



Determination of the Non-Invasive Tear Break-Up Time Cut-Off Point for Diagnosis of Dry Eye Disease and Its Correlation with Other Dry Eye Tests

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Abstract

Objectives: To determine optimal cut-off values for first and average non-invasive tear break-up time (NIBUT-f and NIBUT-av, respectively) in dry eye disease (DED), as well as evaluate their correlation with other tests.

Materials and Methods: This retrospective study included 46 patients with DED and 35 healthy controls. All subjects were assessed using the Ocular Surface Disease Index questionnaire. NIBUT measurements and meibography images were obtained using the Sirius topography device. The conventional diagnostic tests Schirmer-I, tear break-up time (TBUT), ocular surface staining (OSS), Marx line score, and lid wiper epitheliopathy (LWE) grade were performed.

Results: The cut-off values of NIBUT-f and NIBUT-av for DED diagnosis were identified as 10.7 and 12.2 seconds, respectively. The area under the curve (AUC) was 0.93 (95% confidence interval [CI]: 0.889–0.992) for NIBUT-f and 0.92 (95% CI: 0.872–0.985) for

NIBUT-av. For the differentiation between evaporative and mixed DED subtypes, the NIBUT-av cut-off value was 7.2 seconds, with an AUC of 0.63 (95% CI: 0.512–0.743). NIBUT-av showed a positive correlation with TBUT ($p<0.001$, $r=0.905$) and Schirmer-I ($p<0.001$, $r=0.403$) but a negative correlation with OSS ($p<0.001$, $r=-0.700$), meibomian gland loss ($p<0.001$, $r=-0.601$), LWE grade ($p<0.001$, $r=-0.597$), and Marx line score ($p<0.001$, $r=-0.539$).

Conclusion: NIBUT is a highly sensitive and specific non-invasive diagnostic tool for DED that correlates with other ocular surface and meibomian gland function tests.

Keywords: Non-invasive tear break-up time (NIBUT), dry eye disease, cut-off value

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Introduction

The Tear Film and Ocular Surface Dry Eye Workshop II (TFOS DEWS II) defines dry eye disease (DED) as a multifactorial condition of the ocular surface characterized by disrupted tear film homeostasis, accompanied by ocular symptoms. The etiology involves tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities.¹ The global estimated prevalence of DED ranges from 5% to 50%, reflecting variations across different populations.² DED is increasingly extending beyond the typical adult demographic to affect younger individuals, indicating a rising potential for widespread impact in the years ahead.³ According to the TFOS DEWS II diagnostic algorithm, initial triage questions and a comprehensive analysis of risk factors precede the performance of specific diagnostic tests to confirm DED.⁴ Diagnostic testing involves assessments of symptomatology



as well as the measurement of homeostasis markers, which are critical in establishing a definitive diagnosis. To this end, tear film osmolarity, ocular surface staining (OSS), and tear break-up time (TBUT) are employed to assess tear film homeostasis. A positive finding in any of these tests, in conjunction with screening questionnaires, conclusively confirms a diagnosis of DED.⁴

TBUT is defined as the duration between a complete blink and the first disruption in the tear film.⁴ The initial method for assessing TBUT (using fluorescein) was introduced nearly 50 years ago and has become established in clinical practice.⁵ This technique involves instilling sodium fluorescein dye into the tear film, allowing disruptions to be detected using a slit-lamp biomicroscope under cobalt blue light. A benchmark of 10 seconds has been traditionally accepted as the threshold between a normal and unstable tear film.⁵ However, fluorescein inherently alters tear film stability, and measurement variability is highly dependent on the practitioner's technique, which impedes the reliability of the TBUT protocol.^{6,7,8}

Accordingly, non-invasive tear break-up time (NIBUT) methods have emerged as the preferred method over conventional TBUT, and the use of TBUT is recommended only in cases where NIBUT is not feasible or accessible.⁹ NIBUT is the interval from the completion of a full blink to the first disruption in the reflection of a pattern (such as grid, mire, or Placido disc) projected onto the tear film.^{10,11} A NIBUT cut-off value of 10 seconds has been suggested as indicative of DED in examinations of the reflection of an illuminated grid pattern.¹¹ However, studies have presented divergent findings regarding cut-off values obtained through automated measurement systems, with some reporting shorter^{12,13} or longer^{14,15,16} durations. In addition, modern devices incorporating videokeratography systems analyze videos to derive first and average NIBUT values.^{17,18} Despite these advancements, there is no consensus in the literature regarding the most suitable device, reference value, or appropriate cut-off threshold.

