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Article

Spatial Distribution of Noninvasive Break Up Times and Clinical Relevance in Healthy Participants and Mild Dry Eye

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Purpose: Noninvasive keratograph break up times (NIKBUTs) are preferred to dye-based methods to evaluate tear stability in translational medicine. We analyzed the NIKBUTs in different regions of the precorneal tear by using a common imaging technology and explored potential correlations with clinical parameters.

Methods: We tested NIKBUTs of 120 participants (62.5% females, aged 61.0 ± 13.8 years) with the Keratograph 5M, with standardized symptoms, ocular surface evaluation, and tear lipid layer interferometry. NIKBUTs were obtained from color maps in up to 165 spatial zones corresponding to 7 concentric rings.

Results: The lowest NIKBUT of tested zones averaged 7.8 ± 7.4 seconds (median, 4.5; range, 1.5–24 seconds), with the lowest NIKBUT measuring <2 seconds in many inferior zones. A mean of 5 zones had broken up by 2 seconds compared to a mean of about 50 zones by 10 seconds. NIKBUTs in specific inferior peripheral zones were significantly directly correlated to tear lipid thicknesses. The receiver operating characteristics for detecting reduced tear lipid thickness were better than overall NIKBUTs for participants with readings in these zones. Weaker correlations of NIKBUTs with symptoms were observed in two other zones. Overall, the NIKBUT displayed by keratograph was not significantly associated with any clinical parameters.

Conclusions: Decreased NIKBUTs in specific peripheral locations may be associated with lower lipid thicknesses. Future measurements of NIKBUTs should ideally be determined in smaller defined zones than current maps.

Translational Relevance: An understanding of how to evaluate tear stability allows a more robust clinical evaluation of new drugs and medical devices for dry eye.

Introduction

Dry eye is a chronic multifactorial disease of the tear and ocular surface, characterized by tear instability. This is an increasing problem that has a major impact on patients' visual function and quality of life, with symptoms that adversely hinder their ability to carry out daily activities, such as driving and reading. Tear instability and evaporative losses are major components of dry eye, which incur morbidity but can be addressed with eye drops that increase tear stability (through reducing tear evaporation or promoting tear structure stability).[1–4] In addition, tear stability is clinically relevant because it affects clinical decisions related to contact lens use.[5]

Currently, the main clinical test for tear stability is fluorescein break up time (FBUT). However, this is highly subjective and is variable depending on factors like amount of dye and brightness of light used to visualize the eye. Currently, there is a recommendation by the Tear Film and Ocular Surface Society's dry eye workshop (TFOS DEWSII) to use noninvasive keratograph break up times (NIKBUTs), wherever possible, for the assessment of tear stability, instead of just the FBUTs.[6] A previous study has shown that NIKBUTs, measured by Keratograph 4 (Oculus, Wetzlar, Germany) may have regional differences in people with

Table 1. List of Clinical Characteristics of Participants ($n = 120$)

Parameter	Mean ± SD	Median (Range)
Age, years	61.0 ± 13.8	62.5 (26–92)
Women, % (number)	62.5 (75)	
SPEED (dry eye symptoms)	9.2 ± 6.3	8 (0–28)
Conjunctival redness (average, from Oculus)	1.3 ± 0.5	1.3 (0.6–2.9)
Lipid layer thickness, nm	66.2 ± 24.5	65.5 (23.0–100.0)
NIKBUT (first break up, from Oculus K5)	9.7 ± 6.5	8.4 (1.9–25.0)

cataracts and dry eye.[7] Earlier studies not using the Keratograph instrument have also found spatial differences in tear film over time.[8–12]

The most common method of measuring NIKBUT in contemporary practice is using the Keratograph 5 (K5; Oculus) instrument. The NIKBUT algorithms from the K5 instrument are proprietary, and clinicians are uncertain of how they are derived. We have previously shown that K5 NIKBUTs do not correspond to NIKBUTs with Tomey RT-5000.[13] In a recent paper, the NIKBUT was evaluated in four quadrants, and they found that by using K5 in dry eye participants, the inferonasal quadrant had the most frequent tear break up. In addition, the break up times were moderately positively correlated to Schirmer test values ($r = 0.47$), and negatively correlated to fluorescein staining scores ($r = -0.46$).[14] A study showed in 24 participants that the average NIKBUT using K5 was 8.2 ± 3.5 seconds.[15] In people with diabetes, another study reported that NIKBUT was positively correlated to corneal nerve density ($r = 0.28$, $P = 0.04$) and marginally negatively correlated to age ($r = -0.28$, $P = 0.05$).[16] There has been no study that correlated NIKBUT from K5 with ocular symptoms or with other signs, such as conjunctival redness. In addition, one study[7] described NIKBUT in 60-degree-wide regions, but none of the reported studies have examined NIKBUT in smaller specific zones or regions of the cornea in relation to symptoms.

The advantages of evaluating smaller zones of break up not only include more detailed positional information on the break up but also allow the assessment of break up areas of different sizes (based on the number of zones affected) and the speed of break up (based on the change of size of the break up areas within a period of time). These parameters potentially have a functional impact on vision and quality of life, which is additional to the break up time threshold. We, therefore, analyzed the NIKBUTs in different regions of the tear film by using a Placido ring-based imaging technology (K5) in a cross-sectional study of healthy and mild dry eye participants and specifically aimed to, first, describe NIKBUT results in participants and their potential relation to clinical parameters. Second, we described spatial differences in tear break up in these participants and the association of NIKBUT in specific zones with clinical parameters.

Methods

Study Design and Participants

This was a cross-sectional study conducted at the Singapore Eye Research Institute, Singapore. The study was approved by the institutional review board of Singapore Health Services and complied with the Tenets of Declaration of Helsinki for human research. Informed written consent had been obtained from all participants. We recruited 120 participants (62.5% women and mean age 61.0 ± 13.8 years) from the eye clinics in the Singapore National Eye Center (Table 1).

Eligibility

Inclusion criteria were adults above 21 years of age who were willing to undergo the study procedures. Exclusion criteria were patients who presented to the clinic for an acute eye problem, such as visual loss or painful eye. Patients with dry eye and significant corneal staining were also excluded.

