TITLE: Capability of the inter-eye differences in osmolarity, break-up time and corneal staining on the diagnostic of dry eye

Running Heah: Inter-eye differences diagnostic of dry eye

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ABSTRACT

Purpose: Inter-eye variability is a recognized characteristic of Dry Eye Disease (DED) and has been proposed as a diagnostic indicator in clinical practice. This study aimed to assess the diagnostic potential of the absolute difference between eyes in three key diagnostic tests recommended by the Tear Film and Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II) Diagnostic Methodology report: tear film osmolarity, Fluorescein Break-Up Time (FBUT), and ocular surface staining.

Methods: A total of 180 participants were included in a cross-sectional study. Before a dry eye examination, participants completed an online self-administered OSDI questionnaire. The TFOS DEWS II diagnostic criteria for DED assessment were used: along with OSDI, osmolarity, FBUT and ocular surface staining were measured in all participants in both eyes following standardized methodology. Based on signs and symptoms, participants were diagnosed as having No DED or DED. After diagnosis, parameters were computed as the right and left eyes' absolute inter-eye difference (|OD-OS|).

Results: Receiver Operating Characteristics analyses for computed parameters were used based on the previous diagnosis. ROC analyses showed that Osmolarity|OD-OS| have a diagnostic capability to differentiate between No DED and DED participants with a cut-off value of 9.5 mOsm/L (AUC = 0.745 ± 0.052, $p < 0.003$), whereas FBUT|OD-OS| and Corneal Stainning|OD-OS| have not (AUC, both $p \geq 0.160$).

Conclusion: The present study found that the Osmolarity|OD-OS| parameter could be used as a diagnostic indicator for DED assessment, while the FBUT|OD-OS| and the Corneal Staining|OD-OS| parameters do not have this capability.

Keywords: Dry Eye Syndromes; Tear Film Osmolarity; Fluorescein Break-Up Time; Ocular Surface Staining; ROC Curve

1. INTRODUCTION

Dry Eye Disease (DED) had been redefined by the Tear Film and Ocular Surface Society in the second Dry Eye Workshop (TFOS DEWS II) as a chronic multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation, damage, and neurosensory abnormalities play etiological roles.[1-3] The DED may reach to 50% of the population of some areas, impairing the quality of life of those suffering from it.[4] The inclusion of 'homeostasis' in the actual definition implies that DED may be not caused by any single factor but rather by an imbalance of several different systems working in concert.

Tear film parameters show little variation, either over time or between eyes in healthy patients, while as the body loses control during an ocular surface disease, the tear film homeostasis loss is reflected as increasing in time or eye-to-eye value changes of the diagnostic test.[3] Repeated measurements over a while in several parameters such as osmolarity has been reported as low and stable in healthy participants, nonetheless, DED patients showed an increase and unstable readings in short periods.[5-7] Indeed, the variation and increase in diagnostic tests values (heteroscedasticity) is a statistical characteristic of DED indicators because of the physiopathological processes that are implied in the disease, which could be considered a clinical indication of the inherent tear film homeostasis loss.[8] Furthermore, inter-eye variability may serve as a distinctive characteristic of DED, wherein elevated variations in the metrics across eyes could be leveraged in clinical settings; notably, lower disparities might indicate temporary effects within compensatory mechanisms.[9,10]

The present study aimed to explore the diagnostic capability of the absolute inter-eye differences of the three main diagnostic criteria tests proposed by the TFOS DEWS II Diagnostic Methodology report: tear film osmolarity, tear film Fluorescein Break-Up Time (FBUT) and fluorescein ocular surface staining.

2. MATERIALS AND METHODS

2.1. Sample

A total of 180 participants (mean ± standard deviations [SDs] = 49.4 ±14.1 years) who attended the Optometry Clinic were included in the study. All of them were participants who came to the centre for an ocular surface examination referred by their medical doctor, or the health service of the institution based on dry eye complaints. No participant was using any kind of medication or artificial tears at the time of the study. Participants were excluded if they had a prior history of ocular surgery (including refractive surgery or eyelid tattooing), ocular infections, Meibomian Gland Dysfunction (MGD), blepharitis, glaucoma or general autoimmune or systemic diseases (previous diagnosis of diabetes mellitus, sarcoidosis, Sjögren's syndrome, Lupus, Crohn's disease, Graves' disease, Hashimoto's thyroiditis, Psoriasis, tumours or Multiple sclerosis or active influenza, COVID-19 or pneumonia), were pregnant or breast-feeding or wore contact lenses.[11,12] All the participants gave their written informed consent to be included in the study and all procedures followed were in accordance with the Helsinki Declaration. The study protocol was approved by the Bioethics Committee of the institution.