Therefore, our primary objective in the current study was to determine the optimal cut-off values for both the first and average NIBUT (NIBUT-f and NIBUT-av, respectively) in patients diagnosed with DED, as well as to evaluate the correlation between invasive and non-invasive diagnostic tests in these patients. Furthermore, we compared the results of DED patients and healthy controls across conventional diagnostic tests, including Schirmer-I, TBUT, OSS, Marx line score, lid wiper epitheliopathy (LWE) grade, and meibography.

Materials and Methods

This retrospective study was conducted with the approval of the Marmara University Faculty of Medicine's

Ethics Committee (date: 15.11.2024; protocol code: 09.2024.1296) and in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Patients aged 18 years or older presenting to the Ophthalmology Clinic of Marmara University Pendik Training and Research Hospital with complaints of dry eye were included in the study. The exclusion criteria were the presence of an active ocular infection or allergy, contact lens wear, and a history of ocular surgery within the last 3 months.

All examinations were performed according to a predefined clinical protocol with a fixed sequence to minimize test-to-test interference. Non-invasive assessments, including NIBUT and meibography imaging, were conducted prior to invasive procedures such as Schirmer-I, TBUT, and OSS. This retrospective study included only patients whose clinical examinations were performed by a single ophthalmologist (E.Y.). All Sirius topography measurements were routinely obtained by the same trained technician using the same device and standardized protocol, and the data were retrospectively reviewed for the purposes of this study. Only one eye from each patient was included in the analysis. When both eyes met the inclusion criteria, the eye with more severe clinical findings was selected.

Diagnostic Tests for DED

The Turkish version of the Ocular Surface Disease Index (OSDI) questionnaire, consisting of 12 questions in 3 sections, was administered to all subjects.¹⁹ The OSDI score was calculated according to the following formula:

$$\text{OSDI} = (\text{sum of scores for all questions answered} \times 25) / \text{total number of questions answered}$$

NIBUT measurements were conducted using the Sirius topography device (Costruzione Strumenti Oftalmici, Florence, Italy), which operates on the Placido-disc principle. During the measurement, patients were instructed to blink twice and keep their eyes open for as long as possible, in line with the device's prompts. NIBUT-f and NIBUT-av values were automatically recorded by the device's software. Measurements were repeated three times for each eye, and the average of the three values was calculated.

To assess the meibomian glands, images were captured using the Phoenix Meibography Imaging module of the Sirius topography device following the eversion of the upper and lower eyelids. After the borders of the tarsal conjunctiva and the meibomian glands were manually delineated, the software calculated the percentage of gland loss. The area of meibomian gland loss was graded as

follows: grade 0 indicates no loss, grade 1 indicates a loss of up to 25%, grade 2 indicates a loss of 26-50%, grade 3 indicates a loss of 51-74%, and grade 4 indicates a loss of 75% or more.

After these non-invasive tests, the Schirmer-I test was performed without anesthesia using a Whatman 41 filter paper strip marked up to 35 mm. The rounded tip of the paper was placed in the conjunctival sac at the junction of the temporal and middle thirds of the eyelid without contacting the cornea, to prevent reflex tear activity. Both eyes were evaluated concurrently, and the length of the wetted segment of the filter paper was measured after a duration of 5 minutes.

A fluorescein strip, wetted with a drop of saline and shaken to remove excess liquid, was gently applied to the inferior bulbar conjunctiva to stain the ocular surface. The tear film was subsequently evaluated using a biomicroscope with a cobalt blue filter. Patients were instructed to blink several times and then keep their eyes open for as long as possible. The TBUT was recorded at the first appearance of disruption in the tear film.

OSS was assessed following fluorescein application, using a cobalt blue filter on a biomicroscope. Staining of the nasal conjunctiva, temporal conjunctiva, and cornea was evaluated based on the Oxford grading scheme.²⁰

For the evaluation of LWE, a mixture of 2% fluorescein and 1% lissamine green was applied to the conjunctival sac, and the upper and lower eyelid margins were examined. The lesions were graded on a scale of 0-3 based on the width and height of the staining, as described by Korb et al.²¹

During the fluorescein and lissamine-green staining, the mucocutaneous junction of the lower eyelid was examined under a slit lamp, and the Marx line score (also known as the Yamaguchi score) was calculated.²² The lower eyelid was divided into three imaginary sections to evaluate the relationship between the mucocutaneous junction and the meibomian gland orifices: a score of 0 was assigned if there was no intersection, 1 if the Marx line reached the gland orifices at certain points, 2 if the Marx line intersected with all gland orifices, and 3 if the Marx line extended anterior to the gland orifices. The scores from the inner, middle, and outer sections were summed to calculate the final score.