Study Procedures

Questionnaire

All participants underwent symptom evaluation by the standard procedure for evaluation of eye dryness (SPEED) questionnaire, a previously validated way to assess dry eye symptoms.[17,18]

Keratograph 5

We tested the NIKBUTs and conjunctival redness of participants with the K5. The first NIKBUT image

Table 2. Relationship of NIKBUT (First Break Up) to Other Parameters ($n = 120$)

Parameter	NIKBUT (mean ± SD)	*P* Value
Sex		
Male	10.6 ±7.3	
Female	9.2 ± 6.1	0.275
Age, years		
<60	10.5 ± 7.6	
≥60	9.1 ± 5.7	0.278
SPEED		
≤6	10.1 ± 7.1	
>6	9.4 ± 6.2	0.609
Lipid layer thickness, nm		
<60	8.9 ± 6.4	
≥60	10.4 ± 6.6	0.202
Conjunctival redness		
≤1.3	10.2 ± 7.2	
>1.3	8.5 ± 5.4	0.180

acquired in the right eye was used for further analysis.[13,19–21] Briefly, participants were told to blink a few times and then close their eyes. When they opened their eyes again, they were instructed to look at a fixation light. After a certain time interval set by the machine, which was not alterable by the user (maximum of 24–25 seconds), the examination would cease, regardless of whether a NIKBUT reading was obtained. If an NIKBUT reading had been obtained from the machine and the patient subsequently blinked (before 20 seconds), the procedure was not repeated. Failure to keep the eyelids open for 20 seconds might be due to excessive irritation during the period of eye opening, which could be related to tear stability issues. Repeated testing might further impact tear stability and subsequent results. On such occasions, the zones that had not yet broken up would be assigned as 25 seconds. The study data for first break up NIKBUT displayed by the commercial software were first analyzed. In addition, based on the color maps and legends, region-specific NIKBUTs were analyzed. White-colored zones displayed on the color maps were left blank in the spreadsheet and not analyzed. The degree of ocular redness was determined in an automated fashion and displayed by the K5 software in the R-scan module, and this parameter had previously been shown to be clinically important.[22,23]

Lipiview

Tear film lipid thickness was evaluated with the Lipiview interferometry (TearScience, Morrisville, NC), as described by us previously.[24] Briefly, the mean thickness was derived using an interferometric method over the inferior portion of the cornea, based on an imaging procedure acquired over a 30-second duration. Each color interferometric unit of lipid thickness corresponded to 1 nm. Any value provided as 100+ would be analyzed as 100.

Statistical Analysis

Wherever possible, NIKBUTs were assessed simultaneously in up to 165 pre-corneal zones (121.3 ± 21.0). Parametric unpaired *t*-tests were used to compare the NIKBUT between any two groups that were defined by categorical variables. Spearman correlation coefficients were calculated for analyses involving two continuous variables. The significance threshold (alpha level) was set at 0.05. Receiver operating characteristic (ROC) analysis with area under the curve (AUC) and K-means clustering were performed on Stata13.1 (StataCorp, College Station, TX). In K-means clustering, untested zones were given a code of "99."

Unbroken areas were given a value of "25," with the (dis)similarity measure set as "continuous" (L2 or Euclidean option).

Results

Clinical Characteristics of Participants

The clinical and demographic characteristics of the study participants are shown in Table 1. The mean lipid layer thickness was 66.2 ± 24.5 nm; the mean SPEED score, which documented the severity and frequency of dry eye symptoms, was 9.2 ± 6.3 (median, 8; ranging from 0–28); and the mean conjunctival redness was 1.3 ± 0.5. The first break up provided by K5 was 9.7 ± 6.5 seconds (median, 8.4; range 1.9–25.0). This was not significantly associated with age, sex, and the above clinical variables ($P > 0.05$) (Table 2). The lack of significant correlations between the first break up NIKBUT provided by the K5 and the lipid layer thickness, SPEED, and conjunctival redness were also observed (Figs. 1A, 1B, 1C).

NIKBUT in Specific Regions

The mean NIKBUTs over all the tested zones was 14.1 ± 7.7 seconds (median, 14.4; range, 1.5–24.0). This would, of course, be an underestimation of actual mean NIKBUT, as many zones have not

