**IMPORTANCE** Knowledge about the variability of measurements using the TearLab Osmolarity System is necessary when evaluating the clinical utility of readings.

**OBJECTIVE** To examine the variability of tear osmolarity measured by the TearLab Osmolarity System in patients with Sjögren syndrome (SS), patients with blepharitis, and control participants.

**DESIGN, SETTING, AND PARTICIPANTS** Cross-sectional study at a tertiary care academic center from June 13, 2012, to March 21, 2013. Participants included 74 eyes of 37 patients from a volunteer sample (18 patients with SS, 11 patients with blepharitis, and 8 control participants) who were evaluated using the TearLab Osmolarity System, with 3 consecutive osmolarity measurements taken at 1-minute intervals in a session; 15 of these patients had the same measurements taken by the same examiner in 2 additional sessions on the same day (9 AM-10 AM, 12 PM-1 PM, or 3 PM-4 PM). Most patients with SS and patients with blepharitis were taking systemic or topical dry eye medications at the time of enrollment.

**MAIN OUTCOMES AND MEASURES** Mean osmolarity and its variability calculated from a linear mixed model for each disease group that accounts for the variations attributable to different patients, eyes, and sessions and measurement error specific to each disease group.

**RESULTS** Mean tear osmolarity was 307 mOsm/L, 304 mOsm/L, and 301 mOsm/L in the SS, blepharitis, and control groups, respectively ($P = .46$). The error associated with repeated measurements within a session in the patients without dry eye (10.5 mOsm/L [95% CI, 9.0-12.4]) was significantly lower than in the patients with blepharitis (14.6 mOsm/L [95% CI, 12.5-17.5]; $P = .006$) and patients with SS (15.8 mOsm/L [95% CI, 14.2-17.8]; $P < .001$) but a difference in the error of repeated measurements between patients with blepharitis and patients with SS was not identified ($P = .46$).

**CONCLUSIONS AND RELEVANCE** There was increased variability attributable to error in repeated measurements in patients with SS and patients with blepharitis compared with control participants. The high variability of TearLab osmolarity readings in all groups makes the clinical interpretation of measurements unclear.
The prevalence of dry eye disease (DED), which can significantly impair visual acuity, workplace productivity, and quality of life, has been estimated to affect 14.4% of the general population and increases with age. The 2007 Report of the Dry Eye Workshop (DEWS) defined DED as a multifactorial disease accompanied by increased osmolarity of the tear film and inflammation of the ocular surface. Hyperosmolarity is thought to activate inflammatory pathways that lead to epithelial damage, tear instability, and ocular discomfort.

Elevated tear osmolarity is considered an objective marker of DED. However, despite evidence that tear osmolarity may be helpful in the diagnosis of DED, challenges still remain for implementation in clinical practice. Historically, tear osmolarity testing in the clinic was impractical due to the difficulty of tear collection and analytic procedures that required laboratory facilities. The Osmolarity System (TearLab Corp) is available and appealing because of its ease of use and ability to give quick in-office results. The system uses single-use test cards that simultaneously collect and analyze the osmolarity of 50 nL of tears using electrical impedance. Unlike other methods for measuring tear osmolarity, such as freezing-point depression, the TearLab system does not require transporting tear samples to a separate device that risks evaporation and concentration of samples.

However, the clinical utility of tear osmolarity as measured by the TearLab system has been called into question due to the variability of measurements and lack of correlation with symptoms or fluorescein staining of the cornea. As with any instrument, it is important to understand the variability of measurements to allow for useful clinical interpretation of readings. Variability of measurements can be due to a variety of sources including the operator, instrument, patient, and disease. Tear osmolarity has been reported to vary to a greater degree in patients with DED compared with those without DED. However, a high degree of variability of TearLab osmolarity measurements has been reported in 5 patients without DED, more information regarding the intra- and intersession variability in DED is needed.