DED Diagnosis and Classification

DED diagnosis required OSDI ≥ 13 plus at least one positive homeostasis marker (TBUT < 10 s or OSS positivity). Patients diagnosed with DED were subsequently categorized into three subtypes: aqueous-deficient, evaporative, and mixed. The diagnostic algorithm and DED subgroup classifications are summarized in [Table 1](#).

Statistical Analysis

A post-hoc power analysis was conducted using G*Power software based on the observed difference in NIBUT-av values between the DED group (7.7 ± 3.4 s, $n=46$) and the control group (15.1 ± 2.9 s, $n=35$). The calculated effect size was large (Cohen's $d=2.42$). With this effect size, the post-hoc analysis yielded a statistical power of 1.00 at $\alpha=0.05$.

All data in this study were analyzed using SPSS v20 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to describe patient characteristics, with data presented as mean \pm standard deviation. The Shapiro-Wilk test was employed to assess the normality of the data distribution. Variables with normal distribution were analyzed using parametric tests, whereas variables not conforming to normality were analyzed using non-parametric tests. An independent-samples t-test was performed to compare continuous variables for the evaporative and the mixed types of DED. Receiver operating characteristic (ROC) curve analysis was conducted to determine the optimal cut-off values of NIBUT-f and NIBUT-av for the diagnosis of DED, and sensitivity, specificity, and the area under the curve (AUC) were calculated. For ROC analyses, optimal cut-off values were selected by maximizing the Youden index ($J = \text{sensitivity} + \text{specificity} - 1$), which identifies the operating point offering the best combined sensitivity and specificity. The correlations between NIBUT-av and other DED diagnostic tests were assessed using Spearman's rank correlation analysis. To further evaluate independent associations, a multivariate linear regression analysis was performed with NIBUT-av as the dependent variable and TBUT, Schirmer-I test, OSS score, Marx line score, LWE grade, and meibomian gland loss as independent variables. A *p* value of less than 0.05 was considered statistically significant.

Table 1. DED subgroup classification

Aqueous-deficient	Evaporative	Mixed
Schirmer-I test < 10 mm F-TBUT < 10 s	Meibomian gland loss \geq grade 1 LWE \geq grade 1 Marx line score ≥ 1	Criteria of both groups
DED: Dry eye disease, F-TBUT: Fluorescein tear break-up time, LWE: Lid wiper epitheliopathy		

Results

The study included 83 participants with a mean age of 47.2±15.5 years (range 18-85 years); 54 (65.1%) were female and 29 (34.9%) were male. Of these, 48 patients (57.8%) were diagnosed with DED and 35 (42.2%) were in the control group. Within the DED group, 35 patients (72.9%) were female and 13 (27.1%) were male. In the control group, 19 subjects (54.3%) were female and 16 (45.7%) were male. Among those diagnosed with DED, 2 (4%) had aqueous-deficient DED, 23 (48%) had evaporative DED, and 23 (48%) had mixed DED. The two patients with aqueous-deficient DED were excluded from further analyses due to the small sample size for that subgroup. Therefore, the final analyzed cohort comprised 81 participants (46 with DED and 35 controls).

Table 2 shows the characteristics of DED patients and the control group. Compared to the controls, DED patients exhibited significantly lower NIBUT-av, NIBUT-f, TBUT, and Schirmer-I values, while their OSS score, LWE grade, Marx line score, and meibomian gland loss grade were significantly higher (p<0.001).

Table 3 presents the results of the subgroup analysis of patients with DED, specifically comparing the evaporative and mixed types of DED. The evaporative DED group showed significantly higher NIBUT-av, TBUT, and Schirmer-I test values, but notably lower OSS scores, Marx line scores, and LWE grades.

NIBUT-f and NIBUT-av Cut-Off Values for DED Diagnosis

ROC curve analysis identified the cut-off values for the diagnosis of DED as 10.7 seconds for NIBUT-f and 12.2 seconds for NIBUT-av. The AUC was 0.93 (95% confidence interval [CI]: 0.889-0.992) for NIBUT-f and 0.92 (95% CI: 0.872-0.985) for NIBUT-av (Figure 1). At these cut-off values, the sensitivity was 89% (95% CI: 79.7%-96.6%) and the specificity was 88% (95% CI: 74.0%-95.5%) for both parameters. At the selected operating points for overall DED detection, the Youden index was 0.77 (0.89 + 0.88 – 1) for both NIBUT-f and NIBUT-av. In addition, when ROC analysis was performed to differentiate evaporative and mixed DED subtypes, the cut-off value for NIBUT-av was determined to be 7.2 seconds; the sensitivity, specificity,

