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# The Role of Multisystem Disease in Composition of Autologous Serum Tears and Ocular Surface Symptom Improvement

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Short Title: The Role of Multisystem Disease in Composition of Autologous Serum

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**Abstract:**

Purpose: Autologous serum tears (AST) contain growth factors and vitamins similar to those in healthy tears and are an effective treatment option for ocular surface disease. This study determined the differences in composition of AST in patients with systemic diseases versus patients with localized ocular surface diseases.

Method: A prospective observational cohort study was performed on 53 patients with either systemic diseases affecting the ocular surface (Group I) or localized ocular surface diseases (Group II) who were prescribed AST. Concentrations of epidermal growth factor (EGF), interleukin 8 (IL-8), fibronectin, vitamin A, and tumor necrosis factor-α (TNF-α) were determined through ELISA assays from patients in both groups. The Ocular Surface Disease Index (OSDI) scores were calculated prior to and 6 weeks after initiation of treatment with AST for new patients.

Results: The average concentration of EGF in Group I (29.39 pg/ml ± 52.85 pg/ml) was significantly lower than in Group II (88.04 pg/ml ±113.75 pg/ml) ($p<0.05$). Levels of fibronectin, IL-8, and vitamin A were similar in both groups. There was a 24% reduction in OSDI score 6 weeks after initiation in Group I compared to a 36% reduction reported in Group II ($p=0.065$). The OSDI score before and after treatment was reduced significantly in all subjects ($p = 0.002$).

Conclusion: Serum tears are a promising therapy for management of ocular surface disease and associated symptoms. The differences between levels of EGF in patients with localized ocular surface disease and systemic inflammatory disease may account for differences in therapeutic outcome.

**Keywords:** Autologous Serum Tears; Ocular Surface Disease; Systemic Inflammatory Disorders; Epidermal Growth Factor

**Funding:** This work was supported by the Illinois Society for the Prevention of Blindness.

## 1. Introduction:

The tear film is a complex product of the main and accessory lacrimal glands, goblet cells located on the ocular surface, and the meibomian glands of the eyelids. The tear film plays a critical role in maintaining a healthy ocular surface environment by providing growth factors, vitamins, electrolytes, and neuropeptides that support the growth and migration of epithelial cells (1). A myriad of systemic diseases that affect the ocular surface and local ocular surface diseases can negatively impact the tear film and its composition. These include mucoaqueous deficiency such as Sjögren's syndrome and goblet cell destruction, as well as ocular adnexal abnormalities such as lagophthalmos or meibomian gland dysfunction (2). Tear film instability can lead to a variety of ocular symptoms including dryness, irritation, light sensitivity, foreign body sensation, red eyes, and vision loss.

While the symptoms of ocular surface disease do not necessarily correlate with disease severity, there are various treatments to alleviate them. The current first line therapy for the management of ocular surface disease is the use of lubricating artificial tears. Artificial tears, however, are not the ideal substitute for natural tears as they lack the complex composition of water, salts, hydrocarbons, proteins, and lipids of natural tears (3,4). Other FDA approved treatment options for inflammatory causes of decreased tear production and dry eye include anti-inflammatory agents cyclosporine (Restasis®) and Lifitegrast (Xiidra®), respectively. Blood derived products such as autologous serum tears (AST) were developed because they have similar biochemical properties to those of human tears (5). AST contain similar growth factors, cytokines, vitamins, and nutrients found in natural tears. These include epidermal growth factor (EGF), fibronectin, and vitamin A, all of which support epithelial cell growth and migration (6).

The effectiveness of AST has been reported in multiple studies for the management of systemic and local diseases that affect the ocular surface. This list includes the following: Sjögren's syndrome, secondary Sjögren's syndrome, ocular graft versus host disease, Stevens Johnson syndrome, mucus membrane pemphigoid, post LASIK dry eye, and severe dry eye (7-15). Interestingly, the results from multiple studies have failed to show significant improvement in objective clinical findings such as tear break up time, Schirmer testing, or ocular surface staining (7-12). However, many studies have demonstrated an improvement in subjective symptomatic measures of dry eye, including one by Ali, et al. that showed both objective and subjective improvement in patients with systemic autoimmune disease (7-12, 16). There are a few studies that compare the outcomes of ASTs between two different subgroups, one comparing primary and secondary Sjögren's and another comparing chronic Stevens-Johnson disease and non-autoimmune dry eye (17, 18). Existing studies, including the ones mentioned above, either investigated a specific indication or they grouped all indications together without comparing efficacy between the different indications.