Before the study, the sample size was calculated based on TFOS DEWS II Diagnostic Methodology report recommended diagnostic tests.[1] For the sample size calculation, the software PS Power and Sample Size Calculations Version 3.1.2 (Copyright© by William D. Dupont and Walton D. Plummer) was used.[13] The SD reported in the literature on the symptomatology status (Ocular Surface Disease Index [OSDI] score), tear film osmolarity, tear film FBUT, and fluorescein corneal staining was assumed to be 6.7, 4.8 mOsm/L, 2.9 s, and 2 respectively.[1,14-17] To have 80% power for a significance level of α =0.05 (Type I error associated) to detect a clinical difference between no DED and DED participants of 7.3, 5 mOsm/l, 5 s and 1 respectively, the minimum number of subjects required in each group was 60, 54, 36 and 51 respectively to have a relative ratio control/experimental of 1:1. The highest of those values was used as the reference to the sample size (60 participants) to accomplish a more reliable study. A higher sample was recruited to find a greater impact on the results.

2.2. Study Design and Diagnostic Criteria

A battery of clinical procedures was conducted on both eyes of all participants according to the TFOS DEWS II Diagnostic Methodology report.[1] The first eye to be measured was randomly selected. Tests were always performed in the same order, from the least to the most invasive [1]: tear film osmolarity, tear film FBUT and fluorescein corneal staining. The diagnostic tests cut-off values for the DED diagnosis are stated on Table 1.[1] Participants were classified as DED if a positive symptomology result (OSDI score ≥13) combined with a positive result on at least one clinical test in diagnosing dry eye was accomplished in either of the eyes, while were diagnosed as No DED if no one of the inclusion criteria falls out of the cut-off criteria in any of the participant's eyes.[1]

All these measurements were performed and recorded by the same examiner who was unaware of the results of the questionnaire, in a single session, to avoid interexaminer or intersession variability.[18,19] During all protocols, the instruments and material used were kept in the same humidity- and temperature-controlled room (temperature 20-23°C, humidity 50-60%).[5] Upon arrival, participants were allowed to rest for 5–10 min to adapt to the ambient conditions prior to measurements.

2.3. Evaluation Procedures

All protocol procedures were summarized in Table 2.

2.3.1. Symptomatology Assessment

The DED symptomatology was quantified by a self-administered OSDI questionnaire by scanning a QR code provided before the examination [20]; questions were asked concerning a 1-week recall period following the standardized interview model.[21] The scores obtained were computed on a scale of 0 to 100 points by the researchers according to the published standardized guidelines, where higher scores represent greater disability.[20]

2.3.2. Tear Film Osmolarity

The tear film osmolarity was measured by the TearLab osmometer (TearLab Corp, San Diego, CA, United States).[22] The participants were requested to sit with their heads tilted and their eyes looking at the ceiling. Then, the instrument probe was placed on the lower tear meniscus of the randomly selected eye until a beep was emitted, signalling that the sample was collected. The TearLab converts the electrical impedance of the sample into osmolarity (mOsm/L), which was displayed on the device screen. The contralateral eye was measured after a 5–10 min interval to avoid inter-eye interference following the same protocol.[5] In all cases, the same test card lot number was used in both eyes. Quality control electronic check card was used regularly to verify the correct stats of the system (if the reading was 334 ±3 mOsm/L, the pen was working correctly).

2.3.3. Fluorescein Break-Up Time

The FBUT was assessed by the Keratograph 5M (Oculus Optikgerate GmbH, Wetzlar, Germany) using the fluorescein function.[23] The participants were instructed to properly place at the device chin rest and to look up, then, in all cases, a fluorescein strip hydrated with saline solution was applied to the patient's eye in the bulbar conjunctiva area in order to maintain a method to avoid interference in the inter-patient evaluation results.[24] Once the dye was applied, the participants were instructed to blink normally to ensure the adequate distribution of the fluorescein; then, participants were requested to blink three times keeping the eyes open the long as possible until the end of the test.[25] The time interval between the last blink and the appearance of the first black spot on the corneal surface was defined as the FBUT. This entire procedure was videorecorded and repeated three times for each participant.[25] These recorded videos were extracted, and the FBUT was calculated by a second masked examiner with the open-source software VistualDub64 which converts the video into frames (8 frames = 1s), improving the temporal resolution. The final values were computed based on the mean of the three measurements performed.[25]