Table 2. Test results of the DED and control groups

	DED group (n=46)	Control group (n=35)	p
NIBUT-av (s)	7.7±3.4 (2.7-17)	15.1±2.9 (5.1-17)	<0.001
NIBUT-f (s)	5.9±3.7 (1.1-17)	14.6±3.4 (4.7-17)	<0.001
TBUT (s)	6.2±2.6 (1-14)	12.2±2.6 (5-16)	<0.001
Schirmer-I (mm)	9.8±5.4 (1-21)	15.3±4.7 (6.5-26)	<0.001
OSS score	0.91±0.98 (0-5)	0.02±0.16 (0-1)	<0.001
Marx line score	3.9±2.7 (0-9)	1.3±1.7 (0-6)	<0.001
LWE grade	0.66±0.78 (0-3)	0.04±0.18 (0-1)	<0.001
Meibomian gland loss grade	1.6±0.67 (0-4)	0.8±0.65 (0-3)	<0.001

Data presented as mean ± standard deviation (range). DED: Dry eye disease, NIBUT-av: Average non-invasive tear break-up time, NIBUT-f: First non-invasive tear break-up time, TBUT: Tear break-up time, OSS: Ocular surface staining, LWE: Lid wiper epitheliopathy

Table 3. DED subgroup analysis

	Evaporative (n=23)	Mixed (n=23)	p
NIBUT-av (s)	8.5±4.2 (2.4-17)	6.5±3.4 (2.2-17)	0.015
NIBUT-f (s)	6.3±4.9 (1.1-17)	5.1±3.7 (1.1-17)	0.698
TBUT (s)	7.2±2.7 (3-14)	5.1±2.5 (1-10)	0.003
Schirmer-I (mm)	14.8±4.3 (10-25)	4.9±2.2 (1-9)	<0.001
OSS score	0.5±0.6 (0-3)	1.3±1.1 (0-5)	<0.001
Marx line score	3.2±2.5 (0-9)	5.0±2.4 (1-9)	0.028
LWE grade	0.3±0.5 (0-2)	1±0.8 (0-3)	<0.001
Meibomian gland loss area (%)	29.7±12.7 (8.7-66.5)	33.5±13.2 (14.5-69.4)	0.796

Data presented as mean ± standard deviation (range). DED: Dry eye disease, NIBUT-av: Average non-invasive tear break-up time, NIBUT-f: First non-invasive tear break-up time, TBUT: Tear break-up time, OSS: Ocular surface staining, LWE: Lid wiper epitheliopathy

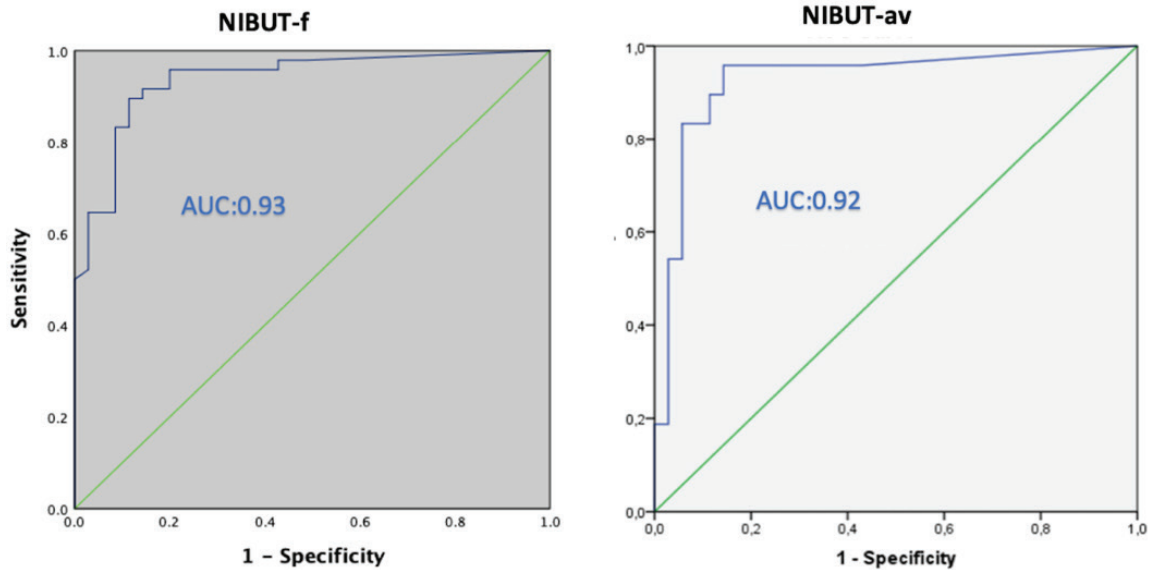


Figure 1. Receiver operating characteristic (ROC) curve analysis for first and average non-invasive break-up times (NIBUT-f and NIBUT-av) in dry eye disease patients
AUC: Area under the ROC curve

and AUC were 56% (95% CI: 36.8%-74.4%), 58% (95% CI: 40.9%-72.0%), and 0.63 (95% CI: 0.512-0.743), respectively (Figure 2). In the DED subgroup ROC analysis, the Youden index at the selected cut-off was 0.14 (0.56+0.58-1), reflecting limited discriminatory performance.

