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Within Session Repeatability Of The Tear Lab Osmolarity Test And Correlation With Other Clinical Tests For Dry Eye

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WITHIN SESSION REPEATABILITY OF THE TEAR LAB OSMOLARITY TEST AND CORRELATION WITH OTHER CLINICAL TESTS FOR DRY EYE

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ABSTRACT

Background

The human tear film consists of three primary layers that work in conjunction to provide protection and nutrition to the cornea. When a layer is disrupted, the entire tear film loses its integrity and fails to function normally.

Dry eye is the most common cause of tear film disruption. Among several methods to characterize dry eye is measurement of tear osmolarity. The TearLab Osmometer is commonly used clinically for this purpose. Previous studies in this lab with an early version of the TearLab showed a lack of consistency of repeat measurements and a limited range of osmolarity, preventing meaningful comparisons with other clinical test results. The current study investigated the reliability of a newer version of the TearLab and compares results with those of other clinical tests for dry eye.

Methods

Thirty participants were recruited for the study, which consisted of one 45-minute clinical visit. Participants completed an Ocular Surface Disease Index questionnaire followed by: visual acuity, six consecutive osmolarity measurements in each eye, non-invasive tear break-up time (NIBUT), Schirmer I test, anterior segment assessment with slit lamp, fluorescein and Lissamine green vital dye staining, and fluorescein tear break-up time (TBUT).

Statistical analyses were run to explore the repeatability of TearLab measurements, to look for ordering effects and to compare results of individual and averaged measurements with other dry eye tests.

Results

The newer TearLab was significantly more reliable than the original version, but also showed a small range of values across a group of normal, aqueous-deficient and evaporative dry eye patients. The third osmolarity measurement correlated best to the mean of the rest for both the right and left eyes. For all ways of expressing TearLab results, no strong correlations were found with other clinical tests for dry eye.

Conclusion

Despite repeatable measurements, the TearLab osmometer lacked the dynamic range to differentiate between different types and severities of dry eye. It also lacked significant correlations with other clinical tests for dry eye. Other tests, apart from the Schirmer I test, showed better inter-test correlations.

Keywords: TearLab, osmolarity, dry eye, clinical dry eye tests

DEDICATION

This master's project and thesis is dedicated to my parents, to whom I owe everything. In the end, the culmination of all my life's work as a reflection of my gratitude will still be insufficient when compared to all they have given me. Though a meager paragraph seems hardly an appropriate first step, I hope they know how much I love them and how much joy they have brought into my life.

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BACKGROUND

Normal Ocular Surface

The normal ocular surface of the human eye consists of a compound tear film overlying the globe, which contains tear-producing and maintaining structures[1].

Cornea

The cornea is located directly under the tear film and serves as the primary refracting unit of the eye[2]. This is accomplished by maintaining the transparency through precise, regular spacing between stromal components of the cornea and avascularity[1]. Due to its avascular nature, the cornea receives its oxygen and nutrient supply primarily through the tear film, anteriorly, and aqueous humor, posteriorly[1]. It is continuous with the conjunctiva and sclera[1].

Conjunctiva

The conjunctiva lines the insides of eyelids and covers the sclera. It lubricates the eye by secreting mucous and contributes to the tear film, secondary to the lacrimal gland. The lacrimal gland is located inside the temporal portion of the upper eyelid and is the primary contributor to the aqueous layer of the tear film. The gland is expressed with each blink of the eye. The lacrimal accessory glands of Krause and Wolfring, which secrete out to the ocular surface, also aid the lacrimal gland in tear production.

Tear Film

A normal tear film is comprised of three layers: the outer lipid layer, the principal aqueous layer, and the innermost mucous layer. The lipid layer is essential to maintaining the integrity of the overall tear film and is primarily produced by meibomian glands, which reside in the tarsal plate. The lipids provide a hydrophobic barrier between the tears and the outer environment to prevent tears from evaporating from the corneal surface. The aqueous layer, as previously stated, is primarily produced by the lacrimal gland and supplemented by glands of Krause and Wolfring. However, the accessory glands provide the basal, maintenance volume of tears and the lacrimal gland is responsible only for reflex tearing. This layer is hydrophilic and is bordered on either side by hydrophobic layers. The inner hydrophobic layer is comprised of mucins, secreted by conjunctival goblet cells. The mucins coat the epithelium of the cornea, allowing for an even distribution of the tears over the cornea. It also protects the cornea from pathogens that may enter the cornea through the precorneal tear film via external environment[3]. These three layers work in conjunction to prevent evaporation and subsequent hyperosmolarity, maintain an even spread across the corneal surface with each blink, and protect the underlying cornea from pathogens and environmental exposure[4].

Abnormal Ocular Surface

An abnormal ocular surface occurs due to several factors, such as inflammatory or environmental factors, that interrupt the integrity of the tear film[5]. Osmolarity is a measurement of the osmotically active elements in a solution[6], which consist of electrolytes, such as sodium, chloride, bicarbonate ions, potassium, and other similar

electrolytes[7]. These electrolytes maintain the pH of the tear film and stabilize osmolarity[8]. The average osmolarity of normal human tears is measured to be 290 mOsm/L, which is isotonic to a 0.9% NaCl solution. However, patients with dry eye tend to have higher tear osmolarity - 316 mOsm/L or more - and the magnitude of "hyperosmolarity" appears to increase with the severity of dry eye[5]. Tear hyperosmolarity is reported to be a primary cause of ocular surface inflammation, causing an inflammatory cascade, and ocular surface damage[5].

Dry eye results from a multisystem dysfunction that includes the lacrimal glands, the eyelids and its accompanying glands, the ocular surface, and motor and sensory nerves[5]. Dry Eye Disease (DED) has been defined by the Definition and Classification committee of the Dry Eye Workshop taskforce[5]:

> *Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.*

DED affects between 7.4% and 33.7% of the US population[6]. There are essentially two types of dry eye: aqueous deficient and evaporative[5] .

Aqueous Deficient Dry Eye

Aqueous-deficient dry eye (ADDE) is caused by insufficient production of the aqueous portion of the tear film. The primary clinical test for ADDE is the Schirmer test. This is measured as the amount of wetting of a length of filter paper, with the rounded

end folded over and inserted between the lower lid and inferior bulbar conjunctiva. The wetting length is measured after a 5 minute period during which the patient sits with eyelids gently closed[9]. The Schirmer test is described in greater detail in the Experimental Design and Methods.

Evaporative Dry Eye

Evaporative dry eye (EDE) occurs when the lipid portion of the tear film, originating primarily from meibomian glands, is inadequate. This may be due to meibomian gland dysfunction (MGD)[10] creating blocked glands, called inspissation, or meibomian gland dropout. The loss of lipid layer integrity results in greater exposure of the aqueous portion and, therefore, an unstable tear film with faster evaporation rate[11]. EDE creates an unstable tear film and manifests signs such as a decreased tear break-up time (TBUT). TBUT is measured as the time taken after a blink for the tear film to become discontinuous. It can be measured either with or without the addition of topical sodium fluorescein dye.[12, 13] TBUT is described in more detail in the Experimental Design and Methods section.

In more severe dry eye in particular, patients will often elicit positive results for both aqueous deficiency (Schirmer test) and evaporative dry eye (TBUT). In this case, the test providing the lower value of wetting length and TBUT is often used to classify the primary cause.

Additional tests commonly used to characterize dry eye include symptom questionnaires such as the Ocular Surface Disease Index (OSDI), which was validated in 2000 as a dry eye survey[14], external slit lamp examination of the ocular surface and

adnexa[15], ocular surface vital dye staining with sodium fluorescein and Lissamine green[16-18], meibomian gland function tests[10, 11, 19-22] and others. All of these clinical tests will be discussed in more detail later.

Tear Osmolarity and its Measurement

In earlier studies, tear osmolarity measuring devices were based upon the ratio of the number of solute particles (electrolytes, proteins, etc.) to the number of solvent molecules (aqueous fluid) in the tears. The measurement was therefore based on the "colligative" properties of the tears. Colligative properties by definition are independent of the type or types of solute molecules present in the solution[23]. Measurement of osmolarity based on colligative properties can be achieved by measuring the change in freezing point of the solvent caused by the presence of the solute molecules. This is the basis of the "freezing point depression" method[23]. Alternatively, vapor pressure osmometers measure change in osmotic pressure and lowering of vapor pressure by solute particles in solution[24]. Vapor pressure is related to the tendency of particles to escape from the liquid in which they are dissolved in a closed system. It can be thought of as a measure of potential evaporation rate or volatility of the solution. Vapor pressure decreases as the concentration of dissolved particles in a given volume of solvent increases because the intermolecular forces between solute molecules become stronger[25]. There are several drawbacks to these types of tear osmolarity measurements. First, tear volume requirements are substantial, typically being in the 1-5 μL or higher range. This precludes *in situ* measurement. Second, these tests can be time consuming. For these reasons, tear osmolarity measurements based on colligative properties of tears are not

clinically viable[26].

It has been reported that tear osmolarity measurements can also be obtained based on non-colligative properties, such as electrical impedance[27]. While electrical impedance is related to resistance, it applies to resistance to electron flow for an alternating current, which includes not only amplitude, but also phase. Impedance is a property pertaining only to charged particles and therefore cannot account for the presence or abundance of non-charged particles in a solution[28]. This was considered an issue by the FDA in the initial FDA trial of the TearLab (OcuSense) osmometer[29]. The FDA specifically requested that "in addition to the analytical method comparisons to other forms of osmometry produced for the 510(k), the TearLab be tested in clinical practice to determine whether the non-colligative impedance-based method used to measure osmolarity produced measurements in people consistent with previously published studies".[29]

A calibration of the TearLab versus a Wescor vapor pressure osmometer was conducted using a series of calibration (contrived tear) solutions. Results showed that the TearLab underestimated the Wescor value by an average of 8%. The equation for the best fit regression line was TearLab osmolarity = (0.9146 × Wescor osmolarity) + 23.061 mOsm/L[30]. This brings into question the relationship between electrical impedance and vapor pressure-derived osmolarity. However, several studies have since been conducted and the conclusions generally support the use of electrical impedance as a measure of tear osmolarity. Conclusions were based on comparison with results obtained with freezing point depression or vapor pressure osmometry[23, 24, 27].

Clinical Tests for Dry Eye

Historically, objective determination of whether or not a patient has DED has been an issue in clinical practice. This was due primarily to the lack of a widely accepted "gold standard" method of diagnosing DED and a lack of consensus of what the cut-off values for dry eye tests should be. The DEWS report summarized the diagnostic criteria for DED in 2007[5].

At that time, the standard-of-care tests used to diagnose DED were the OSDI questionnaire, visual symptoms, fluorescein Tear Break-Up Time (TBUT), Schirmer I Test, corneal and conjunctival staining with sodium fluorescein dye, tear meniscus assessment, and meibomian gland assessment[5] . These tests gave clinicians the ability to grade the presence and severity of DED in patients[5]. A new "gold standard" for dry eye testing was established with the introduction of the TearLab osmometer, which was validated in 2008. Tear osmolarity has been reported by the manufacturer to have the least variability over time amongst tests for dry eye[31]. Conversely, the clinical use of the TearLab osmometer is viewed by a subset of researchers to be of limited value due to variability in measurements and disagreement within the research community about the appropriate threshold value for dry eye[32]. In terms of a simple classifier of presence or absence of dry eye, the DEWS report determined 316 mOsm/L to be a valid threshold [5]. In contrast, the TearLab company suggests an osmolarity of 308 mOsm/L as its dry eye threshold for the TearLab instrument[33]. Some groups have viewed this as a limitation in the range of the instrument, thereby decreasing its power to differentiate between types of patients based on osmolarity[34].

Despite these issues, the TearLab manufacturer claims that the TearLab

osmometer constitutes a new gold standard for clinical testing of dry eye and has produced several recent reports to support this position. In a multicenter study of mild, moderate and severe dry eye categorized according to the DEWS Report severity index[5], Lemp et al[33] reported that the TearLab test had greater diagnostic performance than five other clinical tests: TBUT, corneal staining, conjunctival staining, Schirmer test, and meibomian gland grading. Greatest sensitivity in differentiating normal from mild or moderate dry eye was found using a threshold of 308 mOsm/L. Specificity was greatest when a higher threshold of 315 mOsm/L was used. Lemp's group found the best compromise between sensitivity (73%) and specificity (92%) with a threshold of 312 mOsm/L[33]. In an extended version of the study in which more centers were added[35], the correlation between each clinical test and disease severity was determined. Osmolarity showed the highest correlation ($R^2 = 0.55$), followed by conjunctival staining ($R^2 = 0.47$), corneal staining ($R^2 = 0.43$), OSDI ($R^2 = 0.41$), meibomian score ($R^2 = 0.37$), TBUT ($R^2 = 0.30$), and Schirmer score ($R^2 = 0.17$). The group did, however, acknowledge that 63% of patients were poorly classified by combinations of clinical thresholds.

Interestingly, other groups report very different findings. One reason for this may be that the approach typically used in these reports places more emphasis on standard clinical test results, while the above TearLab reports present a more skeptical view of the same clinical tests. A study by Massof and McDonnell (2011)[36] proposed a model of dry eye disease "severity" as a single variable (which they called Θ) based on scaled clinical observations that is monotonically related to (always changing in the same direction as) clinical indicators of severity. In their model, Schirmer wetting length, Meibomian score, TBUT, OSDI score, conjunctival staining score, and corneal staining score all agreed

with the predictions of the monotonic severity model. However, TearLab tear osmolarity values did not agree with the predictions of the model, instead showing a nonmonotonic relationship. Massof and McDonnell[36] concluded that the relationship between tear osmolarity and dry eye disease severity is either significantly distorted by confounding variables (perhaps poor repeatability as discussed below), or does not exist. Similarly, Messmer et al[37] found that six clinical tests for dry eye, OSDI, corneal fluorescein staining, conjunctival Lissamine green staining, TBUT, Schirmer score, and the presence of blepharitis or meibomitis were not correlated with tear osmolarity.

The TearLab has been criticized for variability in results and an inability to show a broad enough osmolarity range to differentiate between control and dry eye patients. This is especially true for borderline or mild dry eye patients[32]. Messmer et al[37] found a tear osmolarity of 308.9 ± 14.0 mOsm/L in dry eye patients versus 307.1 ± 11.3 mOsm/L in controls. Patients were defined as having dry eye in their study if they met at least three of the following criteria: OSDI score > 15, corneal fluorescein staining in the interpalpebral area, conjunctival Lissamine green staining in the interpalpebral area, TBUT < 7 s, Schirmer wetting length < 7 mm in 5 min, presence of blepharitis or meibomitis.

In the PI's lab, early experience with the original version of the TearLab in other dry eye studies was not a positive one. This was based on several observations about the device: (i) it showed a high degree of variability when running repeat measurements on a given patient, (ii) it was prone to calibration failure, despite using it strictly according to manufacturer's instructions, i.e. maintaining the device in a constant location and adopting the recommended warm-up and equilibration procedures, and (iii) the range of

osmolarity readings, despite the instrument's variability, was relatively narrow. A combination of the above factors precluded any meaningful differentiation among patient groups based on osmolarity.

More recently, a newer version of the TearLab was obtained from the manufacturer. Given the experience with the earlier version of the instrument, it was considered essential to evaluate the newer device for calibration consistency, measurement stability and repeatability, and to conduct an initial evaluation of its potential to differentiate among dry eye groups. The intent was not to conduct a comprehensive dry eye study, which was beyond the scope of the current project. Rather, the purpose of the current study was to determine if the newer version of the TearLab device showed greater potential to classify dry eye patients than the previous model.

SPECIFIC AIMS AND STUDY HYPOTHESES

Specific Aim 1

To determine the ability of the TearLab to produce consistent measurements of tear osmolarity, and to determine if a single measurement, as recommended by the manufacturer, provides an equivalent result to repeated measurements in both control and dry eye patients.

Specific Aim 2

To determine the correlation between TearLab results and results of clinical tests commonly used to differentiate dry eye patients from non-dry eye patients.

Study Hypothesis

The study hypothesis consists of two parts, each addressing one of the specific aims.

Study Hypothesis 1

A single measurement of tear osmolarity with the TearLab produces an equivalent result to six measurements in terms of classifying the patient's dry eye status by osmolarity.

Null Hypothesis 1

A single measurement of tear osmolarity with the TearLab does not produce an equivalent result to six measurements in terms of classifying the patient's dry eye status by osmolarity.

Study Hypothesis 2

The most reliable value or mean value for tear osmolarity as determined by Specific Aim 1 shows significant correlations with other clinical tests for dry eye.

Null Hypothesis 2

The most reliable value or mean value for tear osmolarity as determined by Specific Aim 1 does not show significant correlations with other clinical tests for dry eye.

JUSTIFICATION OF AIMS

Specific Aim 1

The current recommendation by the TearLab manufacturer, consistent with the protocol for insurance coverage, is to perform one TearLab osmolarity measurement per eye. Based on experience with an early version of the TearLab, it is anticipated that a single measurement, by failing to capture the variability in the tear osmolarity reading, will be unable to provide the true diagnostic potential of tear osmolarity. An alternative method is to take multiple readings and perform statistical analyses for each patient: range of values, mean and standard deviation, investigation of trends (e.g. ordering effects), determine if a particular reading in the sequence agrees best with the average of the remaining values across a group of patients, and other tests. This study will use within-session repeat measurements of tear osmolarity to test for the above trends.

Specific Aim 2

It is anticipated that repeated measurements of tear osmolarity within one clinical session should provide a more reliable TearLab value for osmolarity along with a measure of test variability for each patient. Assuming that a more reliable osmolarity value is obtained, this should provide a sound basis for correlating tear osmolarity with other clinical dry eye tests. Demonstrating repeatability of osmolarity measurements will enable a true assessment of its correlation with other clinical test results.

EXPERIMENTAL DESIGN AND METHODS

Study Design

Thirty study participants were enrolled: 10 non-dry eye participants, 10 ADDE participants, and 10 evaporative dry eye participants. Each participant was given one clinical dry eye evaluation, lasting approximately 45 minutes. 20 patients with clinically-defined dry eye were determined to be a minimum valid sample population based on a study by Suzuki et al, in which they recruited 19 dry eye patients[38]. The study found a weak, but significant correlation between TearLab osmolarity and Schirmer score[38].

Exclusion criteria included the following: infectious conditions of the eye, non-dry eye ocular surface disease, recent ocular surgery, allergies to the ingredients of sodium fluorescein, Lissamine green, or 0.9% saline, and the inability or unwillingness to follow instructions during clinical testing.

The study was designed to compare a single measurement of TearLab osmolarity with six repeat measurements to differentiate the three patient groups and to determine the correlation between tear osmolarity and other tests for dry eye.

Clinical Evaluation

After the patient signed an Informed Consent form, tests were performed in the following sequence. A patient history form was completed to obtain any pertinent medical or ocular history. The Ocular Surface Disease Index (OSDI) questionnaire with

12 items to attain a subjective assessment of dry eye severity was administered[39]. Visual acuity, with or without correction depending on the patient's refractive status, was measured using a Snellen chart viewed at 20 feet in a darkened room. Tear osmolarity readings were taken using the TearLab osmometer. The TearLab osmometer was maintained in the same location and was turned on the entirety of the study. The device was calibrated per manual specifications every morning (Tearlab Manual, Revision C, 2012)[30].

The TearLab device includes two separate pens to which the osmolarity measuring cards can be attached for tear osmolarity measurement. The pen used on each patient and the initial eye to obtain measurements were both randomly selected. Six consecutive measurements were then obtained from the first eye followed by six from the contralateral eye. Each measurement, including sample collection and readout of the result was completed within less than the allotted 60 seconds, and the next measurement was commenced immediately after completion of the previous. The sampling procedure followed the manufacturer's standard directions. After attaching a test card to the pen and the green indicator light illuminated, the patient was instructed to look "up and away" from the direction of the pen. The investigator inserted the tip into the inferior marginal strip of the tear film on the border of the middle and outer thirds of the eye. When sufficient tear fluid had been collected, the TearLab indicator light turned off and it emitted an audible beep. The pen was immediately docked into the measuring station, and the tear osmolarity measurement was made[30].

Tear film stability was next measured with the non-invasive break-up time (NIBUT) test, using a slit lamp-attached TearScope (Keeler Instruments Inc., Broomall,

PA). The TearScope projects a concentric, grid-like pattern onto the tear film, and the clinician uses the built-in timer to obtain the time from blink to initial break in the grid pattern. This indicates initial tear film break-up and is recorded as NIBUT.

The Schirmer I test, which measures aqueous tear production, was conducted without the use of anesthesia by placing calibrated filter paper strips at the border of the mid and lateral third of the lower lid margin between the palpebral and bulbar conjunctiva of each eye with a 5-mm notch bent onto the palpebral conjunctiva and the remaining length of the strip protruding down below the eyelid. The participant was instructed to close his or her eyes and, after 5 minutes, the Schirmer strip was removed and the tear wetting length recorded, in millimeters, using the imprinted Schirmer strip scale. The Schirmer test generally measures the total gross tear volume and is typically reported to be > 10 mm/5 minutes in normal patients. The threshold for aqueous-deficient dry eye varies between studies, values of < 10 mm/5 minutes, < 7 mm/5 minutes and < 5 mm/5 minutes being reported by different study groups[40]. In the current study, the severity of aqueous-deficient dry eye patients used the following cutoff measurements: <10mm/5min for mild/moderate ADDE and ≤5mm/5min for severe ADDE.

General ocular health was next observed using a slit lamp. The principal emphasis was for clinical findings that may contribute to dry eye. Corneal evaluation included signs of desiccating stress or disease. The conjunctiva was examined for erythema, concretions, or other ocular surface irregularities that may contribute to vital dye staining. The puncta were assessed for patency and the meibomian glands examined for inspissation and dropout. The clinician looked for eyelid irregularities and malposition, which may adversely affect complete coverage of the eyes with tears with blinking. The

greatest volume of tears is found at the eyelid margin, and the tear meniscus height was measured at the center of the lower eyelid meniscus using a Zeiss graticule eyepiece attached to the slit lamp. A tear meniscus height of 1 mm or more is considered normal.

Vital dye staining scores of the conjunctiva and cornea were measured using the Oxford Scale for both sodium fluorescein and Lissamine green. The sodium fluorescein strip was moistened with a drop of sterile saline and then applied to the inferior palpebral conjunctiva. The patient was instructed to blink multiple times. After 1 minute to let the dye infiltrate the apoptotic cells, the clinician graded the nasal and temporal conjunctiva and cornea using the Oxford scale based on a range from zero (no staining) to five (extensive staining). This procedure was then repeated on the contralateral eye. A slit lamp filter, Boston #7503, was used to enhance contrast of the observed staining pattern. Similarly, Lissamine green dye was placed onto the eye using a pre-wetted strip. For this dye, a white light illumination source was used with a Tiffen Red (Kodak Wratten #25 equivalent) 1, to enhance contrast.

