold assay buffer and immediately frozen as described previously [38].

2.4. Tear inflammatory molecule analysis

The concentrations of 17 molecules: epidermal growth factor (EGF); interferon (IFN)-gamma; interleukin (IL)-1b; interleukin-1 receptor antagonist (IL-1RA); IL-2; IL-4; IL-6; IL-8; IL-10; IL-12p70; IL-13; IL-17A; interferon gamma-induced protein 10 (IP-10); monocyte chemoattractant protein (MCP)-1; regulated on activation, normal T cell expressed and secreted (RANTES), tumor necrosis factor (TNF)-alpha and matrix metalloproteinase-9 (MMP-9) (inactive zymogen and active forms) were measured using a commercial immunobead-based assay (HCYTO-60 Milliplex, Merck Millipore, USA) with a Luminex IS-100 equipment

(Luminex Corporation, Austin, Texas, USA). The samples were analyzed according to the manufacturer's protocol following a reduced volume protocol, as previously described [38]. Molecule concentrations were analyzed as base-2 log-transformed variables. Cytokine levels below the limit of detection were imputed using the robust regression on order statistics (robust ROS) method introduced by Helsel and Cohn [39]. Limits of detection and detection rates are shown in table A1 (Appendix A).

2.4. Data analysis

Quantitative variables were expressed as mean ± standard deviation (SD). Median and interquartile range (IQR) were used to summarize distributions of ordinal variables. Data analysis was performed using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria).

2.4.1. Clustering in Response to CADE Effect

Forty-one clinical and molecular variables were evaluated before and after the 90-minutes exposure to CADE. Additionally, SANDE 2 that was obtained immediately after CADE, and blink rates measured during CADE, were also computed for the analysis. These variables were used to identify and describe different response profiles (clusters) to CADE exposure among CL wearers recruited. The CADE effect for each clinical parameter was computed as the relative change from pre-exposure baseline values except for SANDE 2 and blink rates. To consider the minimum and maximum boundary values, the rate of change per individual was calculated as the relative difference between post- and pre-exposure values with respect to the maximum change over the considered

146 times. In case of tear molecules, the CADE effect was quantified by log2 fold
147 change (FC) as previously described [40].

148 In a first pre-processing step, two criteria were used to remove uninformative
149 variables in the clustering stage: low relevance and high redundancy. Related to
150 the first criterion, from the initial 49 variables, only those that showed relevant
151 changes were selected. Thus, for the clinical variables measured before and after
152 CADE exposure, only those that showed at least a 10% change in 50% of the CL
153 wearers were included. In case of SANDE 2 variables, only those showing a
154 change of 0.5 units in at least 50% of the sample were included. For blink rates,
155 they were included only if showed a coefficient of variation above 10%. And
156 regarding tear molecules, they were considered only if they showed a 0.5 log2
157 FC (up or down). The second criterion (high redundancy) was applied to the
158 remaining variables to avoid including in the subsequent analysis those variables
159 showing high relationship ($r > 0.75$) among them. Detailed information about the
160 selection procedure can be found in Appendix B of the supplementary material.
161 Later, a principal component (PC) analysis was performed for further reducing
162 overlap in the remaining variables with the aim of producing PCs as previously
163 detailed [40]. In the present study, we kept the PCs necessary to explain at least
164 95% of the total data variability. To avoid bias as much as possible in the PC
165 analysis, a Box and Cox transformation was performed to reduce skewness prior
166 to the application of PC analysis.

167 The next stage of the analysis was the performance of an unsupervised
168 hierarchical agglomerative clustering analysis using the PCs previously identified.
169 Our purpose in this step was to group CL wearers that showed similar responses
170 (clinical and tear molecular) to CADE exposure into the same cluster. We started

using a bottom-up approach, wherein each response initially starts in its own cluster, and then, iteratively, clusters are joined by taking the two most similar response together and merging them. The process continues until just one cluster is formed obtaining a hierarchical tree. We computed the similarity between clusters based on the Euclidian distance. At each iteration, the Ward's minimum variance criterion was used to decide what clusters should be merged together and becoming a single cluster. The Ward´s method aims to find the pair of clusters that leads to minimum increase in total within-cluster variance after merging. The optimal number of clusters, K, was chosen based on the principle that K is the optimal solution if the decrease of variance between K-1 and K clusters is much greater than the one between K and K+1 clusters. The final clustering was consolidated by the K-means algorithm, using the partition obtained from the K-cut of the hierarchical tree as the initial partition of this procedure.

To facilitate the interpretation of the final partition, a profile analysis was conducted, including a descriptive summary of all clinical and molecular variables, and testing the differences among groups by a one-way of analysis of variance (ANOVA). When one-way ANOVA assumptions were not met, either normal distribution and/or equal variance, the Kruskal-Wallis and Welch ANOVA was performed, respectively. Post-hoc comparisons were performed using Student's t-tests, Welch's t-test or Mann–Whitney U test when required. For all of them, the false discovery rate adjusted p-value was applied [41].

2.4.2. Predictors of Cluster Membership in Response to CADE

The aim was to determine what baseline variables may have been contributing to membership in a particular cluster previously found. All clinical and tear

196 molecular variables in pre-exposure visit, as well as sex, age, CL power, corneal
197 keratometry and Schirmer I were considered as possible predictors.

198 A logistic regression model was applied to assess the relationship between
199 cluster membership and each prediction variable previously mentioned. Odd-ratio
200 (OR) estimation was used to quantify the association between the response
201 cluster and these variables. Variables associated with cluster membership at the
202 10% significance level were initially identified as potential predictors. Then,
203 potential predictors were evaluated simultaneously to fit a multiple logistic
204 regression model. The selection of final predictors to be included in the
205 multivariable model was performed by exhaustive search optimizing the Akaike
206 information criterion. Multicollinearity of the fitted model was checked using the
207 variance inflation factor, whose values should not exceed 5 because it can be
208 considered a sign of multicollinearity problems.

209 The leave-one-out-cross-validation (LOOCV) procedure was used to
210 estimate the prediction accuracy of the final model. The Brier score was used as
211 global measure of the model accuracy, while the goodness of fit was checked
212 using the Hosmer–Lemeshow test. In addition, the receiver operation
213 characteristic (ROC) curve analysis was used to assess the discriminate ability
214 of the model. The final model was evaluated according to the area under the ROC
215 curve (AUC). Sensitivity and specificity of the model were obtained by setting an
216 optimal threshold such that the probability of accurate diagnosis was highest.

217 **3. RESULTS**

218 Forty-seven participants (29 females and 18 males) were recruited with a mean
219 age of 27.7 ± 7.7 (range, 18-45) years. Their average corrected distance visual

220 acuity was -0.06 ± 0.05 logMAR. Their mean myopic and astigmatic refractive
221 error was -3.12 ± 1.1 D and -0.23 ± 0.44 D, respectively.

222 **3.1. Clustering in Response to CADE**

223 Table 1 summarizes the values of the clinical and tear molecular parameters
224 assessed before and after 90 minutes of exposure to CADE. Data corresponding
225 to SANDE 2 and blink rates recorded after and during CADE exposure,
226 respectively, are also included.

227 From the 49 initial variables (Table 1), limbal hyperemia (superior and
228 inferior), corneal staining (Oxford scheme), central, nasal, temporal and superior
229 corneal staining (CCLRU scheme), superior limbal staining, blurred vision item in
230 SANDE version 1, comfort and blurred vision items in SANDE version 2, and tear
231 levels of IL-17A, IP-10 and TNF-alpha were not considered for further statistical
232 analysis because they were non-informative according to the relevance criterion
233 (Appendix B). In addition, according to redundancy criterion, for blink rates, only
234 the 25-30 minutes interval was considered because the rest of the intervals were
235 highly correlated. The 31 variables finally included after both pre-processing
236 steps are detailed in Table 1 (last column). Detailed information about the
237 selection procedure can be found in Appendix B of the supplementary material.
238 The 31 informative variables were centered, scaled and skewness-corrected and
239 a PC analysis was performed on them. PC analysis discovered 19 statistically-
240 independent dimensions (PCs), which together explained 95.4% of the total
241 variation observed after CADE exposure.