Correlation of NIBUT with Other Dry Eye Diagnostic Tests

Figure 3 illustrates the correlations between NIBUT-av and other DED diagnostic tests. A statistically significant and very strong positive correlation was observed between NIBUT and TBUT ($r=0.905$, 95% CI: 0.843-0.940, $p<0.001$). NIBUT also showed a moderate positive correlation with Schirmer-I test values ($r=0.403$, 95% CI: 0.202-0.575, $p<0.001$). In contrast, significant negative correlations were found between NIBUT and OSS score ($r=-0.700$, 95% CI: -0.809 to -0.553, $p<0.001$), meibomian gland loss ($r=-0.601$, 95% CI: -0.733 to -0.434, $p<0.001$), LWE grade ($r=-0.597$, 95% CI: -0.730 to -0.425, $p<0.001$), and Marx line score ($r=-0.539$, 95% CI: -0.690 to -0.359, $p<0.001$).

A multivariate linear regression analysis was performed to identify independent predictors of NIBUT-av. The overall model was statistically significant ($R^2=0.795$, $p<0.001$). Among the evaluated parameters, TBUT emerged as the only independent predictor of NIBUT-av ($\beta=0.907$, $p<0.001$). Other variables, including Schirmer-I test, OSS

score, Marx line score, LWE grade, and meibomian gland loss, were not independently associated with NIBUT-av (all $p>0.05$) (Table 4).

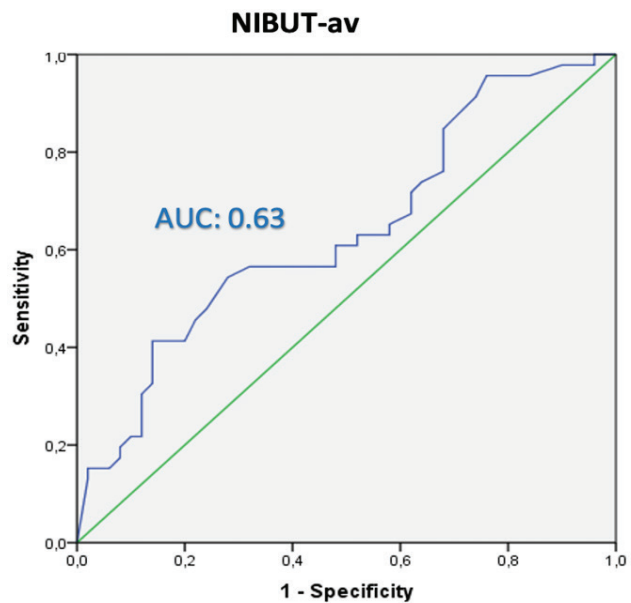


Figure 2. Receiver operating characteristic (ROC) curve analysis for average non-invasive break-up time (NIBUT-av) in evaporative dry eye disease patients
AUC: Area under the ROC curve