Concurrently with the assessment of fluorescein staining, the standard fluorescein tear breakup time (TBUT) test was also conducted. A sodium fluorescein strip was moistened with a drop of sterile saline, and the strip was applied to the patient's inferior palpebral conjunctiva as described above. After the fluorescein solution mixed with the tear film to form a green fluorescent pattern across the corneal surface, a slit lamp biomicroscope with cobalt blue illumination was used to view the pattern. The patient was instructed to blink and then keep the eye open as long as possible while the clinician observed the fluorescent pattern. The time taken for the first break to occur was recorded

as the TBUT. Patients with TBUT <10 seconds were classified as mild/moderate EDE, and patients with TBUTs<5 seconds were classified as severe EDE.

Statistical Analysis

Analyses were designed to explore the repeatability of the TearLab measurements and the advantages of taking six measurements versus one. They were also used to correlate the results of a single TearLab measurement with other clinical tests. This was repeated for each replicate measurement. These analyses addressed the possibility of an "ordering effect" or "temporal effect" on the TearLab value, such as whether stimulated tearing affected sequential measurements, and whether there was a specific replicate measurement that correlated best with the other clinical tests. A comparison was then conducted of the correlation between the mean of six osmolarity measurements and other clinical tests.

Internal consistency was explored to determine the influence of within subject variability of TearLab results versus correlation with other clinical tests. To visualize the effect, variability was plotted against results of other clinical tests. Cronbach's Alpha was used to explore correlations among the six TearLab measurements with the ultimate goal of identifying the value that correlates best with the mean of the other five measurements. This value would be flagged as the most reliable single measure. The procedure for Cronbach's alpha test consisted of correlating measurement 1 with the mean of measurements 2-6, then correlating measurement 2 with the mean of measurements 1, 3-6, and so on. Intra-class correlation analysis was used as an alternative to Cronbach's Alpha to see if the outcomes of the two analyses agreed. Intra-class correlations are most

often used to determine consistency among “raters.” An example of this is if six different doctors assessed the severity of liver damage on a given patient with cirrhosis. Intra-class correlation analysis would be able to identify raters who are not consistent with the rest. For the current study, multiple measurements, not “raters,” were compared to find which measurement best agreed with the mean of the rest.

Further analyses were also conducted to further explore correlations among clinical test results and to relate them to tear osmolarity findings.

RESULTS

Demographics of Study Participants

Initial participant inclusion criteria for the study were intended to fulfill the power analysis criterion of at least 19 participants with no less than 50% classified as dry eye by commonly accepted criteria. This was deliberately expanded to incorporate a relatively equal number of normal control, predominantly aqueous-deficient, and predominantly evaporative dry eye patients, again based on commonly accepted clinical criteria. Table 1 shows the age distribution of study participants by group. Analysis of variance (ANOVA) of mean group ages shows no significant differences among groups. Gender distribution was biased towards females, as is the overall population trend, with seven females in the control group, nine in the ADDE group, and seven in the EDE group.

Table 1 – Demographics of Patients based on Initial Classification of Dry Eye

Group	**Normal**	**ADDE**	**EDE**	
n	10	9	11	
Mean Age (± sd)	52.4 ± 14.1	53.2 ± 13.0	48.0 ± 21.7	
ANOVA				
Source of Variation	DF	SS	MS	F	P
Between Groups	2	168.74	84.37	0.283	0.756
Residual	27	8041.96	297.85		
Sjögren's Syndrome	n = 0	n = 0	n = 4	
Systemic Conds	1.30 ± 1.49	2.39 ± 2.29	2.32 ± 2.33	ns
Systemic Meds	1.50 ± 1.78	1.56 ± 2.24	1.91 ± 1.92	ns
Rx Dry Eye Meds	0	0	0	ns

Power of ANOVA (age) with alpha = 0.05, 0.049

ADDE = aqueous-deficient dry eye; EDE = evaporative dry eye; n = number of participants; sd = standard deviation, conds = conditions; meds = medications.

One of the most surprising findings when participants were grouped by primary cause of dry eye (Schirmer test for ADDE; TBUT for EDE) was that all four participants with rheumatologist-confirmed diagnoses of Sjögren's Syndrome were classified as EDE (Table 1). This was the case despite no reported prescription dry eye medications (e.g. Lotemax or Restasis) for any patients. All four Sjögren's Syndrome patients were using only palliative artificial tears and/or gels. To investigate distribution of important systemic conditions and medications, participants received a score out of a maximum of seven, one point for each of the following systemic conditions: hypertension/elevated cholesterol; diabetes; thyroid condition; other autoimmune condition (excluding Sjögren's Syndrome), asthma, cancer, anxiety/depression. Results are shown as mean ± standard deviation in Table 1. While the data were not normally distributed, there were no significant inter-group differences in the number of reported systemic conditions by Kruskal-Wallis ANOVA on Ranks. Similarly, patients were assigned scores out of seven based on the number of systemic conditions (from the above list) for which they were currently taking prescription medications. Again there were no significant intergroup differences by Kruskal-Wallis ANOVA on Ranks. As stated above, no participants from any of the three groups were currently taking any prescribed dry eye medications. However, as expected, the majority of patients from the ADDE and EDE groups were regularly using palliative artificial tears or gels.

Specific Aim 1

Repeatability of TearLab Osmolarity Measurements

Table 2 shows descriptive statistics for the six consecutive measurements of tear osmolarity taken in each patient's right eye. No trend towards increasing or decreasing osmolarity during the collection sequence is evident from the data and the range of mean values is relatively small. For one participant with a prosthetic right eye, no tests were conducted on this eye, resulting in 29 right eye and 30 left eye measurements for the overall study group.

Table 2 – Right Eye Osmolarity Repeat Measurement Statistics (6 replicates)

Measurement	N	Mean	Stdev	Sum	Min	Max
Osmolarity_OD_1R	29	297.52	9.87	8628	279	323
Osmolarity_OD_2R	29	298.24	11.21	8649	282	320
Osmolarity_OD_3R	29	297.69	11.93	8633	278	328
Osmolarity_OD_4R	29	298.52	9.28	8657	282	319
Osmolarity_OD_5R	29	299.55	10.66	8687	279	328
Osmolarity_OD_6R	29	300.14	13.01	8704	281	333

Osmolarity_OD_1R = first osmolarity measurement from the right eye; N = number of eyes, StDev = standard deviation of mean, Min = minimum, Max = maximum.

Cronbach's Alpha test is based on deletion of one of the 6 repeat osmolarity measurements (e.g. Repeat 1) for all patients and calculating the mean of the 5 remaining values. This mean is then compared to the single measurement excluded from the mean calculation and the process is repeated for the second through sixth repeat measurement. Correlations between each single osmolarity measurement and the mean of the remaining measurements by Cronbach's Alpha test are listed in Tables 3 and 4. According to this analysis for the right eye, measurement 3 correlated best with the mean of the other five values, followed by measurement 5.

Table 3 – Right Eye Osmolarity Cronbach's Alpha with Deleted Variables

	Raw Variables		Standardized Variables	
Deleted Variable	**Correlation with Total**	**Alpha**	**Correlation with Total**	**Alpha**
Osmolarity_OD_1R	0.664	0.889	0.653	0.981
Osmolarity_OD_2R	0.666	0.889	0.671	0.889
Osmolarity_OD_3R	**0.816**	**0.865**	**0.820**	**0.866**
Osmolarity_OD_4R	0.651	0.891	0.646	0.892
Osmolarity_OD_5R	**0.791**	**0.870**	**0.782**	**0.872**
Osmolarity_OD_6R	0.780	0.973	0.782	0.872

Osmolarity_OD_1R = first osmolarity measurement from the right eye

Table 4 – Pearson Correlation between Deleted Osmolarity Measurement and Mean of Remaining Values (Right Eye)

	OD 1R	OD 2R	**OD 3R**	OD 4R	**OD 5R**	OD 6R
Osm OD 1R	1.0000	0.4540	**0.6462**	0.3801	**0.5843**	0.6637
P value	-	0.0134	**0.0002**	0.0420	**0.0009**	<.0001
Osm OD 2R	0.4540	1.0000	**0.6215**	0.6038	**0.5891**	0.5230
P value	0.0134	-	**0.0003**	0.0005	**0.0008**	0.0036
Osm OD 3R	0.6462	0.6215	**1.0000**	0.6489	**0.7139**	0.6780
P value	0.0002	0.0003	**-**	0.0001	**<.0001**	<.0001
Osm OD 4R	0.3801	0.6038	**0.6489**	1.0000	**0.5251**	0.5451
P value	0.0420	0.0005	**0.0001**	-	**0.0034**	0.0022
Osm OD 5R	0.5842	0.5891	**0.7139**	0.5251	**1.0000**	0.7680
P value	0.0009	0.0008	**<.0001**	0.0034	**-**	<.0001
Osm OD 6R	0.6637	0.5230	**0.6780**	0.5451	**0.7680**	1.0000
P value	<.0001	0.0036	**<.0001**	0.0022	**<.0001**	-

Osm OD 1R and OD 1R = first osmolarity measurement from the right eye; P value = significance level of the correlation

Table 5 shows the descriptive statistics for the six consecutive tear osmolarity measurements taken in each patient's left eye. No trends towards increasing or decreasing

osmolarity during the collection sequence are evident. Similarly to the right eye, the range of mean osmolarity values is relatively small in the left eye. However, the maximum values of left eyes measurements were consistently higher than for the right eye.

Table 5 – Left Eye Osmolarity Repeat Measurement Statistics

Measurement	N	Mean	Stdev	Sum	Min	Max
Osmolarity_OS_1L	30	303.80	15.10	9114	280	346
Osmolarity_OS_2L	30	304.17	16.20	9125	281	346
Osmolarity_OS_3L	30	298.33	8.45	8950	285	316
Osmolarity_OS_4L	30	300.40	11.49	2012	285	323
Osmolarity_OS_5L	30	301.20	10.64	9036	284	331
Osmolarity_OS_6L	30	301.27	12.95	9038	281	341

Osmolarity_OS_1L = first osmolarity measurement from the left eye; N = number of eyes, StDev = standard deviation of mean, Min = minimum, Max = maximum.

Correlations between each individual left eye tear osmolarity measurement and the mean of the remaining measurements by Cronbach's Alpha test are described in Tables 6 and 7. According to the analysis, measurement 3 correlated best with the mean of the other five values.

Table 6 – Left Eye Osmolarity Cronbach's Alpha with Deleted Variables

	Raw Variables		Standardized Variables	
Deleted Variable	**Correlation with Total**	**Alpha**	**Correlation with Total**	**Alpha**
Osmolarity_OS_1L	0.623	0.869	0.637	0.888
Osmolarity_OS_2L	0.707	0.856	0.716	0.875
Osmolarity_OS_3L	**0.851**	**0.843**	**0.839**	**0.856**
Osmolarity_OS_4L	0.748	0.844	0.753	0.870
Osmolarity_OS_5L	0.730	0.849	0.738	0.872
Osmolarity_OS_6L	0.602	0.867	0.617	0.891

Osmolarity_OS_1L = first osmolarity measurement from the left eye

Table 7 – Pearson Correlation between Deleted Osmolarity Measurement and Mean of Remaining Values (Left Eye)

	OS 1L	OS 2L	**OS 3L**	OS 4L	OS 5L	OS 6L
Osm OS 1L	1.0000	0.4540	**0.6462**	0.3801	0.5843	0.6637
P value	-	0.0134	**0.0002**	0.0420	0.0009	<.0001
Osm OS 2L	0.4540	1.0000	**0.6215**	0.6038	0.5891	0.5230
P value	0.0134	-	**0.0003**	0.0005	0.0008	0.0036
Osm OS 3L	0.6462	0.6215	**1.0000**	0.6489	0.7139	0.6780
P value	0.0002	0.0003	**-**	0.0001	<.0001	<.0001
Osm OS 4L	0.3801	0.6038	**0.6489**	1.0000	0.5251	0.5451
P value	0.0420	0.0005	**0.0001**	-	0.0034	0.0022
Osm OS 5L	0.5842	0.5891	**0.7139**	0.5251	1.0000	0.7680
P value	0.0009	0.0008	**<.0001**	0.0034	-	<.0001
Osm OS 6L	0.6637	0.5230	**0.6780**	0.5451	0.7680	1.0000
P value	<.0001	0.0036	**<.0001**	0.0022	<.0001	-

Osm OS 1L and OS 1L = first osmolarity measurement from the left eye; P value = significance level of the correlation.

Specific Aim 2

Correlations between TearLab Osmolarity Measurements and other Clinical Tests

Table 8 shows the individual osmolarity measurements of the right eye compared to measurements of other clinical tests for dry eye. The individual osmolarity measurements are averaged across the group. Osmolarity measurement 2 was shown to correlate significantly with the OSDI questionnaire, but none of the other osmolarity measurements showed this correlation.

Table 8 – Pearson Correlation between Individual Osmolarity Measurements and other Clinical Tests for Dry Eye (Right Eye)

	OSDI	NIBUT	TBUT	Schirm	TMH	NaFl	LissG
Osm OD 1R	0.0205	0.2095	0.1683	-0.1219	0.0503	0.1657	0.0544
P value	0.9159	0.2753	0.3829	0.5287	0.7955	0.3902	0.7792
Osm OD 2R	0.4313	-0.2231	-0.1272	-0.1613	-0.2700	0.2440	0.2005
P value	0.0195	0.2448	0.5110	0.4033	0.1567	0.2020	0.2970
Osm OD 3R	0.0899	0.2052	0.2425	-0.1628	-0.0748	-0.0561	-0.0641
P value	0.6428	0.2857	0.2050	0.3987	0.6996	0.7723	0.7413
Osm OD 4R	0.2816	-0.1371	-0.1207	0.0101	0.0006	0.0292	0.2821
P value	0.1389	0.4784	0.5329	0.9587	0.9976	0.8805	0.1381
Osm OD 5R	0.2661	0.1681	-0.0619	-0.3002	-0.0838	-0.0283	0.0993
P value	0.1630	0.3834	0.7498	0.1136	0.6655	0.8842	0.6082
Osm OD 6R	0.0586	0.1521	-0.0601	-0.1409	0.0389	-0.0357	-0.1335
P value	0.7627	0.4308	0.7570	0.4659	0.8413	0.8542	0.4900

Osm OD 1R = first osmolarity measurement from the right eye; OSDI = Ocular Surface Disease Index questionnaire; NIBUT = non-invasive tear break-up time; TBUT = fluorescein tear break-up time; Schirm = Schirmer I test; TMH = tear meniscus height; NaFl = fluorescein vital dye staining; LissG = Lissamine green vital dye staining

Table 9 continues from Table 8 with correlations between osmolarity measurement of the right eye and other clinical tests for dry eye.

Table 9 – Pearson Correlation between Osmolarity Measurements and other Clinical Tests for Dry Eye, cont. (Right Eye)

	DEWS Severity (Higher)	DEWS Severity OD
Osm OD 1R	-0.0559	-0.0490
P value	0.7734	0.8007
Osm OD 2R	0.2694	0.3436
P value	0.1576	0.0680
Osm OD 3R	-0.1568	-0.0762
P value	0.4165	0.6943
Osm OD 4R	0.1266	0.1683
P value	0.5129	0.3828
Osm OD 5R	0.1525	0.2298
P value	0.4298	0.2305
Osm OD 6R	-0.0051	0.0279
P value	0.9792	0.8859

Osm OD 1R = first osmolarity measurement from the right eye; DEWS Severity (Higher) = the higher value between the right and left eye from DEWS severity grading; DEWS Severity OD = Dews severity grading of the right eye.

Table 10 and Table 11 show the individual osmolarity measurements of the left eye compared to measurements of other clinical tests for dry eye. The individual osmolarity measurements are averaged across the group. There are four significant correlations between individual osmolarity measurements and other clinical test results. Osmolarity measurement 2 and measurement 5 have a significant correlation with the OSDI questionnaire score. Osmolarity measurement 4 is close to having a significant correlation with OSDI. All other clinical tests correlate with one or zero osmolarity repeats.

Table 10 – Pearson Correlation between Osmolarity Measurements and other Clinical Tests for Dry Eye (Left Eye)

	OSDI	NIBUT	TBUT	Schirm	TMH	NaFl	LissG
Osm OS 1L	0.2278	-0.0523	-0.2749	-0.3290	-0.1733	0.1976	0.3010
P value	0.2260	0.7836	0.1415	0.0758	0.3597	0.2952	0.1061
Osm OS 2L	0.3815	-0.1274	-0.2076	-0.2360	-0.2616	0.1489	0.2069
P value	0.0375	0.5024	0.2709	0.2094	0.1625	0.4324	0.2727
Osm OS 3L	0.2030	-0.0441	-0.1760	-0.2189	-0.2497	0.3047	0.4050
P value	0.2820	0.8172	0.3521	0.2452	0.1833	0.1016	0.0264
Osm OS 4L	0.3275	-0.1620	-0.1640	-0.0839	-0.0727	0.1456	0.3011
P value	0.0772	0.3923	0.3864	0.6592	0.7025	0.4426	0.1059
Osm OS 5L	0.3826	-0.1913	-0.2285	-0.2647	-0.2712	0.3241	0.2668
P value	0.0369	0.3114	0.2246	0.1575	0.1471	0.0806	0.1541
Osm OS 6L	0.0788	-0.2774	-0.3827	-0.1266	-0.1871	0.0907	-0.0045
P value	0.6791	0.1378	0.0368	0.5052	0.3221	0.6335	0.9810

Osm OS 1L = first osmolarity measurement from the left eye; OSDI = Ocular Surface Disease Index questionnaire; NIBUT = non-invasive tear break-up time; TBUT = fluorescein tear break-up time; Schirm = Schirmer I test; TMH = tear meniscus height; NaFl = fluorescein vital dye staining; LissG = Lissamine green vital dye staining.

Table 11 – Pearson Correlation between Osmolarity Measurements and other Clinical Tests for Dry Eye, cont. (Left Eye)

	DEWS Severity (Higher)	DEWS Severity OS
Osm OS 1L	0.2628	0.2869
P value	0.1606	0.1243
Osm OS 2L	0.2895	0.2812
P value	0.1207	0.1323
Osm OS 3L	0.2526	0.2462
P value	0.1781	0.1898
Osm OS 4L	0.2150	0.2269
P value	0.2540	0.2279
Osm OS 5L	0.2625	0.3260
P value	0.1611	0.0787
Osm OS 6L	0.1048	0.1099
P value	0.5814	0.5633

Osm OS 1L = first osmolarity measurement from the left eye; DEWS Severity (Higher) = the higher value between the right and left eye from DEWS severity grading; DEWS Severity OS = Dews severity grading of the left eye.

Correlations between Mean Tear Osmolarity (TearLab) and Other Clinical Test Results

Table 12 shows the correlations between mean osmolarity and other clinical tests for dry eye. No significant correlations are seen between tear osmolarity and other clinical tests for either eye. Trends approaching significance were evident for the relationship between mean osmolarity in the left eye and OSDI score ($p<0.067$), and mean osmolarity and DEWS severity score in the left eye ($p<0.097$).

Table 13 shows correlations between the "most representative" individual osmolarity measurement from Cronbach's Alpha, osmolarity measurement 3 and other clinical tests results. Lissamine green staining correlates significantly ($p<0.026$), but nothing else shows a significant correlation.

Table 12 - Correlations between Mean Tear Osmolarity and Other Clinical Test Results

Dependent Variable	Independent Variable	R	R^2	P	Normal Dist^n (P>0.05)	Constant Variance (P>0.05)	Power (α=0.05)
Me Osm OD	OSDI	0.228	0.052	0.234	0.445	0.661	0.219
Me Osm OS	OSDI	0.338	0.114	0.067	0.345	0.944	0.448
Me Osm OD	TBUT OD	0.011	0.000	0.957	0.526	0.699	0.028
Me Osm OS	TBUT OS	0.303	0.092	0.102	0.042*	0.590	0.369
Me Osm OD	NIBUTOD	0.084	0.007	0.667	0.751	0.548	0.063
Me Osm OS	NIBUTOS	0.177	0.031	0.350	0.261	0.778	0.151
Me Osm OD	Schirm OD	0.183	0.034	0.342	0.793	0.725	0.155
Me Osm OS	Schirm OS	0.268	0.072	0.152	0.301	0.347	0.298
Me Osm OD	TMH OD	0.070	0.005	0.717	0.710	0.383	0.055
Me Osm OS	TMH OS	0.252	0.063	0.180	0.111	0.385	0.267
Me Osm OD	NaFl OD	0.059	0.004	0.760	0.561	0.585	0.049
Me Osm OS	NaFl OS	0.239	0.057	0.204	0.104	0.970	0.243
Me Osm OD	LissG OD	0.073	0.005	0.705	0.076	0.056	0.056
Me Osm OS	LissG OS	0.295	0.087	0.114	0.253	0.818	0.352
Me Osm OD	DEWS S H	0.062	0.004	0.751	0.478	0.718	0.005
Me Osm OS	DEWS S H	0.291	0.085	0.118	0.197	0.976	0.344
Me Osm OD	DEWSS OD	0.126	0.016	0.513	0.603	0.918	0.095
Me Osm OS	DEWSS OS	0.309	0.096	0.097	0.188	0.993	0.382

Me Osm OD = mean osmolarity in the right eye; Me Osm OS = mean osmolarity in the left eye; OSDI = Ocular Surface Disease Index questionnaire; TBUT OD = fluorescein tear break-up time in the right eye; TBUT OS= fluorescein tear break-up time in the left eye; NIBUT OD = non-invasive tear break-up time in the right eye; NIBUT OS = non-invasive tear break-up time in the left eye; Schirm OD= Schirmer I test in the right eye; Schirm OS= Schirmer I test in the left eye; TMH OD = tear meniscus height in the right eye; TMH OS = tear meniscus height in the left eye; NaFl OD = fluorescein vital dye staining in the right eye; NaFl OS = fluorescein vital dye staining in the left eye; LissG OD = Lissamine green vital dye staining in the right eye; LissG OS = Lissamine green vital dye staining in the left eye; DEWS S H = the higher value between the right and left eye from DEWS severity grading; DEWSS OD = Dews severity grading of the right eye; DEWSS OS = Dews severity grading of the left eye.