242 After applying an unsupervised hierarchical agglomerative clustering
243 procedure for the 19 PCs, three clusters were found. All CL users within each

cluster showed similar response to CADE exposure. The same number (n=22) CL wearers were assigned to each Cluster 1 and 2 (46.8%; 95%CI: 32.4%, 61.8%), and only three CL wearers were classified into Cluster 3 (6.4%; 95%CI: 1.7%, 18.6%). Table 2 summarizes differences among clusters with respect to all clinical and tear molecular parameters evaluated. There were not significant differences among the three clusters regarding age (p=0.42) and sex (p=0.35).

The three clusters showed a significant (p≤0.05) increase in total limbal hyperemia, limbal staining (nasal, inferior and total) and CL dehydration after CADE (Table 2). CL users that were classified into Cluster 1 (n=22) mostly showed significant (p≤0.05) higher worsening of corneal and limbal staining, as well as increased blur vision, dryness and discomfort (SANDE 1 and 2) after CADE (Table 2). In addition, these Cluster 1 CL wearers showed significant (p≤0.05) reduced EGF, and increased IL-4 and IL-6 tear levels (Table 2). Cluster 2 (n=22) membership was mainly characterized by the absence of changes in corneal staining and symptoms in response to CADE (SANDE 1 and 2 scores did not change significantly (p>0.05). However, IL-12p70, MCP-1 and RANTES post-exposure tear levels significantly (p≤0.05) increased in Cluster 2 (Table 2). Finally, Cluster 3 members (n=3) were mainly characterized by significant (p≤0.05) higher blink rates (mean values > 70) and large post-exposure changes (reduction of the levels) in several tear molecules assessed (Table 2).

3.2. Predictors of Cluster Membership

Cluster 3 was excluded from the prediction analysis considering that only 3 CL wearers (6.38% of the sample) showed this same response to CADE. Each pre-exposure variable separately was used as independent variable in a binary logistic regression model to predict Cluster 1 (higher subjective and objective

worsening after CADE exposure) membership. Potential predictors, that is, variables associated with Cluster 1 at the 10% significance level, were the following: global corneal staining (Oxford scheme), Schirmer I test, and tear concentrations of IL-12p70 and RANTES (Figure 1).

An exhaustive search to select the best subset of potential predictors for the final multiple model was performed. The best models by number of variables included in them, based on the lower value of the Akaike information criterion, are showed in Table C1 (Appendix C). The model based on two variables, corneal staining and IL-12p70 tear levels, was identified as the optimal model. The OR for Cluster 1 membership for corneal staining was 0.16 (p=0.008. 95% CI: 0.04-0.62) and for IL-12p70 tear concentration was 1.63 (p=0.01. 95% CI: 1.11-2.41). The outcomes of the internal validity of this corneal staining- and IL-12p70-based model (using the LOOCV procedure) are detailed in Table 3. The Brier Score obtained supported the accuracy of the model, and the Hosmer-Lemeshow test indicated the lack of serious calibration problems (Table 3). The model obtained an AUC of 0.75, with a sensitivity of 81.8% (95% CI: 65.7-97.9) and a specificity of 77.3% (95% CI: 59.7-94.7).

To apply these outcomes in the clinical setting, a simple decision rule including the estimated cut-off values for corneal staining and tear IL-12p70 levels are detailed in Table 4. This rule, created to provide the straight forward cut-off values for corneal staining and tear IL-12p70 levels, obtained a sensitivity of 81.8% (95% CI: 61.5-97.7) and a specificity of 77.3% (95% CI: 56.6-89.9).

4. DISCUSSION

The main goal of clinicians, researchers and industry in current and neophyte CL wearers is enable them to continue using CL comfortably as long as possible. Thus, several strategies have been recommended to avoid the appearance of CLD, otherwise it can result in CL wear discontinuation and finally, in CL drop out. Therefore, researchers have aimed to determine the clinical factors that could predict CLD in new and current CL users [13-17], or even characterized those who ceased CL wear [18]. However, CLD might have an inflammatory nature, as previously reported [23-27]. Thus, our aim was to study for the first time if tear inflammatory biomarkers could characterize and specially, predict the worsening of the ocular surface and the CLD symptoms when CL users are exposed to indoor adverse environments.

The present study shows that it should be expected an objective ocular surface worsening and inflammatory upregulation when monthly silicon hydrogel CL wearers are exposed to adverse conditions. However, after CADE exposure, CLD was only reported in around half of the CL wearers. And these CL users might be the ones suffering CLD more frequently on a daily basis under indoor adverse conditions (i.e. office buildings). Moreover, this subjective response could be predicted based on corneal integrity and tear IL-12p70 levels.

In our study, we observed that when monthly silicone hydrogel CL users were exposed to adverse conditions, they showed a common basic response to CADE: an increase of limbal staining and hyperemia (Table 2). In addition to this common response, we found three different types of ocular response, regardless of age and sex. The expected response of a common monthly silicone hydrogel CL user when undergoing adverse conditions could be similar to the one showed by Cluster 1 and 2 members (94% of the CL users recruited were classified within

318 these two clusters in equal proportion). The main difference between Cluster 1
319 and 2 members were that CL wearers belonging to Cluster 1 showed a higher
320 objective and subjective worsening of the ocular surface after CADE exposure.
321 Cluster 1 membership was mainly characterized by a slight increase in corneal
322 staining (predominantly inferior), and specially, an increase in CL wear
323 symptoms. Additionally, Cluster 1 CL users showed a significant decrease in EGF
324 and an increase in IL-4 and IL-6 tear levels after CADE exposure. These changes
325 observed in Cluster 1 showed an objective increase of the inflammatory status of
326 the lachrymal functional unit. This subjective worsening of the ocular surface
327 symptoms accompanied by a variation of these tear molecules has been
328 previously reported in other studies. For instance, a previous study found a
329 negative correlation between EGF tear concentrations and ocular surface
330 symptoms (as measured with the ocular surface disease index (OSDI)
331 questionnaire) in a group composed of CL wearers and healthy subjects [42].
332 Besides, other authors [43,44] have reported a positive association between
333 increasing IL-4 and IL-6 levels and elevated ocular surface symptoms (OSDI
334 questionnaire) in dry eye disease patients.

335 In contrast to Cluster 1, Cluster 2 members coped better with the exposure
336 to an indoor adverse condition. Despite Cluster 2 CL wearers showed also
337 reduced pre-lens BUT after CADE exposure (similar to Cluster 1 members) and
338 an increase in IL-12p70, MCP-1 and RANTES tear levels (which indicates the
339 presence of an inflammatory response in their ocular surface), they did not report
340 any worsening of CL wear symptoms (Table 2). These results might indicate that
341 this increase in the inflammation status was no related or was not large enough
342 to produce a worsening of CL wear symptoms in these CL users. Consequently,

Cluster 2 members are the CL wearers less likely to suffer CLD when wearing a monthly silicone hydrogel CL under daily life indoor adverse conditions. In contrast, Cluster 1 CL wearers might be the ones more likely to suffer CL symptoms when being exposed to desiccating environments. This finding is very important because indoor adverse conditions (e.g. office-like environments) can produce ocular symptoms regardless of the geographical location [45]. Thus, our outcomes could be applied to monthly silicone CL wearers globally. Consequently, the main clinical goal should be to detect these type of CL wearers as soon as possible to prevent CL wear discontinuation.

A small proportion of our CL users (6%; n=3) was grouped into Cluster 3. In this cluster, we observed not only a significant decrease on one third of the tear molecules assessed after CADE exposure, but also, these decreases on the tear molecule concentrations were significantly different in comparison with the other two clusters (Cluster 1 and 2) in the vast majority of the molecules studied (Table 2). Besides, Cluster 3 members showed an elevated blink frequency, around 80 blinks per minute during the CADE exposure. This value is much higher than the one previously reported in soft CL wearers undergoing also an adverse exposure [46]. This high blink rate might help CL users to maintain pre-lens tear film, and it could help to avoid CLD because of the constant CL rewetting. In fact, these CL users did not show a significant worsening of the CL wear symptoms after CADE exposure (Table 2). Besides, the elevated blink rate observed in Cluster 3 during CADE could be responsible for the high reduction detected in several tear molecules (Table 2). Nonetheless, Cluster 3 might only resemble the ocular surface response of around 6% of the monthly CL wearers population.