ASTs have been shown to have minimal side effects overall, however the possible side effect of limbitis in the treatment of patients with underlying immunologic systemic diseases should be considered, as originally reported by Welder et al (19). Contamination of ASTs with various bacteria and ocular infections because of the use of AST have been reported in previous studies, but is very rare (20).

Prior studies on serum only, the source of AST, have shown that cytokines, growth factors, and other mitogens are quite variable in serum concentration between healthy controls and those with systemic conditions like SJS (21). Serum from patients with systemic conditions have higher levels of inflammatory mediators, which could be present in their serum and affect

both the composition and the efficacy of the AST. Additionally, it is unknown how the levels of growth factors, nutrients, and vitamins in AST vary between different conditions. There are currently no studies that have compared the composition of AST and the improvement in symptomology based on a patient's underlying condition.

The purpose of this study was to determine if there is a difference in the composition of AST between patients with systemic diseases with dysregulation of the immune system and/or inflammation, and those with local ocular surface diseases. We hypothesize that there is a significant difference in the composition of AST between patients with systemic diseases that affect that ocular surface disease and those with localized ocular surface disease.

**2 Methods:**

A prospective, observational cohort study involving 53 patients with ocular surface diseases requiring AST as part of their treatment was carried out in Chicago, Illinois, USA between November 2017 and November 2018. The tenets of the Declaration of Helsinki were followed and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. Institutional Review Board (IRB) approval was obtained from the University of Illinois at Chicago (Chicago, IL, USA) and Loyola University Chicago Health Sciences Division (Maywood, IL, USA). Patients were recruited at the Illinois Eye and Ear Infirmary, University of Illinois at Chicago (Chicago, Illinois, USA). The AST were also compounded at the same institution per their established protocol. A total of 53 patients with ocular surface diseases requiring AST as part of their treatment were recruited over the stated time period. Informed consent was obtained at the time of recruitment. The diagnoses of the specific ocular surface disease and the indication for prescribing of AST were provided by

cornea specialists in the Chicagoland area prior to referral for AST. Information collected included age, sex, and past medical history of systemic immune mediated disorders such as Sjögren's Syndrome (primary and secondary), ocular graft versus host disease (oGVHD), Stevens Johnson syndrome (SJS) or Toxic Epidermal Necrolysis (TEN), lupus, rheumatoid arthritis (RA), uveitis, chemical burn, and herpes keratitis. The patient's blood was drawn at the Illinois Eye and Ear Infirmary and a 2-mL aliquot of 20% AST was obtained from each patient at the time of compounding. The aliquot was placed in a sterile 5 cc vial and transported on wet ice to Loyola in an insulated container. The samples were stored at -40oC in the Ophthalmology Research Laboratory in the Center for Translational Research and Education (CTRE) at Loyola where all ELISA testing was performed. ELISA assays were run for vitamin A (LSBio LS-F25655-1 Seattle, WA), fibronectin (Thermo Fischer BMS2028 Waltham, MA), epidermal growth factor (EGF) (Thermo Fischer KHG0062 Waltham, MA), tumor necrosis factor- α (TNFα) (Thermo Fischer 88-7346-88 Waltham, MA), interleukin-8 (IL-8) (Thermo Fischer 88-8086-88 Waltham, MA), interferon γ (IFNγ) (Thermo Fischer 88-7316-88 Waltham, MA), and interleukin 17 (IL-17) (Thermo Fischer KAC1591 Waltham, MA) using the manufacturer's protocol.