Table 13 - Correlations between Tear Osmolarity (Msmt. 3) and Other Clinical Results

Dependent Variable	Independent Variable	R	R^2	P	Normal Dist^n (P>0.05)	Constant Variance (P>0.05)	Power (α=0.05)
Osm (3) OD	OSDI	0.090	0.008	0.643	0.148	0.523	0.067
Osm (3) OS	OSDI	0.203	0.041	0.282	0.048*	0.295	0.187
Osm (3) OD	TBUT OD	0.242	0.059	0.205	0.291	0.055	0.242
Osm (3) OS	TBUT OS	0.176	0.031	0.352	0.010*	0.091	0.150
Osm (3) OD	NIBUTOD	0.205	0.008	0.286	0.364	0.110	0.184
Osm (3) OS	NIBUTOS	0.044	0.002	0.817	0.111	0.167	0.042
Osm (3) OD	Schirm OD	0.163	0.027	0.399	0.271	0.174	0.131
Osm (3) OS	Schirm OS	0.219	0.048	0.245	0.246	0.192	0.211
Osm (3) OD	TMH OD	0.075	0.006	0.700	0.246	0.883	0.057
Osm (3) OS	TMH OS	0.250	0.062	0.183	0.074	0.749	0.263
Osm (3) OD	NaFl OD	0.056	0.003	0.772	0.209	0.071	0.047
Osm (3) OS	NaFl OS	0.305	0.093	0.102	0.144	0.771	0.373
Osm (3) OD	LissG OD	0.064	0.004	0.741	0.342	0.009*	0.051
Osm (3) OS	LissG OS	0.405	0.164	0.026*	0.056	0.866	0.607
Osm (3) OD	DEWS S H	0.157	0.025	0.417	0.444	0.235	0.124
Osm (3) OS	DEWS S H	0.253	0.064	0.178	0.013*	0.481	0.268
Osm (3) OD	DEWSS OD	0.076	0.006	0.694	0.271	0.444	0.058
Osm (3) OS	DEWSS OS	0.246	0.061	0.190	0.019*	0.516	0.257

Osm (3) OD = osmolarity measurement 3 in the right eye; Osm (3) OS = osmolarity measurement 3 in the left eye; OSDI = Ocular Surface Disease Index questionnaire; TBUT OD = fluorescein tear break-up time in the right eye; TBUT OS= fluorescein tear break-up time in the left eye; NIBUT OD = non-invasive tear break-up time in the right eye; NIBUT OS = non-invasive tear break-up time in the left eye; Schirm OD= Schirmer I test in the right eye; Schirm OS= Schirmer I test in the left eye; TMH OD = tear meniscus height in the right eye; TMH OS = tear meniscus height in the left eye; NaFl OD = fluorescein vital dye staining in the right eye; NaFl OS = fluorescein vital dye staining in the left eye; LissG OD = Lissamine green vital dye staining in the right eye; LissG OS = Lissamine green vital dye staining in the left eye; DEWS S H = the higher value between the right and left eye from DEWS severity grading; DEWSS OD = Dews severity grading of the right eye; DEWSS OS = Dews severity grading of the left eye.

Correlations among other Clinical Test Results using Regression Analysis

1. DEWS Severity (Higher Eye) vs OSDI

Results for the regression analysis and analysis of variance (ANOVA) of the regression are listed in Table 14. A statistically significant correlation was found between DEWS Severity and OSDI ($p<0.001$), using the eye with higher severity score for each patient. ANOVA confirmed the significance of the regression with good statistical power, and data distribution parameters showed a normal distribution and constant variance. Figure 1 shows a plot of the correlation between DEWS Severity for the eye with higher severity score and OSDI.

Table 14: Regression Analysis and ANOVA for DEWS Severity (Higher Eye) vs OSDI

Regression Equation		DEWS Severity (Higher Eye) =1.053 + (0.0253 × OSDI) (N = 30, R = 0.722, R^2 = 0.522)			
Regression		Coefficient	Std. Error	t	P
	Constant	1.053	0.103	10.239	<0.001
	OSDI	0.0253	0.00457	5.529	<0.001
ANOVA	DF	SS	MS	F	P
Regression	1	5.651	5.651	30.566	<0.001
Residual	28	10.828	0.373		
					P
Normality Test (Shapiro-Wilks)			Passed		0.867
Constant Variance Test			Passed		0.391
Power of test with alpha = 0.05, 0.997					

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

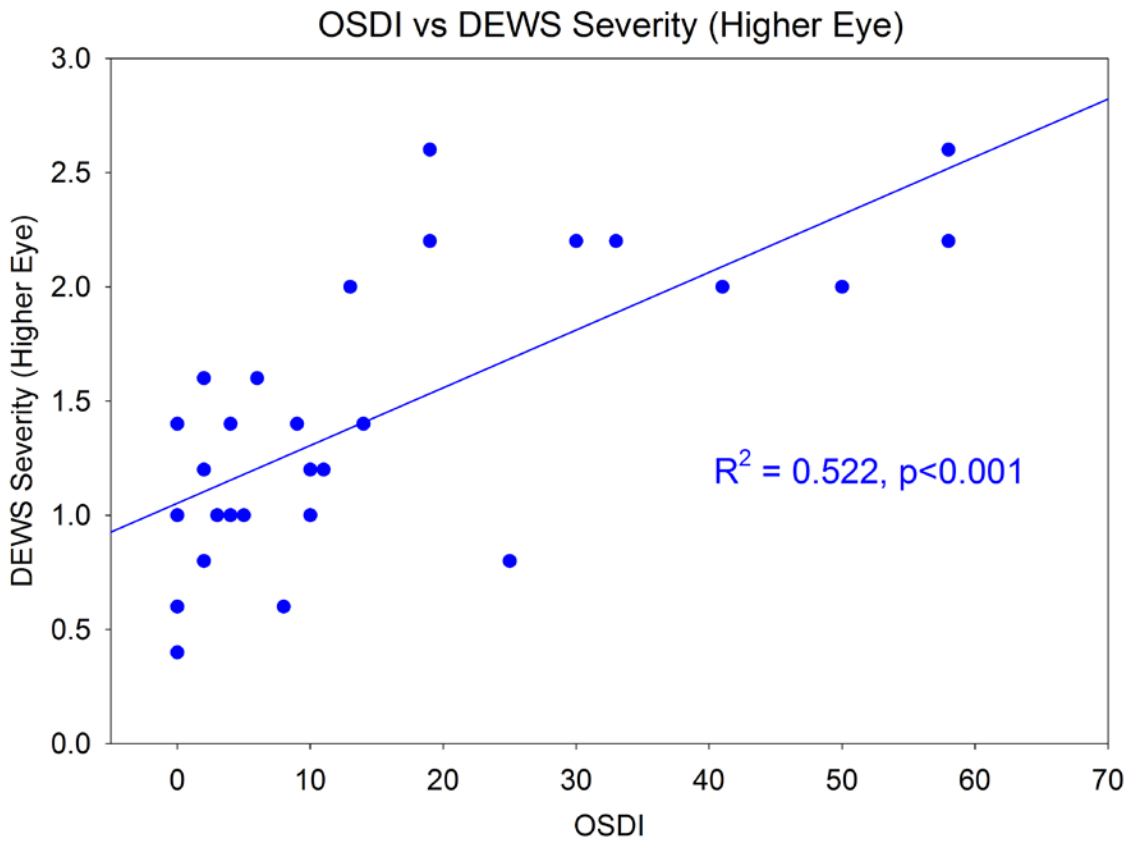

Figure 1 - DEWS Severity grading for the eye with higher severity score versus OSDI (Ocular Surface Disease Index score) for each patient showing a statistically significant correlation (R^2 = 0.522, p<0.001).

2. DEWS Severity (OD) vs OSDI

Results for the regression analysis and analysis of variance (ANOVA) of the regression are listed below (Table 15). A statistically significant correlation was found between OSDI and DEWS Severity of the right eye ($p<0.001$). ANOVA confirmed the significance of the regression with good statistical power and data distribution parameters showed a normal distribution and constant variance. Figure 2 shows a plot of the correlation between DEWS Severity for the right eye and OSDI.

Table 15: Regression Analysis and ANOVA for DEWS Severity (OD) vs OSDI

Regression Equation		DEWS Severity (OD) = 0.936 + (0.0241 × OSDI) (N = 30, R = 0.702, R^2 = 0.493)			
Regression		Coefficient	Std. Error	t	P
	Constant	0.936	0.104	8.996	<0.001
	OSDI	0.0241	0.00462	5.218	<0.001
ANOVA	DF	SS	MS	F	P
Regression	1	5.154	5.154	27.225	<0.001
Residual	28	5.301	0.189		
					P
Normality Test (Shapiro-Wilks)			Passed		0.455
Constant Variance Test			Passed		0.558
Power of test with alpha = 0.05, 0.995					

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

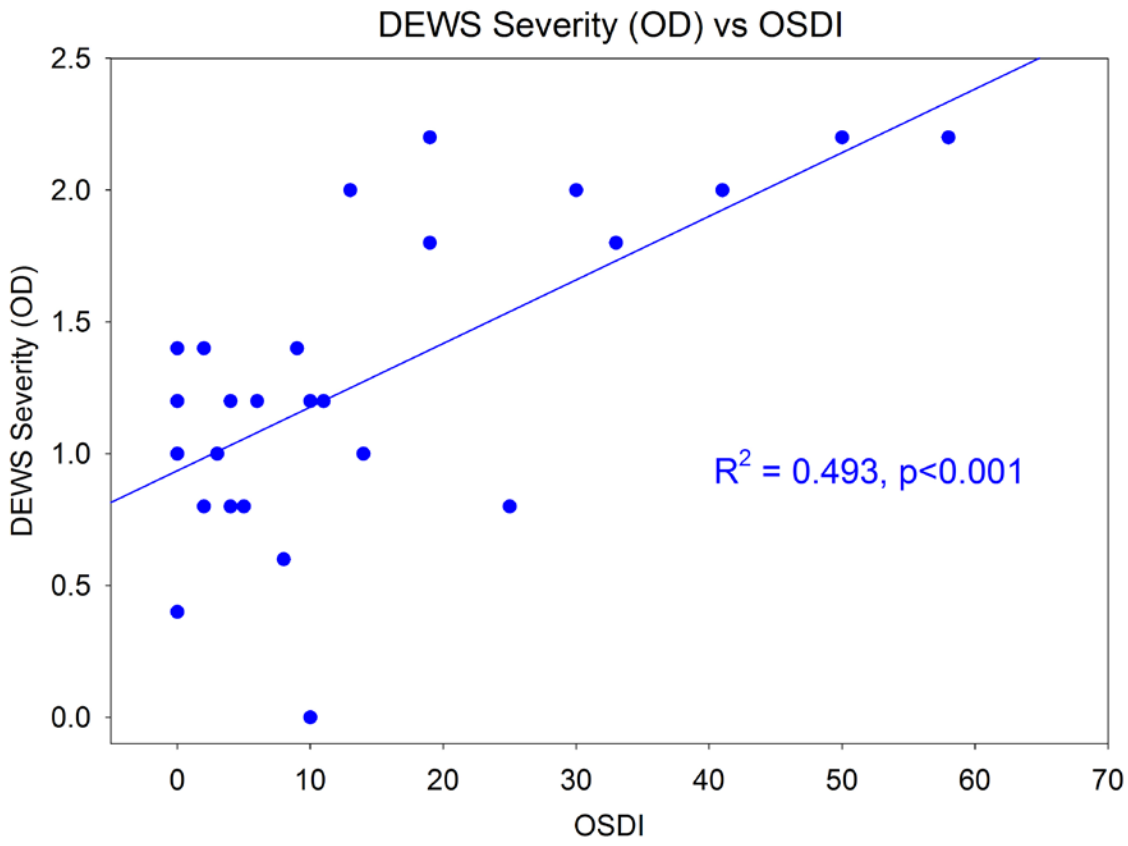

Figure 2 - DEWS Severity grading for the right eye versus OSDI (Ocular Surface Disease Index score) for each patient showing a statistically significant correlation ($R^2 = 0.493$; $p<0.001$).

3. DEWS Severity (OS) vs OSDI

Results for the regression analysis and analysis of variance (ANOVA) of the regression are listed below (Table 16). A statistically significant correlation was found between OSDI and DEWS Severity of the left eye ($p<0.001$). ANOVA confirmed the significance of the regression with good statistical power, and data distribution parameters showed a normal distribution and constant variance. Figure 3 shows a plot of the correlation between DEWS Severity of the left eye and OSDI.

Table 16: Regression Analysis and ANOVA for DEWS Severity (OS) vs OSDI

Regression Equation		DEWS Severity (OS) = 0.890 + (0.0263 x OSDI) (N = 30, R = 0.745, R^2 = 0.555)			
Regression		Coefficient	Std. Error	t	P
	Constant	0.890	0.100	8.870	<0.001
	OSDI	0.0263	0.00446	5.904	<0.001
ANOVA	DF	SS	MS	F	P
Regression	1	6.144	6.144	34.860	<0.001
Residual	28	4.935	0.176		
					P
Normality Test (Shapiro-Wilks)			Passed		0.250
Constant Variance Test			Passed		0.175

Power of test with alpha = 0.05, 0.999

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

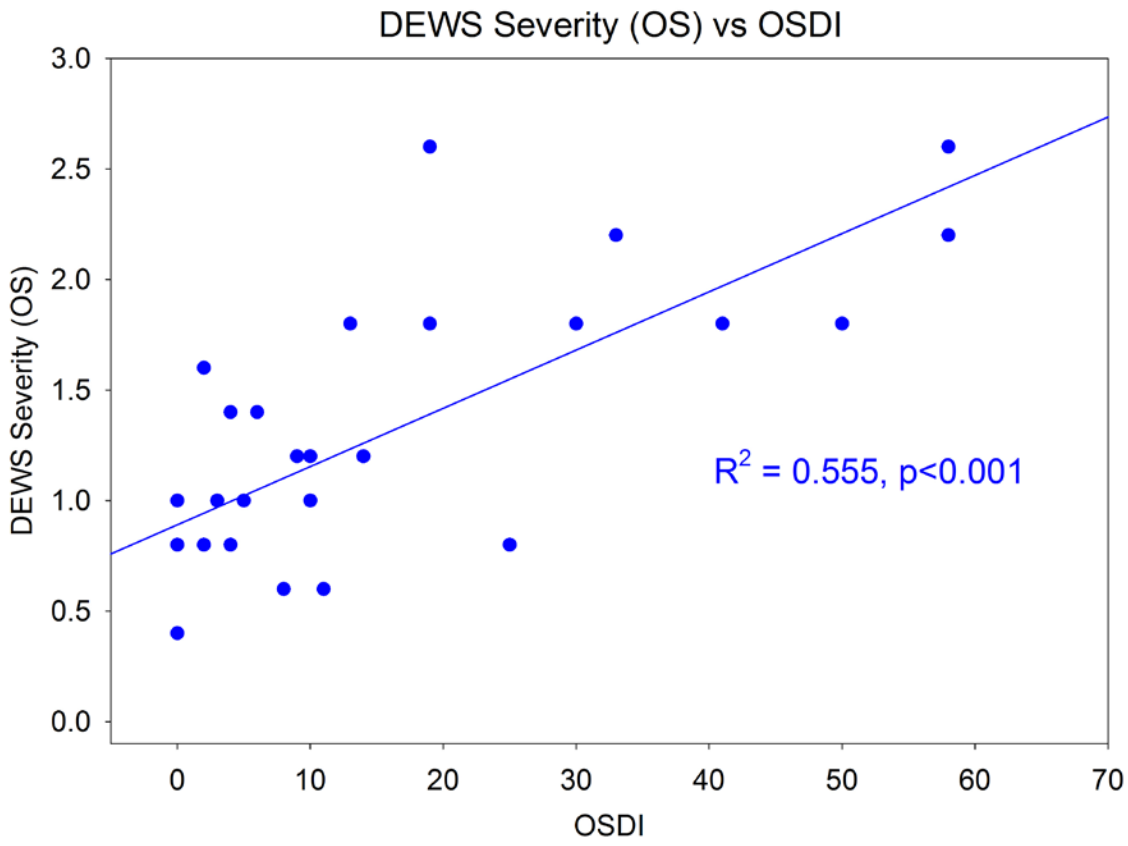

Figure 3 - DEWS Severity grading for the left eye versus OSDI (Ocular Surface Disease Index score) for each patient shows a statistically significant correlation (R^2 = 0.555; $p<0.001$).

4. NIBUT (OD) vs OSDI

Results for the regression analysis and analysis of variance (ANOVA) of the regression for NIBUT in the right eye versus OSDI are listed in Table 17. The Pearson correlation was not statistically significant ($p<0.063$). ANOVA confirmed a lack of significance of the regression and revealed poor statistical power for the test. While variance was equal in both groups, data distribution was not normal. The Spearman rank order correlation was therefore also calculated and found to be not statistically significant ($p<0.079$). Figure 4 shows a plot of the correlation between NIBUT (OD) and OSDI (upper) and a residual plot of (lower), which revealed a non-systematic distribution of residuals.

Table 17: Regression Analysis and ANOVA for NIBUT (OD) vs OSDI

Regression Equation		NIBUT (OD) = 17.177 – (0.194 × OSDI) (N = 29, R = 0.350, R^2 = 0.122)			
Regression		Coefficient	Std. Error	t	P
	Constant	17.177	2.281	7.532	<0.001
	OSDI	–0.194	0.1000	–1.939	0.063, ns
ANOVA	DF	SS	MS	F	P
Regression	1	331.98	331.96	3.760	0.063
Residual	27	2383.67	88.28		
					P
Normality Test (Shapiro-Wilks)			Failed		0.008
Constant Variance Test			Passed		0.105
			R	R^2	P
Spearman Rank Order Correlation			0.331	0.110	0.079, ns

Power of test with alpha = 0.05, 0.461

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

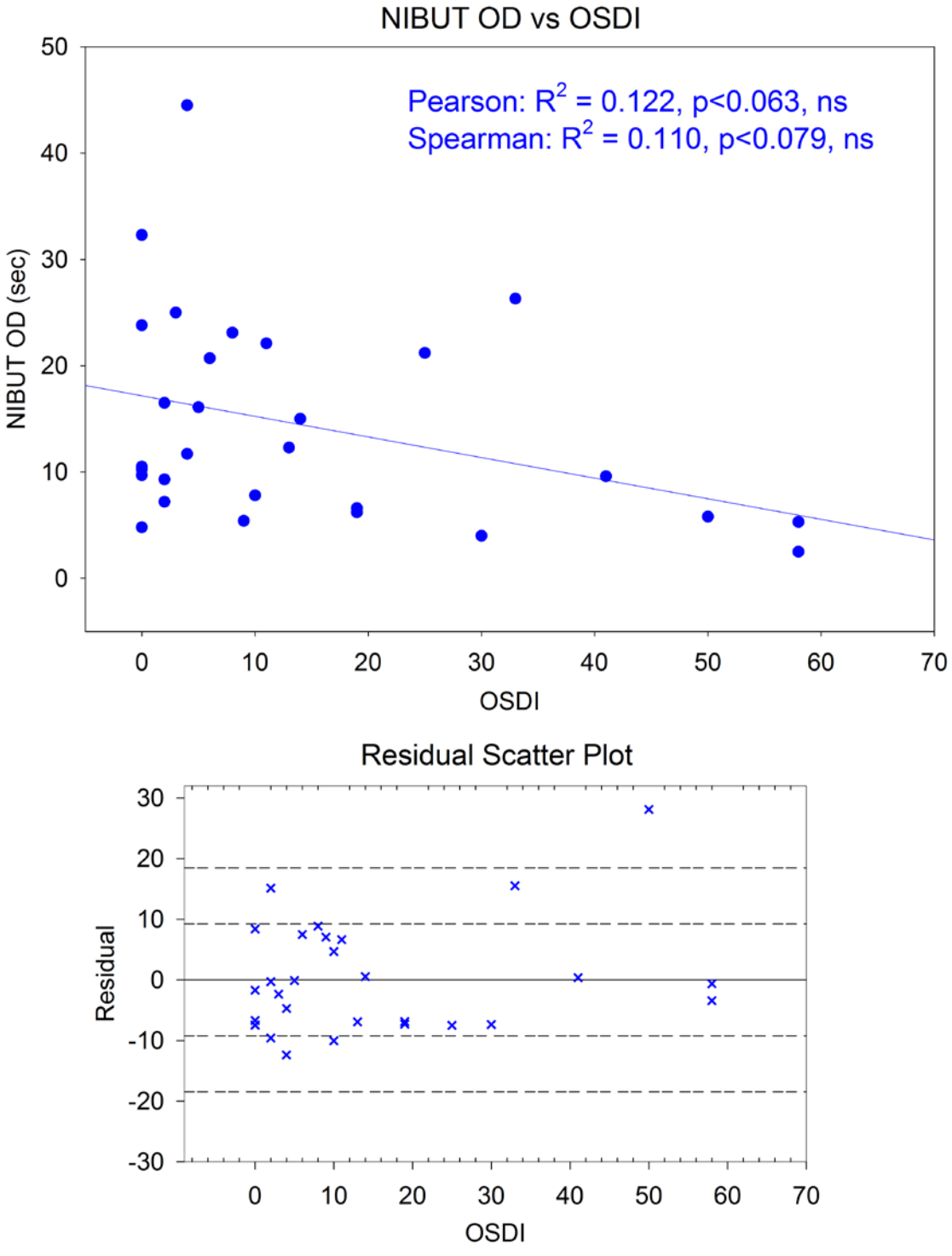

Figure 4 – Upper: NIBUT of the right eye versus OSDI for each patient shows non-statistically significant Pearson ($p<0.063$) and Spearman correlations $p<0.079$).
Lower: residual scatterplot showing a predominantly random pattern of residuals, supporting a lack of other factors contributing to the correlation.

To further investigate the NIBUT (OD) vs OSDI relationship, NIBUT (OD) data was log transformed then plotted against OSDI. Regression analysis and ANOVA showed an improvement in the correlation to statistical significance ($p<0.009$), data was normally distributed, and statistical power of the test improved to 0.749. This is shown in Table 18. Figure 5 is a plot of the correlation between log transformed NIBUT (OD) and OSDI.