When we analyzed what clinical and tear cytokines were able to predict the response of CL wearers to CADE, we observed that the likelihood of feeling CL wear symptoms increased (Cluster 1 CL users) with baseline lower corneal staining and higher IL-12p70 tear levels. The sensitivity and specificity values of this predictive model based on clinical and tear molecular variables were around 80%, consequently, our findings were very reliable. Previous authors have found several clinical factors able to predict the appearance of CLD in new and current CL wearers [13-17], and even those responsible for CL drop out [18]. However, to our knowledge, no previous studies showed that the inflammatory status of the lachrymal functional unit in CL wearers can predict the worsening of CL wear symptoms in response to adverse environment conditions. Currently, there is available a commercial test to detect tear MMP-9 (InflammaDry; Rapid Pathogen Screening, Inc, Sarasota, FL) [47]. Thus, if the required translational research is performed, a new commercial point of care could be developed to measure tear IL-12p70 levels, allowing clinicians to easily detect Cluster 1-type CL wearers in their daily clinical setting. Thus, CLD interventions could be performed earlier and CLD discontinuation rates could be reduced.

The present study has several limitations. First, we did not include in our sample individuals above 45 years old because most of them require multifocal fittings, and CL wear discontinuation rates are higher in this group of CL users because they report visual problems [48]. Thus, we did not recruit possible presbiopic volunteers to avoid including a confounding factor when subjectively assessing vision after CADE exposure. Second, we fitted our volunteers a silicone hydrogel monthly replacement CL, therefore, further studies are needed to provide evidence regarding the validity of our outcomes in CL users fitted with

different CL materials or replacement schedules (i.e. daily disposable CL). Nonetheless, monthly replacement CL fitting is still the most prescribed option [49], and silicone hydrogel CL prescriptions are continuously increasing in comparison with conventional hydrogel ones [50]. Finally, we used a slightly modified version of SANDE 1 and 2 questionnaires [37] to assess subjective visual changes before and after 90-minutes CADE exposure. We administered this modified instrument because the most common used questionnaire to assess CLD ask about symptoms “during a typical day in the past 2 weeks”, thus, it could not be used for our study [51].

5. CONCLUSIONS

In conclusion, we showed that the response of soft CL wearers to an indoor adverse environment is mainly grouped into two clusters despite sharing a basic common response. It should be expected a worsening of the ocular surface and an upregulated inflammatory response. However, the response will be mainly differentiated by the subjective perception of the CL wearer, and there will be CL wearers who suffer a worsening of CL symptoms and others who will not. In addition, the specific type of response to CADE exposure can be predicted based on the baseline corneal integrity and the inflammatory status of the lachrymal functional unit. The ability to predict this response is really important because those CL users likely to suffer higher CL wear symptoms should be provided with CLD interventions as soon as possible, otherwise CL wear discontinuation and drop out would happen.

Disclosure/Conflict of Interest Statement

No conflicting relationship exists for any author. Disclosures of Dr. Margarita Calonge are the following: Research/clinical trials contracts, consultantships, advisory boards and/or lectures for Novaliq, Chiesi, Santen, Kala, Johnson and Johnson, and Horus Pharma laboratories.

REFERENCES

1. Dumbleton K, Caffery B, Dogru M, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the subcommittee on epidemiology. Invest Ophthalmol Vis Sci. 2013;54(11):20-36.
2. Dumbleton K, Woods CA, Jones LW, Fonn D. The impact of contemporary contact lenses on contact lens discontinuation. Eye Contact Lens. 2013;39(1):93-99
3. Pesudovs K, Garamendi E, Elliott DB. A quality of life comparison of people wearing spectacles or contact lenses or having undergone refractive surgery. J Refract Surg. 2006;22(1):19-27.
4. Plowright AJ, Maldonado-Codina C, Howarth GF, Kern J, Morgan PB. Daily disposable contact lenses versus spectacles in teenagers. Optom Vis Sci. 2015;92(1):44-52.
5. Asbell PA. Contact Lens Discomfort: Can We Prevent Dropout? Eye Contact Lens. 2017;43(1):1.
6. Papas EB. Contact lens technology to 2020 and beyond: a review of recent patent literature. Clin Exp Optom. 2017 Sep;100(5):529-536.
7. Hampton D, Green JA, Robboy M, Eydelman M. Food and Drug Administration Efforts to Mitigate Contact Lens Discomfort. Eye Contact Lens. 2017;43(1):2-4.
8. Nichols KK, Redfern RL, Jacob JT, Nelson JD, Fonn D, Forstot SL, Huang JF, Holden BA, Nichols JJ; members of the TFOS International Workshop on Contact Lens Discomfort. The TFOS International Workshop on Contact Lens Discomfort: report of the definition and classification subcommittee. Invest Ophthalmol Vis Sci. 2013;54(11):TFOS14-9.

9. Navascues-Cornago M, Morgan PB, Maldonado-Codina C. Effect of Three Interventions on Contact Lens Comfort in Symptomatic Wearers: A Randomized Clinical Trial. PLoS One. 2015;10(8):e0135323
10. González-Méijome JM, Parafita MA, Yebra-Pimentel E, Almeida JB. Symptoms in a population of contact lens and noncontact lens wearers under different environmental conditions. Optom Vis Sci. 2007;84(4):296-302.
11. Maruyama K, Yokoi N, Takamata A, Kinoshita S. Effect of environmental conditions on tear dynamics in soft contact lens wearers. Invest Ophthalmol Vis Sci. 2004;45(8):2563-8.
12. González-García MJ, González-Sáiz A, de la Fuente B, Morilla-Grasa A, Mayo-Iscar A, San-José J, Feijó J, Stern ME, Calonge M. Exposure to a controlled adverse environment impairs the ocular surface of subjects with minimally symptomatic dry eye. Invest Ophthalmol Vis Sci. 2007;48(9):4026-32.
13. Pult H, Purslow C, Berry M, Murphy PJ. Clinical tests for successful contact lens wear: relationship and predictive potential. Optom Vis Sci. 2008;85(10):E924-9.
14. Pult H, Murphy PJ, Purslow C. A novel method to predict the dry eye symptoms in new contact lens wearers. Optom Vis Sci. 2009;86(9):E1042-50.
15. Best N, Drury L, Wolffsohn JS. Predicting success with silicone-hydrogel contact lenses in new wearers. Cont Lens Anterior Eye. 2013;36(5):232-7.
16. Sulley A, Young G, Hunt C. Factors in the success of new contact lens wearers. Cont Lens Anterior Eye. 2017;40(1):15-24.

17. Siddireddy JS, Tan J, Vijay AK, Willcox M. Predictive Potential of Eyelids and Tear Film in Determining Symptoms in Contact Lens Wearers. Optom Vis Sci. 2018;95(11):1035-1045.
18. Pucker AD, Jones-Jordan LA, Marx S, Powell DR, Kwan JT, Srinivasan S, Sickenberger W, Jones L. Clinical factors associated with contact lens dropout. Cont Lens Anterior Eye. 2019;42(3):318-324.
19. Martín-Montañez V, López-Miguel A, Arroyo C, Mateo ME, González-Méijome JM, Calonge M, González-García MJ. Influence of environmental factors in the in vitro dehydration of hydrogel and silicone hydrogel contact lenses. J Biomed Mater Res B Appl Biomater. 2014;102(4):764-71.
20. Calonge M, Pinto-Fraga J, González-García MJ, Enríquez-de-Salamanca A, López-de la Rosa A, Fernández I, López-Miguel A. Effects of the External Environment on Dry Eye Disease. Int Ophthalmol Clin. 2017;57(2):23-40.
21. Tesón M, López-Miguel A, Neves H, Calonge M, González-García MJ, González-Méijome JM. Influence of Climate on Clinical Diagnostic Dry Eye Tests: Pilot Study. Optom Vis Sci. 2015;92(9):e284-9.
22. Nichols JJ, Willcox MD, Bron AJ, Belmonte C, Ciolino JB, Craig JP, Dogru M, Foulks GN, Jones L, Nelson JD, Nichols KK, Purslow C, Schaumberg DA, Stapleton F, Sullivan DA; members of the TFOS International Workshop on Contact Lens Discomfort. The TFOS International Workshop on Contact Lens Discomfort: executive summary. Invest Ophthalmol Vis Sci. 2013;54(11):7-13.
23. Willcox MD, Zhao Z, Naduvilath T, et al. Cytokine changes in tears and relationship to contact lens discomfort. Mol Vis 2015;21:293–305.