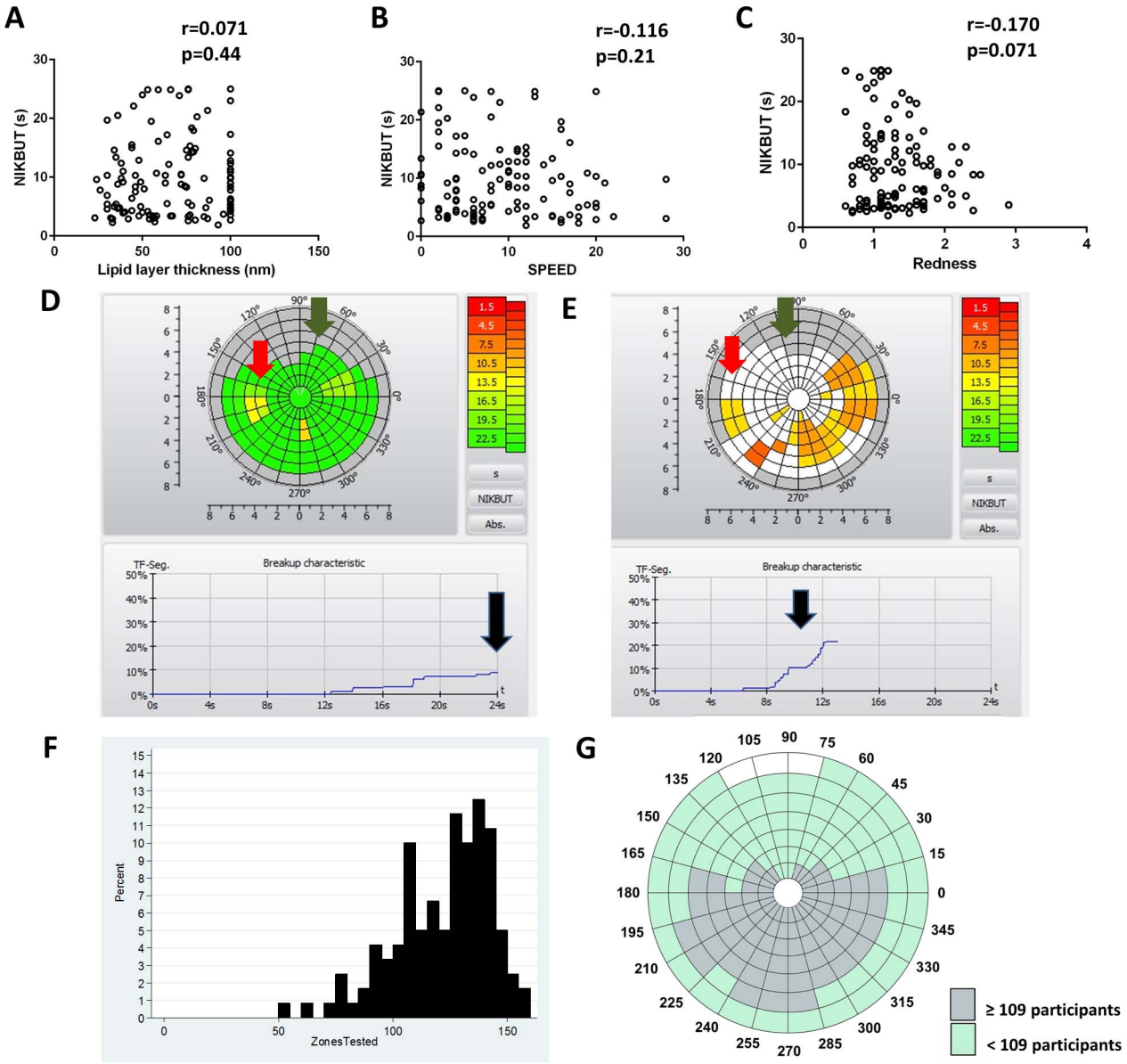

Figure 1. (A–C) Scatter diagrams showing first break up NIKBUT from Oculus K5 and relationship with (A) Tear lipid layer thickness (from Lipiview interferometer), (B) SPEED score, and (C) conjunctival redness score (from Oculus K5). Each circle represents one patient's right eye result. (D and E) Examples of tear maps from K5. (D, *top*) Tear break up occurs before 24 seconds for all regions tested (*red arrow* points to the *greenish region*). (D, *bottom*) *Black arrow* shows that all regions have broken up by 24 seconds. (E, *top*) *White-colored region* has not broken up by the time test has been interrupted (*red arrow*). (E, *bottom*) *Black arrow* shows that the test was interrupted at about 13 seconds (see text for discussion). (D and E, *top*) Unevaluated zones are shown in *grey* (*green arrow* pointing to the *greyish region*). (F) Histogram showing the number of zones that were tested (with valid results) in the 120 patients that underwent NIKBUT evaluation with the Oculus K5. (G) Diagram showing the corneal zones with valid results in more than 109/120 (above 90%) of participants (*grey zones*).

broken up at the end of the test procedure (Fig. 1D). We also calculated the minimum NIKBUT in every zone for each participant, and this had an average of 7.8 ± 7.4 seconds (median, 4.5; range, 1.5–24). For some reason, this was less than the first break up times provided by the K5 software. The number of zones with measurable NIKBUT varied tremendously between participants, with the mode corresponding to about 140 zones according to the histogram (Fig. 1E). The mean number of zones with measurable NIKBUTs per participant was 121.3 ± 21.0 (median, 126; range, 54–160).

There were 81 zones where more than 109 participants had measurements (Fig. 1G). Figure 1G

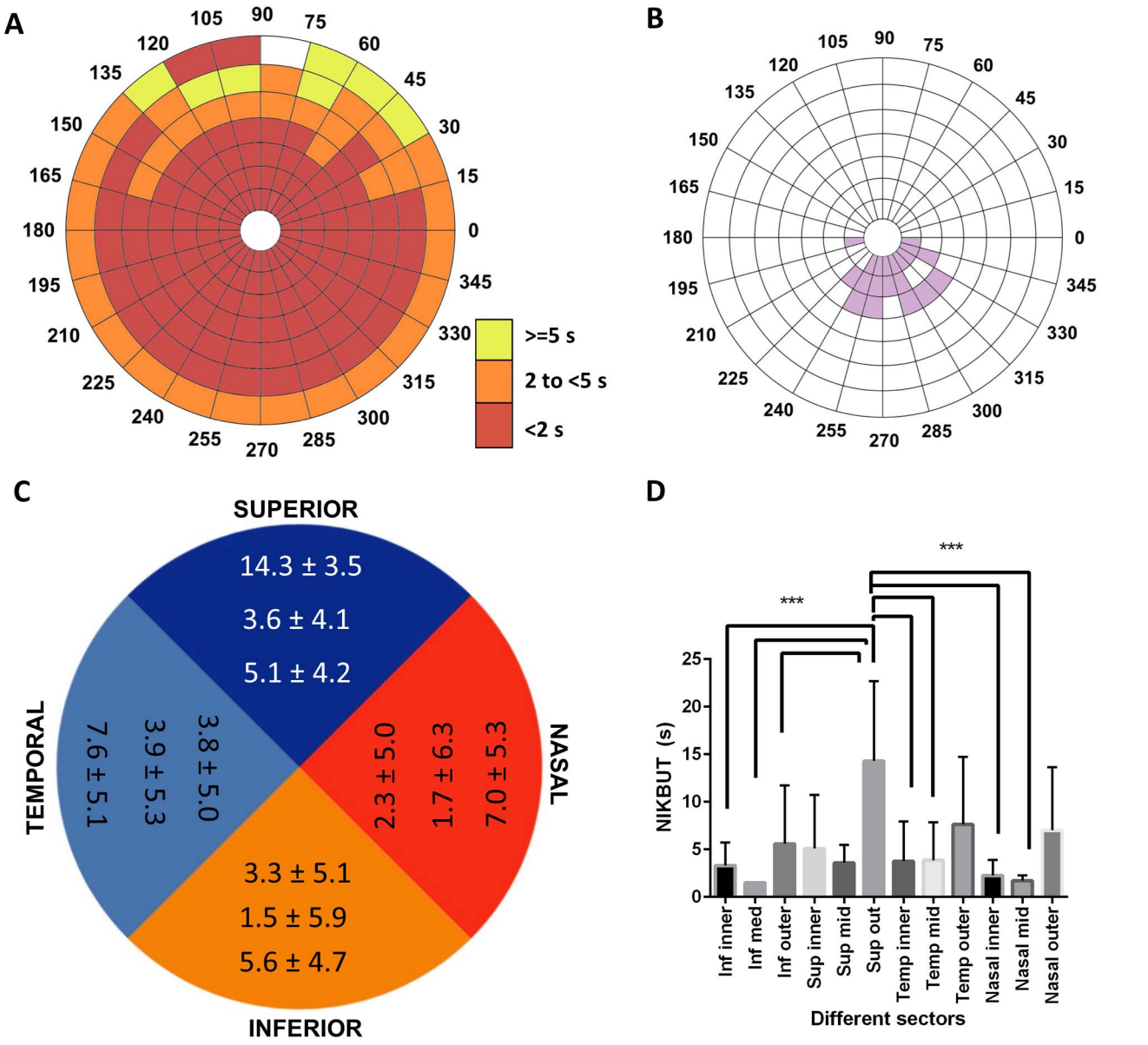

Figure 2. (A) Tear break up times over different locations (showing the fastest tear break up time among all the participants in that zone). (B) Diagram showing location of the 20 corneal zones where all 120 participants achieved valid readings of less than 20 seconds (*purple*). (C) Mean and SD of NIKBUT of 12 sectors of the cornea. This consists of four spatial locations in different colors (labeled outside the circle), each further subdivided into three areas per sector according to distance from the center: summarized from the ring NIKBUT from inner two rings, intermediate two rings, and outer three rings, respectively. (D) Bar chart showing the NIKBUT analysis of variance and post-hoc analysis; statistically significant results shown in brackets above. Height of each bar represents the mean of each sector in (A), and the error bars represent standard deviation. ***$P < 0.001$.

shows that the zones with more than 109 participants (90% of study sample) who obtained measurements were mainly inferior to the fixation axis, and none of the zones in the most peripheral seventh ring was able to record findings for at least 109 participants (colored grey). In general, the shortest NIKBUT readings (<2 seconds) within each zone were obtained in the inferior half of the tested area but, surprisingly, not in the most peripheral zones (Fig. 2A).

On further analysis, 9/120 (7.5%) of the tested individuals had NIKBUTs of above 20 seconds in every tested zone (data not shown). Interestingly 74/120 (62%) participants had break up before 20 seconds in every zone tested. Figure 2B shows the locations of

the 20 zones where these 74 participants were tested. In these 20 zones, 46/120 (38%) participants had NIKBUTs of >20 seconds. Among these 20 zones, the zone with the most variability between patients was inferiorly just nasal to the midline along the third ring from the center (SD of 8.31 seconds), but even the zone with the least variability still showed a rather high SD of 7.68 seconds.