Table 18: Regression Analysis and ANOVA for Log NIBUT (OD) vs OSDI

Regression Equation		Log NIBUT (OD) = 1.178 – (0.194 × OSDI) (N = 29, R = 0.475, R^2 = 0.225)			
Regression		Coefficient	Std. Error	t	P
	Constant	1.178	0.0661	17.818	<0.001
	OSDI	-0.00812	0.00290	–2.802	0.009
ANOVA	DF	SS	MS	F	P
Regression	1	0.582	0.582	7.853	0.009
Residual	27	2.002	0.0741		
Total	28	2.584	0.0923		
					P
Normality Test (Shapiro-Wilks)			Passed		0.582
Constant Variance Test			Passed		0.805

Power of test with alpha = 0.05, 0.749

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

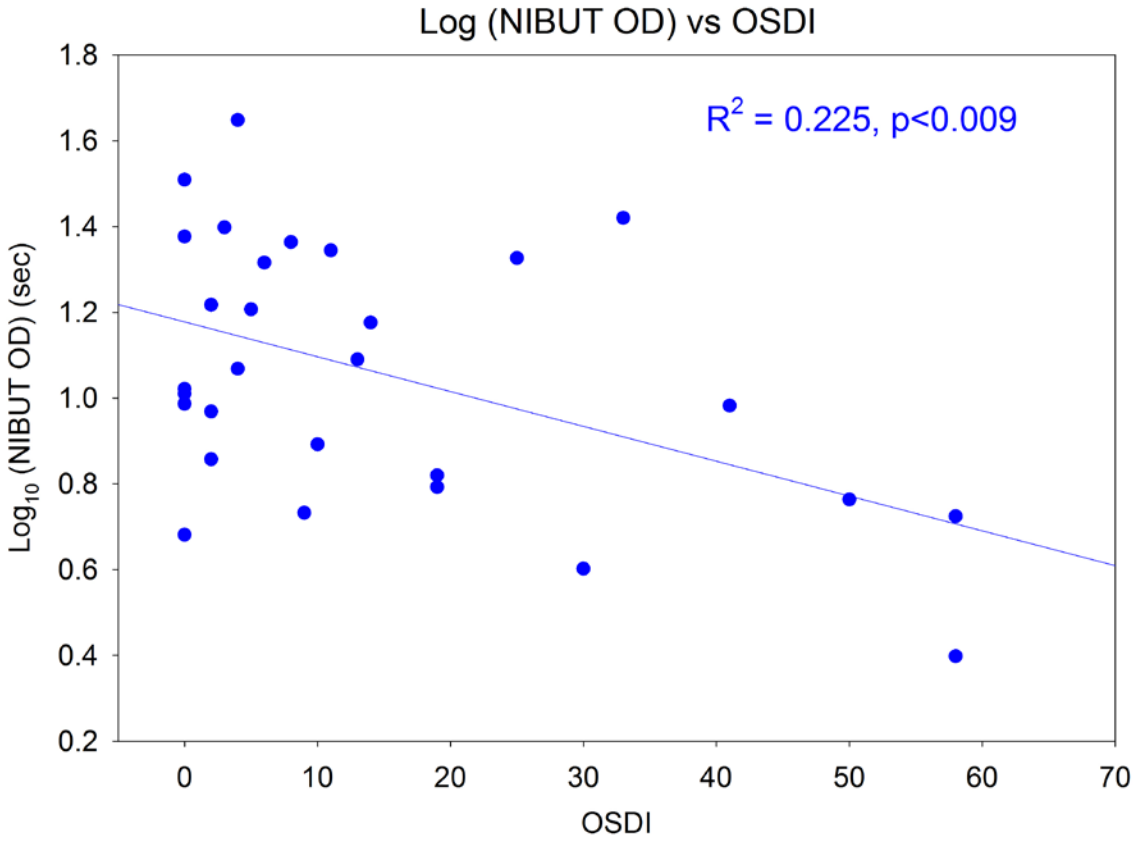

Figure 5 - Log transformed NIBUT of the right eye versus OSDI for each patient resulted in a statistically significant correlation (R^2 = 0.225; p<0.009).

5. NIBUT (OS) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed below (Table 19). ANOVA confirmed the significance of the regression with good statistical power. The Pearson correlation was statistically significant ($p<0.011$). Again, because the data were not normally distributed, the Spearman Rank Order correlation between NIBUT (OS) and OSDI was also calculated. This slightly improved statistical significance of the relationship ($p<0.005$), indicating that ranking of NIBUT data may be more appropriate in this case. Figure 6 (upper) is a plot of the correlation between OSDI and NIBUT of the left eye. The lower residual plot indicates some heteroscedasticity in the linear model and that a data transformation may improve the relationship.

Table 19: Regression Analysis and ANOVA for NIBUT (OS) vs OSDI

Regression Equation		NIBUT OS = 20.001 - (0.271 × OSDI) (N = 30, R = 0.459, R^2 = 0.211)			
Regression		Coefficient	Std. Error	t	P
	Constant	20.001	2.229	8.974	<0.001
	OSDI	-0.271	0.0991	-2.737	0.011
ANOVA	DF	SS	MS	F	P
Regression	1	650.53	650.53	7.489	0.011
Residual	28	2432.30	86.87		
					P
Normality Test (Shapiro-Wilks)			Failed		0.005
Constant Variance Test			Passed		0.070
			R	R^2	
Spearman Rank Order Correlation			0.247	0.110	0.005

Power of test with alpha = 0.05, 0.732

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

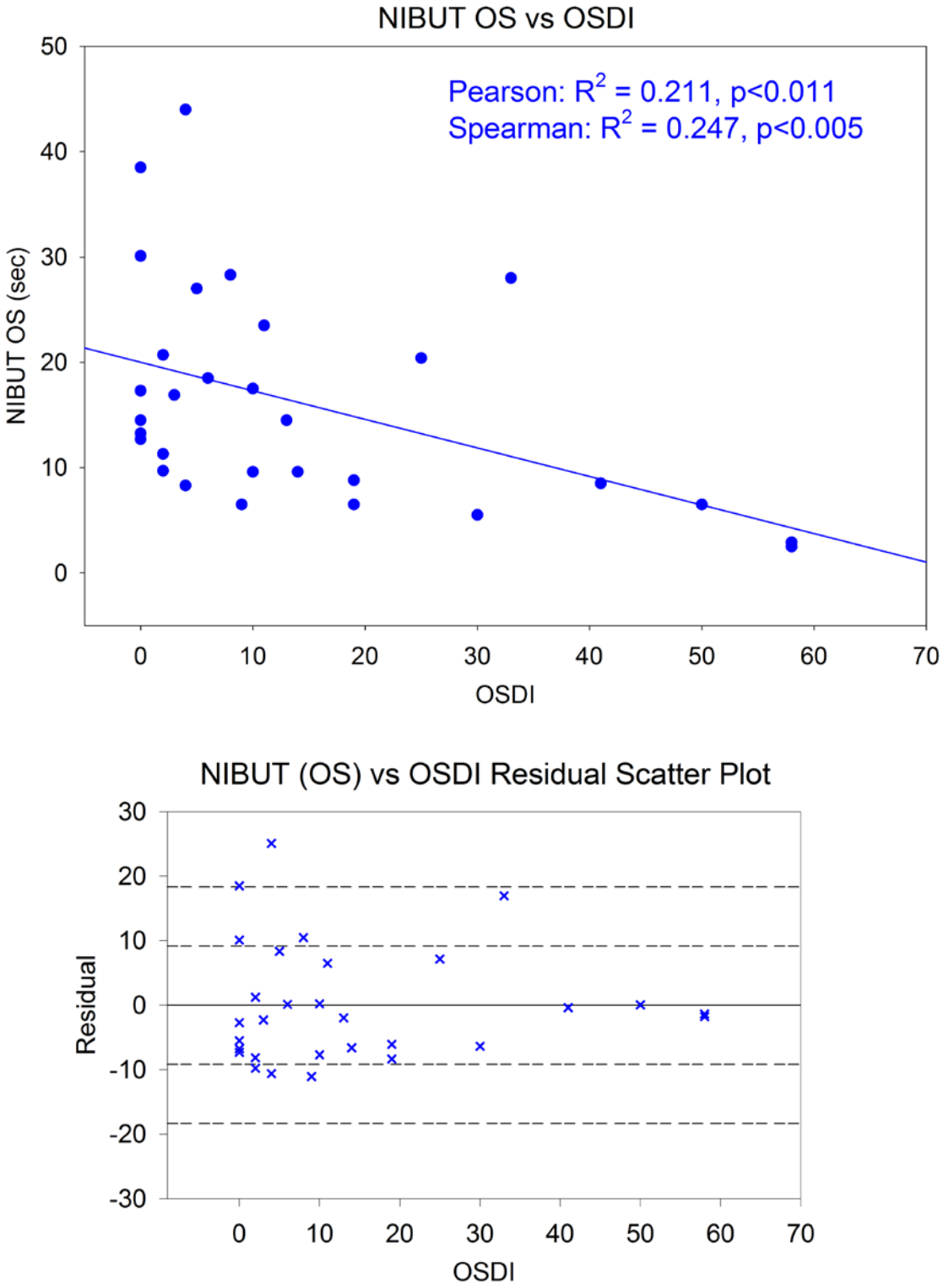

Figure 6 – Upper: NIBUT of the left eye versus OSDI score shows statistically significant Pearson ($p<0.011$) and Spearman $p<0.079$) correlations. Lower: residual scatterplot shows heteroscedasticity of the data, indicating that a linear regression may not be optimal.

To further investigate the NIBUT (OS) vs OSDI relationship, NIBUT data was log transformed then plotted against OSDI. Regression analysis and ANOVA showed a substantial improvement in the correlation with good statistical significance ($p<0.001$), normal data distribution, and improved statistical power of the test (0.980). This is shown in Table 20. Figure 7 is a plot of the correlation between log transformed NIBUT (OS) and OSDI.

Table 20: Regression Analysis and ANOVA for Log NIBUT (OS) vs OSDI

Regression Equation		Log NIBUT OS = 1.278 – (0.013 × OSDI) (N = 30, R = 0.649, R^2 = 0.422)			
Regression		Coefficient	Std. Error	t	P
	Constant	1.278	0.0563	22.687	<0.001
	OSDI	–0.0113	0.0025	–4.518	<0.001
ANOVA	DF	SS	MS	F	P
Regression	1	1.131	1.131	20.408	<0.001
Residual	28	1.552	0.0554		
Total	29	2.684	0.0925		
					P
Normality Test (Shapiro-Wilks)			Passed		0.582
Constant Variance Test			Passed		0.805
Power of test with alpha = 0.05, 0.980					

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

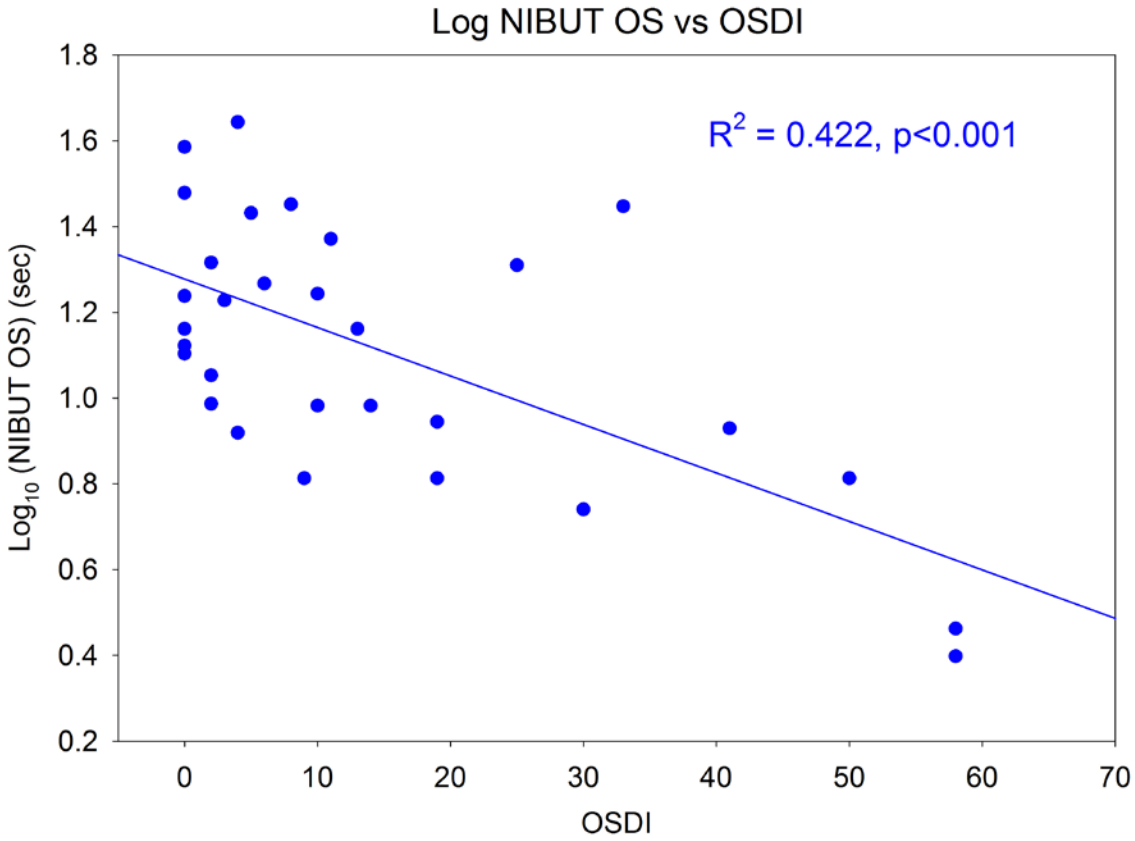

Figure 7 - Log transformed NIBUT of the left eye versus OSDI for each patient resulted in a statistically significant correlation (R^2 = 0.422; p<0.001).

6. TBUT (OD) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed in Table 21. The Pearson correlation was not statistically significant ($p<0.083$). Because data were not normally distributed, the Spearman rank order correlation was calculated and found to be significant ($p<0.007$). Again this indicates a ranking of tear break-up time data may be more appropriate than the actual values. Figure 8 shows a plot of the correlation between TBUT of the right eye and OSDI. The lower residual plot again indicates some heteroscedasticity in the linear model and that a data transformation may improve the relationship.

Table 21: Regression Analysis and ANOVA for TBUT (OD) vs OSDI

Regression Equation		TBUT OD = 9.117 – (0.119 × OSDI) (N = 29, R = 0.327, R^2 = 0.107)			
Regression		Coefficient	Std. Error	t	P
	Constant	9.117	1.515	6.017	<0.001
	OSDI	–0.119	0.0664	–1.799	0.083, ns
ANOVA	DF	SS	MS	F	P
Regression	1	126.077	126.077	3.235	0.083
Residual	27	1052.372	38.977		
					P
Normality Test (Shapiro-Wilks)			Failed		<0.001
Constant Variance Test			Passed		0.189
			R	R^2	P
Spearman Rank Order Correlation			0.495	0.245	0.007

Power of test with alpha = 0.05, 0.410

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

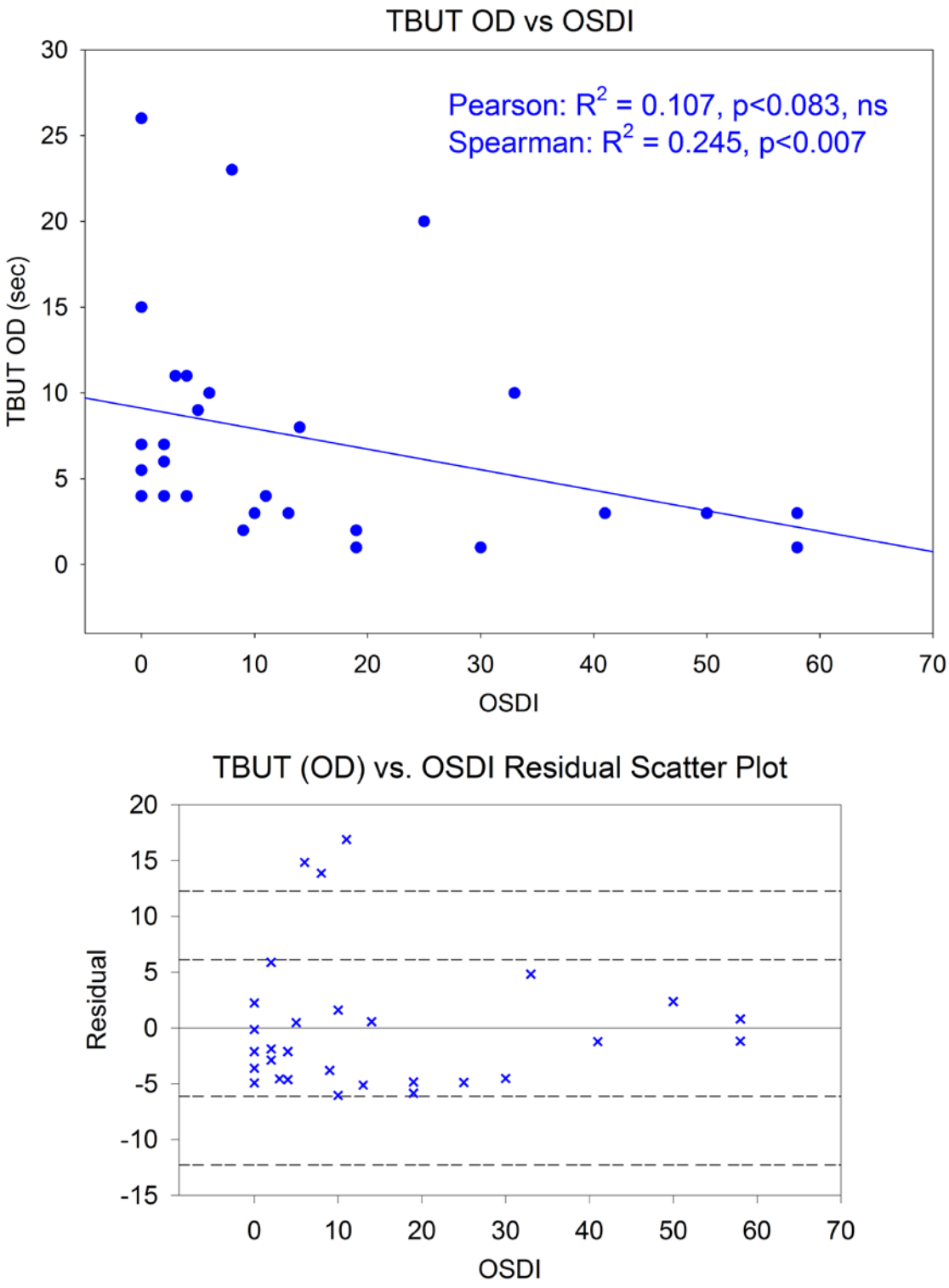

Figure 8 - Upper: TBUT (OD) versus OSDI shows a statistically insignificant Pearson ($p<0.083$) and significant Spearman ($p<0.007$) correlation.
Lower: the residual scatterplot shows a somewhat heteroscedastic pattern of residuals, the scatter decreasing with increasing OSDI.

To further investigate the TBUT (OD) vs OSDI relationship, the TBUT data was log transformed then plotted against OSDI. Regression analysis and ANOVA showed an improvement in the Pearson correlation to statistical significance ($p<0.009$), data was normally distributed, and statistical power of the test improved to 0.749. This is shown in Table 22. The correlation for log transformed data was no better than the Spearman correlation ($p<0.007$) for ranked linear data. Figure 9 is a plot of the correlation between log transformed TBUT (OD) and OSDI.

Table 22: Regression Analysis and ANOVA for Log TBUT (OD) vs OSDI

Regression Equation	$\log_{10}(\text{TBUT OD})) = 0.865 - (0.0104 \times \text{OSDI})$ ($N = 29$, $R = 0.479$, $R^2 = 0.229$)				
Regression		Coefficient	Std. Error	t	P
	Constant	0.865	0.0840	10.295	<0.001
	OSDI	-0.0104	0.00368	-2.832	0.009
ANOVA	DF	SS	MS	F	P
Regression	1	0.961	0.961	8.021	0.009
Residual	27	3.236	0.120		
Total	28	4.198	0.150		
					P
Normality Test (Shapiro-Wilks)			Passed		0.863
Constant Variance Test			Passed		0.107
Power of test with alpha = 0.05, 0.757					

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

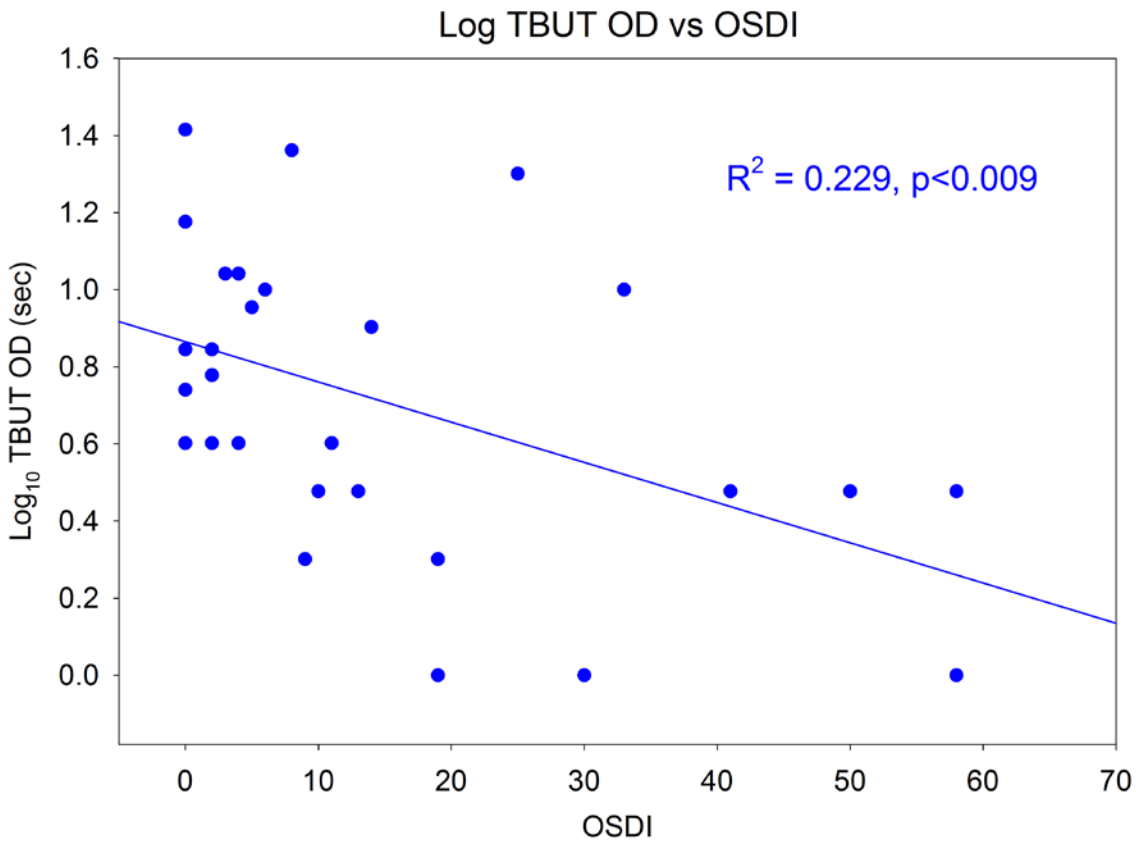

Figure 9 - Log transformed TBUT of the right eye versus OSDI for each patient resulted in a statistically significant Pearson correlation (R^2 = 0.422; p<0.001). However, significance did not improve over the Spearman correlation from linear data

7. TBUT (OS) vs OSDI

Results for the regression analysis and ANOVA of the regression are shown below (Table 23). A statistically significant correlation was found between TBUT in the left eye and OSDI ($p<0.020$). ANOVA confirmed the significance of the regression with reasonable, but less than ideal, statistical power. While data groups exhibited constant variance, distribution parameters were significantly different from normal. Spearman correlation was therefore determined and resulted in a substantial improvement in statistical significance ($p<0.002$), again indicating that rank ordering of tear break-up time may be more appropriate than using actual data values. Figure 10 shows a plot of the correlation between TBUT of the left eye and OSDI.