24. Masoudi S, Stapleton FJ, Willcox MD. Contact Lens-Induced Discomfort and Protein Changes in Tears. Optom Vis Sci. 2016 Aug;93(8):955-62.
25. Willcox MD. Is There a Role for Inflammation in Contact Lens Discomfort? Eye Contact Lens. 2017 Jan;43(1):5-16.
26. López-de la Rosa A, García-Vázquez C, Fernández I, Arroyo-del Arroyo C, Enríquez-de-Salamanca A, González-García MJ. Substance P level in tears as a potential biomarker for contact lens discomfort. Ocul Immunol Inflamm. 2019 (in press).
27. Gad A, Vingrys AJ, Wong CY, Jackson DC, Downie LE. Tear film inflammatory cytokine upregulation in contact lens discomfort. Ocul Surf. 2019;17(1):89-97.
28. von Thun Und Hohenstein-Blaul N, Funke S, Grus FH. Tears as a source of biomarkers for ocular and systemic diseases. Exp Eye Res. 2013 Dec;117:126-37.
29. Roy NS, Wei Y, Kuklinski E, Asbell PA. The growing need for validated biomarkers and endpoints for dry eye clinical research. Invest Ophthalmol Vis Sci. 2017;58(6):BIO1-BIO19.
30. Di Zazzo A, Micera A, De Piano M, Cortes M, Bonini S. Tears and ocular Surface disorders: Usefulness of biomarkers. J Cell Physiol. 2019 Jul;234(7):9982-9993.
31. Hagan S, Martin E, Enríquez-de-Salamanca A. Tear fluid biomarkers in ocular and systemic disease: potential use for predictive, preventive and personalized medicine. EPMA J. 2016 Jul 13;7:15.
32. López-de la Rosa A, González-García MJ, Calonge M, Enríquez-de-Salamanca A. Tear Inflammatory Molecules in Contact Lens Wearers: A

Literature Review. Curr Med Chem. 2019. doi: 10.2174/0929867326666190409152921.

33. Tesón M, González-García MJ, López-Miguel A, Enríquez-de-Salamanca A, Martín-Montañez V, Benito MJ, Mateo ME, Stern ME, Calonge M. Influence of a controlled environment simulating an in-flight airplane cabin on dry eye disease. Invest Ophthalmol Vis Sci. 2013;54(3):2093-9
34. Efron N, Morgan PB & Katsara SS. Validation of grading scales for contact lens complications. Ophthalmic Physiol Opt 2001; 21: 17–29.
35. Terry RL, Schnider CM, Holden BA et al. CCLRU standards for success of daily and extended wear contact lenses. Optom Vis Sci 1993; 70: 234–243.
36. López-de la Rosa A, Martín-Montañez V, López-Miguel A, Fernández I, Calonge M, González-Méijome JM, González-García MJ. Ocular response to environmental variations in contact lens wearers. Ophthalmic Physiol Opt. 2017;37(1):60-70.
37. Schaumberg DA, Gulati A, Mathers WD, Clinch T, Lemp MA, Nelson JD, Foulks GN, Dana R. Development and validation of a short global dry eye symptom index. Ocul Surf. 2007;5(1):50-7.
38. Enríquez-de-Salamanca A, Castellanos E, Stern ME, Fernández I, Carreño E, García-Vázquez C, et al. Tear cytokine and chemokine analysis and clinical correlations in evaporative-type dry eye disease. Mol Vis 2010;16:862-73.
39. Helsel DR, Cohn TA. Estimation of descriptive statistics for multiply censored water quality data. Water Resour Re. 1988;24:1997-2004.
40. Fernández I, López-Miguel A, Enríquez-de-Salamanca A, Tesón M, Stern ME, González-García MJ, Calonge M. Response profiles to a controlled

adverse desiccating environment based on clinical and tear molecule changes. Ocul Surf. 2019 Mar 29. pii: S1542-0124(18)30462-2.

41. Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B 57, 289–300.
42. López-de la Rosa A, Martín-Montañez V, López-Miguel A, Calonge M, Enríquez-de-Salamanca A, González-García MJ. Corneal Sensitivity and Inflammatory Biomarkers in Contact Lens Discomfort. Optom Vis Sci. 2016;93(8):892-900.
43. Massingale ML, Li X, Vallabhajosyula M, Chen D, Wei Y, Asbell PA. Analysis of inflammatory cytokines in the tears of dry eye patients. Cornea. 2009;28(9):1023-7.
44. Liu R, Gao C, Chen H, Li Y, Jin Y, Qi H. Analysis of Th17-associated cytokines and clinical correlations in patients with dry eye disease. PLoS One. 2017;12(4):e0173301.
45. Wolkoff P, Kärcher T, Mayer H. Problems of the "outer eyes" in the office environment: an ergophthalmologic approach. J Occup Environ Med. 2012;54(5):621-31.
46. Kojima T, Matsumoto Y, Ibrahim OM, Wakamatsu TH, Uchino M, Fukagawa K, Ogawa J, Dogru M, Negishi K, Tsubota K. Effect of controlled adverse chamber environment exposure on tear functions in silicon hydrogel and hydrogel soft contact lens wearers. Invest Ophthalmol Vis Sci. 2011;52(12):8811-7.

47. Messmer EM, von Lindenfels V, Garbe A, Kampik A. Matrix Metalloproteinase 9 Testing in Dry Eye Disease Using a Commercially Available Point-of-Care Immunoassay. Ophthalmology. 2016;123(11):2300-2308
48. Sulley A, Young G, Hunt C. Factors in the success of new contact lens wearers. Cont Lens Anterior Eye. 2017;40(1):15-24.
49. Jones D, Woods C, Jones L, Efron N, Morgan P. A sixteen year survey of Canadian contact lens prescribing. Cont Lens Anterior Eye. 2016;39(6):402-410.
50. Morgan PB, Efron N, Helland M, Itoi M, Jones D, Nichols JJ, van der Worp E, Woods CA. Twenty first century trends in silicone hydrogel contact lens fitting: an international perspective. Cont Lens Anterior Eye. 2010;33(4):196-8.
51. Chalmers RL, Begley CG, Moody K, Hickson-Curran SB. Contact Lens Dry Eye Questionnaire-8 (CLDEQ-8) and Opinion of Contact Lens Performance. Optom Vis Sci 2012;89:1435–1442.

TABLES

Table 1. **Clinical data and tear molecule levels before and 90 minutes after exposure to a controlled adverse desiccating environment (CADE).** CADE effect for each clinical parameter was computed as the relative change (percentage) from pre-exposure time. For each tear molecule level this effect (change) was quantified by log2-Fold change.

	Before CADE	**90-minutes after CADE**	**CADE effect**	
Parameters	**Mean ± SD or *Median ± IQR***	**Mean ± SD or *Median ± IQR***	**Mean ± SD**	**Informative variable**
Limbal hyperemia (Efron, 0-4 each area)				
Nasal	*1 ± 0*	*2 ± 1*	29.4 ± 26.7	✓
Temporal	*1 ± 0*	*2 ± 1*	43.6 ± 52.8	✓
Superior	*1 ± 1*	*1 ± 0*	10.6 ± 32.9	-
Inferior	*1 ± 0.5*	*1 ± 1*	19.2 ± 25.8	-
Total	*4 ± 2*	*6 ± 1*	32.4 ± 24.7	✓
CL dehydration (0-100)	34.1 ± 2.1	26.2 ± 1.4	-72.4 ± 10.1	✓
Tear osmolarity (mOsm/l)	327.0 ± 21.4	320.4 ± 20.0	-13.9 ± 30.8	✓
Phenol red thread test (mm)	16.7 ± 7.0	18.1 ± 6.4	7.7 ± 40.5	✓
Pre-lens BUT (secs)	7.1 ± 3.2	5.8 ± 1.8	-26.7 ± 32.3	✓
Corneal staining (Oxford, 0-5)	*0 ± 0*	*1 ± 1*	15.2 ± 32.2	-
Corneal staining (CCLRU, 0-4 each area)				
Central	*0 ± 0*	*0 ± 0*	2.8 ± 24.9	-
Nasal	*0 ± 0*	*0 ± 1*	7.8 ± 26.9	-
Temporal	*0 ± 0*	*0 ± 0*	4.2 ± 17.5	-
Superior	*0 ± 0*	*0 ± 0*	0.0 ± 33.0	-
Inferior	*0 ± 0*	*1 ± 2*	22.9 ± 37.2	✓
Total	*0 ± 1*	*2 ± 3*	17.5 ± 32.6	✓
Limbal staining (CCLRU, 0-4 each area)				
Nasal	*1 ± 0*	*2 ± 0.5*	31.6 ± 27.4	✓
Temporal	*1 ± 0*	*2 ± 1*	25.2 ± 27.6	✓
Superior	*1 ± 0*	*1 ± 1*	26.2 ± 33.5	-
Inferior	*1 ± 0*	*2 ± 1.5*	41.1 ± 45.3	✓
Total	*4 ± 0*	*7 ± 2*	31.6 ± 22.1	✓
SANDE 1 (0-10)				
Dryness	1.2 ± 1.3	3.2 ± 2.4	19.7 ± 35.9	✓