2.3.4. Corneal Staining

The corneal staining was assessed by the Keratograph 5M (Oculus Optikgerate GmbH, Wetzlar, Germany) using the fluorescein function immediately after the FBUT measurement.[23] To observe

the possible damage in the entire cornea, the participants were requested to look at a red target in the centre of the device, subsequently, the participants were requested to look to the right, left, up and down to assess the paracentral areas; the upper lid was slightly manipulated to evaluate the upper part of the cornea during the look down.[26,27] A second masked examiner evaluated the extracted videos from this test using the Oxford Scheme, a six-scheme grade that denotes the ocular surface damage severity: mild (stage 0 or 1), moderate (stage 2 or 3) or severe (stage 4 or 5).[26,27]

2.4. Statistical analysis

SPSS statistical software v.25.0 for Windows (SPSS Inc., Chicago, IL, United States) was used for data analysis. Significance was set at a $p \leq 0.05$ for all statistical tests. The inter-eye differences parameters employed for the analysis were calculated as the absolute difference between values obtained from both participant's eyes (|OD-OS|), being named as Osmolarity|OD-OS|, FBUT|OD-OS| and Corneal Staining|OD-OS|.[9,28] Before analysis, the normal distribution of the data was checked using the Kolmogorov-Smirnov test [29]; all parameters showed a non-normal distribution (Kolmogorov-Smirnov, all $p \leq 0.001$). The median, Interquartile Range (IQR), minimum and maximum values were displayed as descriptive statistics, while parametric statistics (mean and SD) are also reported to enable comparisons with other studies and clinical results. Differences in the values obtained in each parameter between groups were analysed with the Mann-Whitney U test for non-parametric variables.[29]

The optimal threshold for discriminating between Non DED and DED in diagnostic terms was determined through Receiver Operating Characteristics (ROCs) procedures.[12,30] For each hypothetical threshold value (ranging from the lowest to the highest value observed in the study population), sensitivity and specificity of the test were calculated. Subsequently, these results were graphed with sensitivity plotted against (1-specificity). The predictive classification model's discriminatory capability was quantified as the Area Under the Curve (AUC), ranging from 0 (no predictive power) to 1 (almost perfect predictive power), along with its SD. Moreover, upper and lower 95% Confidence Intervals (CI) of the AUC were provided (mean ± 1.96×SD).[12,29]

Additionally, Youden's J statistic (J = sensitivity + specificity - 1) was calculated to ascertain the optimal numerical criterion for each ROC curve.[12,29]

In a subsequent revaluation, a cross-validation procedure specifically targeting parameters exhibiting diagnostic potential (AUC, $p \leq 0.05$) to assess the validity of the obtained cut-off values was conducted on an 80% random sampling. The variables were then transformed into a new dichotomous parameter, utilizing the calculated cut-off values (0 representing a negative diagnosis, and 1 indicating a positive diagnosis). The association between the new parameter and the initial diagnosis (TFOS DEWS II Diagnostic Methodology report) was examined using Cramer's V, which ranges from 0 (no predictive ability) to 1 (perfect predictive ability).[12]

3. RESULTS

The Non-DED group showed an age median (IQR) = 40.0 (32.0-52.0) years and an OSDI score median (IQR) = 10.42 (6.25-11.96); meanwhile, the DED group showed an age median (IQR) = 53.0 (41.0-61.0) years and an OSDI score median (IQR) = 29.55 (20.83-41.37). Descriptive statistics for all the measurements performed in the study on each subgroup were provided in Table 3. There was found a statistical difference in the Age, OSDI and Osmolarity|OD-OS| between groups (Mann-Whitney U, all $p \leq 0.020$), whereas no statistical difference was found in the FBUT|OD-OS| or Corneal Staining|OD-OS| values (Mann-Whitney U, both $p \geq 0.160$).

3.1. Assessment of Osmolarity|OD-OS| Cut-Off Values to Differentiate between No DED and DED Participants

ROCs procedures showed that Osmolarity|OD-OS| has a significant diagnostic capability to differentiate between participants´ diagnostic (AUC = 0.745 ±0.052, p =0.003, 95% CI = 0.643–0.847, Figure 1). By calculating the Youden's index (Youden's J statistic = 0.491), there was found a cut-off value for the Osmolarity|OD-OS| of 9.5 mOsm/L (specificity: 100.0%; sensitivity: 49.1%) to differentiate No DED from DED participants (Figure 1). The cross-validation analysis on a random sampling of 80% showed an association of the calculated Osmolarity|OD-OS| cut-

off value with the previous proposed diagnostic criteria of the TFOS DEWS II to differentiate between No DED and DED participants (Cramer's V = 0.258, p =0.003).