The earliest NIKBUTs at different distances from the central axis outward and at different sectors of the cornea are provided in Figure 2C. On analysis of variance and post-hoc testing, NIKBUT in the superior peripheral sector was higher than any sector inferiorly ($P < 0.001$). The NIKBUT in the superior peripheral sector was also higher than in the superior middle, temporal, and nasal middle inner sectors ($P < 0.001$) (Fig. 2D).

Associations between NIKBUT and Clinical Parameters

Two corneal locations had NIKBUTs positively correlated to lipid layer thickness ($r > 0.6$); these were peripheral and near to the horizontal line (Figs. 3A and 3D). These zones could be located within the area where Lipiview performed the lipid layer assessment. Two locations had NIKBUTs negatively correlated to SPEED scores ($r < -0.3$). These were mainly in the peripherally (Figs. 3B and 3E). Two other zones had NIKBUTs negatively correlated to conjunctival redness ($r < -0.2$), but the association was weaker than the case of tear lipid thickness. These two adjacent zones were superior and close to the visual axis (Figs. 3C and 3F). No single zone was correlated to all three clinical parameters. In contrast, as mentioned above, the first NIKBUT computed by K5 was not significantly associated with lipid thickness, SPEED, or conjunctival redness (all $-0.2 < r < 0.2$) (Figs. 1A–C). Because the correlations with lipid thickness were stronger than with the other two parameters (Figs. 3D–F), we explored whether NIKBUTs in single zones were able to predict a reduced tear lipid thickness of less than 60 nm. This was performed with ROC analyses in two inferior zones and one nasal zone (Figs. 4A–D), achieving a better AUC than the first break up NIKBUT provided by K5 (Fig. 4E). Nevertheless, it is important to note that none of these peripheral zones achieved measurements in all participants, unlike the more central zones displayed in Figure 2B. To illustrate the utility of the ROC in Figure 4B, we found that by using a threshold of 12 seconds (Fig. 2B) or more at this zone, the sensitivity of detecting a lipid thickness above 60 nm was 93%, with a specificity of 62.5%. However, for the adjacent area analyzed in Figure 4C, using the same NIKBUT threshold, the sensitivity and specificity dropped to 85% and 56%, respectively.

When a peripheral nasal zone was evaluated (corresponding to Fig. 3B, colored dark blue), the NIKBUT was significantly lower in those who were symptomatic (SPEED > 6) compared to those who were not (Fig. 5A). In contrast, the first break up from the K5 (Fig. 5B) was not different between participants with symptoms or not. In addition the distribution of single-region NIKBUTs (Fig. 5C) was different from that for the first break up (Fig. 5D) in that the former was skewed to the left, whereas the latter was skewed to the right.

Although the NIKBUTs of some specific zones (Figs. 3, 4, and 5) were highly correlated to clinical parameters, not many participants had acquired readings in those zones, for reasons discussed later. For example, the two locations in Figures 3A and D had readings for only 10 to 20 participants each. To increase the usefulness of routine clinical testing with specific zones, additional zones may have to be used. We used logistic regression (Figs. 6A–D) to determine if NIKBUTs from an additional one or two zones could explain a greater variance of the clinical parameter, for example tear lipid thickness. We found that by using three zones (positions shown in Fig. 6D in yellow), up to 85% of the variance of lipid thickness could be explained (Fig. 6A). Compared to the case of lipid thickness, adding NIKBUT from a few zones did not increase by much the variances of the SPEED or conjunctival redness (Figs. 6B and C). Adding more than three zones to these regression models did not significantly increase the variances of the clinical parameters (data not shown).

Next, we wanted to display the zones that had related NIKBUT values because zones within the same cluster may be providing redundant information and it may not be efficient to investigate NIKBUTs of zones within the clusters separately. K-means clustering showed that the zones of NIKBUT could be divided into four clusters, and their positions are shown in Figure 6E.

We were also interested in the size of the area that had broken up at various time intervals after eye opening. The number of zones that have broken up is a measure of the area of tear break up, keeping in mind that more peripheral zones occupy a larger area than the more central zones. At 2 seconds, a mean of