	Before CADE	90-minutes after CADE	CADE effect	
Parameters	**Mean ± SD or *Median ± IQR***	**Mean ± SD or *Median ± IQR***	**Mean ± SD**	**Informative variable**
Comfort	8.9 ± 0.9	7.7 ± 1.7	-17.0 ± 32.0	✓
Blurred vision	1.0 ± 1.4	1.5 ± 1.8	2.2 ± 32.0	-
SANDE 2 (-5/+5)				
Dryness	-	0.7 ± 1.6	-	✓
Comfort	-	-0.2 ± 1.4	-	-
Blurred vision	-	0.2 ± 1.2	-	-
Blink rates (Blinks/min)				
Intervals				
5-10 minutes	-	40.7 ± 22.7	-	-
25-30 minutes	-	43.2 ± 22.3	-	✓
55-60 minutes	-	45.0 ± 23.4	-	-
85-90 minutes	-	45.1 ± 23.2	-	-
Average	-	43.5 ± 22.2	-	-
Tear molecule levels (pg/mL)				
EGF	1050.0 ± 699.5	949.2 ± 746.7	-0.27 ± 0.99	✓
IFN-gamma	83.7 ± 101.1	79.5 ± 81.1	0.06 ± 1.40	✓
IL-1beta	24.0 ± 34.0	19.9 ± 22.8	-0.04 ± 1.88	✓
IL-1RA	2529.1 ± 3856.8	1984.5 ± 2906.9	-0.24 ± 1.90	✓
IL-2	38.4 ±56.0	37.3 ± 47.9	0.07 ± 1.72	✓
IL-4	120.5 ± 198.6	130.5 ± 216.8	0.14 ± 2.55	✓
IL-6	43.7 ± 45.9	48.0 ± 41.5	0.24 ± 1.28	✓
CXCL8/IL-8	120.2 ± 117.5	112.7 ± 122.4	-0.17 ± 1.07	✓
IL-10	79.0 ± 140.3	71.6 ± 97.9	0.05 ± 1.50	✓
IL-12p70	162.7 ± 263.5	168.1 ± 219.7	0.2 ± 1.71	✓
IL-13	69.4 ± 93.4	59.1 ± 73.6	-0.13 ± 1.96	✓
IL-17A	19.8 ± 38.6	19.7 ± 30.6	0.32 ± 1.72	-
IP-10	23354.4 ± 18512.7	30616.3 ± 72387.6	-0.16 ± 2.00	-
MCP-1	592.5 ± 1069.3	504.5 ± 701.2	-0.10 ± 1.54	✓
RANTES	125.3 ± 134.3	122.8 ± 134.9	-0.15 ± 1.45	✓
TNF-alpha	29.0 ± 34.1	29.3 ± 31.0	0.06 ± 1.11	-
MMP-9	3101.0 ± 10503.0	1860.1 ± 5061.6	-0.22 ± 2.30	✓

SD= Standard deviation; IQR= Interquartile range; BUT = Break-up time; SANDE = symptom assessment in dry eye questionnaire; EGF = Epidermal growth factor; IFN-gamma = interferon - gamma; IL-1beta= Interleukin-1beta; IL-1RA = Interleukin-1 Receptor Antagonist; IL-2 = Interleukin-2; IL-4 = Interleukin-4; IL-6 = Interleukin-6; IL-8 = Interleukin-8; IL-10 = Interleukin-10; IL-12p70 = Interleukin-12p70; IL-17A = Interleukin-17A; IP-10 = interferon-gamma– Induced Protein-10; MCP-1 = monocyte chemoattractant protein 1; RANTES =

Regulated on Activation, Normal T cell Expressed and Secreted; TNF = tumor necrosis factor ; MMP-9 = matrix metalloproteinase-9.

Table 2. Clinical and tear molecular changes for each cluster after the controlled adverse desiccating environment (CADE) exposure. Demographic data is also provided. For clinical and molecular parameters, description of relative change is shown. Mean and standard deviation is used to summarize quantitative variables. For sex, the percentage of males (and its 95% confidence interval) is calculated. The clusters are compared by Fisher's exact test and equality of proportions hypothesis tests for pairwise comparisons. Arrows indicate the direction of statistically significant ($p \leq 0.05$) changes. Regarding ANOVA p-values, significant values ($p \leq 0.05$) are denoted in bold font, and borderline values ($0.05 < p < 0.1$) are in italics.

	Cluster 1 (n=22)	**Cluster 2 (n=22)**	**Cluster 3 (n=3)**	**ANOVA**			
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Global p-value**	**Post hoc comparison**		
					Cluster 1 vs 2	Cluster 1 vs 3	Clusters 2 vs 3
Demographic variables							
Age (years)	28.6 ± 7.7	26.3 ± 7.6	31.7 ± 9.3	0.42	0.51	0.51	0.51
Sex (% males)	45.5% (25.1%; 67.3%)	31.8% (14.7%; 54.9%)	33.3% (1.8%; 87.5%)	0.79	1	1	1
Clinical parameter changes (%)							
Limbal hyperemia (Efron)							
Nasal	36.4 ± 27.5 ↑	22.0 ± 24.9 ↑	33.3 ± 28.9	0.19	0.23	0.85	0.73
Temporal	45.5 ± 57.6 ↑	38.6 ± 48.6 ↑	66.7 ± 57.7	0.68	0.67	0.67	0.67
Superior	11.4 ± 40.6	11.4 ± 26.4	0.0 ± 0.0	0.73	0.90	0.73	0.73
Inferior	24.2 ± 28.5 ↑	12.1 ± 21.3 ↑	33.3 ± 28.9	0.18	0.27	0.56	0.27
Total	37.2 ± 28.8 ↑	26.0 ± 20.1 ↑	44.4 ± 13.9 ↑	0.24	0.29	0.61	0.29
CL dehydration	-69.4 ± 9.4 ↓	-74.9 ± 9.7 ↓	-76.6 ± 16.2 ↓	0.15	0.22	0.36	0.78
Tear osmolarity	-7.1 ± 19.8	-17.4 ± 34.2 ↓	-38.3 ± 62.5	0.45	0.62	0.62	0.62
Phenol red thread test	29.2 ± 35.3 ↑	-15.1 ± 35.4	16.8 ± 23.1	**0.002**	**0.002**	0.53	0.21
Pre-lens BUT	-26.7 ± 27.4 ↓	-24.0 ± 36.9 ↓	-45.3 ± 34.5	0.36	0.64	0.33	0.33
Corneal staining (Oxford)	27.3 ± 33.9 ↑	3.8 ± 28.1	11.1 ± 19.3	**0.048**	**0.044**	0.59	0.70
Corneal staining (CCLRU)							
Central	7.6 ± 27.1	-3.0 ± 22.8	11.1 ± 19.3	0.31	0.48	0.82	0.54