3.2. Assessment of FBUT |OD-OS | cut-Off Values to Differentiate between No DED and DED Participants

ROCs procedures showed that FBUT|OD-OS| has no significant diagnostic capability to differentiate between participants´ diagnostic (AUC = 0.617 ± 0.088, *p* = 0.160, 95% CI = 0.586–0.648, Figure 2).

3.3. Assessment of Corneal Staining |OD-OS| Cut-Off Values to Differentiate between No DED and DED Participants

ROCs procedures showed that Corneal Staining|OD-OS| has no significant diagnostic capability to differentiate between participants´ diagnostic (AUC = 0.508 ±0.076, p = 0.952, 95% CI = 0.359–0.657, Figure 3).

4. DISCUSSION

In the present study, the absolute inter-eye difference value diagnostic capability of the three main diagnostic criteria tests proposed by the TFOS DEWS II Diagnostic Methodology report was analysed.[1] An inter-eye variability of the results during DED assessment has been proposed as a characteristic that increases with disease severity, which has been recommended as a feature that clinicians should specifically be looking at diagnosis.[1,9,10] To the author's knowledge, from those parameters, the only previously studied was the inter-eye osmolarity difference; however, most of those studies have calculated this difference as a tangential analysis to the main objective of their research.[5,6,9,31-34]

The sample analysis in the present study was composed of participants with previous dry eye complaints who attended an ocular surface examination at the centre referred by their medical doctor or the health service of the institution. Although the FBUT|OD-OS| and the Corneal Staining|OD-OS| showed no difference between groups and no reliable diagnostic cut-off criteria,

there should be noted that inter-eye differences median and IQR values in the three parameters studied were clinically higher in the DED group than in the No DED group. However, it also should be noted that despite the general trend of the data, there were spare participants in the No DED group (low symptomatology and no sign over the cut-off criteria in any eye) that still showed high inter-eye differences (e.g. No DED group FBUT|OD-OS| maximum value = 8.75s), or even the opposite situation in the DED group (e.g. No DED group Osmolarity|OD-OS| minimum value = 0.00 mOsm/L). The DED condition was associated with various manifestations, such as non-obvious disease involving ocular surface signs without related symptoms (including the neurotrophic conditions where with the presence of dysfunctional sensation exists) and those with symptoms but without demonstrable signs on the ocular surface (including neuropathic pain).[1,2,8] This issue has been established as a limitation in all the studies which pursue obtaining reliable diagnostic tests, therefore, strict inclusion criteria and interprofessional collaboration are essential for a proper diagnosis and management of the patients. Similar to other conditions, could be proposed that the DED patient's quality of life may be beneficiated from regular multidisciplinary management of different practice areas (e.g., neurologist, endocrinologist, or vision specialists).[35] In addition, this issue also remarks on the importance of searching for new diagnostic and reliable criteria during the routine diagnosis of ocular surface diseases that avoid false negative or positive diagnostics.

From the studied parameters, the present analysis found that only the Osmolarity|OD-OS| showed a diagnostic capability to diagnose the DED in the same direction that the criteria proposed by the TFOS Diagnostic methodology subcommittee report.[1] Previous reports have suggested that inter-eye differences beyond the threshold of 8 or 9.2 ±9.3 mOsm/l should be considered an indication of the tear film homeostasis loss that occurs with DED.[8,28] The absolute inter-eye osmolarity difference found as a cut-off criterion in the present study was near this value (Osmolarity|OD-OS| = 9.5 mOsm/L). Along with the stability, since the initial reports and definitions of the disease,[36] tear film hyperosmolarity has been considered the main trigger and core mechanism in dry eye physiopathology.[3] Here was confirmed that, far from a single measurement where a higher osmolarity in the tear film is recorded, the inter-eye variability of the tear osmolarity may be also

greater in DED than in healthy patients; therefore, this characteristic seems to increase with disease severity and could be recommended as a feature that clinicians should specifically be looking upon diagnosis.[1,9]

Contrary to this finding, although “film instability” and “ocular surface inflammation and damage” were included as key etiological factors in the definition of the disease, FBUT|OD-OS| and the Corneal Staining|OD-OS| have not shown a diagnostic capability for DED diagnosis.[2] Those parameters seem to evolve and worsen in the same proportion in both eyes at the same time, without being affected by the homeostasis loss in the contralateral eye. Previous reports found differences in the values of both, FBUT and Corneal Staining evaluated with the Oxford Scheme, between open-angle glaucoma-diagnosed eyes treated medically and the fellow eyes treated surgically of trabeculectomy; during the medication process, worse results are reported.[37] It could be hypothesised that conditions (pathologies or medications) that alter the homeostasis of one eye have no influence on the damage or stability of the other. This issue may be controversial and requires further analysis and research.