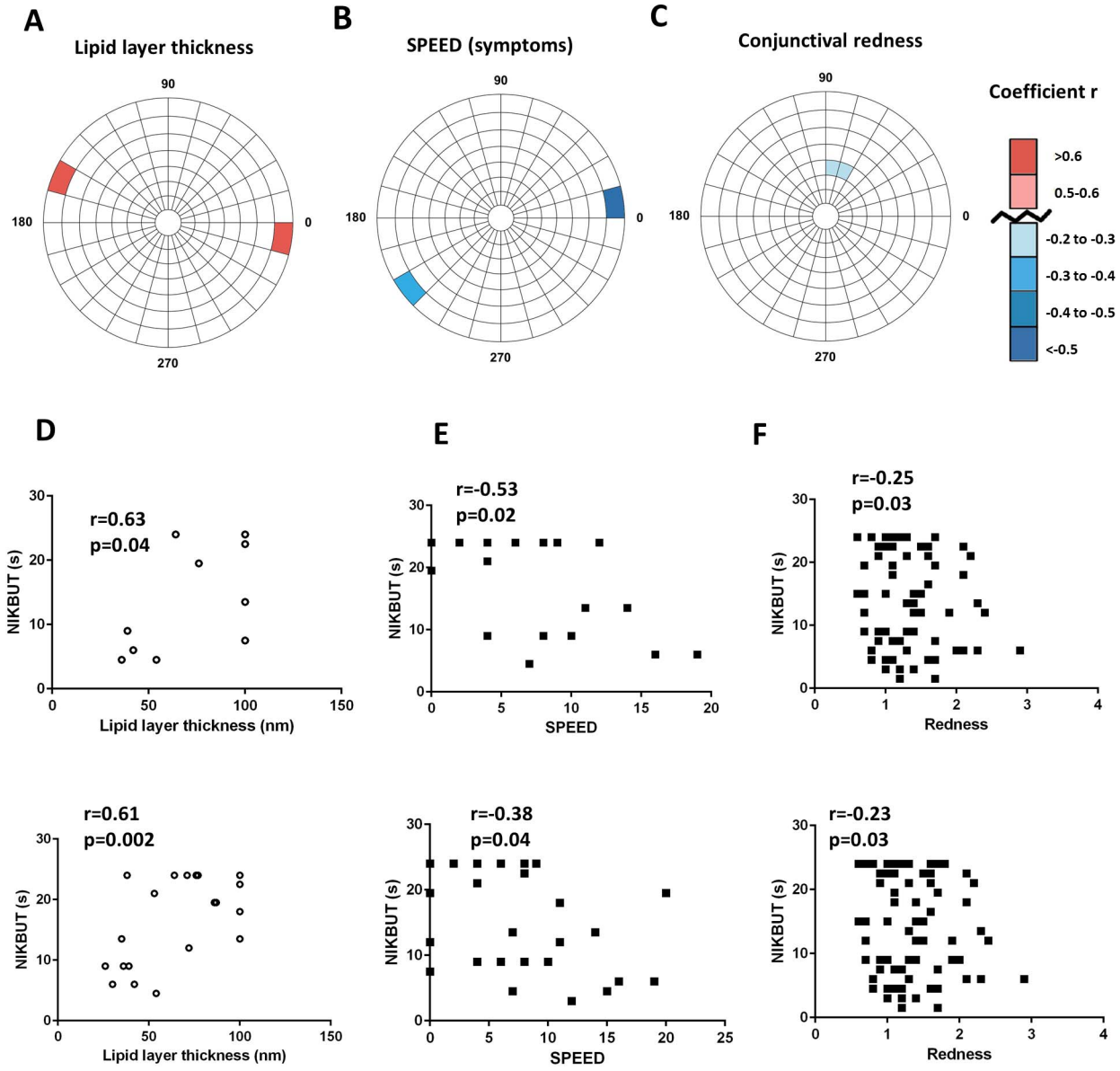

Figure 3. Correlation of NIKBUT in specific zones with clinical parameters. (A, D) With lipid layer thickness, (B, E) with SPEED score, which documents dry eye symptoms, and (C, F) with conjunctival redness. Scatter diagrams showing NIKBUT of specific zones and relationship with lipid thickness (D), with SPEED (E), and with conjunctival redness (F). Each symbol represents one participant and all are $P < 0.05$. In (D and E), the scatter diagram for the zone that showed a stronger association is shown on *top*, whereas the scatter diagram for the other colored zone that showed a relatively weaker correlation was shown *below*. *r*, correlation coefficient.

5 zones had broken up compared to a mean of about 50 zones by 10 seconds (Fig. 7A). The area of tear break up, thus, increased fairly rapidly between 2 and 10 seconds after eye opening. At 2 seconds (Fig. 7B), the cumulative frequency graph showed that up to 90% of the participants (y axis) had less than 10 to 20 zones with tear break up (x axis). In Figures 7C and 7D, the curves have shifted to the right, indicating that at 5 and 10 seconds after eye opening, a more extensive area of tear break up involving many more zones had occurred. In fact, by 5 seconds (Fig. 7C), up to 90% (y-axis) of participants had break up of 110 zones (x axis). Thus the NIKBUT profile showed that the size of the tear break up occurred more than 5-fold between 2 and 5 seconds.

Discussion

In this study, we described the minimum tear break up times in each tested zone of the NIKBUT by using

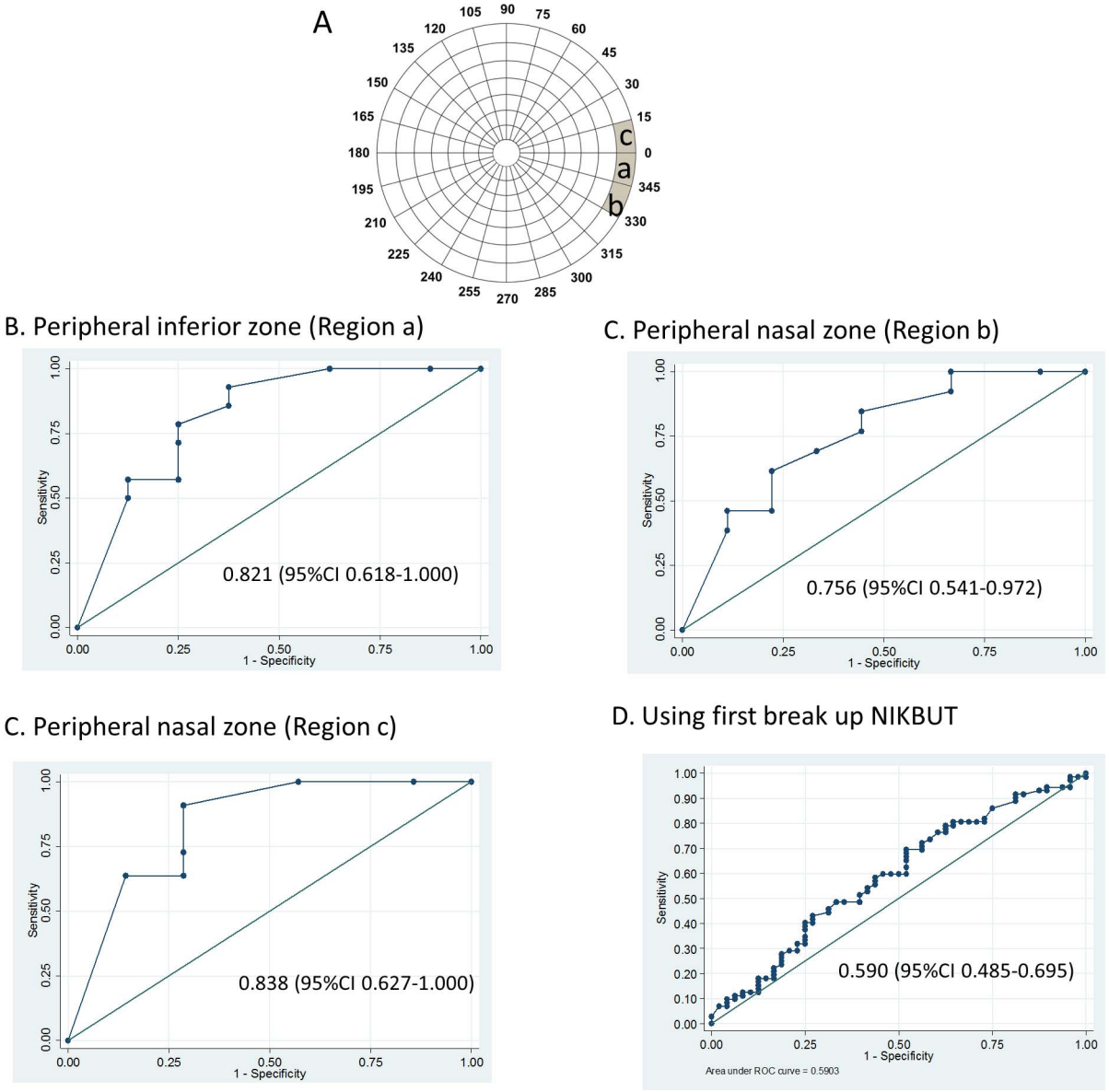

Figure 4. Specific zones were evaluated for NIKBUT (A) and their relationship to tear lipid thickness. ROC analysis for the NIKBUT in specific peripheral zones (B–D) and overall first NIKBUT (E) to predict the presence of a thin tear lipid layer (defined as <60 nm on Lipiview interferometry). AUC and 95% confidence intervals are shown below the ROC curve. Diagonal, reference line. Each *dot* represents one participant's result.

the Oculus K5. The initial break-up of the tear film occurs inferiorly below the visual axis. Unlike the overall first tear break up NIKBUT provided by the Oculus software, which did not correlate significantly with clinical parameters, some specific zones had NIKBUTs correlated to tear lipid thickness, and to a lesser extent, the symptoms of dry eye. The superior outer sector of the NIKBUT test area achieved higher NIKBUTs than sectors in the inferior, nasal, and temporal sector in the inner and mid areas. This may be related to less exposure of the superior sector or that this region is last to be exposed on opening the eye.