	Cluster 1 (n=22)	Cluster 2 (n=22)	Cluster 3 (n=3)	ANOVA			
	Mean ± SD	Mean ± SD	Mean ± SD	Global p-value	Post hoc comparison		
					Cluster 1 vs 2	Cluster 1 vs 3	Clusters 2 vs 3
Nasal	6.8 ± 28.5	6.8 ± 24.5	22.2 ± 38.5	0.64	1	0.54	0.54
Temporal	6.8 ± 23.4	2.3 ± 10.7	0.0 ± 0.0	0.59	0.80	0.80	0.80
Superior	0.0 ± 26.7	0.0 ± 40.8	0.0 ± 0.0	0.97	0.97	0.97	0.97
Inferior	43.6 ± 31.1 ↑	3.0 ± 33.4	16.7 ± 28.9	**0.0006**	**0.0004**	0.27	0.49
Total	32.4 ± 26.7 ↑	2.0 ± 31.5	22.2 ± 38.5	**0.0057**	**0.004**	0.54	0.41
Limbal staining (CCLRU)							
Nasal	39.4 ± 23.9 ↑	18.2 ± 23.0 ↑	72.2 ± 25.5 ↑	**0.0004**	**0.007**	**0.03**	**0.002**
Temporal	31.8 ± 28.1 ↑	17.4 ± 20.9 ↑	33.3 ± 57.7	0.19	0.25	0.92	0.51
Superior	32.6 ± 33.5 ↑	18.9 ± 30.1 ↑	33.3 ± 57.7	0.38	0.55	0.97	0.73
Inferior	53.0 ± 39.4 ↑	21.2 ± 43.4 ↑	100.0 ± 0.0 ↑	**0.004**	**0.03**	**0.05**	**0.02**
Total	39.8 ± 21.4 ↑	19.4 ± 15.1 ↑	61.0 ± 18.6 ↑	**0.0005**	**0.004**	*0.07*	**0.01**
SANDE 1							
Dryness	31.7 ± 28.5 ↑	8.9 ± 37.2	11.5 ± 60.2	*0.09*	0.10	0.52	0.90
Comfort	-26.5 ± 31.8 ↓	-5.4 ± 29.6	-32.9 ± 29.3	*0.06*	*0.08*	0.73	0.22
Blurred vision	14.3 ± 27 ↑	-10.2± 34.6	3.3 ± 4.5	**0.03**	**0.03**	0.55	0.55
SANDE 2 (changes in units: -5 to +5)							
Dryness	1.2 ± 1.3 ↑	0.1 ± 1.6	1.4 ± 1.7	**0.03**	**0.04**	0.83	0.22
Comfort	-0.6 ± 1.0 ↓	0.3 ± 1.6	-0.9 ± 1.1	**0.05**	*0.07*	0.71	0.20
Blurred vision	0.6 ± 1.0 ↑	-0.3 ± 1.3	0.3 ± 0.3	*0.06*	*0.07*	0.68	0.68
Tear molecule levels changes (Log2-fold-changes)							
EGF	-0.55 ± 0.80 ↓	0.29 ± 0.67	-2.40 ± 0.28 ↓	**0.0001**	**0.002**	**0.008**	**0.008**
IFN-gamma	0.5 ± 1.25	0.01 ± 0.15	-2.74 ± 1.07 ↓	**0.0003**	0.18	**0.0002**	**0.0008**
IL-1beta	0.35 ± 1.88	-0.16 ± 1.45	-1.94 ± 3.88	0.37	0.38	0.38	0.38
IL-1Ra	0.05 ± 1.30	0.02 ± 1.69	-4.33 ± 2.94	**0.0002**	0.94	**0.0001**	**0.0001**
IL-2	0.24 ± 1.84	0.26 ± 1.17	-2.65 ± 2.51	0.12	0.51	0.18	0.12
IL-4	1.21 ± 2.01 ↑	-0.25 ± 2.30	-4.77 ± 0.86 ↓	**0.005**	*0.07*	**0.02**	**0.02**
IL-6	0.54 ± 1.18 ↑	0.33 ± 0.86	-2.66 ± 1.23	**0.01**	0.50	**0.001**	**0.001**
IL-8	0.01 ± 0.62	-0.05 ± 1.16	-2.40 ± 0.57 ↓	**0.02**	0.91	**0.02**	**0.02**
IL-10	0.00 ± 1.25	0.45 ± 1.33	-2.46 ± 2.33	**0.004**	0.28	**0.007**	**0.003**
IL-12p70	0.01 ± 1.64	0.80 ± 1.40 ↑	-2.77 ± 1.08 ↓	**0.001**	*0.09*	**0.007**	**0.001**
IL-13	0.02 ± 1.86	0.27 ± 1.38	-4.24 ± 2.25	**0.0003**	0.61	**0.0002**	**0.0002**
IL-17A	0.68 ± 1.87	0.24 ± 1.10	-1.74 ± 3.30	*0.07*	0.38	*0.06*	*0.09*
CXCL10/ IP-10	-0.24 ± 0.84	0.59 ± 1.62	-5.01 ± 3.95	*0.09*	0.12	0.17	0.17
MCP-1	-0.40 ± 1.40	0.61 ± 1.04 ↑	-3.15 ± 1.38	**<0.0001**	**0.009**	**0.001**	**<0.0001**
CCL5/ RANTES	-0.25 ± 1.03	0.49 ± 0.81 ↑	-4.15 ± 1.30 ↓	**0.0008**	**0.009**	**0.009**	**0.009**
TNF-alpha	0.38 ± 1.04	0.08 ± 0.74	-2.37 ± 1.11	**0.0001**	0.27	**<0.0001**	**0.0001**

	Cluster 1 (n=22)	Cluster 2 (n=22)	Cluster 3 (n=3)	ANOVA			
	Mean ± SD	Mean ± SD	Mean ± SD	Global p-value	Post hoc comparison		
					Cluster 1 vs 2	Cluster 1 vs 3	Clusters 2 vs 3
MMP-9	-0.39 ± 1.47	0.51 ± 2.35	-4.38 ± 3.12	**0.03**	0.37	**0.02**	**0.03**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**				
Blink rate (Blinks/min)							
Interval							
5-10 min	39.3 ± 20.8	37.7 ± 22.6	72.3 ± 17.2	**0.03**	0.80	**0.02**	**0.02**
25-30 min	41.6 ± 18.5	39.6 ± 22.4	80.8 ± 17.5	**0.03**	0.67	**0.02**	**0.02**
55-60 min	42.6 ± 20.2	42.7 ± 23.0	80.1 ± 28.1	*0.08*	0.96	**0.05**	**0.05**
85-90 min	45.8 ± 20.7	39.7 ± 22.2	79.3 ± 24.5	*0.06*	0.31	*0.07*	*0.07*
Average	42.3 ± 19.0	39.9 ± 22.0	78.1 ± 21.7	**0.05**	0.61	**0.04**	**0.04**

SD= Standard deviation; IQR= Interquartile range; CI=Confidence interval; TBUT = Tear film break-up time; SIDEQ = Single-item score dry eye questionnaire; OSDI = Ocular surface disease index; EGF = Epidermal growth factor; CX3CL = Chemokine [C-X3-C motif] ligand; IL-1RA = Interleukin-1 Receptor Antagonist; IL-6 = Interleukin-6; CXCL = Chemokine [C-X-C motif] ligand; IL-8 = Interleukin-8; IP-10 = interferon-γ– Induced Protein-10; MCP-1 = monocyte chemoattractant protein 1; CCL = Chemokine [C-C motif] ligand; RANTES = Regulated on Activation, Normal T cell Expressed and Secreted; VEGF = Vascular endothelial growth factor; MMP-9 = matrix metalloproteinase-9.

Table 3. Internal validation of corneal staining and IL-12p70 based model to predict cluster 1 membership. Brier score is a measure of accuracy that ranges from 0, for a perfect model, to 1. We used the Hosmer-Lemeshow test as calibration measure. This test provides significant ($p<0.05$) results when assessing badly calibrated models. As discrimination indexes we used the area under the ROC curve (AUC), sensitivity, and specificity.