The purpose of this study was to examine the variability of tear osmolarity measurements using the TearLab Osmolarity System in patients with and without DED, including patients with Sjögren syndrome (SS) or patients with blepharitis. Because the initiation of DED is thought to be due to a breakdown of compensatory mechanisms leading to transient changes in tear stability, we hypothesized that there would be increased variability among patients with DED compared with those without DED. In addition, we set out to quantify the degree of variability that could be expected if repeated measurements were taken within the same session and also throughout the day in a given patient.

Methods

Patients who were 18 years or older were recruited from the ophthalmology and rheumatology practices at an academic center over 9 months, from June 13, 2012, to March 21, 2013. Patients with blepharitis were enrolled if they had a history of blepharitis and had clinical signs of at least moderate blepharitis (using an abbreviated grading system). Patients with SS were diagnosed by the American-European Consensus Group criteria or the American College of Rheumatology criteria. Control participants were enrolled if they did not have dry eye symptoms (Ocular Surface Discomfort Index [OSDI; Allergan, Inc] <13) and had no diagnosis of DED, SS, or blepharitis. The study was approved by the institutional review board at the University of Pennsylvania and was compliant with the Health Insurance Portability and Accountability Act. Written informed consent was obtained from all participants prior to participation. This study also adhered to the tenets of the Declaration of Helsinki.

Exclusion criteria for all patients included a history of any significant ocular surface disease (other than DED) or ocular inflammation, thyroid eye disease, diabetes mellitus, active ocular infection, active ocular allergy, ocular surgery within 1 year, punctal plug placement within 30 days, evidence of eyelid deformity or abnormal eyelid movement disorder, use of eyedrops (excluding artificial tears), contact lens use within 1 month, pregnancy or lactation, birth control or hormone therapy, abnormal nasolacrimal drainage, evidence of a systemic disease known to affect tear production (excluding SS in that subgroup), or use of artificial tears within 2 hours of enrollment. Patients were also excluded if they had initiated or altered the dose of chronic systemic medication known to affect tear production within 30 days of testing.

For all patients, demographic information, medical history, and ocular history were obtained. Subjective symptoms of dry eye were assessed using the OSDI.

Tear Osmolarity Testing and Ocular Surface Examinations

Tear osmolarity was measured in each eye with the TearLab Osmolarity System. To ensure proper functioning of the instrument, the system was calibrated at the start of each day of testing in accordance with the manufacturer’s instructions. Using the same pen for all readings, 3 consecutive osmolarity measurements were taken at 1-hour intervals in each eye of each patient (for all 37 patients), always starting with the right eye first.

A subgroup of 15 patients agreed to have 3 measurements per eye taken at each of the 3 sessions throughout the day (9 AM-10 AM, 12 PM-1 PM, or 3 PM-4 PM) by the same examiner to assess the intersession variability during the course of the day. This resulted in a total of 402 individual tear osmolarity measurements from 37 participants.

All patients also underwent a slitlamp examination, vital dye staining with fluorescein and lissamine green, tear breakup time assessment, and unanesthetized Schirmer testing.

Statistical Methods

Differences in ocular surface parameters between patients with SS, patients with blepharitis, and control participants were estimated using linear regression with generalized estimating equations to account for intereye correlation.

Tear osmolarity was modeled using a hierarchical linear mixed-effects model, with disease group and age considered fixed effects. Differences among patients, eyes, and sessions were
considered successively nested random effects (ie, patients within the disease group, eyes within patients, and sessions within eyes) and differences among repeated measurements within a session were considered the residual variance, which was allowed to be of different magnitude for each of the 3 disease groups. Model specification and output for the variance estimates are shown in the eFigure in the Supplement. \( P \) values for each random effect and residual variance estimate were calculated using Wald z tests. \( P \) values for comparisons between residual variance estimates of each disease group were calculated using the Monte Carlo simulation with 10,000 samples.