The main strength of the present study was the high total sample size recruited (180 participants). In addition, it should be noted that participants were diagnosed under restrictive and accurate diagnostic criteria according to the TFOS DEWS II Diagnostic Methodology.[1] On the other hand, this criterion is established in the TFOS report (positive symptomatology combined with a positive result on at least one clinical test in diagnosing dry eye) it might have limited the results; some participants have been positively diagnosed as DED based on only a cut of criteria (e.g. an osmolarity in either of eye ≥308 mOsm/l) which implies that the other studied inclusion criteria are "normal" (e.g., which could implied no differences between eyes (e.g., FBUT in both patient´s eyes ≥10 s) and the subsequent no significant diagnostic capability on this parameter.

In summary, the present study found that Osmolarity|OD-OS| may be used as a diagnostic indicator for DED, whereas FBUT|OD-OS| and Corneal Staining|OD-OS| seem to do not have this capability; inter-eye differences in tear hyperosmolarity should be also considered a clinically useful estimate for tear homeostasis. The present study proposed a cut-off value of

Osmolarity|OD-OS| to differentiate between the No DED and DED participants that should be validated in further research in a different cohort of No DED versus DED participants.

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DECLARATION OF INTEREST STATEMENT

The authors report there are no competing interests to declare.

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TABLES

Table 1. Summary of the battery of clinical procedures performed [1].

Classification	Symptoms	Signs
No DED	OSDI score < 13	All conditions must be filled: - Osmolarity in both eyes < 308 mOsm/l - FBUT in both eyes ≥ 10 s - Corneal staining (Oxford Scheme) in both eyes < 2
DED	OSDI score ≥ 13	Only one condition must be filled: - Osmolarity in either of the eyes ≥ 308 mOsm/l - FBUT in either of the eyes < 10 s - Corneal staining (Oxford Scheme) in either of the eyes ≥ 2

OSDI = Ocular Surface Disease Index; FBUT = Fluorescein Break-Up Time

445 Table 2. Summary of the battery of clinical procedures performed.

Parameter	Material	Method
Symptomatology Assessment	OSDI questionnaire.	Participants access the online survey by QR code. The questionnaire was self-administered.
Osmolarity	TearLab (TearLab, San Diego, CA, United States).	A probe was placed on the lower tear meniscus.
Fluorescein Break-Up Time	Oculus Keratograph 5M (Oculus Optikgerate GmbH, Wetzlar, Germany).	Instillation of fluorescein (fluorescein strip hydrated with saline solution) onto inferotemporal bulbar conjunctiva. A video was recorded and extracted for analysis by counting frames with the VirtualDub software by a second masked examiner.
Corneal Staining	Oculus Keratograph 5M (Oculus Optikgerate GmbH, Wetzlar, Germany).	Performed immediately after FBUT video recording. A video was recorded for analysis of the different corneal areas and evaluated by a second masked examiner.

446 OSDI = Ocular Surface Disease Index; FBUT = Fluorescein Break-Up Time

447 Corneal Staining|OD-OS| values.

Table 3. Descriptive statistics and analysis of differences (Mann-Whitney U test, non-parametric parameters) of the No DED and DED groups. Median and IQR are ported (non-parametric parameter), while parametric statistics (mean and SD) are also reported to enable comparisons with other studies and clinical results.

		Osmolarity (mOsm/L)			FBUT (seconds)			Corneal Staining (Oxford Scheme)		
		Eye Value	|OD-OS|		Eye Value	|OD-OS|		Eye Value	|OD-OS|	
		Mean ± SD	Mean ± SD	Median (IQR)	Mean ± SD	Mean ± SD	Median (IQR)	Mean ± SD	Mean ± SD	Median (IQR)
No DED	OD	303.0 ± 6.0	4.5 ± 3.3	4.0 (2.0-8.0)	15.35 ± 4.08	2.96 ± 2.21	2.17 (1.32-3.69)	0.3 ± 0.5	0.5 ± 0.5	0.0 (0.0-1.0)
	OS	301.9 ± 4.4			14.89 ± 2.46			0.6 ± 0.5		
DED	OD	323.1 ± 20.5	12.5 ± 12.2	9.0 (4.0-15.0)	7.19 ± 5.80	3.14 ± 3.76	3.38 (0.92-5.93)	0.8 ± 1.0	0.6 ± 0.7	0.0 (0.0-1.0)
	OS	325.8 ± 19.7			7.61 ± 6.85			0.8 ± 1.0		
	p	-	-	0.002	-	-	0.160	-	-	0.915

DED = Dry Eye Disease. OD = Oculus Dexter. OS = Oculus Sinister. SD = Standard Deviation. IQR = Interquartile Range. OSDI = Ocular Surface Disease Index. FBUT = Fluorescein Break-Up Time. |OD-OS| = Inter-eye absolute difference

FIGURES

Figure 1.