Table 23: Regression Analysis and ANOVA for vs TBUT (OS) vs OSDI

Regression Equation		TBUT (OS) = 10.042 – (0.151 × OSDI) (N = 30, R = 0.424, R^2 = 0.180)			
Regression		Coefficient	Std. Error	t	P
	Constant	10.042	1.370	7.330	<0.001
	OSDI	-0.151	0.0609	–2.478	0.020
ANOVA	DF	SS	MS	F	P
Regression	1	201.483	201.483	6.138	0.020
Residual	28	919.092	32.825		
					P
Normality Test (Shapiro-Wilks)			Failed		<0.001
Constant Variance Test			Passed		0.066
			R	R^2	P
Spearman Rank Order Correlation			0.539	0.291	0.002

Power of test with alpha = 0.05, 0.652

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

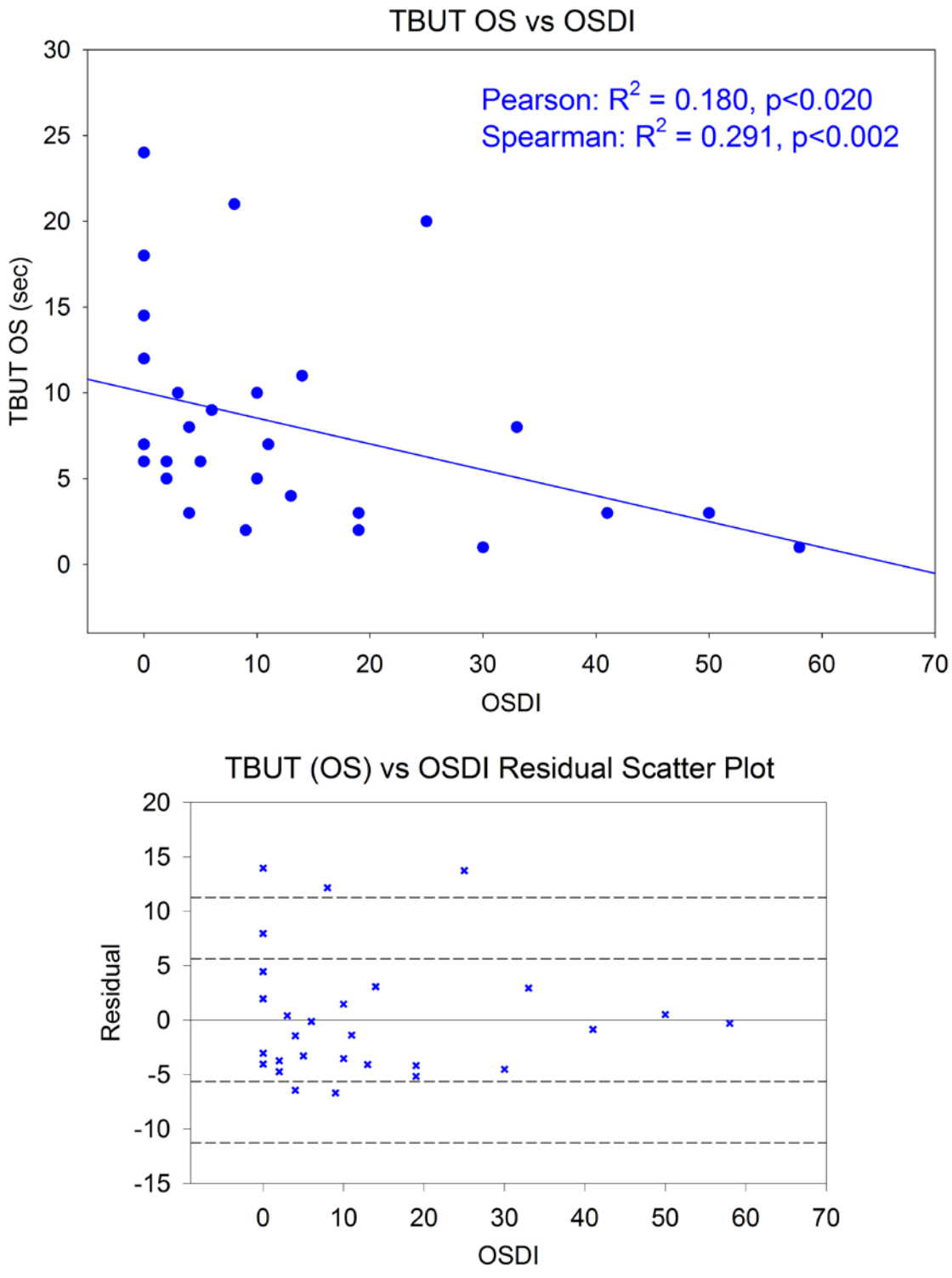

Figure10 – Upper: TBUT (OS) versus OSDI shows statistically significant Pearson ($p<0.020$) and Spearman ($p<0.002$) correlations.
Lower: the residual scatterplot shows a heteroscedastic pattern of residuals, the scatter decreasing with increasing OSDI.

As with all other tear break-up time plots, to further investigate the TBUT (OS) vs OSDI relationship, TBUT data was log transformed then plotted against OSDI. Regression analysis and ANOVA showed an improvement in the Pearson correlation to statistical significance ($p<0.009$), data was normally distributed, and statistical power of the test improved to 0.749. This is shown in Table 24. The correlation for log transformed data was no better than the Spearman correlation ($p<0.007$) for ranked linear data. Figure 11 is a plot of the correlation between log transformed TBUT (OD) and OSDI.

Table 24: Regression Analysis and ANOVA for Log TBUT (OS) vs OSDI

Regression Equation		$\log_{10}(\text{TBUT OD})) = 0.952 - (0.0138 \times \text{OSDI})$			
		(N = 30, R = 0.628, R^2 = 0.394)			
Regression		Coefficient	Std. Error	t	P
	Constant	0.952	0.0729	13.060	<0.001
	OSDI	-0.0138	0.00324	-4.270	<0.001
ANOVA	DF	SS	MS	F	P
Regression	1	1.695	1.695	18.237	<0.001
Residual	28	2.603	0.093		
Total	29	4.298	0.148		
					P
Normality Test (Shapiro-Wilks)			Passed		0.762
Constant Variance Test			Passed		0.567

Power of test with alpha = 0.05, 0.970

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

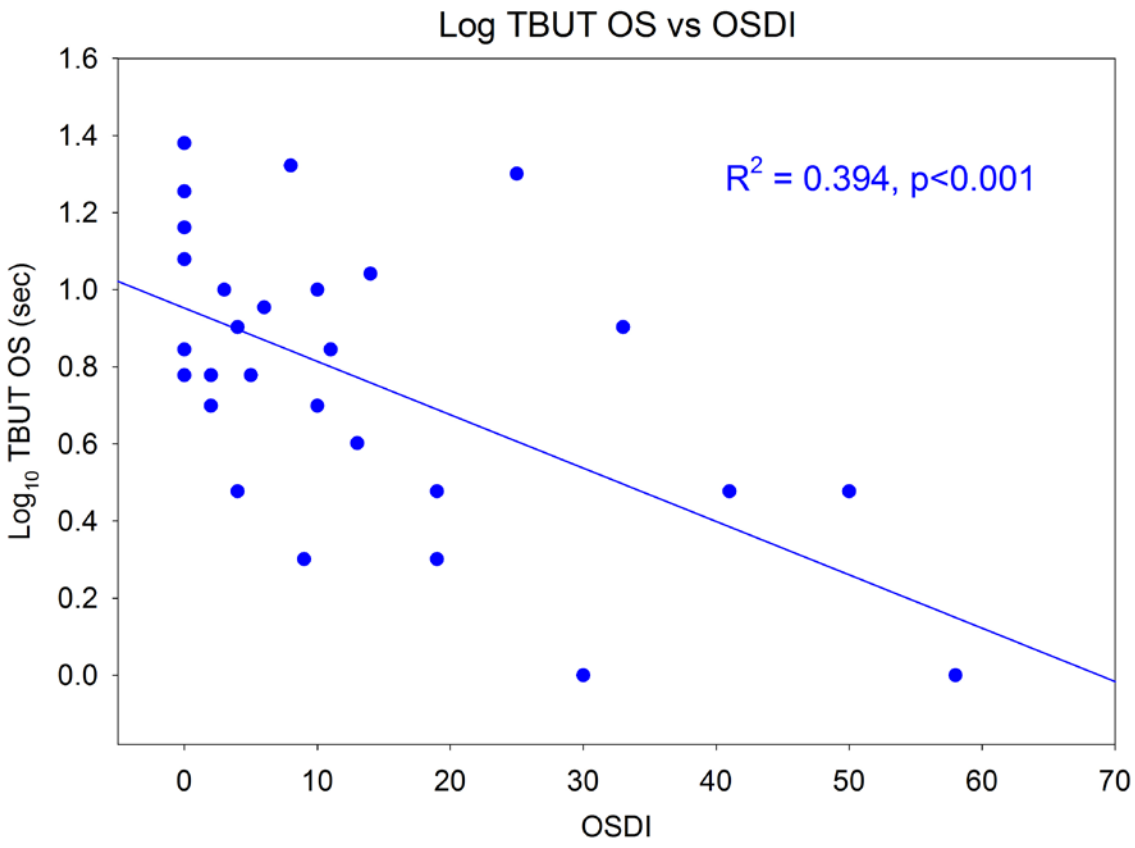

Figure 11 - Log transformed TBUT of the left eye versus OSDI for each patient resulted in a statistically significant Pearson correlation (R^2 = 0.394; p<0.001). This was a marginal improvement on the Spearman correlation for the linear data.

8. Schirmer Score (OD) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed in Table 25. Data distribution differed significantly from normal and group variances were unequal. The correlation between Schirmer score of the right eye and OSDI was not statistically significant by Pearson (p=0.217) or Spearman (p=0.195) correlation. ANOVA confirmed the lack of significance of the regression and poor statistical power of the test. A plot of the correlation between Schirmer score (OD) and OSDI is shown in Figure 12 (upper) and a residual plot (lower). No transformations improved the correlation between Schirmer score and OSDI. Removing maximum wetting lengths (35 mm) achieved in less than 5 minutes similarly produced no improvement.

Table 25: Regression Analysis and ANOVA for Schirmer Score (OD) vs OSDI

Regression Equation		Schirmer OD = 19.858 - (0.159 × OSDI) (N = 29, R = 0.237, R^2 = 0.056)			
Regression		Coefficient	Std. Error	t	P
	Constant	19.858	2.872	6.914	<0.001
	OSDI	-0.159	0.126	-1.265	0.217, ns
ANOVA	DF	SS	MS	F	P
Regression	1	224.250	224.250	1.601	0.217
Residual	27	3780.992	140.037		
					P
Normality Test (Shapiro-Wilks)			Failed		0.006
Constant Variance Test			Failed		0.019
			R	R^2	P
Spearman Rank Order Correlation			0.247	0.061	0.195

Power of test with alpha = 0.05, 0.233

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

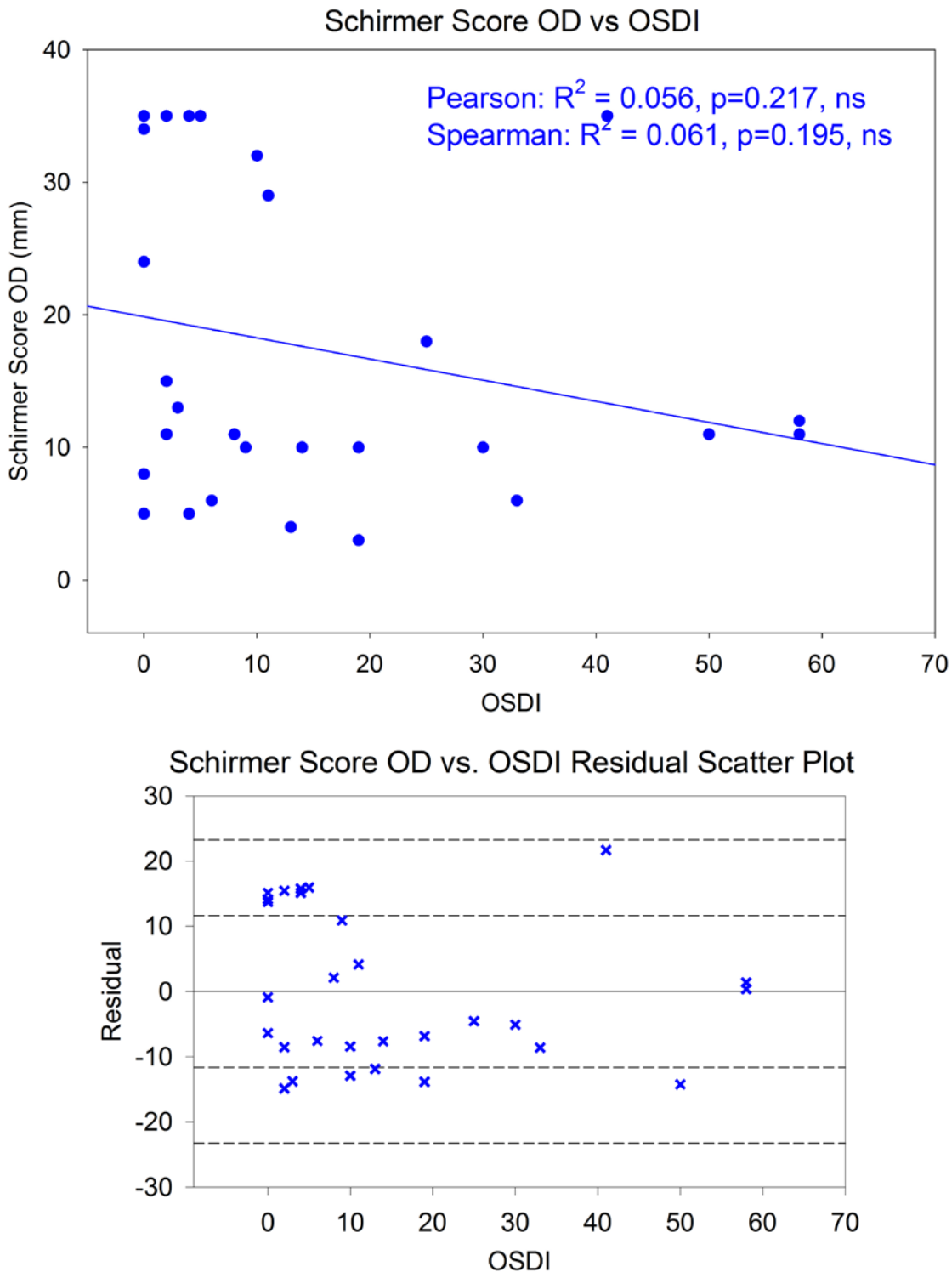

Figure 12 – Upper: Schirmer score of the right eye versus OSDI shows a statistically insignificant Pearson (p=0.217) and Spearman (p=0195) correlation. Lower: the residual scatterplot shows a more or less random pattern of residuals.

9. Schirmer Score (OS) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed below (Table 26). The correlation between Schirmer score in the left eye and OSDI was not statistically significant ($p<0.635$). ANOVA confirmed the lack in significance of the regression and poor statistical power of the test. Data distribution parameters were significantly different from normal, but group variances were equal. Figure 13 shows a plot of the correlation between Schirmer Score (OS) and OSDI. As was the case for the right eye, no transformations improved the correlation between Schirmer score and OSDI. Removing maximum wetting lengths (35 mm) achieved in less than 5 minutes also produced no improvement.

Table 26: Regression Analysis and ANOVA for Schirmer Score (OS) vs OSDI

Regression Equation		Schirmer OS = 17.568 - (0.0551 × OSDI) (N = 30, R = 0.091, R^2 = 0.008)			
Regression		Coefficient	Std. Error	t	P
	Constant	17.568	2.585	6.797	<0.001
	OSDI	-0.0551	0.115	-0.480	0.635, ns
ANOVA	DF	SS	MS	F	P
Regression	1	26.928	26.928	0.231	0.635
Residual	28	3270.439	116.801		
					P
Normality Test (Shapiro-Wilks)			Failed		0.003
Constant Variance Test			Passed		0.671
			R	R^2	P
Spearman Rank Order Correlation			0.229	0.052	0.222, ns

Power of test with alpha = 0.05, 0.068

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

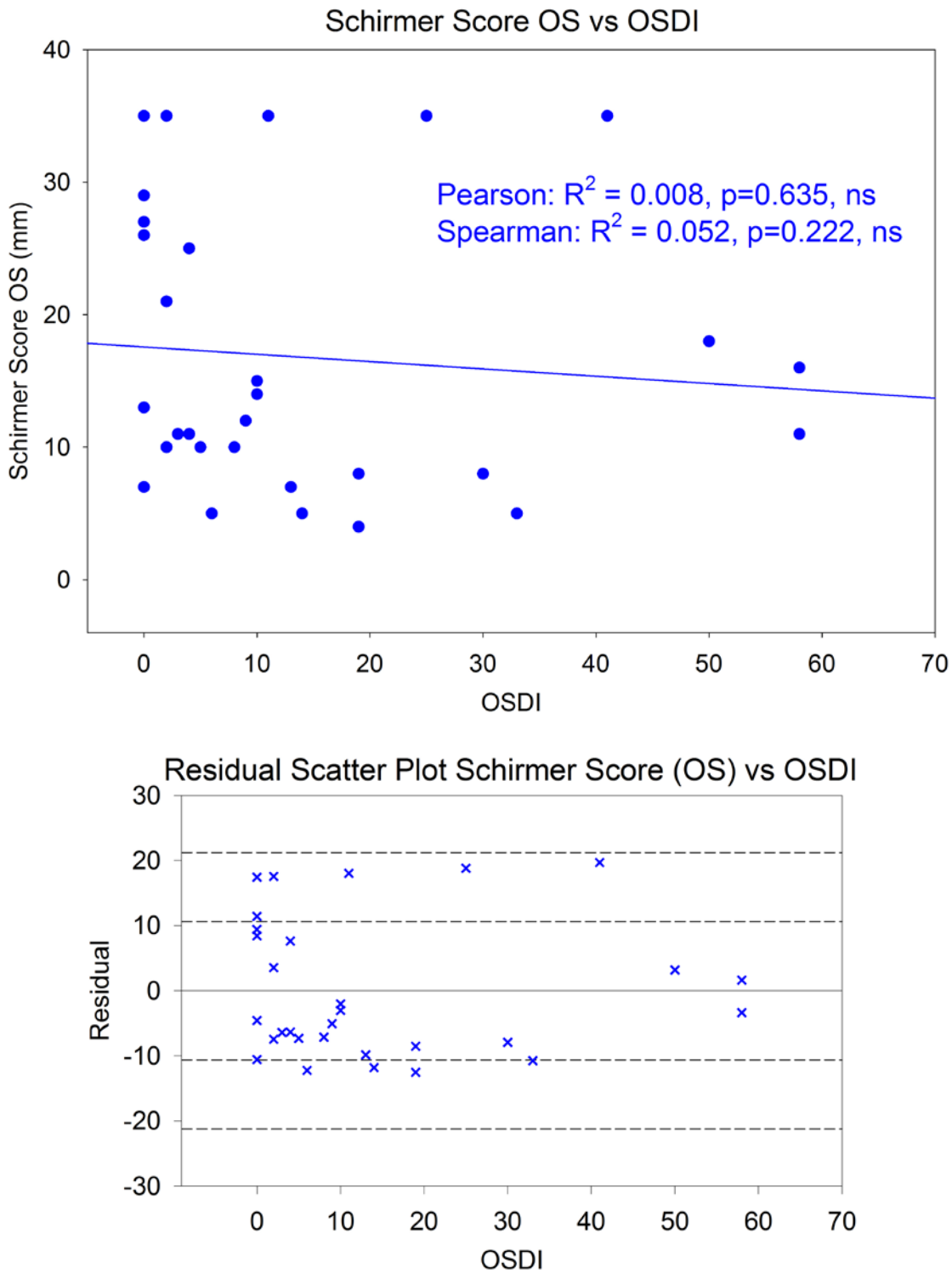

Figure 13 – Upper: Schirmer score of the left eye versus OSDI shows statistically insignificant Pearson (p=0.635) and Spearman (p=0.222) correlations. Lower: the residual scatterplot shows a random pattern of residuals.

10. Tear Meniscus Height (OD) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed below (Table 27). A statistically significant correlation was found between the tear meniscus height in the right eye and OSDI ($p<0.022$). ANOVA confirmed the significance of the regression with good statistical power and data distribution parameters showed a normal distribution and constant variance. Figure 14 is a plot of the correlation between tear meniscus height (OD) and OSDI.