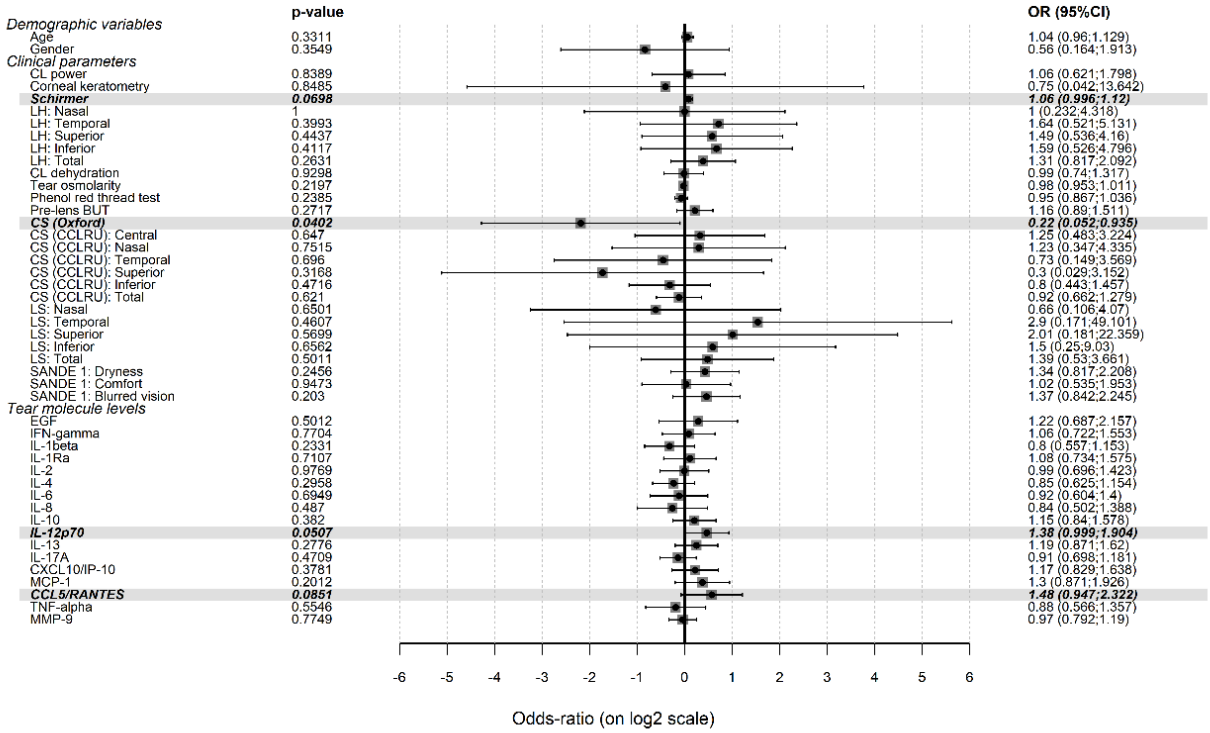
Accuracy	**Calibration**	**Discrimination**		
Brier Score (95% CI)	**Hosmer-Lemeshow p-value**	**AUC (95% CI)**	**Sensitivity (%) (95% CI)**	**Specificity (%) (95% CI)**
0.1994 (0.1292, 0.2721)	0.06	0.75 (0.59, 0.91)	81.8 (65.7, 97.9)	77.3 (59.8, 94.8)

Table 4. Simple decision rule to apply the study outcomes in the clinical setting.

Baseline corneal staining score	**Baseline tear IL-12p70 levels (pg/mL)**	**Cluster membership predicted**
0	≤32	2 (No increase in CL discomfort after CADE)
	>32	1 (Increase in CL discomfort after CADE)
>0	No matter the value	2 (No increase in CL discomfort after CADE)

FIGURE LEGENDS

Figure 1. Potential predictors of Cluster 1 (worsening after CADE) membership. Associations between each pre-exposure variable and Cluster 1 membership are shown. The x-axis is the base-2 logarithmic odds ratio (OR) estimated by binary logistic regression analysis. The 95% confidence intervals for log2 odds ratio are plotted as horizontal lines. The vertical bold line represents the no association value. For each pre-exposure variable, positive values (right to the bold vertical line) mean positive association between the variable and Cluster 1 membership, while negative values (left to the bold vertical line) mean negative association. Variables associated with Cluster 1 membership at the 10% significance level were considered possible potential predictors (bold italic text and shadow).



BUT = break-up time; CCL = chemokine [C-C motif] ligand; CI = confidence interval; CL = contact lens; LS = limbal staining; CS = corneal staining; LH = limbal hyperemia; EGF = epidermal growth factor; IFN = interferon; IL = interleukin; IL-1RA = interleukin-1 receptor antagonist; IP = induced protein; MCP = monocyte chemoattractant protein; MMP = matrix metalloproteinase; OR = odds ratio; RANTES = regulated on activation, normal T cell expressed and secreted; TNF = tumor necrosis factor.

APPENDIX A

Table A1. Limit and percentage of detection of the 17 tear molecules analyzed in tear samples.

	Limit of detection (pg/ml)	Rate of detection (%) (95% CI)	
		Pre-exposure	Post-exposure
EGF	1.23	97.9 (87.28 ; 99.89)	95.7 (84.27 ; 99.26)
IFN-gamma	1.23	83 (68.65 ; 91.86)	87.2 (73.56 ; 94.7)
IL-1beta	1.05	51.1 (36.26 ; 65.7)	48.9 (34.3 ; 63.74)
IL-1RA	1.23	97.9 (87.28 ; 99.89)	95.7 (84.27 ; 99.26)
IL-2	1.16	59.6 (44.31 ; 73.29)	55.3 (40.24 ; 69.54)
IL-4	1.23	66 (50.6 ; 78.72)	63.8 (48.48 ; 76.94)
IL-6	1.12	72.3 (57.13 ; 83.91)	74.5 (59.36 ; 85.58)
CXCL8/IL-8	1.23	95.7 (84.27 ; 99.26)	93.6 (81.44 ; 98.34)
IL-10	1.23	66 (50.6 ; 78.72)	61.7 (46.38 ; 75.12)
IL-12p70	1.10	83 (68.65 ; 91.86)	89.4 (76.11 ; 96.02)
IL-13	1.23	66 (50.6 ; 78.72)	66 (50.6 ; 78.72)
IL-17A	1.19	25.5 (14.42 ; 40.64)	31.9 (19.52 ; 47.25)
CXCL10/ IP-10	1.23	100 (90.59 ; 100)	100 (90.59 ; 100)
CCL2/MCP-1	1.23	97.9 (87.28 ; 99.89)	91.5 (78.73 ; 97.24)
CCL5/ RANTES	1.23	87.2 (73.56 ; 94.7)	85.1 (71.08 ; 93.31)
TNF-alpha	1.18	57.4 (42.26 ; 71.43)	57.4 (42.26 ; 71.43)
MMP-9	1.23	91.5 (78.73 ; 97.24)	93.6 (81.44 ; 98.34)

CI=Confidence interval; EGF = Epidermal growth factor; IFN-g = interferon - g; IL-1beta= Interleukin-1beta;IL-1RA = Interleukin-1 Receptor Antagonist; IL-2 = Interleukin-2; IL-4 = Interleukin-4; IL-6 = Interleukin-6; IL-8 = Interleukin-8; IL-10 = Interleukin-10; IL-12p70 = Interleukin-12p70; IL-17A = Interleukin-17A; IP-10 = interferon- gamma– Induced Protein-10; MCP-1 = monocyte chemoattractant protein 1; CCL = Chemokine [C-C motif] ligand; RANTES = Regulated on Activation, Normal T cell Expressed and Secreted; TNF= tumor necrosis factor; MMP-9 = matrix metalloproteinase-9.

APPENDIX B

Pre-processing step to remove uninformative variables in the construction of the clusters. Two criteria were used: relevance and redundancy. Except blink rates, variables whose change did not exceed a relevant threshold in the most of the participants were ignored. The threshold definition was dependent on the scale used to measure the variable. For blink rates measurements, the coefficient of variation was used. In addition, among the relevant variables, those highly correlated were considered redundant and were not taken into account in the clustering stage.

Table B1: Relevance criterion. Except for blink rates variables, the percentage of sample that met the corresponding criteria for each variable is showed. According to the relevance criterion, the informative variables were those that showed a percentage of the sample meeting the criteria below 50%. For blink rates measurements, the criterion was based on the coefficient of variation: values below 10% were considered insufficient.