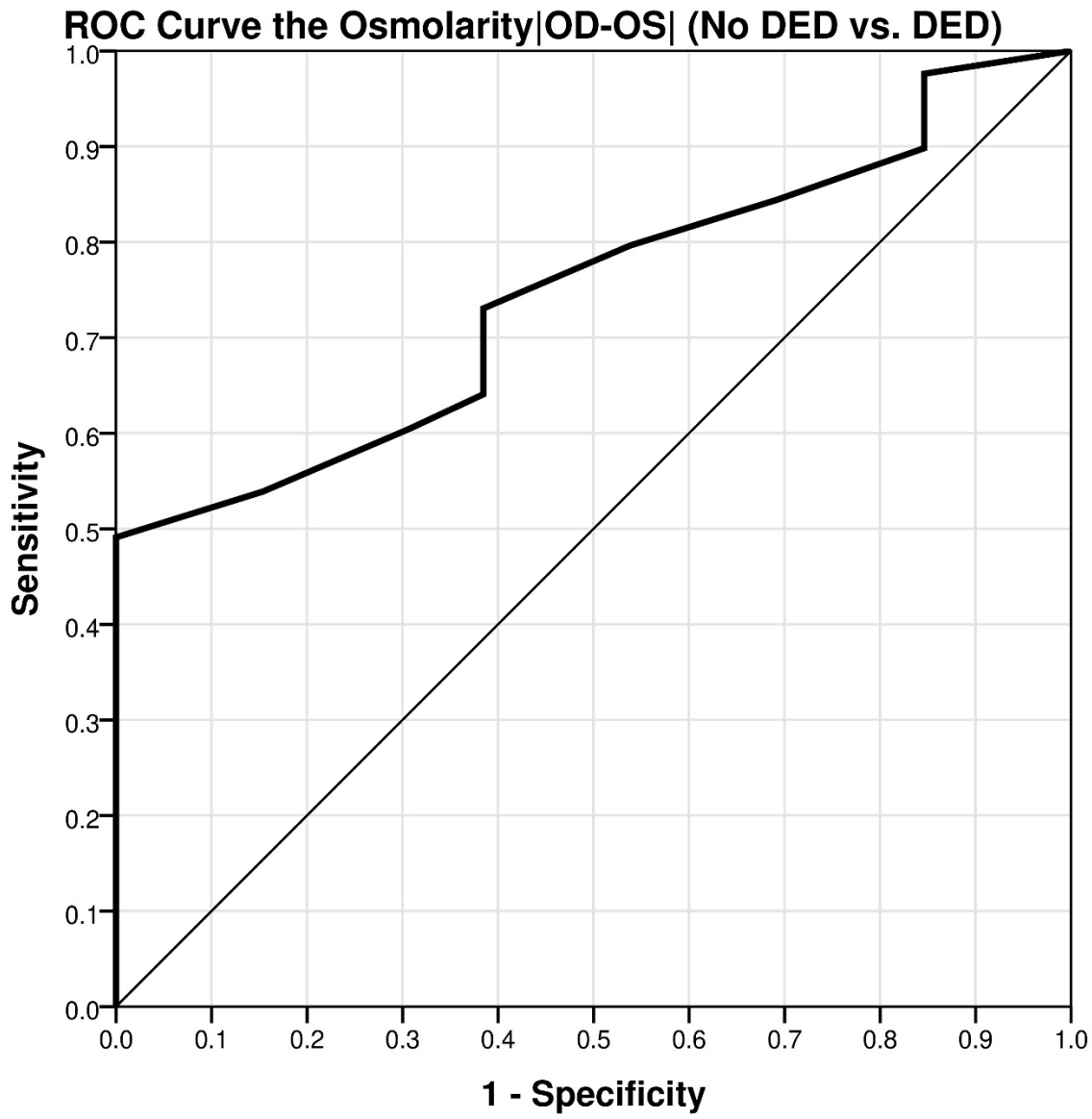

Figure 2.