A previous study found the NIKBUT from the Oculus K4 to vary in people with cataracts and dry eye. That study evaluated NIKBUT within 60-degree sectors but did not explore the correlation of NIKBUT from individual smaller zones of the cornea. The first break up was found to be earlier in inferior peripheral (outer four rings) compared to inferior central (inner five rings).[7] These data cannot be directly compared to the current data (Figs. 2C,

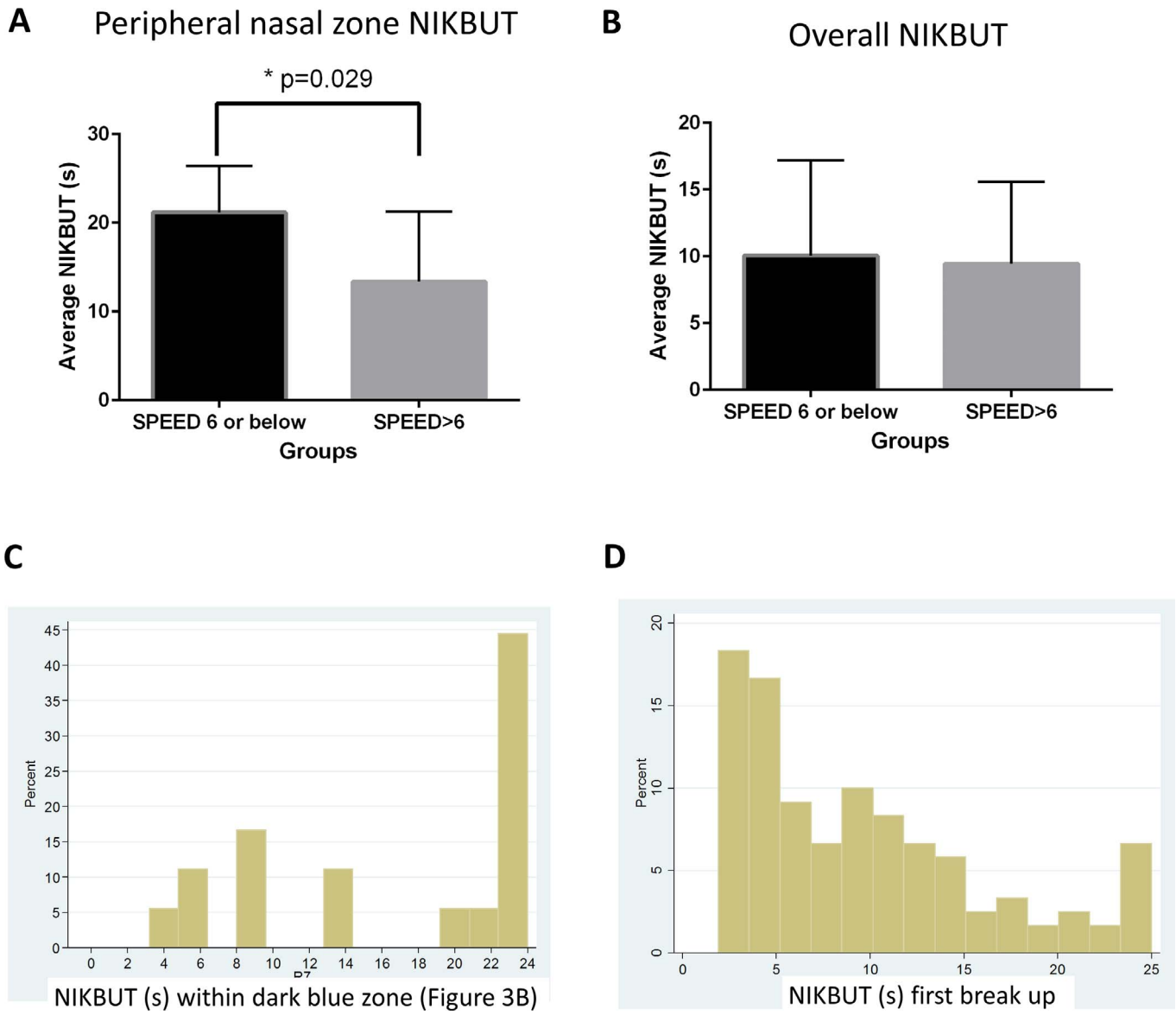

Figure 5. (A and B) Bar charts showing NIKBUT of symptomatic and asymptomatic eyes. (A) NIKBUT for one peripheral nasal zone only (location c in Fig. 4A), and (B) overall first break up NIKBUT. Height of bar: mean; error bar: SD. (C) Histogram of the frequency distribution of NIKBUT over one zone (as in A). (D) Histogram for NIKBUT readings by the Oculus K5 for entire eye.

2D), as the K5 used in the present study produced a total of only seven rings instead of nine rings. Five other studies have investigated the NIKBUT (Table 3), but none has evaluated each individual zone like in the current study.

Most of the inferior zones in the mid and inner areas had valid NIKBUT readings (more than 90% of the participants, grey area in Fig. 1G). However, there was relatively less information in the most peripheral, as well as some superior locations (green area in Fig. 1G). This is reflected by the reduced number of points in the scatter diagrams (Figs. 3D and 3E) as well as in the ROC plots (Figs. 4B–D). The spatial differences may be due to the position of eyelid, premature blinking or ptosis, changes of fixation or instability of fixation, or movement of eye during testing. The NIKBUT in the most peripheral inferior ring may not be measurable in a number of patients because of distortion related to the inferior tear meniscus or occlusion by the lower eyelid. Many regions illustrated with white color on the tear map (Fig. 1E) are not interpretable because they could represent very stable tears (break up times beyond 25 seconds) or lack of NIKBUT due to interruption by blinks. Premature