Fixed effects represent factors that are associated with consistent differences in the true value of the tear osmolarity of an eye, such as an increased level among diseased groups or a decreased level among treated groups. Random effects represent factors that are associated with random differences for which the value of the difference is not of interest but the amount of variance is, such as the differences among patients with the same disease, between a single patient’s eyes, and among sessions in a single eye. Residual variance, or measurement error, represents the variance in the measurements after all the other factors in the model have been accounted for and may be due to causes such as moment-to-moment fluctuations in the value or limitations of the measurement tool. The variance of a series of measurements is the sum of the disease-specific residual variance (measurement error) plus the variance of any applicable random effects (effects). The variance of a difference between 2 independent measurements is the sum of the variability for each measurement. All variance estimates presented hereafter were transformed into standard deviations (SDs) by taking the square root of the variance and are referred to as variability. The total variability from 2 or more sources was calculated by summing their variances first, followed by taking the square root of total variance instead of summing the SD directly.

As suggested by TearLab, a fixed-effects analysis of variance was also performed using only the maximum value of the 2 eyes from the first measurement of each eye at the first session for each patient. All statistical analyses were performed in SAS version 9.3 (SAS Institute Inc).

**Results**

**Characteristics of Study Patients**

Of the 37 patients enrolled in the study, 18 patients had SS (14 patients with primary SS and 4 patients with secondary SS), 11 had blepharitis, and 8 were classified as control participants. Of the 15 patients who completed 3 sessions during the course of the day, 7 patients had SS (39% of the SS group), 2 patients had blepharitis (18% of the blepharitis group), and 6 were control participants (75% of the control group).

**Table 1. Characteristics of Patients With Sjögren Syndrome, Patients With Blepharitis, and Control Participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Participants (n = 8)</th>
<th>Patients With Blepharitis (n = 11)</th>
<th>Patients With Sjögren Syndrome (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>40.4 (12.7)</td>
<td>65.2 (12.7)</td>
<td>55.2 (11.2)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (100)</td>
<td>8 (73)</td>
<td>16 (89)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>7 (88)</td>
<td>11 (100)</td>
<td>12 (67)</td>
</tr>
<tr>
<td>African American</td>
<td>1 (13)</td>
<td>0 (0)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Artificial tear use</td>
<td>0 (0)</td>
<td>8 (73)</td>
<td>17 (94)</td>
</tr>
<tr>
<td>Topical cyclosporine</td>
<td>0 (0)</td>
<td>2 (18)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Fish oil</td>
<td>0 (0)</td>
<td>5 (45)</td>
<td>6 (33)</td>
</tr>
</tbody>
</table>

**Table 2. Clinical Ocular Surface Parameters of Patients With Sjögren Syndrome, Patients With Blepharitis, and Control Participants**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Participants (n = 16 Eyes)</th>
<th>Patients With Blepharitis (n = 22 Eyes)</th>
<th>Patients With Sjögren Syndrome (n = 36 Eyes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSDI</td>
<td>0.83 (0-2.5)</td>
<td>20 (10-31)</td>
<td>37 (25-47)</td>
</tr>
<tr>
<td>Schirmer, mm/5 min</td>
<td>18.5 (13-33)</td>
<td>10.5 (7-15)</td>
<td>11 (7-17)</td>
</tr>
<tr>
<td>TBUT, s</td>
<td>8 (7.33-9.67)</td>
<td>2.67 (2-4.33)</td>
<td>2 (1-4)</td>
</tr>
<tr>
<td>Fluorescein staining</td>
<td>1 (0-1)</td>
<td>2 (1-6)</td>
<td>3 (1-5)</td>
</tr>
<tr>
<td>Lissamine green staining</td>
<td>0 (0-0.5)</td>
<td>3 (0-4)</td>
<td>6 (1.5-9)</td>
</tr>
</tbody>
</table>