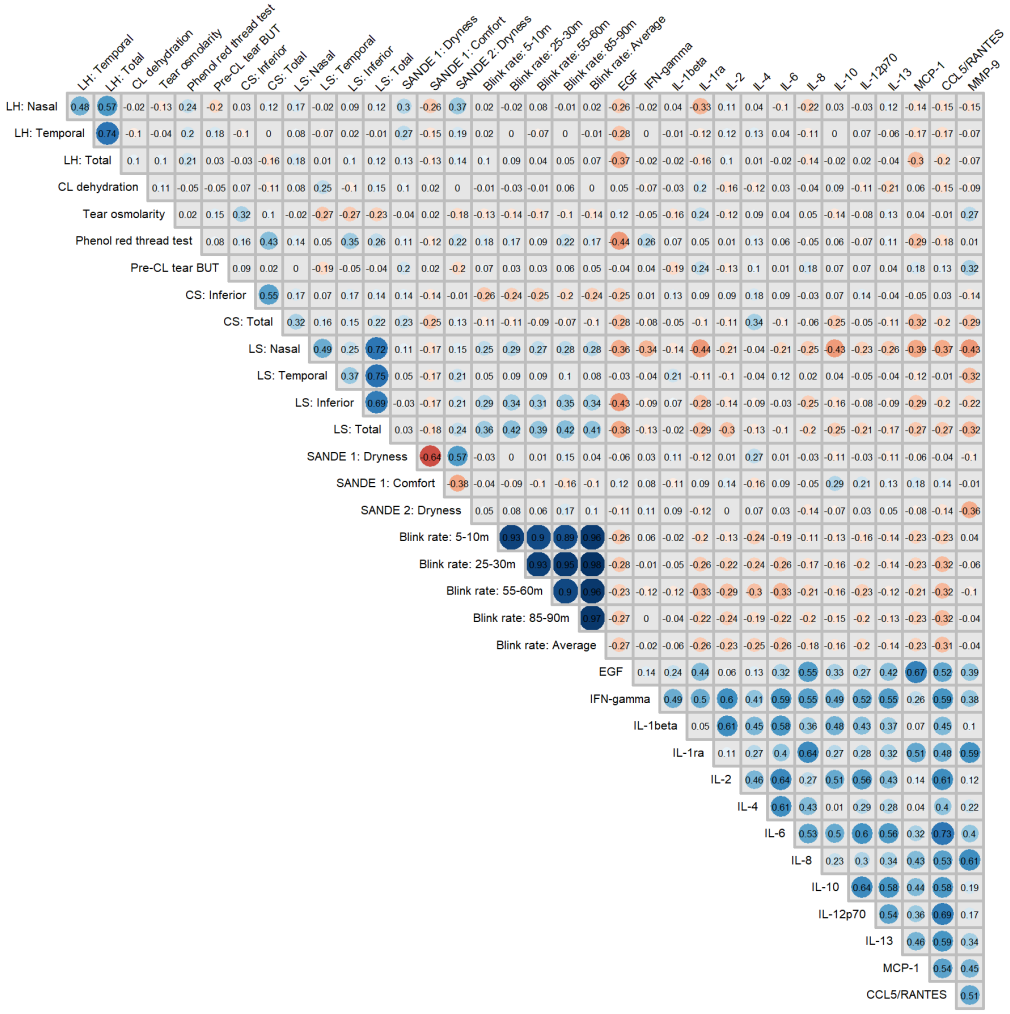
Criteria	Variable	Percentage of the study sample meeting the criteria (95%CI)	Relevant variable
Percentage of change, in absolute value, ≤ 10%	Limbal hyperemia (Efron)		
	Nasal	42.6% (28.57%; 57.74%)	✓
	Temporal	42.6% (28.57%; 57.74%)	✓
	Superior	*66% (50.6%; 78.72%)*	
	Inferior	*59.6% (44.31%; 73.29%)*	
	Total	14.9% (6.69%; 28.92%)	✓
	CL dehydration	0% (0%; 9.14%)	✓
	Tear osmolarity	44.7% (30.46%; 59.76%)	✓
	Phenol red thread test	19.1% (9.65%; 33.73%)	✓
	Pre-lens break-up time	29.8% (17.79%; 45.08%)	✓
	Corneal staining (Oxford)	*55.3% (40.24%; 69.54%)*	

Criteria	Variable	Percentage of the study sample meeting the criteria (95%CI)	Relevant variable
	Corneal staining (CCLRU scheme)		
	Central	*80.9% (66.27%; 90.35%)*	
	Nasal	*78.7% (63.93%; 88.8%)*	
	Temporal	*87.2% (73.56%; 94.7%)*	
	Superior	*83% (68.65%; 91.86%)*	
	Inferior	44.7% (30.46%; 59.76%	✓
	Total	27.7% (16.09%; 42.87%)	✓
	Limbal staining (CCLRU scheme)		
	Nasal	23.4% (12.79%; 38.37%)	✓
	Temporal	44.7% (30.46%; 59.76%)	✓
	Superior	*57.4% (42.26%; 71.43%)*	
	Inferior	38.3% (24.88%; 53.62%)	✓
	Total	23.4% (12.79%; 38.37%)	✓
	SANDE 1		
	Dryness	14.9% (6.69%; 28.92%)	✓
	Comfort	31.9% (19.52%; 47.25%)	✓
	Blurred vision	*59.6% (44.31%; 73.29)*	
Change, in absolute value, ≤ 0.5 units	SANDE 2		
	Dryness	29.8% (17.79%; 45.08%)	✓
	Comfort	*57.4% (42.26%; 71.43%)*	
	Blurred vision	*76.6% (61.63%; 87.21%)*	
Coefficient of variation ≤ 10%	Blink rates intervals		
	5-10 minutes	55.8% (47.64%; 63.9%)	✓
	25-30 minutes	51.7% (44.14%; 59.22%)	✓
	55-60 minutes	52% (44.42%; 59.6%)	✓
	85-90 minutes	51.4% (43.93%; 58.93%)	✓

Criteria	Variable	Percentage of the study sample meeting the criteria (95%CI)	Relevant variable
	Average	51% (43.54%; 58.4%)	✓
Log2 FC ≤ 0.5	EGF	40.4% (26.71%; 55.69%)	✓
	IFN-gamma	42.6% (28.57%; 57.74%)	✓
	IL-1beta	40.4% (26.71%; 55.69%)	✓
	IL-1RA	29.8% (17.79%; 45.08%)	✓
	IL-2	38.3% (24.88%; 53.62%)	✓
	IL-4	23.4% (12.79%; 38.37%)	✓
	IL-6	48.9% (34.3%; 63.74%)	✓
	IL-8	42.6% (28.57%; 57.74%)	✓
	IL-10	38.3% (24.88%; 53.62%)	✓
	IL-12p70	38.3% (24.88%; 53.62%)	✓
	IL-13	40.4% (26.71%; 55.69%)	✓
	IL-17A	*70.2% (54.92%; 82.21%)*	
	IP-10	*57.4% (42.26%; 71.43%)*	
	MCP-1	46.8% (32.37%; 61.77%)	✓
	RANTES	44.7% (30.46%; 59.76%)	✓
	TNF-alpha	*57.4% (42.26%; 71.43%)*	
	MMP-9	12.8% (5.3%; 26.44%)	✓

CI=Confidence interval; FC = Fold-change; EGF = Epidermal growth factor; IFN-gamma = interferon - gamma; IL-1beta= Interleukin-1beta;IL-1RA = Interleukin-1 Receptor Antagonist; IL-2 = Interleukin-2; IL-4 = Interleukin-4; IL-6 = Interleukin-6; IL-8 = Interleukin-8; IL-10 = Interleukin-10; IL-12p70 = Interleukin-12p70; IL-17A = Interleukin-17A; IP-10 = interferon- gamma– Induced Protein-10; RANTES = Regulated on Activation, Normal T cell Expressed and Secreted; TNF = tumor necrosis factor ; MMP-9 = matrix metalloproteinase-9.

Figure B1. Redundancy criterion. Correlogram showing the relationship between each pair of relevant variables previously selected. Correlation coefficients were colored according to theirs values: blue and red for positive and negative values, respectively. Color intensity and bubble size indicate how strong the corresponding two variables are related. According to the criterion of redundancy, the variables whose correlation coefficient is above 0.75 (in absolute value) are defined as redundant. The variables related to the blink rates were removed, except the 25-30 minutes interval one.



BUT = break-up time; CCL = chemokine [C-C motif] ligand; CL = contact lens; LS = limbal staining; CS = corneal staining; LH = limbal hyperemia; EGF = epidermal growth factor; IFN = interferon; IL = interleukin; IL-1RA = interleukin-1 receptor antagonist; IP = induced protein; MCP = monocyte chemoattractant protein; MMP = matrix metalloproteinase; RANTES = regulated on activation, normal T cell expressed and secreted; TNF = tumor necrosis factor.

APPENDIX C

Table C1. The best multiple logistic regression models for Cluster 1 membership by size (number of independent variables). Potential predictors that showed a p-value below 0.1 individually were: corneal staining in Oxford scale, Schirmer I test and baseline levels of IL-12p70 and RANTES. M0, M1, M2, M3 and M4, are a model based on 0, 1, 2, 3 and 4 potential predictors respectively. The best model by size is the one with the lower Akaike information criterion value (last column). The M2 model, based on corneal staining and Interelukin IL-12p70 levels, was the best.

Model	**Baseline Corneal staining (Oxford)**	**Baseline Schirmer-I test**	**Baseline Interleukin-12p70 tear levels**	**Baseline RANTES tear levels**	**Akaike information criterion**
M0					61.00
M1	✓				56.50
M2	✓		✓		**50.86**
M3	✓	✓		✓	50.98
M4	✓	✓	✓	✓	52.19

RANTES = Regulated on Activation, Normal T cell Expressed and Secreted;