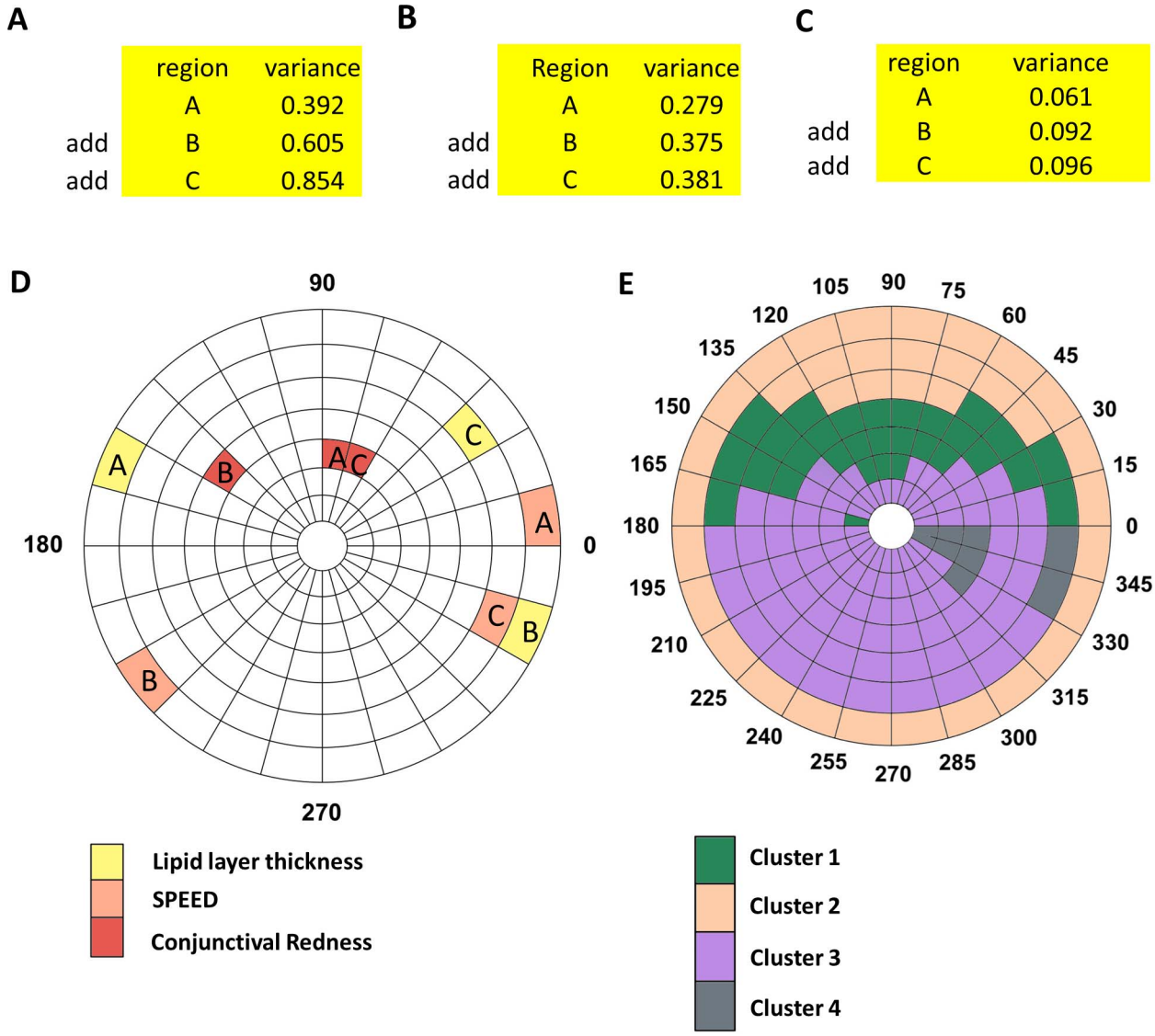

Figure 6. Linear regression analysis to examine the number of zones of NIKBUT and contribution to the variance of (A) lipid layer thickness, (B) SPEED, and (C) conjunctival redness. The selection of the zones was based on the correlation and its significance of individual zones (see main text). Zones that reduce the variance were not selected. The maximal variance that could be explained for each clinical parameter was shown with the addition of each zone with NIKBUT that contributed to the variance of the clinical parameter. For example, in (A) NIKBUT of zone A explained 0.392 of the total variance (r^2) of lipid thickness, and on addition of NIKBUT from zone B to the model, the r^2 increased to 0.605, whereas addition of NIKBUT from zones B and C increased the r^2 to 0.854. (D) The locations of the different zones in (A–C) are shown in three colors, corresponding to the three clinical outcomes investigated. (E) Results of K-means clustering analysis showing four clusters with NIKBUT (see text for details).

blinks can be detected by a shift of the green line graph to the left (Fig. 1D and 1E, bottom plots). Premature blinking or lowering of the eye may indeed be related to ocular dryness and inability to maintain an open lid aperture. It was previously reported that the maximal eye opening time could be reduced in dry eye.[29]

We chose not to repeat the NIKBUT acquisition for any participants who blinked before 20 seconds, as the first break up had been observed by the time of the blink. It is difficult to test NIKBUT repeatedly because repeated testing may result in greater tear instability and potentially more irritative symptoms. In fact, some aspects of the tear morphology, such as meniscal height, may actually be altered just by performing NIKBUT testing.[30] This also explains why we did not assess intrapatient repeatability of NIKBUT.

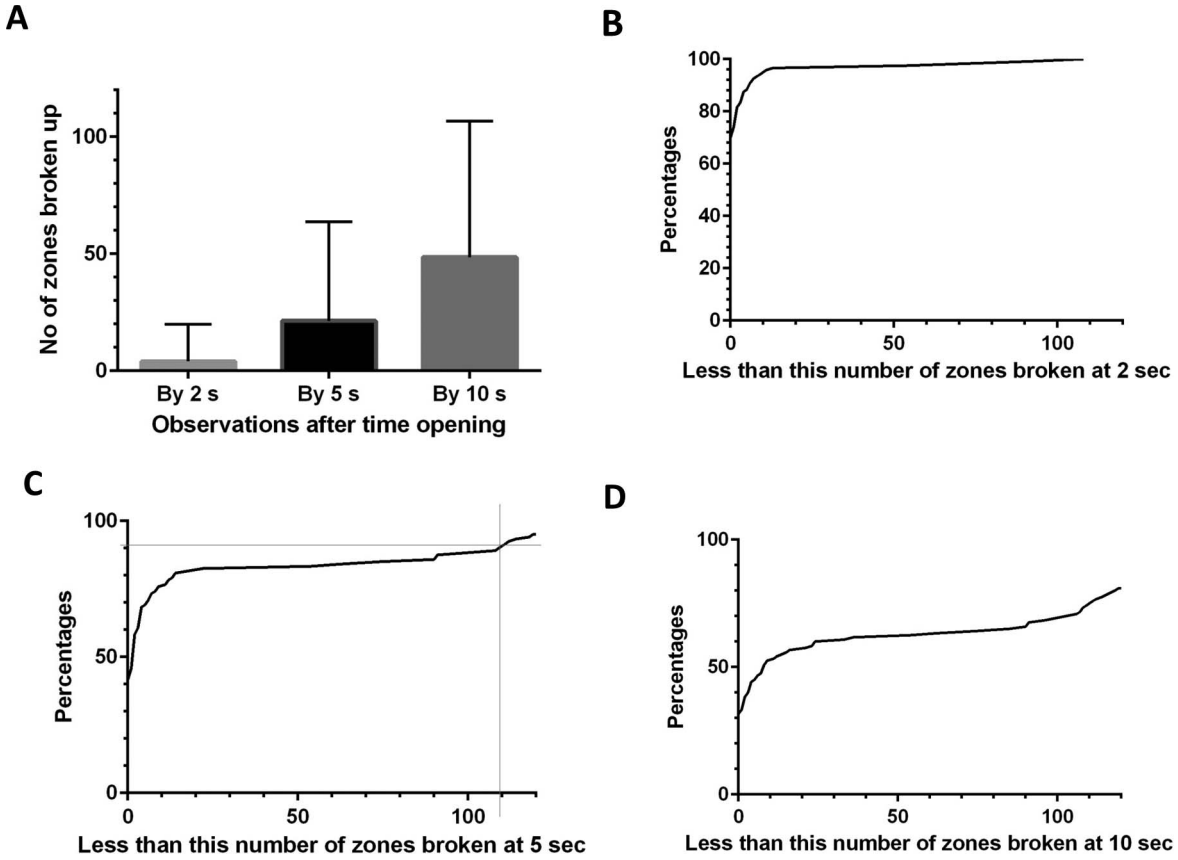

Figure 7. Number of zones that had tear break up over time. (A) Bar chart showing mean number of zones that had tear break up after eye opening. Error bar: SD. (B–D) Cumulative frequency curves that show the percentage of participants that had a certain number of zones of tear break up at 2 seconds (B), 5 seconds (C), and 10 seconds (D).