In addition, 20 patients who were evaluated and received serum tears for the first time were enrolled in a prospective study to evaluate the changes in ocular symptoms associated with the use of the AST. Patients completed a standard validated ocular surface disease index (OSDI) questionnaire both prior to and 6 weeks following the initiation of treatment with AST (22). Results of OSDI questionnaires in addition to information including age, sex, and past medical history were collected and evaluated.

**2.1 Statistical analysis:**

The data is presented as mean ± standard deviation and/or percentage. The statistical analyses were performed using SPSS software version 24. Mean differences between groups were analyzed using t-test, Mann-Whitney U, Chi-Square tests, and one-way ANOVA. P-values less than 0.05 were considered as statistically significant.

## 3. Results

### 3.1 Study Composition

A total of 53 patients were enrolled in the study, of which 52 (104 eyes) were included for analysis. One patient was omitted because the patient had a lacrimal gland lymphoma, which was difficult to classify as systemic or non-systemic at the time of diagnosis. Of those included for analysis, 33 were females (63%) and 19 were males (37%). Of the total participants, 31 were in the systemic disease group (Group I), while 21 were in the localized ocular surface disease group (Group II). The diseases included in Group I are noted in the methods section above (Figure 1). There was no significant difference in terms of gender between the groups ($p = 0.42$). The average age of the patients in the studied population was 53.13±12.82. The average age in Group I and Group II was (53.65±12.85) and (52.38±13.04), respectively. There was no statistically significant difference in terms of age between the two groups ($p=0.73$). The average age of patients with rheumatoid arthritis (RA) (N=7) was 55.43±12.27; dry eye (N=18) was 49.00±9.46; graft-versus-host-disease (GVHD) (N=15) was 54.20±7.74; Stevens-Johnson disease (SJS) (N=3) was 29.67±10.21 and uveitis, alkali-burn, herpes simplex keratitis, lupus, toxic epidermal necrolysis (TEN) (N=5) was 72.00±10.63. When comparing the diseases individually, there was a statistically significant difference in the age for patients ($p=<0.0001$).

There were 20 patients who participated in the OSDI survey before and six weeks after the initiation of serum tears. In this group, there were 12 females and 8 males. The average age was 47.4± 9.28. There were 12 in Group I and 8 in Group II. Of those in Group I, 7 had GVHD. The average age for Group I was 47.83±9.78 and the average age for Group II was 46.75±9.10. There was no statistically significant difference in terms of age between the two groups (p=0.806).

**3.2 ELISA Results**

There was a statistically significant difference (p= 0.015) in the level of EGF between Group I (29.39 pg/ml ± 52.85 pg/ml) and Group II (88.04 pg/ml ±113.75 pg/ml) (Table1). The level of IL-8, Fibronectin and Vitamin A in Group I was 128.41±122.45, 106.01±108.48, 1.97±0.92 and in Group II was 111.3±108.61, 159.12±166.25, 2.35±0.99, respectively. There were no statistically significant differences in the levels of IL-8, fibronectin, and Vitamin A (p= 0.607, p=0.169, p=0.289) (Figure 2). There were no significant differences between the subgroups of systemic diseases and localized dry eye in levels of IL-8, fibronectin, EGF and Vitamin A (Table 2 and Figure 3). In terms of TNF-$\alpha$ levels, 9 out of 31 patients in Group I and 2 out of 21 patients in Group II had detectable levels of TNF-$\alpha$ ($\chi^2$=1.533, p = 0.216).

**3.3 OSDI Results**

The average initial OSDI in the localized ocular surface disease group was 41.041±10.05 and the average initial OSDI in the systemic disease group was 66.81±9.45. After 6 weeks, the average in the localized ocular and systemic disease group was 25.47±7.96 and 50.22±9.58 respectively. There was a 36% reduction in OSDI scores in patients with localized ocular surface disease compared to a 24% reduction in OSDI scores in patients with systemic disease (p=0.065)

which was not statistically significant. Both groups showed an overall reduction in ODSI six weeks post treatment ($p = 0.002$). (Table 3)