Abbreviations: OSDI, Ocular Surface Discomfort Index; TBUT, tear breakup time.
Comparison of Mean Osmolarity Among Groups of Patients

Table 3 shows the estimates mean and 95% CIs of mean tear osmolarity by disease group. In the mixed-effects model, all 402 measurements (3 measurements per session per eye in 22 patients and 9 measurements from 3 sessions per eye in 15 patients) were used in estimating mean values and their SDs. A difference in the mean value among the 3 disease groups (P = .46) was not identified. Similarly, when only the maximum value of the 2 eyes from the first measurement of the first session was used (1 measurement each from 37 patients), a difference in the mean value among the 3 disease groups (P = .60) was not identified.

Sources of Variability

The estimated variability from each component is shown in Table 4. The within-session measurement error in an eye of the control participants (10.5mOsm/L [95% CI: 9.0-12.4]; P < .001) was lower than that in the patients with blepharitis (14.6mOsm/L [95% CI: 12.5-17.5]; P < .001) or patients with SS (15.8mOsm/L [95% CI: 14.2-17.8]; P < .001) but no difference in measurement error was found between patients with blepharitis and patients with SS (control vs blepharitis, P < .001; control vs SS, P = .006; and blepharitis vs SS, P = .46). The between-session random effect was 6.4mOsm/L (P = .006), which was an additional source of variability in addition to the disease-specific variability. The between-eye random effect was low compared with the other measurement errors, was not different from 0, and the between-person random effect was irrelevant to the discussion of variability within a patient; therefore, these effects were not included in the analyses that follow. The Figure shows the individual tear osmolarity measurements from both eyes and all 3 sessions from a representative patient, a control patient with near-median variability in measurement needs to be taken into account for clinical interpretation. For patients without dry eye, the 95% CI for a single measurement is ±1.96 times the SD (SD for control participants = 10.5 so 1.96 × 10.5 = 20.6mOsm/L). For example, with a tear osmolarity reading of 310mOsm/L in a patient without dry eye, the 95% CI ranges from 289.4mOsm/L to 330.6mOsm/L. Because the SD was larger for patients with blepharitis (SD = 14.6) and patients with SS (SD = 15.8), the 95% CIs were also larger (±1.96 × 14.6 = 28.6mOsm/L and ±1.96 × 15.8 = 31.0mOsm/L, respectively). However, regardless of disease group, the 95% CI for a single measurement spanned the range considered normal and the range considered abnormal, which has been described to be from 305mOsm/L to 365mOsm/L.

When a single measurement is taken on the same eye at each of 2 sessions, such as before and after a new treatment, the measurement error for each reading as well as the variability in tear osmolarity between sessions needs to be taken into consideration. Continuing the example of measurements on a patient without dry eye, the difference in 2 mea-

### Table 3. Comparison of Mean Tear Osmolarity Among Groups of Patients With Dry Eye and Control Participants

<table>
<thead>
<tr>
<th>Group</th>
<th>Mixed-Effects Model of All Osmolarity Measurements</th>
<th>Model Using the Maximum Value Between Eyes From the First Measurement of the First Session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td>Control participants</td>
<td>302 (293-310)</td>
<td>.57</td>
</tr>
<tr>
<td>Patients with blepharitis</td>
<td>303 (295-312)</td>
<td></td>
</tr>
<tr>
<td>Patients with Sjögren syndrome</td>
<td>307 (301-312)</td>
<td></td>
</tr>
</tbody>
</table>

dcontrol participants had modified DEWS severity grades of 0 in all but 1 parameter, with half of control participants having a grade of 0 in all parameters.