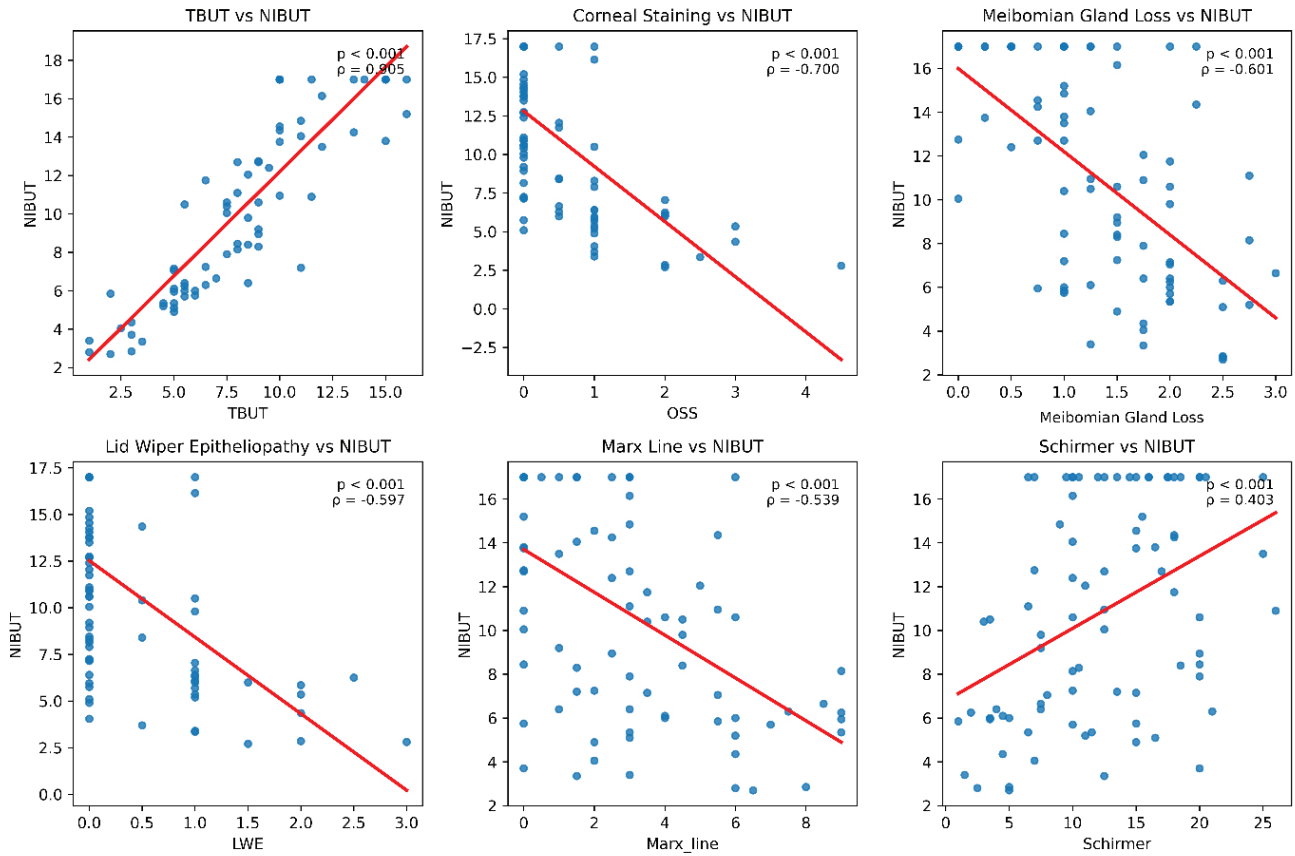


Figure 3. Correlation of non-invasive break-up time (NIBUT) with other dry eye diagnostic tests
 TBUT: Tear break-up time, OSS: Ocular surface staining, LWE: Lid wiper epitheliopathy

Table 4. Multivariate linear regression analysis of factors associated with NIBUT-av			
Variable	β (standardized coefficient)	Standard error	p
TBUT (s)	0.907	0.085	<0.001
Schirmer-I (mm)	-0.041	0.058	0.488
OSS score	-0.732	0.584	0.215
Marx line score	-0.079	0.136	0.568
LWE grade	0.174	0.777	0.825
Meibomian gland loss	-0.842	0.446	0.063

NIBUT-av: Average non-invasive tear break-up time, TBUT: Tear break-up time, OSS: Ocular surface staining, LWE: Lid wiper epitheliopathy

Discussion

This study aimed to determine the optimal NIBUT-f and NIBUT-av cut-off points for DED diagnosis and subgroup differentiation, as well as the correlation of these non-invasive measures with conventional clinical diagnostic methods. While several studies in the literature

have compared NIBUT measurements across different devices,^{23,24,25} we utilized the Sirius topography system for our automated assessments. We found that although the NIBUT cut-off value demonstrated high sensitivity and specificity for diagnosing DED overall, its ability to differentiate between evaporative and mixed subtypes was limited.

The first NIBUT measurement reported in the literature was conducted by Mengher et al.,¹¹ who used an instrument attached to a slit lamp biomicroscope that projected a rectangular grid pattern onto the cornea. Today, automated measurements can be performed using various devices. Based on NIBUT measurements obtained with the Sirius topography device, we determined cut-off values for DED diagnosis to be 10.7 seconds for NIBUT-f and 12.2 seconds for NIBUT-av, with AUC values of 0.93 and 0.92, respectively. We also analyzed the evaporative group specifically, for which the cut-off value was 7.2 seconds with an AUC of 0.63.

Kim et al.²³ investigated NIBUT values, tear break-up locations, and break-up patterns across DED subtypes using a Keratograph 5M. The cut-off values for DED diagnosis were determined to be 4.84 seconds for NIBUT-f and 8.62 seconds for NIBUT-av. In their study, the AUC values for DED diagnosis were reported as 0.671 and 0.640. However, when individual ROC analysis was performed for the aqueous-deficient type and the non-evaporative type, an increase in AUC values was observed. In contrast, in our study, the AUC value decreased when only the evaporative type was evaluated.