**4. Discussion**

Autologous serum tears are an important adjunct therapy for the treatment of dry eye disease in patients with localized ocular surface disease and in patients with systemic diseases that affect the ocular surface. Although there have been reports regarding the variable effectiveness of AST in patients with systemic diseases as opposed to those without, there are currently no dedicated studies investigating the differences in efficacy between the two groups. Our current study reveals that there is a significantly lower epidermal growth factor level and a trend to lower reduction in OSDI in patients with systemic autoimmune disease compared to those with localized ocular surface disease ($p = 0.015$, 0.065 respectively). Despite these differences, both groups showed an overall reduction in ODSI six weeks post treatment ($p = 0.002$). There was no difference in the levels of IL-8, IL-17, fibronectin, or Vitamin A between the two groups. The proportion of patients with detectable levels TNF-$\alpha$ were also not different between the two groups ($\chi^2=1.533$, $p = 0.216$).

Our study is the first to show a difference in EGF between patients with systemic disease and those with localized ocular disease. Previous studies on serum have shown that cytokines, such as INF-$\gamma$, TNF-$\alpha$, and IL-8, are quite variable in serum concentration between healthy controls and those with inflammatory conditions like SJS (21). However, other studies have shown no difference in AST composition between controls and patients with SJS (18). Other studies have compared the components of AST in patients with Sjögren's syndrome specifically and have shown an increased level of hyaluronic acid and transforming growth factor b1 in those with active disease (23).

Epidermal growth factor, a 6 KdA polypeptide mitogen first described by Cohen et al, plays an important role in wound healing by promoting keratinocyte migration and re-epithelialization (24,25). It does so by binding to EGFR, a tyrosine kinase transmembrane protein found in the basal layer of epithelium. With acute injury, there is a rise of free ligands for EGFR, including EGF (25). Binding of EGF ligand to EGFR elicits a signaling transduction cascade that ultimately leads to the migration of keratinocytes and rapid re-epithelialization (26). EGF plays a similar role in corneal epithelium by promoting epithelial migration and proliferation to improve the wound healing process (27). In addition to promoting proliferation of corneal epithelium, EGF plays a vital role in preventing apoptosis (6). Finally, EGF also increases goblet cell number and muc-1 expression, which is a membrane tethered mucin that contributes to tear film stability (28).

The relatively lower levels of EGF in patients with systemic diseases shown in our study are consistent with the previously noted findings that decreased EGF levels are implicated in the pathophysiology of both Sjögren syndrome and chronic GVHD. Azuma et al showed that salivary EGF levels are decreased in Sjögren's syndrome and decreased levels of EGF correlate with the severity of oral symptoms (29). The same group showed that tears in patients with Sjögren's syndrome have significantly less EGF than healthy controls (30). EGF has also been shown to be significantly reduced in patients with chronic GVHD (31).

The exact mechanism for lower levels of serum EGF in patients with systemic conditions remains unknown. These findings could occur due to the higher level of systemic inflammation causing upregulation of EGF receptors (EGFR) and a resulting decrease of free EGF in serum. Other possibilities include the presence of a milieu that leads to increased breakdown of EGF or

its increased endocytosis following binding to EGFR. Regardless, both changes would then be reflected in the levels of EGF in AST.

Additionally, our study found that TNF-α and INF-γ were not detectable in every sample. While nine patients with systemic disease had detectable levels of TNF-α and three with localized ocular surface disease had detectable levels of TNF-α, this was not a statistically significant difference. None of the patients had detectable levels of INF-γ in their autologous serum tears. This could be due to either low levels of both factors in serum, secondary to processing during the production of AST, or stability of these ligands during storage.

Additionally, this is also the first study to compare differences in treatment efficacy with AST in patients with localized ocular surface disease to those with systemic diseases that affect that ocular surface. Existing randomized controlled studies either investigated a specific indication or grouped all indications together without comparing efficacy between the different indications (7-12). Currently, Hwang et al compared primary to secondary Sjögren's syndrome and showed subjective improvement in those with primary Sjögren's (17). While not statistically significant, our study showed a clear trend toward more improvement of OSDI scores in those with localized dry eye disease compared to those with systemic autoimmune disease after 6 weeks of use of AST. This could partly be due to the higher levels of EGF in the autologous serum tears of patients with no systemic disease that ultimately promotes better healing. However, further determination as to whether there is a clinical significance to this trend requires more investigation.