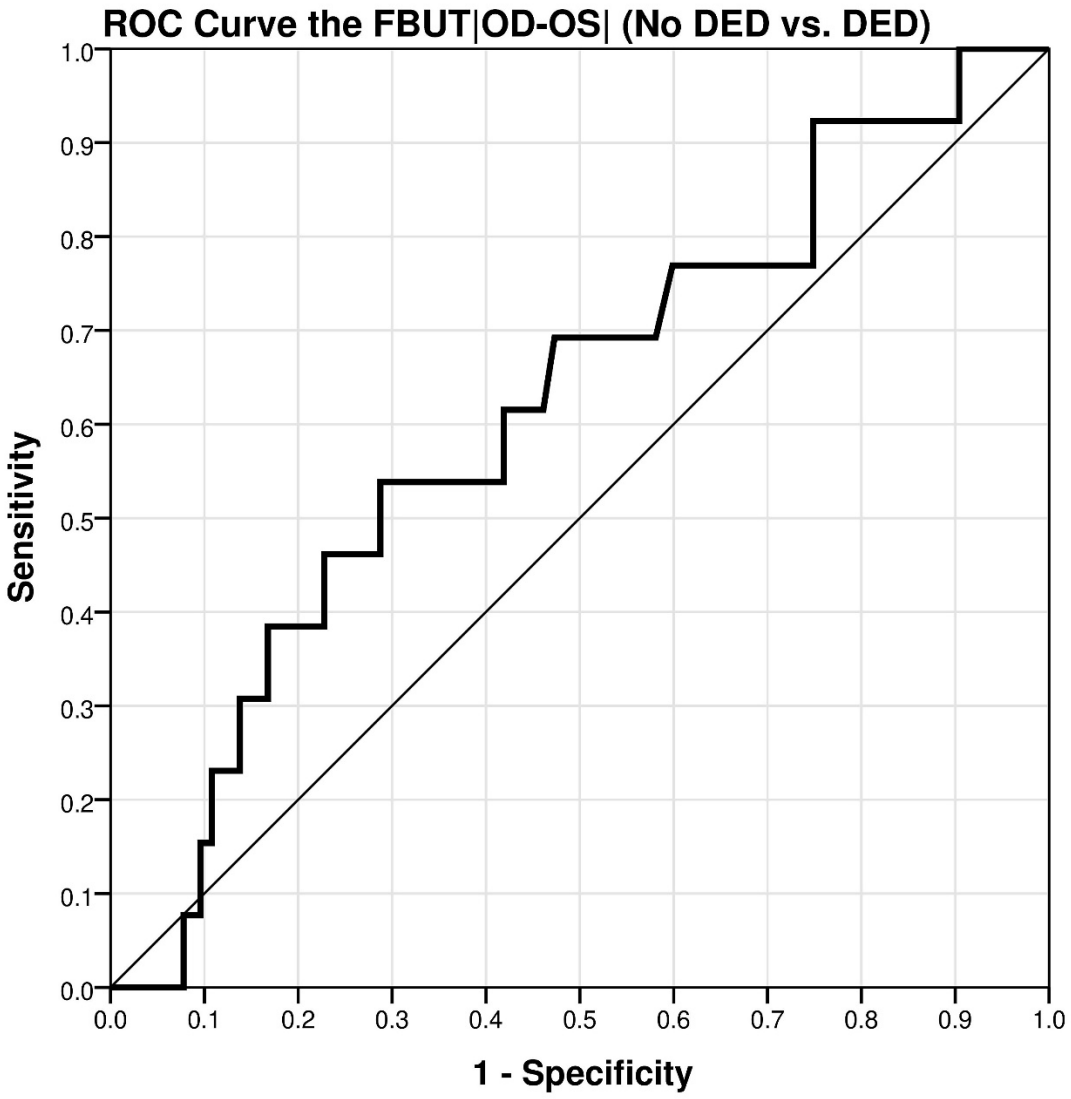
ROC Curve the FBUT|OD-OS| (No DED vs. DED)
Sensitivity
1 - Specificity
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Figure 3.