A study by Hong et al.¹² using a Keratograph reported the NIBUT cut-off value to be 2.65 seconds, with an AUC of 0.825. Additionally, in a study conducted by Muhafiz and Demir¹⁷ with contact lens wearers, a TBUT of less than 10 seconds was accepted as indicative of tear instability. Based on Sirius topography measurements before contact lens wear, the cut-off values for NIBUT-f and NIBUT-av were identified as 8 seconds and 12.65 seconds, respectively. These values are similar to our cut-off values. Furthermore, they reported AUC values of 0.842 and 0.810, respectively.

In a different study conducted with DED patients, NIBUT measurements were performed on the same subjects using both a DR-1 and a Keratograph 5M. The cut-off values for DED diagnosis were found to be 3.9 seconds with the DR-1, compared to 6.31 seconds for NIBUT-f and 8.63 seconds for NIBUT-av with the Keratograph 5M (AUC values of 0.725, 0.720, and 0.748, respectively).²⁴ Further supporting this concept, a recent study by Zeri et al.²⁵ demonstrated that NIBUT measurements obtained from different non-invasive devices are not interchangeable, even within subjects. The authors showed that despite strong correlations between measurements, significant differences exist between devices due to variations in Placido disc configuration, illumination systems, and analysis algorithms. These findings indicate that agreement between devices does not necessarily imply equivalence, and that absolute NIBUT values—and consequently diagnostic cut-off thresholds—are inherently device-dependent. In

this context, the relatively higher cut-off values observed in our study using the Sirius topography system should be interpreted within the technical framework of this specific device. Therefore, our results support the concept of device-specific cut-off values, emphasizing that NIBUT thresholds derived from one platform should not be directly extrapolated to others. This device dependency represents an important limitation in terms of clinical generalizability and highlights the need for either device-specific normative databases or standardization across measurement systems. The notable differences in cut-off values may be attributed to the measurements being taken with different devices. To the best of our knowledge, no other study has determined NIBUT cut-off values using Sirius topography in patients diagnosed with DED, and further studies comparing the measurements of the different devices could provide valuable guidance.

In our study, we compared evaporative and mixed types of DED and observed that in the mixed-type DED group, the NIBUT-av, TBUT, and Schirmer-I values were significantly lower, whereas the OSS, Marx line score, and LWE grade values were significantly higher. However, no significant difference was found between the subgroups for NIBUT-f and meibomian gland loss. Therefore, our findings suggest that NIBUT-av may be a more appropriate option for subgroup analysis than NIBUT-f.

Although there was a significant difference in NIBUT-av values between patients with evaporative and mixed-type dry eye, the relatively low AUC value (0.63) in the ROC analysis reflects the limited ability of NIBUT-av to distinguish between the evaporative and mixed subtypes of DED. Since both subtypes are characterized by tear film instability and consequently low NIBUT values, this is expected to result in significant overlap. However, the mixed subtype exhibited more severe clinical findings, consistent with the coexistence of aqueous deficiency and evaporative mechanisms. The limited discriminatory ability likely reflects the heterogeneous nature of DED, the presence of meibomian gland dysfunction as a dominant component in both groups, and the fact that NIBUT primarily measures tear film stability and may not capture subtype-specific differences. Additionally, the relatively small subgroup size and the device- and algorithm-dependent nature of NIBUT measurements may also have contributed to this poor performance.

In a subgroup analysis of DED patients by Lemp et al.,²⁶ Schirmer-I values for the evaporative and mixed types were reported as 16.3 mm and 3.2 mm, respectively, while the TBUT values were 4.4 seconds and 3.2 seconds, respectively. Additionally, higher grade corneal and conjunctival staining was observed in the mixed type. In

our study, the evaporative and mixed groups had Schirmer-I values of 14.8 mm and 4.9 mm and TBUT values of 7.2 seconds and 5.1 seconds, respectively. In another study, lower Schirmer-I and TBUT values, as well as higher OSS scores, were reported for the mixed type compared to the evaporative type.²³ Although the mentioned studies contain differences in their diagnostic criteria for subgroup analysis, their results are consistent with our findings.