Table 27: Regression Analysis and ANOVA for Tear Meniscus Height (OD) vs OSDI

Regression Equation		Tear Meniscus Height OD = 0.911 – (0.00591 × OSDI) (N = 29, R = 0.424, R^2 = 0.180)			
Regression		Coefficient	Std. Error	t	P
	Constant	0.911	0.0554	16.453	<0.001
	OSDI	-0.00591	0.00243	-2.434	0.022
ANOVA	DF	SS	MS	F	P
Regression	1	0.308	0.308	5.926	0.022
Residual	27	1.405	0.0520		
					P
Normality Test (Shapiro-Wilks)			Passed		0.788
Constant Variance Test			Passed		0.283
Power of test with alpha = 0.05, 0.636					

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

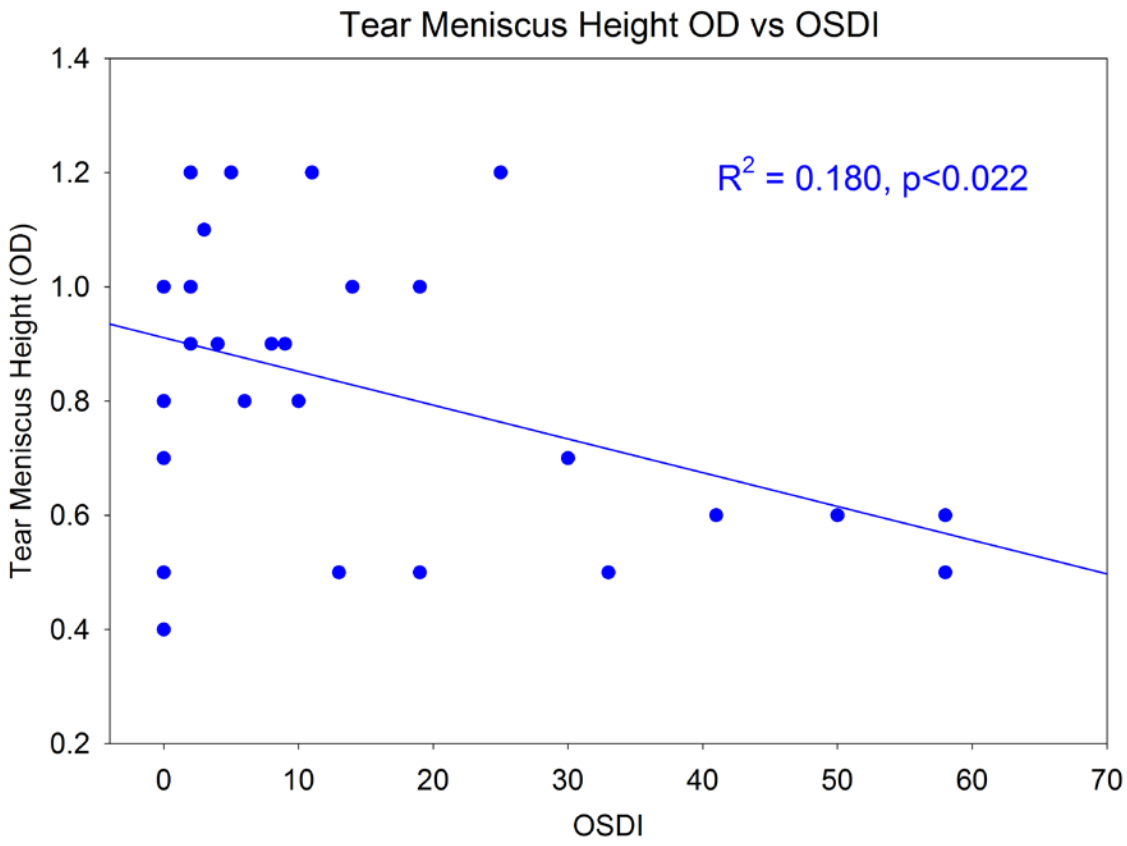

Figure 14 – Tear meniscus height of the right eye versus OSDI (Ocular Surface Disease Index score) for each patient shows a significant statistical correlation (R^2 = 0.180; p<0.022).

11. Tear Meniscus Height (OS) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed below (Table 28). A statistically significant correlation was found between tear meniscus height in the left eye and OSDI ($p<0.042$). ANOVA confirmed the significance of the regression with fair statistical power and data distribution parameters showed a normal distribution and constant variance. Figure 15 shows a plot of the correlation between tear meniscus height (OS) and OSDI.

Table 28: Regression Analysis and ANOVA for Tear Meniscus Height (OS) vs OSDI

Regression Equation		Tear Meniscus Height OS = 0.869 - (0.00545 * OSDI) (N = 30, R = 0.373, R^2 = 0.139)			
Regression		Coefficient	Std. Error	t	P
	Constant	0.869	0.0576	15.091	<0.001
	OSDI	-0.00545	0.00256	-2.129	0.042
ANOVA	DF	SS	MS	F	P
Regression	1	0.263	0.263	4.531	0.042
Residual	28	1.624	0.0580		
					P
Normality Test (Shapiro-Wilks)			Passed		0.679
Constant Variance Test			Passed		0.435
Power of test with alpha = 0.05, 0.531					

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

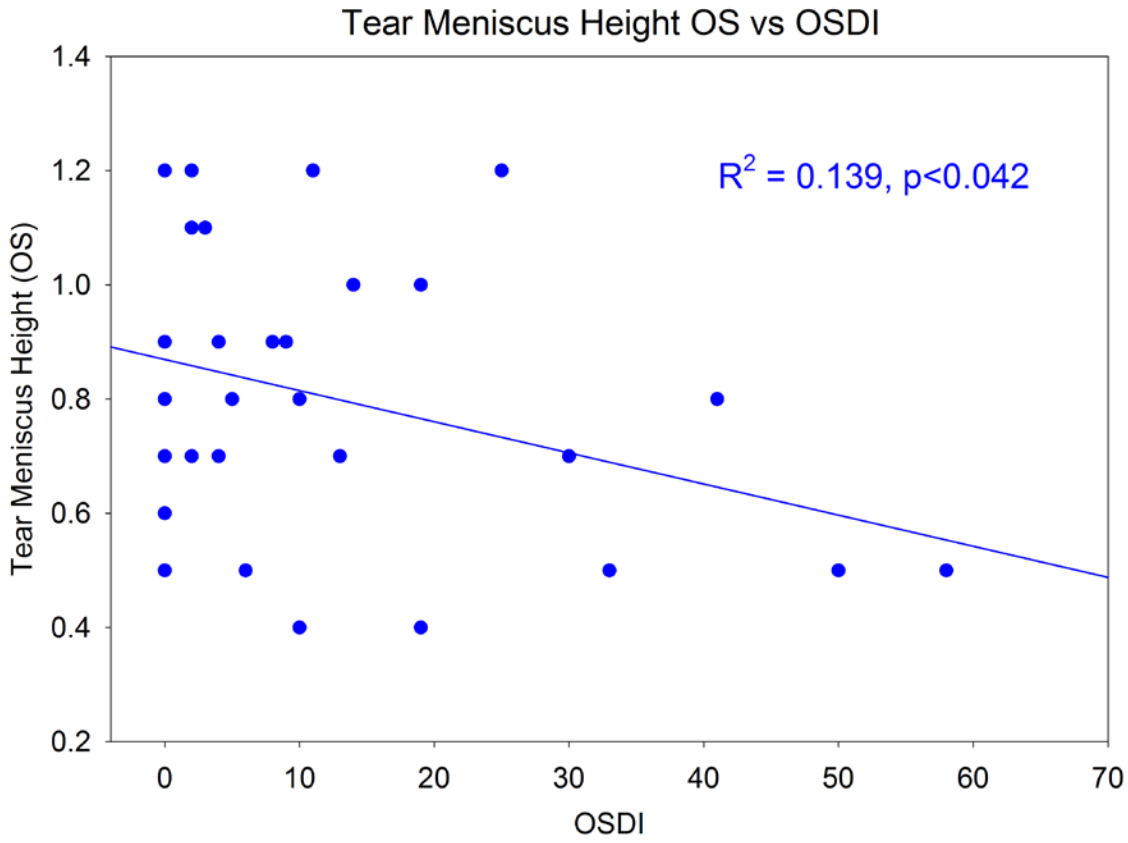

Figure 15 – Tear meniscus height of the left eye versus OSDI (Ocular Surface Disease Index score) for each patient shows a significant statistical correlation (R^2 = 0.139; p<0.042).

12. NaFl (OD) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed below (Table 29). A statistically significant correlation was found between the NaFl staining score in the right eye and OSDI ($p<0.049$). ANOVA confirmed the significance of the regression with fair statistical power and data distribution parameters showed a normal distribution and constant variance. Figure 16 shows a plot of the correlation between NaFl score (OD) and OSDI.

Table 29: Regression Analysis and ANOVA for NaFl (OD) vs OSDI

Regression Equation		NaFl Staining OD = 3.226 + (0.0386 * OSDI) (N = 29, R = 0.369, R^2 = 0.136)			
Regression		Coefficient	Std. Error	t	P
	Constant	3.226	0.427	7.553	<0.001
	OSDI	0.0386	0.0187	2.063	0.049
ANOVA	DF	SS	MS	F	P
Regression	1	13.176	13.176	4.256	0.049
Residual	27	83.582	3.096		
					P
Normality Test (Shapiro-Wilks)			Passed		0.152
Constant Variance Test			Passed		0.803
Power of test with alpha = 0.05, 0.506					

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

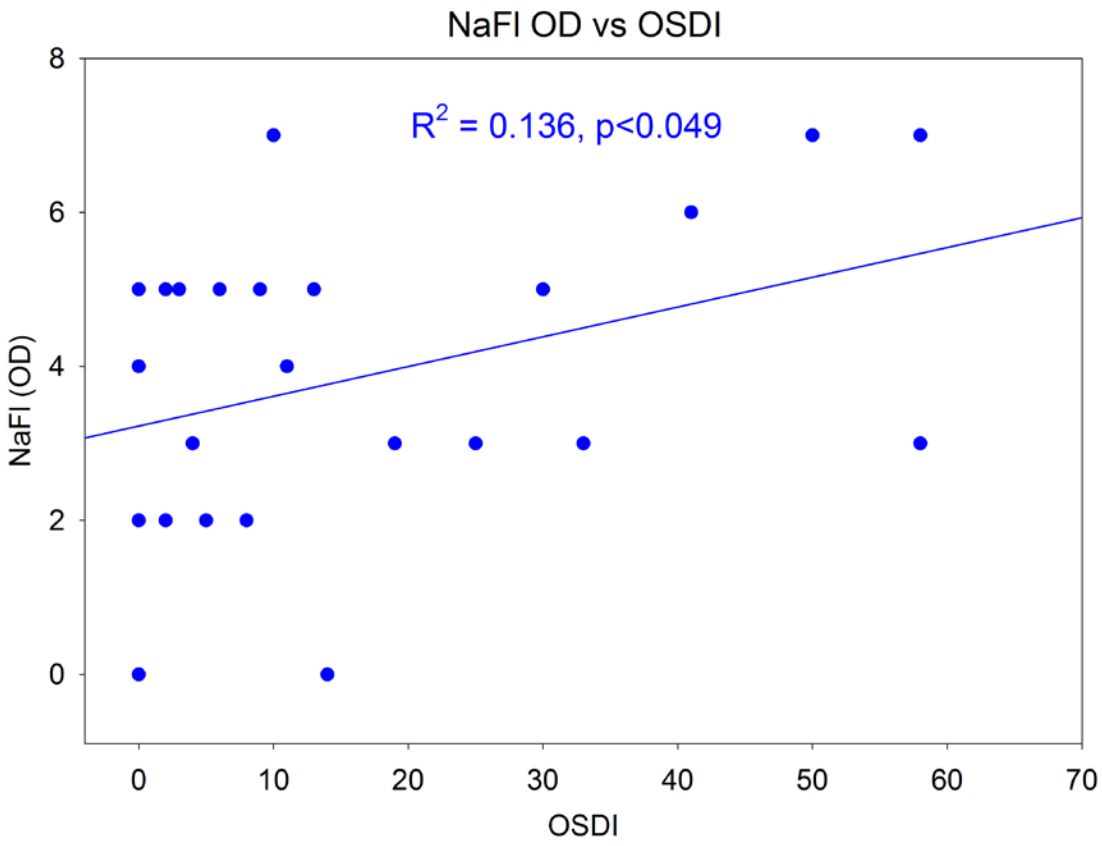

Figure 16 – NaFl staining of the right eye versus OSDI (Ocular Surface Disease Index score) for each patient shows a significant statistical correlation ($R^2 = 0.136$; $p<0.049$).

13. NaFl (OS) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed in Table 30. A statistically significant correlation was found between the NaFl staining score in the left eye and OSDI ($p<0.013$). ANOVA confirmed the significance of the regression with good statistical power and data distribution parameters showed a normal distribution and constant variance. Figure 17 shows a plot of the correlation between NaFl score (OS) and OSDI.

Table 30: Regression Analysis and ANOVA for NaFl (OS) vs OSDI

Regression Equation		NaFl Staining OS = 3.313 + (0.0565 × OSDI) (N = 30, R = 0.450, R^2 = 0.202)			
Regression		Coefficient	Std. Error	t	P
	Constant	3.313	0.477	6.950	<0.001
	OSDI	0.0565	0.0212	2.665	0.013
ANOVA	DF	SS	MS	F	P
Regression	1	28.222	28.222	7.104	0.013
Residual	28	111.244	3.973		
					P
Normality Test (Shapiro-Wilks)			Passed		0.157
Constant Variance Test			Passed		0.287
Power of test with alpha = 0.05, 0.711					

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

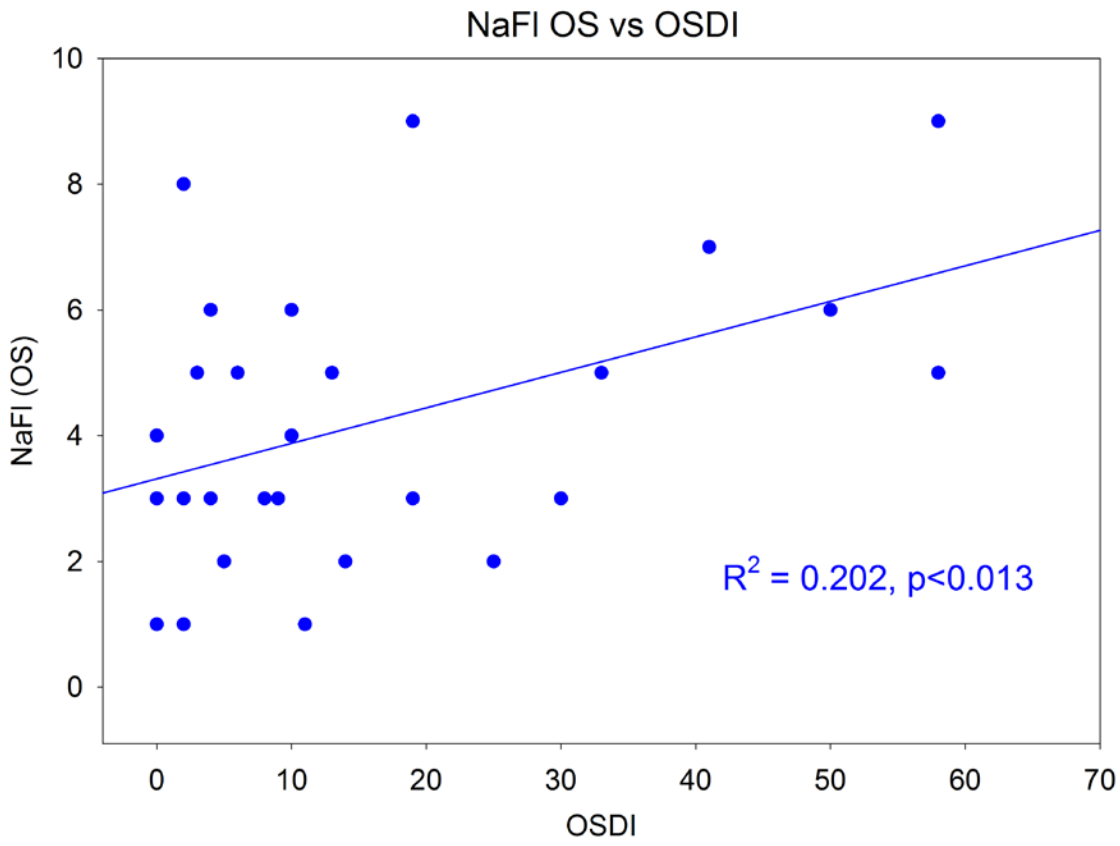

Figure 17 – NaFl staining of the left eye versus OSDI (Ocular Surface Disease Index score) for each patient shows a significant statistical correlation ($R^2 = 0.202$; $p<0.013$).

14. Lissamine Green Score (OD) vs. OSDI

Results for the regression analysis and ANOVA of the regression are listed in Table 31. Data distribution differed significantly from normal, but group variances were equal. Statistically significant Pearson ($p<0.043$) and Spearman ($p<0.013$) correlations were found between Lissamine green staining score in the right eye and OSDI. ANOVA confirmed the significance of the regression with fair statistical power of the test. A plot of the correlation between Lissamine green score (OD) and OSDI is shown in Figure 18 (upper) and a residual plot (lower). Figure 18 shows a plot of the correlation between Lissamine green score (OD) and OSDI.

Table 31: Regression Analysis and ANOVA for Lissamine Green Score (OD) vs OSDI

Regression Equation		LG Staining OD = 1.923 + (0.0358 × OSDI) (N = 29, R = 0.378, R^2 = 0.143)			
Regression		Coefficient	Std. Error	t	P
	Constant	1.923	0.385	4.996	<0.001
	OSDI	0.0358	0.0169	2.121	0.043
ANOVA	DF	SS	MS	F	P
Regression	1	11.308	11.308	4.499	0.043
Residual	27	67.864	2.513		
					P
Normality Test (Shapiro-Wilks)			Failed		0.015
Constant Variance Test			Passed		0.754
			R	R^2	P
Spearman Rank Order Correlation			0.456	0.208	0.013

Power of test with alpha = 0.05, 0.527

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

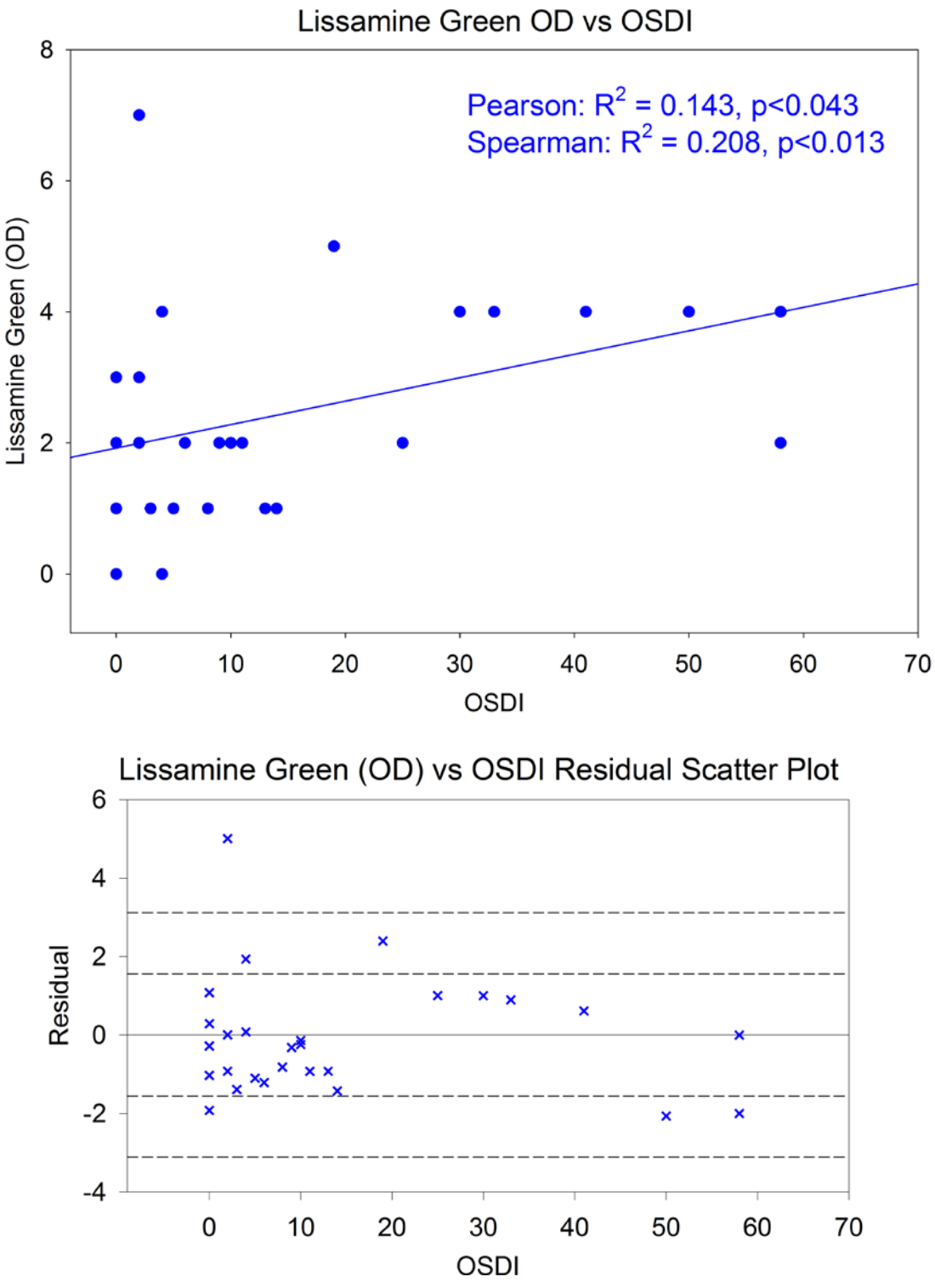

Figure 18 – Upper: Lissamine green staining (OD) versus OSDI shows statistically significant Pearson ($p<0.043$) and Spearman ($p<0.013$) correlations. Lower: residual scatterplot showing a heteroscedastic pattern of residuals, the scatter decreasing with increasing OSDI.

To further investigate the Lissamine Green (OD) vs OSDI relationship, Lissamine Green data was log transformed then plotted against OSDI. Regression analysis and ANOVA showed an improvement in the Pearson correlation ($p<0.016$), data was normally distributed, and statistical power of the test improved to 0.683. This is shown in Table 32. The correlation for log transformed data was not quite at the level of the Spearman correlation ($p<0.013$) for ranked linear data. Figure 19 is a plot of the correlation between log transformed TBUT (OD) and OSDI.