It is interesting that a relatively small number of participants showed an increase in symptoms with shorter NIKBUT in a peripheral zone (Figs. 3B and 5A). In contrast, the first break up NIKBUT in the overall tear film, measured in all the participants, did not vary according to symptoms (Fig. 5B). It is possible that the participants may be heterogenous in terms of the underlying factors that contribute to dry eye symptoms. Some of the dry eye sufferers in Figure 5B may have symptoms unrelated to tear film stability. For example, the symptoms may be related to inflammation or neuropathic factors.

When limiting studies to a few zones, we found that more of the variance of lipid thickness (Fig. 6A) could be explained by NIKBUT compared to either symptoms (Fig. 6B) or conjunctival redness (Fig. 6C). This is not surprising because NIKBUT is an assessment of stability, and lipid morphology is more likely to influence tear stability than symptoms or conjunctival redness.[31] Clustering analysis (Fig. 6E) shows that NIKBUT displayed different characteristics in a more peripheral location compared to a central zone.

Last, we show that there is a huge rate of increase in the area of tear break up between 2 and 5 seconds after eye opening (Figs. 7A–D). This may have clinical relevance as in most people the interblink-interval during many activities is extended beyond 5 seconds, so the high rate of extension of the break up area may induce significant visual blurring or aberrations.

A decrease in NIKBUT in specific locations, should this be measurable, can be more clinically meaningful than the NIKBUT over the entire tested area calculated by the current Oculus software. Tear instability in some peripheral locations seems to be most affected by reduced lipid thickness. We recommend that future versions of the Oculus software provide a separate NIKBUT from the peripheral location (seventh ring). It would be advantageous clinically if technical advances allow more participants to acquire valid NIKBUT measurements in peripheral zones, especially inferiorly.

Table 3. Previous Relevant Publications on NIKBUTs Assessed by Keratograph (table extended on next page)

Study	Participants	Machine/Technology for NIKBUT[a]	Zones Analyzed
Markoulli et al., 2018[15]	24 healthy subjects	Oculus K5 Tearscope Plus	Entire cornea
Wang et al., 2018[1]	22 healthy subjects	Slit lamp-mounted keratoscope[b] Interferometer[b] Keeler keratoscope	Entire cornea
Addelfattah et al., 2015[25]	Ocular surface disease: 223 eyes Control: 73 eyes	Oculus K5 Keeler tearscope	Entire cornea
Downie et al., 2015[26]	28 dry eye patients 17 controls	Placido disc video keratography[b] (manual or automated interpretation)	Entire cornea
Jiang et al., 2014[7]	43 participants with cataracts and dry eye symptoms	Oculus K4	Corneal sectors of 60 degrees each
Best et al., 2012[27]	100 patients with no anterior eye disease	Oculus K (software 2.73 r19) Keeler tearscope	Entire cornea
Gumus et al., 2011[28]	45 dry eye patients 25 asymptomatic controls	Tomey RT-7000	256 points over 11 mire rings

[a] Refers to the Oculus keratograph in the studies which also used handheld tearscopes.
[b] Noncommercial equipment.
[c] Ocular surface disease index (dry eye symptom assessment).

A limitation of the study is that we did not address NIKBUT in cases with extensive corneal staining. Anecdotally, we found that NIKBUT may be more unreliable in such cases, as the reflection of the rings may be highly irregular or distorted due to the corneal epitheliopathy altering the smoothness of the preocular reflecting surface. In practice, we may also have difficulty evaluating patients using FBUTs if the corneal epitheliopathy is highly confluent. As we did not have a tool to measure the spatial changes of the overall tear thicknesses over each part of the tear during the blink cycle, we were unable to explain the spatial differences in the correlation with lipid thicknesses. Without a real-time concurrent videographic monitoring of the eyelid position during the acquisition of NIKBUT, it would be difficult to verify if the more peripheral inferior zones were affected by eyelid interference. There could be large variation in terms of the pathogenesis, treatment, and behavior of the dry eye cases in this study. Sampling of the NIKBUT over different times and days can potentially increase the correlation with symptoms. Rather than using right eye values, ideally the eye used should be randomized.

The current report is only a general exploratory study aiming to evaluate associations between noninvasive tear analysis and some ocular surface parameters. The knowledge of how clinical treatment can affect the NIKBUT in various zones is valuable, but this will require future longitudinal studies. Although it is interesting that small zones of NIKBUT correlated to lipid thicknesses, we do not imply that this strategy can be used to diagnose dry eye because less than 90% of participants had measurable values in those zones. We introduced the interesting concept of area of break up at specific time intervals, for example 5 seconds, and it would be interesting to see if this parameter correlates to visual function in future studies. If so, it may possibly replace the break up time threshold for clinical assessment of tear stability. Future developments could also incorporate automation and fractal analysis.[32] Considering the advantag-

Table 3. Extended

Study	Clinical Outcomes	Main Finding Relevant to Current Discussion
Markoulli et al., 2018[15]	Lipid layer thickness Tearscope Plus Lipiview	NIKBUT for Oculus not interchangeable with Tearscope plus measurement
Wang et al., 2018[1]	Interinstrument agreement	NIKBUT by interferometry correlated to keratoscopy results
Addelfattah et al., 2015[25]	Diagnosis of ocular surface disease	NIKBUT not different between ocular surface disease and control groups
Downie et al., 2015[26]	Tear osmolarity	NIKBUT sensitive and specific for detection of tear hyperosmolarity
Jiang et al., 2014[7]	FBUTs	NIKBUT correlated to fluorescein tear break up time, break up earlier inferiorly in periphery
Best et al., 2012[27]	Symptoms	NIKBUT very weakly correlated to OSDI[c] ($r = -0.19$)
Gumus et al., 2011[28]	Symptoms Schirmer FBUTs	NIKBUT longer than FBUTs

es of NIKBUT over more conventional FBUT measurements in dry eye,[6] research should be continued in this area. Apart from increasing ocular morbidity, the visual blurring related to tear instability may have other psychological effects on patients.[33]

In conclusion, NIKBUT is a noninvasive tool for assessing tear stability based on the evaluation of images of rings on the tear-air interface. There are significant differences in the NIKBUT in different spatial zones of the area assessed. NIKBUT measurements in inferior peripheral zones, although not achievable currently in many participants, may have functional implications.

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