The observed differences between evaporative and mixed DED subgroups may be explained by their underlying pathophysiological mechanisms. Evaporative DED is primarily associated with increased tear evaporation due to meibomian gland dysfunction, while aqueous tear production is relatively preserved, which may result in higher Schirmer-I values and less pronounced ocular surface damage. In contrast, mixed-type DED involves both increased evaporation and aqueous tear deficiency, leading to greater tear film instability, hyperosmolar stress, and more severe ocular surface involvement, as reflected by higher OSS, Marx line, and LWE scores.²⁷

In the current study, NIBUT was compared with other DED tests and a positive correlation was observed with TBUT and Schirmer-I ($r=0.905$, $p<0.001$ and $r=0.403$, $p<0.001$, respectively). However, NIBUT showed negative correlations with OSS, meibomian gland loss, LWE grade, and Marx line score ($r=-0.700$, $r=-0.601$, $r=-0.597$, $r=-0.539$, respectively; all $p<0.001$). A study by Hong et al.¹² produced similar findings, reporting that NIBUT correlated with TBUT and Schirmer-I ($r=0.550$, $p<0.001$ and $r=0.405$, $p<0.001$, respectively). Additionally, Ozulken et al.¹⁵ found that NIBUT measured using Sirius topography correlated with TBUT and Schirmer-I ($r=0.947$, $p<0.001$ and $r=0.166$, $p=0.030$, respectively). Karakılıç and Taşkıran Çömez²⁸ also found a positive correlation between NIBUT and TBUT ($r=0.473$, $p<0.001$).

In addition to correlation analysis, a multivariate regression model was constructed to determine independent predictors of NIBUT. Although several parameters showed significant correlations with NIBUT in univariate analysis, only TBUT remained an independent predictor in the multivariate model. This finding can be explained by the shared pathophysiological basis of DED parameters, particularly tear film instability. TBUT, as a direct measure of tear film break-up, likely reflects the core mechanism underlying NIBUT measurements, whereas other parameters such as OSS, LWE, Marx line score, and meibomian gland loss represent downstream or secondary manifestations. Therefore, the lack of independent association in multivariate analysis does not indicate a lack of relationship but rather suggests overlapping biological pathways among these variables. This result further

supports the validity of NIBUT as a non-invasive surrogate for tear film instability.

Traditionally, TBUT measurement is a subjective method performed invasively using dye or strip application. With advancements in technology, non-invasive measurements can now be performed with objectivity and reproducibility. Future studies conducted with Sirius topography can utilize the cut-off values we obtained, with their high sensitivity and specificity. Its strong correlation with TBUT supports its applicability. Additionally, the NIBUT measurements obtained in our study demonstrated positive correlations with Schirmer-I values while showing negative correlations with meibomian gland loss, OSS score, LWE grade, and Marx line score. These findings highlight the potential of NIBUT as an objective, non-invasive, and effective diagnostic tool for DED.

Study Limitations

This study has several limitations. Due to its retrospective design, formal masking procedures were not implemented. However, clinical examinations and device-based measurements were performed independently by different individuals, and the examining clinician was not aware of the Sirius measurement results at the time of evaluation, which may have reduced potential measurement bias. In addition, inter-observer and intra-observer variability were not prospectively assessed, and therefore some degree of measurement variability cannot be excluded. Furthermore, due to the retrospective nature of the study, only two patients with pure aqueous-deficient DED were identified, which precluded a separate subgroup analysis for this category. Consequently, the exclusion of the aqueous-deficient subgroup of DED patients limits the generalizability of our findings to all subtypes of DED. Another limitation is that tear osmolarity and inflammatory biomarker testing, particularly tear MMP-9, were not assessed. Although our diagnostic approach was based on accepted TFOS DEWS II principles, the absence of these complementary measures may have reduced diagnostic completeness and limited characterization of tear film homeostasis and ocular surface inflammatory status. Consequently, some borderline cases or inflammation-predominant DED phenotypes may have been under-recognized or misclassified.

Conclusion

NIBUT demonstrated high sensitivity and specificity for the diagnosis of DED in our study population. It is a non-invasive and repeatable method that shows good correlation with other tests assessing ocular surface and meibomian gland function. However, its diagnostic performance appears to be lower when distinguishing between DED

subtypes. These findings should be interpreted with caution due to the retrospective design, relatively small sample size, and single-center nature of the study. Larger prospective, multicenter studies are warranted to further validate these findings and better evaluate the clinical utility of NIBUT in subclassifying DED.

Ethics

Ethics Committee Approval: This retrospective study was conducted with the approval of the Marmara University Faculty of Medicine's Ethics Committee (date: 15.11.2024; protocol code: 09.2024.1296) and in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all subjects.

Declarations

Authorship Contributions

Surgical and Medical Practices: E.Y., E.B.V., Concept: S.A.T., A.E.T., Design: S.A.T., A.E.T., Data Collection or Processing: E.Y., E.B.V., Analysis or Interpretation: E.Y., E.B.V., Z.A., Literature Search: E.Y., E.B.V., Z.A., Writing: E.Y., E.B.V., Z.A.

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