Table 32: Regression Analysis and ANOVA for Log Lissamine Green (OD) vs OSDI

Regression Equation		$\log_{10}$(Lissamine Green (OD)) = 0.246 + (0.0609 × OSDI) (N = 29, R = 0.444, R^2 = 0.197)			
Regression		Coefficient	Std. Error	t	P
	Constant	0.207	0.0629	3.281	0.003
	OSDI	0.00711	0.00276	2.577	0.016
ANOVA	DF	SS	MS	F	P
Regression	1	0.447	0.447	6.642	0.016
Residual	27	1.585	0.0634		
Total	28	2.262	0.0808		
					P
Normality Test (Shapiro-Wilks)			Passed		0.078
Constant Variance Test			Passed		0.305
Power of test with alpha = 0.05, 0.683					

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

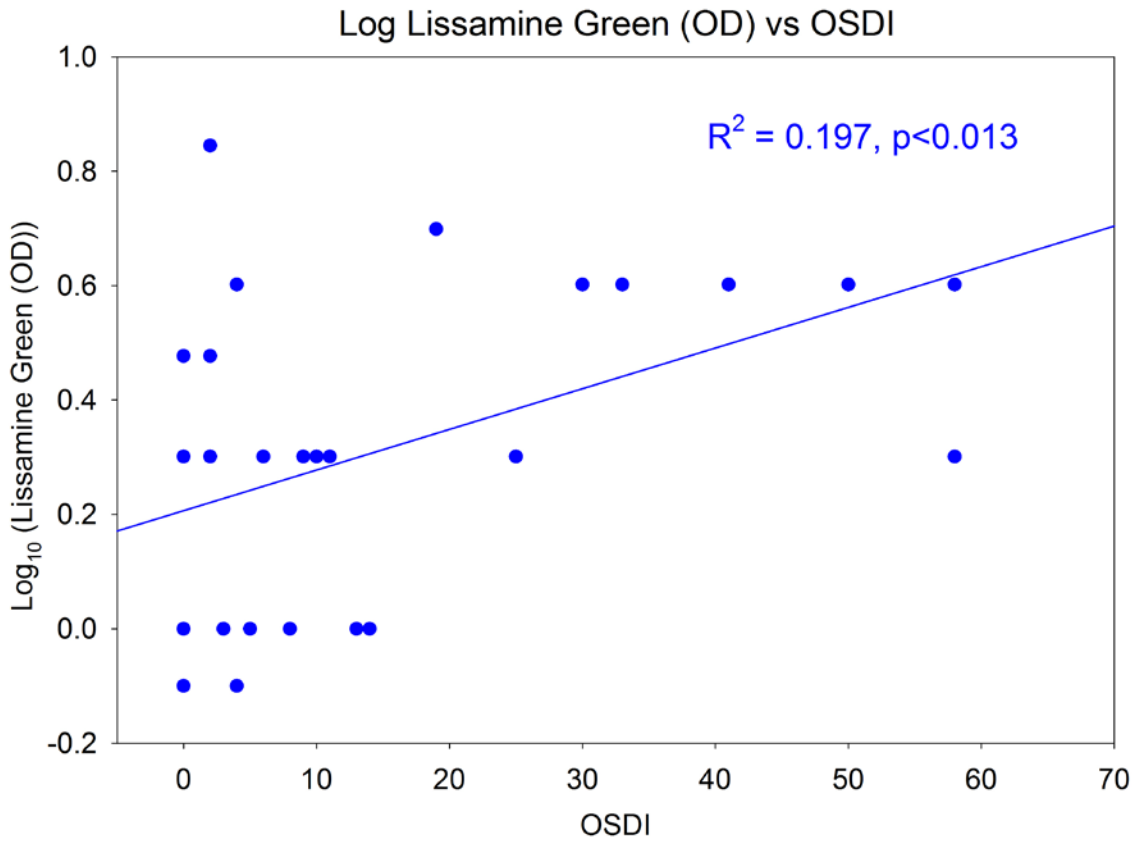

Figure 19 - Log transformed Lissamine Green data of the left eye versus OSDI for each patient resulted in an improved Pearson correlation ($R^2 = 0.197$; $p<0.013$).

15. Lissamine Green Score (OS) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed below (Table 33). Data distribution differed significantly from normal, but group variances were equal. A non-statistically significant Pearson correlation ($p<0.057$) and significant Spearman ($p<0.036$) correlation were found between Lissamine green staining score in the left eye and OSDI. ANOVA confirmed the significance of the regression with fair statistical power of the test. A plot of the correlation between Lissamine green score (OS) and OSDI is shown in Figure 20 (upper) and a residual plot (lower).

Table 33: Regression Analysis and ANOVA for Lissamine Green Score (OS) vs OSDI

Regression Equation		LG Staining OS = 1.823 + (0.0397 × OSDI) (N = 30, R = 0.351, R^2 = 0.123)			
Regression		Coefficient	Std. Error	t	P
	Constant	1.823	0.450	4.049	<0.001
	OSDI	0.0397	0.0200	1.985	0.057
ANOVA	DF	SS	MS	F	P
	1	13.963	13.963	3.940	0.057
Residual	28	99.237	3.544		
					P
Normality Test (Shapiro-Wilks)			Failed		<0.001
Constant Variance Test			Passed		0.189
			R	R^2	P
Spearman Rank Order Correlation			0.387	0.150	0.035

Power of test with alpha = 0.05, 0.479

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

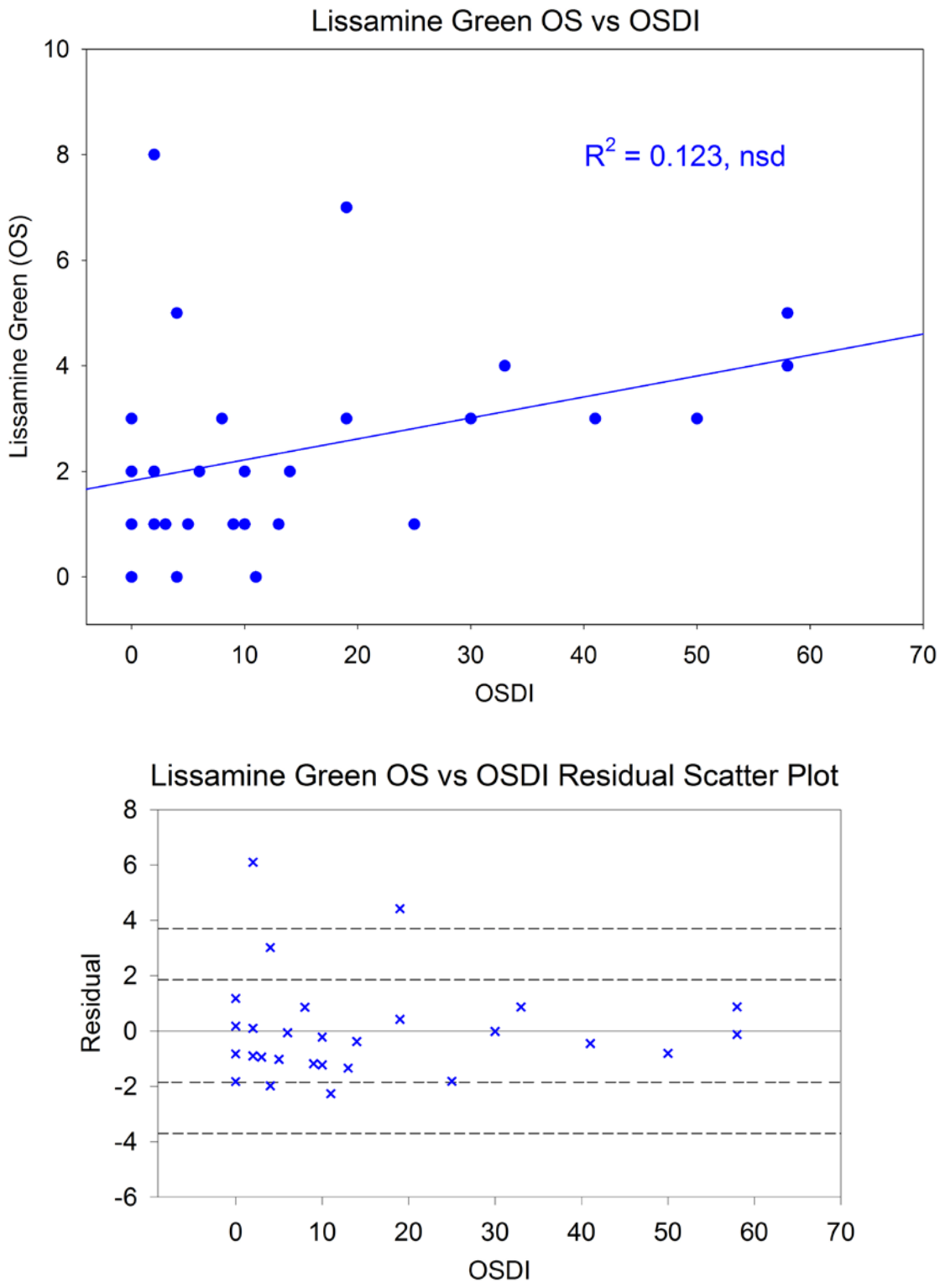

Figure 20 – Upper: Lissamine green staining (OS) vs. OSDI shows non-significant Pearson ($p<0.057$) and significant Spearman ($p<0.036$) correlations. Lower: residual scatterplot showing a heteroscedastic pattern of residuals, the scatter decreasing with increasing OSDI.

As with the right eye, to further investigate the Lissamine Green (OS) vs OSDI relationship, Lissamine Green data transformations were conducted. Interestingly, no transformation produced both normal data distributions, equal variances, and a significant correlation with OSDI. The relationship was therefore not further pursued.

While similar analyses are reported elsewhere in this thesis, analyses were included here for mean TearLab Osmolarity Score versus OSDI to complete all regression/ANOVA analyses of clinical test results versus OSDI.

16. Mean Osmolarity (OD) vs OSDI

Results for the regression analysis and ANOVA of the regression are listed below (Table 34). The correlation between mean osmolarity in the right eye and OSDI was not statistically significant ($p<0.234$). ANOVA confirmed this lack in significance of the regression and poor statistical power for the test. Data distribution parameters showed normal distribution, and there was no significant departure from equal variances in the two groups. Figure 21 shows a plot of the correlation between mean osmolarity (OD) and OSDI.

Table 34: Regression Analysis and ANOVA for Mean Osmolarity (OD) vs OSDI

Regression Equation		OSM Mean OD = 296.911 + (0.116 × OSDI) (N = 29, R = 0.228, R^2 = 0.052)			
Regression		Coefficient	Std. Error	t	P
	Constant	296.911	2.167	137.011	<0.001
	OSDI	0.116	0.0950	1.217	0.234, ns
ANOVA	DF	SS	MS	F	P
Regression	1	118.100	118.100	1.482	0.234
Residual	27	2152.193	79.711		
					P
Normality Test (Shapiro-Wilks)			Passed		0.445
Constant Variance Test			Passed		0.661

Power of test with alpha = 0.05, 0.219

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

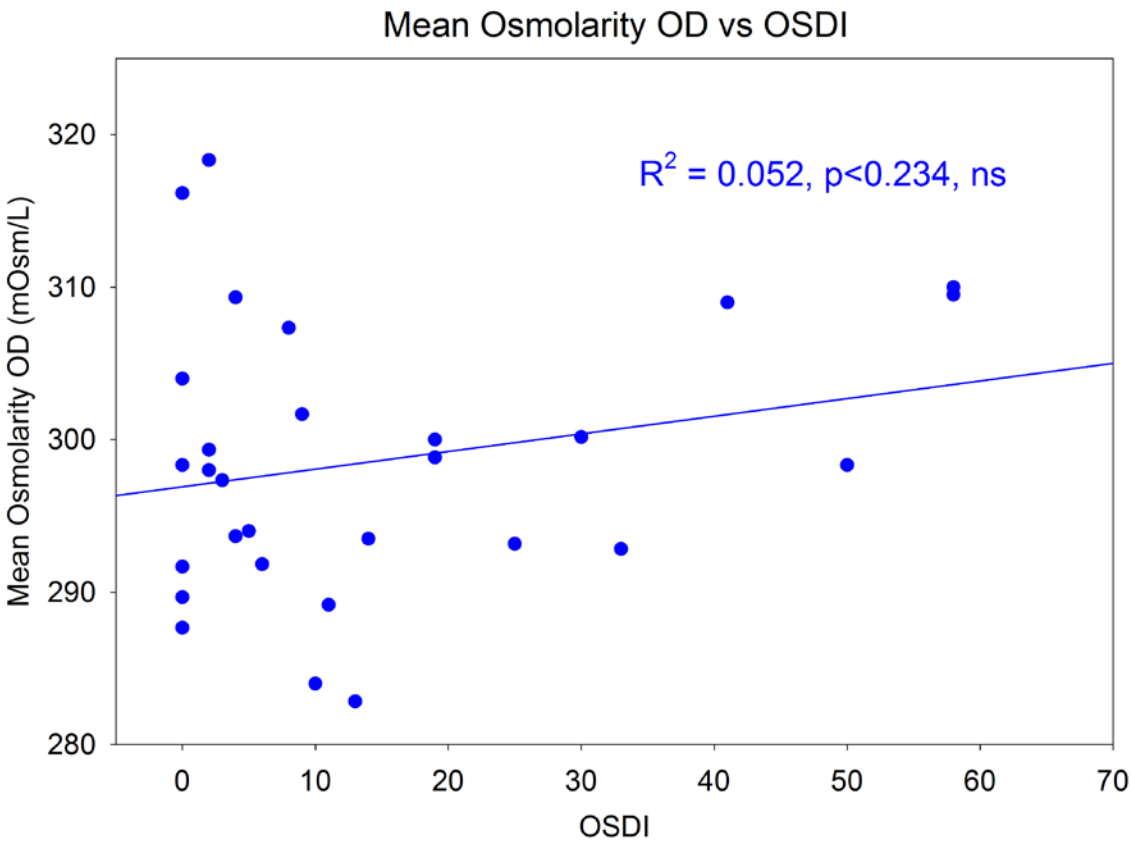

Figure 21 – Mean osmolarity of the right eye versus OSDI (Ocular Surface Disease Index score) for each patient shows an insignificant statistical correlation ($R^2 = 0.052$; $p<0.234$).

17. Mean Osmolarity (OS) vs. OSDI

Results for the regression analysis and ANOVA of the regression are listed below (Table 35). A statistically significant correlation was not found between mean osmolarity of the left eye and OSDI ($p<0.067$). ANOVA confirmed the lack in significance of the regression with poor statistical power. Data distribution parameters showed normal distribution, and constant variance showed there was no significant departure from equal variances in both groups. Figure 22 shows a plot of the correlation between mean osmolarity (OS) and OSDI.

Table 35: Regression Analysis and ANOVA for Mean Osmolarity (OS) vs OSDI

Regression Equation		Osm Mean OS = 298.737 + (0.192 × OSDI) (N = 30, R = 0.338, R^2 = 0.114)			
Regression		Coefficient	Std. Error	t	P
	Constant	298.737	2.272	131.488	<0.001
	OSDI	0.192	0.101	1.902	0.067, nsd
ANOVA	DF	SS	MS	F	P
	1	326.575	326.575	3.618	0.067
Residual	28	2527.207	90.257		
					P
Normality Test (Shapiro-Wilks)			Passed		0.345
Constant Variance Test			Passed		0.944

Power of test with alpha = 0.05, 0.448

t = Student's t value, P = probability of a statistically significant relationship, DF = degrees of freedom, SS = sum of squares, MS = mean square, F = F ratio.

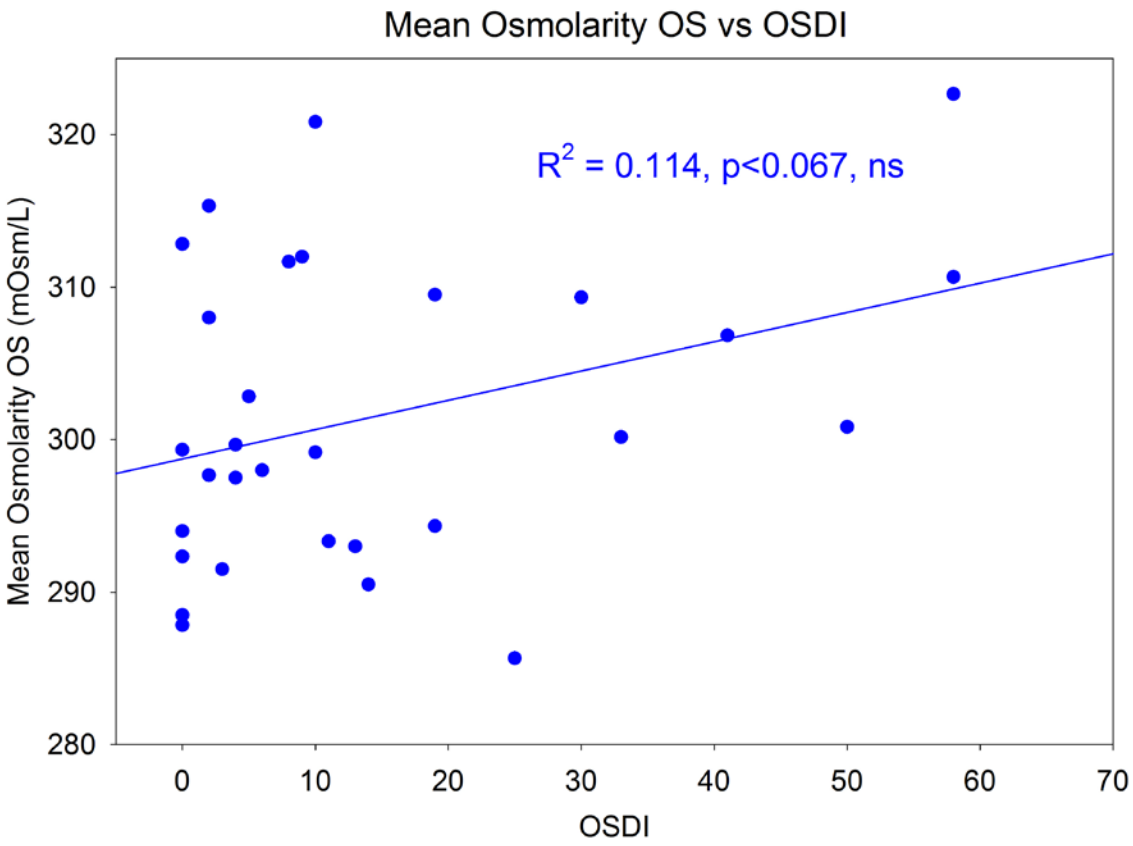

Figure 22 – Mean osmolarity of the left eye versus OSDI (Ocular Surface Disease Index score) for each patient shows an insignificant statistical correlation (R^2 = 0.114; p<0.067).

Differences among Clinically Grouped Data

The intent of Specific Aim 2 was to determine both the relationship between tear osmolarity and other clinical tests for dry eye and the ability of all dry eye tests to classify the study cohort. As an additional way to determine if other clinical tests could be used to group patients, dichotomous groups were defined based on the outcome of each clinical test, further divided by right and left eye (Tables 31 and 32). A series of t-tests were conducted on the grouped data to determine the ability of the clinical results to differentiate among participants based on clinical presentation. For example, participants were grouped based on low (<10) versus high (≥ 10) OSDI score. For data that was not normally distributed, a Mann-Whitney Rank Sum test was substituted in place of a t-test, using median values to test for differences between groups.

In Tables 36 and 37, clinical test results are listed by column, with grouping variables at the top. "Low-high" group means are listed by rows, identified by names in the far left column. More significant correlations are seen when the dry eye tests are grouped in this manner. TBUT and Lissamine green staining show the largest number of significant correlations for the right eye. For the left eye, the greatest number of significant correlations is seen with sodium fluorescein and Lissamine green staining. However, when the significant values for the right eye are compared with those of the left eye, only a small number of tests show the same grouped trends for both eyes. For example, TBUT showed a significant correlation in both eyes with the OSDI "low–high" value. Similarly, TBUT, NaFl staining, and Lissamine green staining showed the greatest number of significant differences between "low" and high "grouped" values; whereas, the Schirmer test did not show any significant correlations with this type of grouping.

Table 36 – Comparison of Clinical Test Results between Clinically Grouped Participants (Right Eye)

Group	OSDI OD	TBUT OD	NIBUT OD	Schirm. OD	TMH OD	NaFl OD	LissG OD	MeOsm OD
OSDI low hi								
t (or MW U)	-	(41.5)	(66.0)	(77.5)	1.57	(73.5)	(55.5)	0.85
P	-	0.006	0.100	0.251	0.202	0.179	0.031	0.403
TBUT OD								
t (or MW U)	(45.0)	-	(31.0)	(90.5)	1.113	–2.61	(52.0)	–0.107
P	0.009	-	0.001	0.538	0.275	0.014	0.019	0.916
NIBUT OD								
t (or MW U)	(66.0)	(22.5)	-	(84.0)	1.240	(69.0)	(56.5)	–1.759
P	0.098	0.001	-	0.389	0.103	0.122	0.034	0.272
Schirm OD								
t (or MW U)	(70.0)	(64.0)	(39.0)	-	0.640	0.357	(58.5)	0.456
P	0.739	0.522	0.056	-	0.527	0.724	0.346	0.652
TMH OD								
t (or MW U)	(54.0)	(56.0)	(57.0)	(86.5)	-	–2.27	(57.5)	–0.888
P	0.044	0.055	0.062	0.587	-	0.032	0.059	0.383
NaFl OD								
t (or MW U)	(104)	(73.5)	(83.0)	(102)	1.643	-	(85.0)	–0.231
P	0.982	0.174	0.348	0.913	0.112	-	0.382	0.819
LissG OD								
t (or MW U)	(59.5)	(39.0)	(59.0)	(98.0)	2.464	–1.31	-	–1.396
P	0.078	0.007	0.076	0.9892	0.020	0.121	-	0.146
MeOsm OD								
t (or MW U)	(90.0)	(61.0)	(58.0)	(91.0)	1.196	–0.72	–1.81	-
P	0.552	0.061	0.046	0.581	0.091	0.396	0.082	-

Groups on left hand column represent the low hi values of each test; OSDI = Ocular Surface Disease Index questionnaire; TBUT OD = fluorescein tear break-up time in the right eye; NIBUT OD = non-invasive tear break-up time in the right eye; Schirm OD= Schirmer I test in the right eye; TMH OD = tear meniscus height in the right eye; NaFl OD = fluorescein vital dye staining in the right eye; LissG OD = Lissamine green vital dye staining in the right eye; MeOsm OD = mean osmolarity values in the right eye.

Table 37 – Comparison of Clinical Test Results between Clinically Grouped Participants (Left Eye)

Group	OSDI OS	TBUT OS	NIBUT OS	Schirm. OS	TMH OS	NaFl OS	LissG OS	MeOsm OS
OSDI low hi								
t (or MW U)	-	(61.5)	(57.5)	(93.5)	–1.25	1.561	(79.5)	0.563
P	-	0.037	0.025	0.453	0.104	0.130	0.175	0.578
TBUT OS								
t (or MW U)	(47.0)	-	(6.0)	(107)	0.672	–2.19	(63.0)	–1.994
P	0.008	-	0.001	0.883	0.507	0.037	0.045	0.371
NIBUT OS								
t (or MW U)	(36.0)	(15.0)	-	(97.0)	0.843	–2.25	(53.5)	–2.083
P	0.002	0.001	-	0.655	0.406	0.032	0.020	0.047
Schirm OS								
t (or MW U)	(57.5)	(79.5)	(84.0)	-	1.173	–0.36	(56.0)	0.186
P	0.157	0.707	0.870	-	0.086	0.723	0.132	0.853
TMH OS								
t (or MW U)	(87.0)	(82.0)	(88.0)	(0.88)	-	(35.0)	–3.31	–0.398
P	0.306	0.219	0.328	0.327	-	0.001	0.003	0.694
NaFl OS								
t (or MW U)	(77.0)	1.919	1.770	(101)	5.251	-	(46.5)	–0.329
P	0.145	0.065	0.088	0.632	0.001	-	0.006	0.744
LissG OS								
t (or MW U)	(60.5)	(66.0)	(73.0)	(78.5)	2.651	–3.39	-	–2.356
P	0.037	0.065	0.121	0.186	0.013	0.002	-	0.026
MeOsm OS								
t (or MW U)	(72.0)	(67.5)	(76.5)	(86.5)	1.364	–1.31	(58.5)	-
P	0.131	0.089	0.189	0.372	0.183	0.202	0.034	-

Groups on left hand column represent the low hi values of each test; OSDI = Ocular Surface Disease Index questionnaire; TBUT OS = fluorescein tear break-up time in the left eye; NIBUT OS = non-invasive tear break-up time in the left eye; Schirm OS= Schirmer I test in the left eye; TMH OS = tear meniscus height in the left eye; NaFl OS = fluorescein vital dye staining in the left eye; LissG OS = Lissamine green vital dye staining in the left eye; MeOsm OS = mean osmolarity values in the left eye.