### Discussion

When using all of the data and accounting for the experimental design with a hierarchical mixed model, the mean tear osmolarity level in patients with blepharitis or patients with SS was slightly higher than in control participants without dry eye, but not to a statistically significant degree. When the maximum tear osmolarity value of the 2 eyes was used as recommended by TearLab, the differences among the estimated means for these groups were smaller (within 5mOsm/L) and not statistically significantly different. One possible explanation is that the majority of patients with SS and patients with blepharitis were taking dry eye treatment at the time of enrollment, which may have improved their DED and decreased their osmolarity to a level that was similar to that of control participants. There may have been a greater difference in mean osmolarity among the groups if none of the patients were receiving treatment. However, this is unlikely because if treatment had improved their DED, we would have expected an improvement in all ocular surface parameters, not just osmolarity.

There were differences among the 3 patient groups in the magnitude of measurement error, with the patients without dry eye having lower measurement error or variability compared with the 2 dry eye groups. This was consistent with previous reports that patients with DED exhibited greater tear instability and, thus, greater variability in osmolarity and that patients without dry eye have less variation of tear osmolarity during the course of the day.
TearLab recommends using the maximum of the patient’s right and left eyes when measuring the tear osmolarity for a patient. However, the variability of a maximum of 2 measurements is no less than the variability of a single measurement, so the CIs for the maximum from a single session or for the difference in the maximum between sessions would only be the same or larger than those cited earlier.

The control participants in our study were younger than the patients with SS and patients with blepharitis; however, because it has been shown that DED increases with age, we would expect this age difference to lead to a bias toward lower tear osmolarity compared with our patients with dry eye. We adjusted for age in our model and sensitivity analyses revealed no change in our results. Studies using age-matched controls would be helpful in examining this further.

It is also possible that some of our control participants had undiagnosed asymptomatic dry eye. While all control participants had minimal to no symptoms as evidenced by an OSDI score of less than 4, some control participants had minimal positive examination findings. However, most control participants had modified DEWS severity grades of 0 in all but 1 parameter, with half having a modified DEWS severity grade of 0 in all parameters. It was rare to find control participants with absolutely no symptoms or signs of dry eye and, therefore, these patients represented real world patients who we would be evaluating every day in the office. Sensitivity analyses by excluding control participants with a single ocular surface parameter greater than a modified DEWS severity grade of 0 showed similar results.

A final limitation was our relatively small sample size. However, because our goal was to assess the variability in individual patients, we were able to accomplish this by taking multiple measurements in a small number of patients.

**Conclusions**

Our study showed that measurements of tear osmolarity taken with the TearLab system in both patients with dry eye and patients without dry eye are highly variable, replicating and extending the results of previous studies of the TearLab system. Consistent with previous studies, we found a higher degree of variability in patients with DED compared with those without dry eye. This variability was likely due to a combination of the variability of the osmolarity in the tear film itself, sampling of a small volume of tears, and variability in the measurement process. However, whatever the cause of the variability, the interpretation of the values after a single measurement was unclear when the 95% CI included both the normal and abnormal range. In clinical practice, this means that in a given patient, a single tear osmolarity measurement cannot be used to distinguish patients with dry eye from patients without dry eye. This variability may not be useful to follow up patients over time. The high variability among TearLab osmolarity readings calls into question its clinical utility.
Variability of Tear Osmolarity in Patients With Dry Eye

ARTICLE INFORMATION
Submitted for Publication: July 10, 2014; final revision received January 9, 2015; accepted January 25, 2015.
Published Online: March 26, 2015. doi:10.1001/jamaophthalmol.2015.0429.

Author Contributions: Dr Bunya had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Obtained funding: Bunya, Massaro-Giordano.
Administrative, technical, or material support: Fuerst, Salvo, Macchi.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Bunya received research supplies from TearLab for this study. No other disclosures were reported.

Funding/Support: This study was funded by grants K12-EY-015398 (Dr Bunya) and P30 EY001583 (Mr Pistilli and Dr Ying) from the National Eye Institute as well as Research to Prevent Blindness (Drs Bunya and Massaro-Giordano).

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank Maureen Maguire, PhD, University of Pennsylvania, for reviewing earlier versions of our manuscript.

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