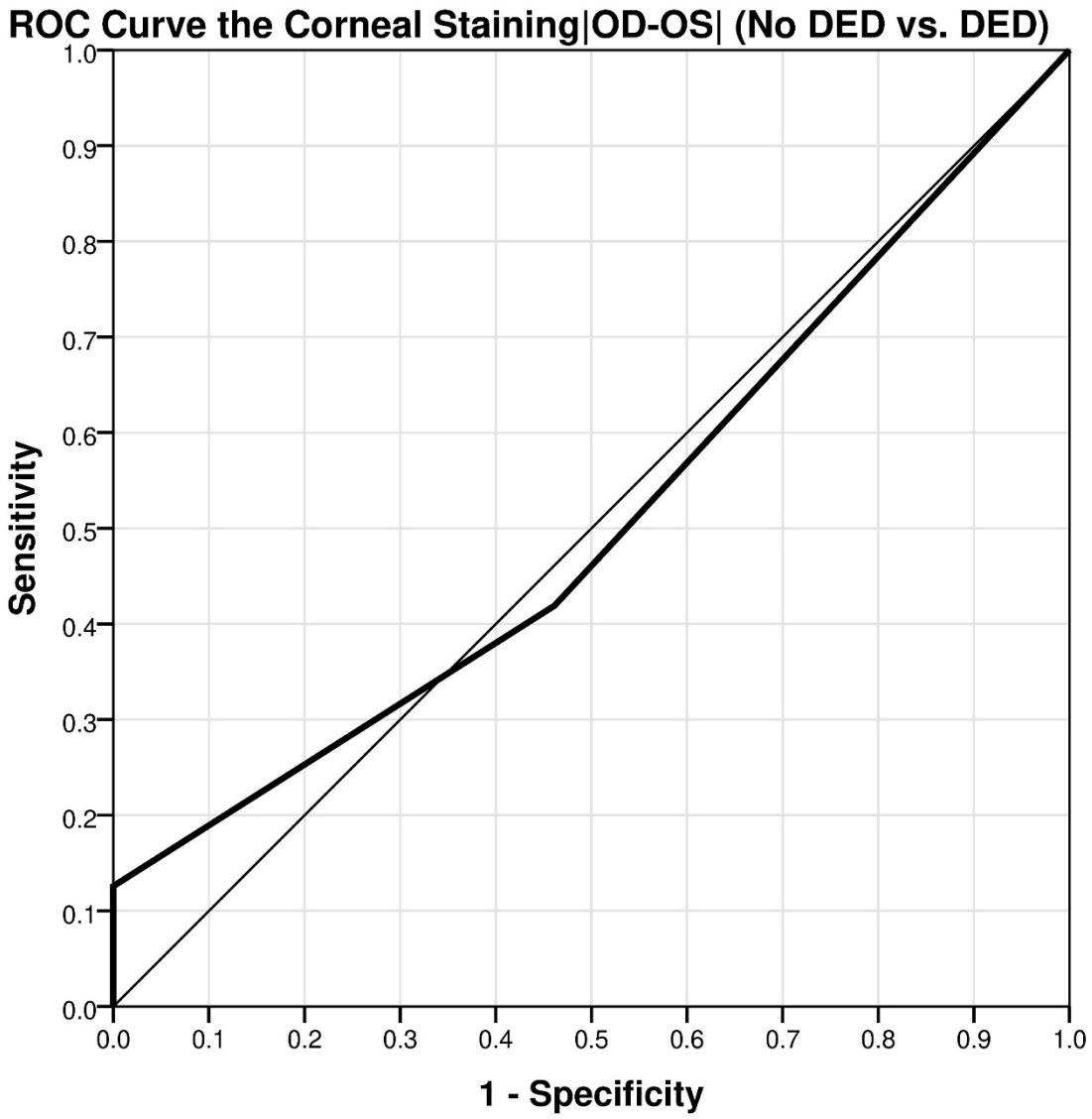
ROC Curve the Corneal Staining|OD-OS| (No DED vs. DED)
Sensitivity
1 - Specificity
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

FIGURE CAPTIONS

Figure 1. ROC curve showing the relationship between sensitivity and specificity of the Osmolarity|OD-OS| (No DED vs. DED) according to theoretical thresholds; for each of the values observed in the study population (from the lowest to the highest in either No DED or the DED group), the sensitivity and sensibility indexes have been calculated and reported in the graph. n = 180. ROC = Receiver Operating Characteristic. DED = Dry Eye Disease. |OD-OS| = Inter-eye absolute difference.

Figure 2. ROC curve showing the relationship between sensitivity and specificity of the FBUT|OD-OS| (No DED vs. DED) according to theoretical thresholds; for each of the values observed in the study population (from the lowest to the highest in either No DED or the DED group), the sensitivity and sensibility indexes have been calculated and reported in the graph. n = 180. ROC = Receiver Operating Characteristic. DED = Dry Eye Disease. |OD-OS| = Inter-eye absolute difference.

Figure 3. ROC curve showing the relationship between sensitivity and specificity of the Corneal Staining |OD-OS| (No DED vs. DED) according to theoretical thresholds; for each of the values observed in the study population (from the lowest to the highest in either No DED or the DED group), the sensitivity and sensibility indexes have been calculated and reported in the graph. n = 180. ROC = Receiver Operating Characteristic. DED = Dry Eye Disease. |OD-OS| = Inter-eye absolute difference.