DISCUSSION

This project had two specific aims, both of which were successfully addressed. The first specific aim provided a more definitive outcome, which was expected based on the number and diversity of enrolled participants.

Specific Aim 1

"Determine the reliability of the TearLab in producing consistent measurements of tear osmolarity and if a single measurement, as recommended by the manufacturer, provides an equivalent result to repeated measurements in both control and dry eye patients."

This aim was considered important because the original version of the TearLab in the PI's lab provided very inconsistent measurements. However, the newer TearLab instrument provided consistent values for tear osmolarity upon repeat measurements within a single session. This was true for both eyes. During the current study, no trends were evident over the course of the six repeat osmolarity measurements in each eye, so temporal or cumulative sampling effects did not appear to be an issue. The results support this by showing little variability across all six measurements of osmolarity of both eyes. While Cronbach's Alpha test demonstrated that the third osmolarity measurement correlated best with the mean of the remaining five measurements, the difference was not sufficient to warrant a recommendation that three measurements be taken per eye.

As mentioned before, an earlier version of the TearLab instrument, used in the same clinic research facility by the PI and other investigators, did not show the same level of consistency. It therefore appears that for the newer version of the TearLab instrument, the consistency issue has been resolved. This is an important outcome because it removes a source of variability that may otherwise have confounded comparisons between tear osmolarity measurements and the results of other clinical tests for dry eye. Therefore, for the current version of the TearLab osmometer, measurements of osmolarity can be considered suitably consistent. Reliability was confirmed to the extent that it performed according to published instrument specifications for expected variability. According to a 2012 report published in IOVS by TearLab, the device underwent extensive testing prior to obtaining conditional FDA approval. For the purposes of assessing "in-house" performance, the following procedures were reported to the FDA (510k Studies)[35]: TearLab calibration – human tears' TearLab matrix effects & interfering substances; internal precision – within run and day to day; internal precision – between instrument; internal precision – lot to lot; linearity; internal method comparison; limits of detection; and external precision – within run and day to day.

However, despite matching the published within-run specifications, the lack of intra-measurement variability within one specific measurement found in the current study(e.g. measurement 1, measurement 2) enables the question as to whether the TearLab osmometer has sufficient dynamic range to differentiate a normal eye from a dry eye, especially in mild to moderate cases. There is an expectation, based on earlier studies using vapor pressure or freezing point depression osmometers, that the range of osmolarity across the normal and dry eye population is much greater[23, 41]. To what extent

this difference in measurement ranges is due to the very different sampling methods between these earlier procedures and the TearLab was not addressed in the current study. Intuitively, the osmolarity measured "in-vitro" in the marginal strip is very different from that of a large ($\geq$ 5uL) tear sample that is removed from the eye and processed, "ex-vitro." Certainly, a small nanoliter range tear volume should be much more representative of the in vivo tear film than a collected and stored sample of much greater volume.

Specific Aim 2

"Determine the correlation between TearLab results and results of clinical tests commonly used to differentiate dry eye patients from non-dry eye patients."

TearLab osmolarity measurements showed some correlations with the outcome of other clinical tests results for dry eye. However, the mean of the six osmolarity measurements for the right eye showed no significant correlations with any other clinical test. The same was true for the left eye, although the OSDI symptom questionnaire showed a significance level of 0.067 for the correlation with osmolarity. Conversely, when patients were grouped based on specific clinical tests, osmolarity did show some significant intergroup differences.

In addition, individual osmolarity measurements did correlate with other clinical tests. In the right eye, the second repeat measurement of osmolarity correlated significantly with the OSDI symptom score, but none of the other repeats were even close to correlating. More correlations were found for the left eye, but they demonstrated a very sporadic pattern in terms of which repeat measurement was correlating. The OSDI score

correlated with osmolarity repeat 2 ($p<0.0375$) and 5 ($p<0.0369$), but none of the other repeats correlated. Lissamine green staining score (OS) correlated with osmolarity repeat 3 ($p<0.0264$), and Fluorescein TBUT (OS) correlated with repeat 6 ($p<0.0368$). Again the lack of consistent correlations across all six osmolarity repeats means that these isolated correlations are of little value.

These findings indicated that, although there was little inter-measurement variability across all osmolarity measurements, there was also little correlation between osmolarity and other clinical tests for dry eye. In general, the other clinical tests exhibited better and more frequent correlations amongst themselves. A noticeable exception was the Schirmer test. Therefore, despite acceptable repeatability of the TearLab measurement, mean osmolarity measurement appeared in this limited study to lack the clinical potential to differentiate between different types of dry eye and different severity levels.

One reason for the lack of more systematic correlations is almost certainly the limited range of tear osmolarity measured across the study group. For any single set of osmolarity readings in the right eye, the largest range was found in measurement 6 with a minimum osmolarity of 281mOsm/L and a maximum of 333mOsm/L, a 52mOsm/L difference. Osmolarity measurement 3 of the right eye, which Cronbach's Alpha determined to correlate best with the mean of the other measurements, showed a 50mOsm/L difference between the maximum and minimum measurement values. In the left eye, measurement 1 showed the largest variance with a minimum osmolarity of 280mOsm/L and a maximum of 346mOsm/L, a 66mOsm/L difference. Measurement 3, which also correlated best in the left eye, showed a 31mOsm/L variance. Given the good

internal consistency of repeat TearLab measurements and the almost sporadic significant correlations between individual osmolarity measurements and other clinical tests, the lack in dynamic range in the measured osmolarities is clearly a limiting factor. Again, it should be stressed that this may have been due to the small number of patients selected for each dry eye classification (normal, aqueous deficient and evaporative), and may also be attributable to a lack in range of severe cases in each dry eye category.

Correlations among other Clinical Tests for Dry Eye

More significant correlations were seen amongst the other clinical tests for dry eye compared to those with tear osmolarity. However, this was not universal and some tests failed to elicit correlations. The OSDI questionnaire as a symptom-based assessment was compared to all other clinical tests for dry eye. DEWS severity of the higher eye, right eye, and left eye all showed statistical significance with OSDI. However, both NIBUT and TBUT failed to correlate with OSDI for the right eye, yet they both showed significant correlation with OSDI for the left eye. This may be an ordering effect, because both tests were first conducted on the right eye and perhaps the fellow eye was more indicative of the patients' symptomatology as it would correlate in the real world. The second eye tested, the left eye, would have undergone a break-up time twice for both tests, because the first break-up time was measured on the right eye. The Schirmer I test did not correlate with OSDI for either eye and was the only test that failed to show any correlation to OSDI score. Tear meniscus height and sodium fluorescein staining correlated with OSDI score for both eyes. Lissamine green staining in the right eye correlated significantly with OSDI, while the left eye did not.

When comparing the grouped low versus high clinical score values, more individual significant correlations were seen amongst the clinical dry test. TBUT and Lissamine green staining showed the largest number of significant correlations for the right eye, and sodium fluorescein staining and Lissamine green staining correlated most with the low versus high values in the left eye. As mentioned previously, the individual correlations of each eye in general did not correspond with those found for the contralateral eye. Encompassing the values for both eyes, TBUT, NaFl staining, and Lissamine green staining showed the greatest number of significant differences between the low and high grouped values. Similarly to the grouped correlations between clinical tests and OSDI, the Schirmer score did not elicit a significant correlation with any low high values.

Comparisons with other Studies using the TearLab Osmometer

Repeatability

Prior to acquisition of the newer TearLab instrument used in the current study, Dr. Benjamin Sullivan traveled to the PI's lab to review findings with the original device. During his visit, he reported that the original instrument being used in the PI's laboratory was one of the earliest designs and that both calibration and consistency issues had been reported by some investigators. In addition, some of the earlier batches of cards, manufactured in Australia and shipped to the United States, had shown batch-to-batch inconsistency. According to Dr. Sullivan, both issues were resolved with a newer TearLab instrument design and with improved quality control procedures in card manufacture and shipping (Dr. Benjamin Sullivan, Chief Scientific Officer, TearLab

Corporation, Personal Communication, May, 2013). Following Dr. Sullivan's visit, a newer version of the TearLab was provided and installed by TearLab personnel, and replacement batches of test cards were also provided. The newer instrument and the replacement test cards were used in the current study.

As reported in the Results section of the current study, a substantial improvement in repeatability was found after changing from the older version of the TearLab to the more recent design.

Interestingly, some groups continue to report substantial variability with repeat measurements using the TearLab, while others report good repeatability. High variability of repeat measurements is often cited as a confounding factor, limiting the ability to correlate tear osmolarity findings with the results of other clinical tests for dry eye[42, 43]. Sebbag et al (2014)[43] conducted osmolarity tests and repeatability studies on a canine dry eye/control cohort. Repeat intra-session TearLab measurements revealed high variability and poor to moderate repeatability and reproducibility. Limits of agreement were wide (95%) and intraclass correlation coefficients < 0.75 were found.

Conversely, Sullivan et al[31] reported that the TearLab had the lowest degree of variability amongst clinical dry eye tests, including the Ocular Surface Disease Index questionnaire, Schirmer test, tear break-up time, staining, and meibomian gland integrity score[31]. He also observed that, similarly to the current study, tear osmolarity demonstrated a lower range than expected when compared to other clinical dry eye tests. Despite the low variability of repeat measurements and, more importantly, the lower dynamic range, Sullivan et al still concluded that tear osmolarity did differ significantly between mild, moderate and severe dry eye patients.[31] Other groups report variability

and suggest ways to compensate for its influence. Khanal and Millar[44] conducted repeat measurements of tear osmolarity both within session and on a diurnal basis, three measurements being taken within each session at 9 AM, 12 noon and 4 PM on two consecutive days. They reported individual variations up to 35 mOsm/L for consecutive tear osmolarity readings. However, when averaged over the three repeats within each session, osmolarity values were considered reliable at the 95% confidence level. A power analysis based on the variability of their data indicated that three repeat measurements would be required to obtain reliable data for a study with fewer than 50 subjects, but one measurement would be adequate for a group of 490 or more subjects[44]. In the same study, the authors concluded that diurnal variations were not an issue. Across the study group, when the three repeat measurements for 9 AM were compared with those for 12 noon and those for 4 PM, no significant diurnal variation was noted[44].

Correlations between Osmolarity and other Clinical Dry Eye Tests

Multiple studies have been conducted to compare TearLab osmometer findings with other clinical dry eye tests in terms of consistency and variability. The current study did not find that there was sufficient differentiation between mild, moderate and severe dry eye based on tear osmolarity, again due to the lack of range of values.

Li et al[45] compared tear meniscus height (TMH) with tear osmolarity using a diurnal sampling method on dry eye and control patients. Osmolarity and TMH were measured at 2 hour intervals between 8:30 AM and 4:30 PM. The mean tear osmolarity of the dry eye patients was 304.0 ± 10.8 mOsm/L, and the mean tear osmolarity of the normal subjects was 298.0 ±14.2 mOsm/L ($P > 0.05$). Over the course of 8 hours, the

average measured osmolarities of the dry eye group varied by approximately 21.9 ± 13.5 mOsm/L (range, 6–43 mOsm/L), and the average measured tear osmolarities of the normal group varied by approximately 21.0 ± 9.2 mOsm/L (range, 8–35 mOsm/L).

A second study by Sullivan investigated the clinical utility and accuracy of grading dry eye severity with various clinical dry eye tests, including the TearLab osmometer[35]. The study's results showed that tear osmolarity was the most accurate test in grading severity of dry eye, as shown by the highest correlation coefficient, followed by conjunctival staining, corneal staining, OSDI, meibomian score, tear break-up time, and Schirmer test[35]. This second study by Sullivan was not consistent with the first in terms of findings, because in the first study, corneal and conjunctival staining failed to differentiate between mild, moderate and severe dry eye[31, 35].

In the current study, participants were also categorized into low-high groups to explore the ability of each clinical test to classify patients. Once classified, differences were sought between high and low groups in terms of their correlation with other clinical tests. Comparing all the clinical dry eye tests to each other in this way, osmolarity was a poor discriminator compared to the other tests, only Schirmer test groupings showing lower discrimination. Overall, the low dynamic range of tear osmolarity values, as also seen in Sullivan's study[31], made it difficult to differentiate between mild, moderate and severe dry eye groups.

Other study findings also concur with those of the current study. Amparo compared symptomology (OSDI), corneal staining, and tear osmolarity in groups of patients previously documented as having various degrees of DED, and they found that tear osmolarity did not differentiate patients based on symptoms or corneal staining

across all DED subgroups of [46]. They did find significant correlations between clinical tests, as did the current study. Corneal staining score correlated with symptoms (OSDI) in DED patients [46]. Messmer et al also found that tear osmolarity could not distinguish between dry eye and control groups and that osmolarity did not correlate with any other clinical dry eye tests[37]. Contrary to Sullivan's explanation for the lack in correlation[31], Messmer concluded that the TearLab was less reliable than other clinical tests for differentiating dry eye groups[37].

Similarly to Messmer's findings, the current study indicated that tear osmolarity was unable to distinguish groups based on dry eye severity, and thus, was not useful for grading dry eye type or severity.

Several studies found TearLab osmometer measurements to be inconsistent and their value unclear. For example, Bunya et al administered three repeat measurements within the same clinical visit in 1-minute intervals[47]. They found that the difference between Sjögren's syndrome patients, blepharitis patients, and the control group was 6mOsm/L, a difference that was not statistically significant. In addition, they noted that the control group showed substantially greater consistency of repeat measurements than either of the dry eye groups[47]. The current study found that repeat measurements did not yield to high error or variability and that the tear osmolarity measurements were largely consistent. However, both studies agree that the range between different categories of dry eye is narrow. Another important note is that when Bunya et al followed the manufacturer's recommendation to use the higher osmolarity value measured between the two eyes, the difference between the control and dry eye groups' mean values decreased[47].

Perhaps the study that provided the greatest number of parallels with the current study was that of Szalai et al[32]. Three study groups were used, non-Sjögren's dry eye, Sjögren's dry eye and normal control patients. Mean tear osmolarity across the three groups was remarkably consistent at 296.8 (non-Sjögren's dry eye), 303.4 (Sjögren's dry eye), and 303.5 mOsm/L (control group). According to the TearLab threshold for hyperosmolarity 15%, 23% and 16% respectively of patients in the three study groups were abnormal (hyperosmolarity)[32]. Also consistent with the current study, other clinical test results showed greater ability to differentiate the dry eye patient groups from the normal controls. Schirmer scores, corneal staining score, TBUT, and meibomian gland status differed significantly in the dry eye groups compared to the normal control group (P , 0.0001). Finally, as with the current study, Szalai found no significant correlations between tear osmolarity and any other clinical test for dry eye[32].

A study of canine dry eye in beagles, a breed with a high incidence of Sjögren's-like severe dry eye also brought into question the ability of the TearLab to correctly classify dry eye based on severity[43]. Although the study cohort was small, mean tear osmolarity was significantly higher ($p<0.0001$) in normal dogs (337.4 ± 16.2) than dogs with untreated dry eye (306.2 ± 18.0). Interestingly, treated dry eye dogs elicited a higher osmolarity (330.5 ± 13.7) than untreated dogs. Repeated measurements revealed high variability and overall poor to moderate repeatability and reproducibility (wide 95% limits of agreement, intraclass correlation coefficients < 0.75), although this was improved by taking three successive measurements at each session. A positive correlation existed between STT-1 and tear osmolarity measurements of all dogs combined (Pearson's correlation test, $p = 0.04$, $r = 0.62$).

Study Limitations

The main challenge for this study was to recruit study participants with a sufficiently broad range of dry eye severity – in particular those with severe dry eye. Due to the scope of the study and the limitation to a single 45 minute clinic visit, participants were not asked to suspend any dry eye or autoimmune disease treatments prior to the clinic visit. Contact lens wearers make up a significant fraction of the real world dry eye population. For this reason daily wear patients were accepted into the study prior and were not instructed to modify their normal daily wear routine. This insured that participants represented the typical normal and dry eye demographic in a routine clinical setting, with the same presentation of dry eye signs and symptoms as would be seen in clinical practice.

Another limitation and potential criticism of the single study visit format is that the effects of diurnal variations in tear osmolarity could not be addressed in this study. This was intentional because diurnal trends were outside the scope of the primary aim: Specific Aim 1. With the emphasis on within-visit repeatability of tear osmolarity measurements, diurnal trends represent an irrelevant variable. In addition, single session measurement of tear osmolarity is the norm for clinical practice; not the discovery of diurnal trends in individual patients.

An additional variable was introduced with the acceptance of both contact lens wearers and non-contact lens wearers into the study. It could be argued that contact lens wearers should have been excluded. However, given the popularity of contact lenses for both patient groups, this would have biased the study away from the real world of clinical practice.

A similar argument could be made to have dry eye patients discontinue all treatments for a period for example of 2 weeks. That is to establish a "wash-out" period. This was considered both unnecessary and impractical for several reasons. The study cohort included several patients on immunosuppressant and other medications. Some were Sjögren's syndrome patients. Patients rely on these medications for symptom relief and for longer-term therapeutic benefits for a variety of underlying autoimmune diseases and other conditions. In addition, the time required to truly "wash out" the effects of longer acting medications such as Restasis would be much longer than a standard two week wash-out period.

Environmental control of temperature, humidity, air-flow, and other factors in the examination room were as consistent as possible within the limitations of a centrally air conditioned and reheated building with modular reheat controls for temperature regulation. No specific environmental controls were added in the study examination room. A separate thermometer was used to monitor room temperature throughout the study. Temperature remained relatively constant in the low 70° range for the entire duration of the study. Larger fluctuations due to a system malfunction did not occur at any time. The vast majority of patient visits were conducted in a single summer, and humidity, along with temperature, remained relatively constant. The TearLab osmometer was maintained in the same room in the same location on the countertop and remained switched on for the entire duration of the study. The instrument was calibrated daily according to manufacturer instructions.

Follow-up Questions Raised by the Study

The following issues were identified during the study and are discussed in terms of potential solutions to those issues or possible follow-up studies:

1. The TearLab osmometer was found in this study to elicit a very narrow dynamic range which limited its ability to discriminate dry eye patients from controls, and to obtain a measure of dry eye severity. Would an alternate tear osmolarity measuring device with larger dynamic range solve this problem? There are currently no alternatives to the TearLab osmometer that would be practical and clinically feasible for on-eye measurement or in-office use. Alternative devices using colligative or other properties, such as vapor pressure or freezing point depression osmometers, are not developed to the extent that they could provide on-eye measurement. In addition, they are not likely to do so in the foreseeable future because of their tear volume requirements, the need to collect a tear sample, and their use of controlled measurement chambers. There are no other instruments that could provide on-eye measurements. In the future, a device based on alternative technology to the above methods and to simple impedance, may be able to provide on-eye measurements with greater dynamic range.
2. Is the range of tear osmolarity intrinsically limited when measured on-eye? This would be difficult to test, considering that osmolarity measurements taken from a collected tear sample, ex-vitro, introduce many new variables when compared to on-eye measurement. The variables include, but are not limited to, reflex stimulus with prolonged lid/conjunctival contact with a

collection tube, evaporation of the tear sample over the course of collection, and changes in tear composition during collection, transfer, and osmolarity measurement. Until an alternate device capable of providing in-vivo tear osmolarity measurements becomes available, these issues cannot be addressed.

Future Directions

Future directions to expand upon the current study:

1. A larger study of dry eye patients with a deliberately greater number of true normals and true severe dry eye patients (e.g. high OSDI, high DEWS score, consistently low T/NIBUT, low Schirmer score, and high staining scores).
2. A study of diurnal variation with a smaller number of repeat measurements but more measurements throughout the day (e.g. two repeat measurements, three times a day). This would ideally be a 30-day study with a sample of both males and females to contrast any differences in fluctuation of osmolarity between the genders throughout the 30-day period. The foreseeable problem with this study would be that many of the other clinical tests for dry eye (e.g. Schirmer, TBUT, etc.) would not be amenable to rapid or within-day repeats. Except for the NIBUT test, most other clinical tests would not be truly repeatable due to the invasive nature of the tests. OSDI would not be meaningful as a repeatable test within a given session, though it could vary throughout the day.

CONCLUSION

Dry eye is a multifactorial disease stemming from several different etiologies. As a result, it is difficult to have one clinical test that can diagnose all the different types of dry eye disease. Tear osmolarity is one method, and the TearLab osmometer has been heralded as the "gold standard" for diagnosing dry eye.

The change to a new model of the TearLab osmometer has resulted in substantially more consistent and repeatable osmolarity measurements across a series of six measurements per eye in a sample population of 30 dry and non-dry eye participants. This study found that a single measurement is sufficient in obtaining the patient's tear osmolarity, although the third measurement in a series of six was found to correlate best with the mean of the six according to statistical analysis using the Cronbach's Alpha test.

Despite the repeatability of tear osmolarity measurements, the range of osmolarities across the entire study group was very narrow precluding the discrimination of dry eye severity or even differentiating dry eye from non-dry participants. While the number of severe dry eye patients was small in the study, there were sufficient mild to moderate patients to conclude that the TearLab osmometer will have difficulty differentiating these patients from those without dry eye.

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APPENDIX

INFORMED CONSENT IRB APPROVAL FOR PROTOCOL X130626011

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator:	SHIN, PEARL
Co-Investigator(s):	MORROW, ERIKA MARIE THAN, TAMMY P
Protocol Number:	**X130626011**
Protocol Title:	*Within Session Repeatability of the Tear Lab Osmolarity Test and Correlation with Other Clinical Test for Dry Eye*

The IRB reviewed and approved the above named project on 7-18-14. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 7-18-14

Date IRB Approval Issued: 7-18-14

IRB Approval No Longer Valid On: 7-18-15

Marilyn Doss

Marilyn Doss, M.A.
Vice Chair of the Institutional Review
Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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