Our study reaffirms that AST provides subjective improvement for both patients with localized ocular surface disease and systemic disease. Previous studies have looked at OSDI scores after use of AST and found AST to lead to subjective improvement in symptoms for dry

eye patients (7-12). This is also consistent with a recent study which showed 85.1% of patients with systemic autoimmune diseases reported a significant decrease in ocular symptoms (16).

The results of this study may help to guide the treatment algorithms for patients with systemic diseases and dry eye symptoms. As there seems to be a greater decrease in symptoms for those patients with localized ocular surface disease who have higher levels of EGF in their autologous serum, EGF could be investigated as a potential therapeutic target in the future.

We acknowledge several limitations to this study. It is a nonrandomized study with samples collected at a single center. Additionally, all of the study subjects who were willing to donate serum tears were long-term users of autologous serum tears and this might introduce a bias as those who benefited were more likely to be compliant with therapy. As a result, we were also unable to correlate the improvement in ODSI with EGF levels as most first time patients were unwilling to donate AST but were willing to take the surveys. In the current study, availability of samples limited the number of components of autologous serum tears we were able to measure and only those factors with well validated ELISA protocols were used.

Lower levels of EGF might explain the differences in ODSI in patients with systemic disease associated ocular surface disorders when compared to those with localized ocular surface disease. While there is a difference, both groups seem to benefit from AST and this should not limit the prescription of AST to those with systemic disease associated ocular surface disorders.

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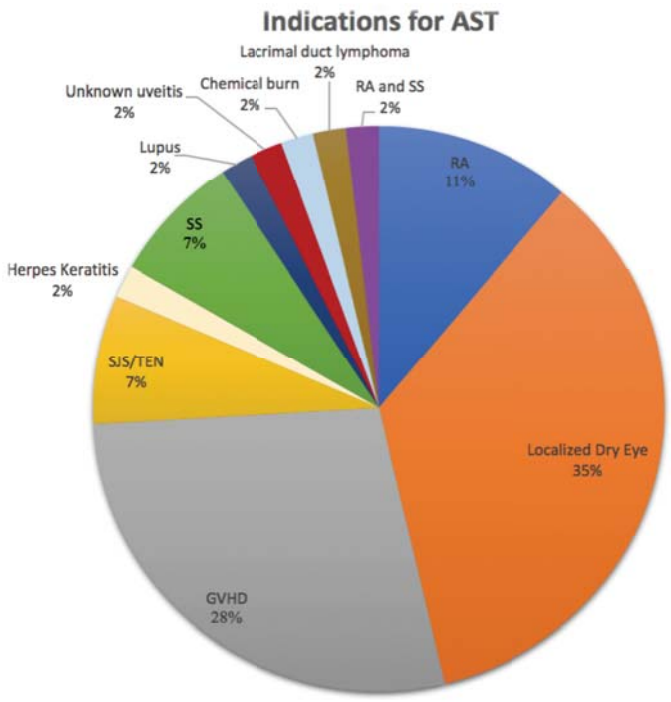


Figure 1: Indications for AST

| | Systemic Inflammatory Diseases (N=31) | Localized Diseases (N=21) | P-Value |
|---|---|---|---|
| Age (y) | 53.65±12.85 | 52.38±13.04 | 0.731 |
| IL-8 (pg/ml) | 128.41±122.45 | 111.3±108.61 | 0.607 |
| Fibronectin (ug/ml) | 106.01±108.48 | 159.12±166.25 | 0.169 |
| Vitamin A (mg/ml) | 1.97±0.92 | 2.35±0.99 | 0.289 |
| EGF (pg/ml) | 29.39±52.85 | 88.04±113.75 | 0.015 |

Table 1: ELISA Composition Differences between Systemic and Localized Disease

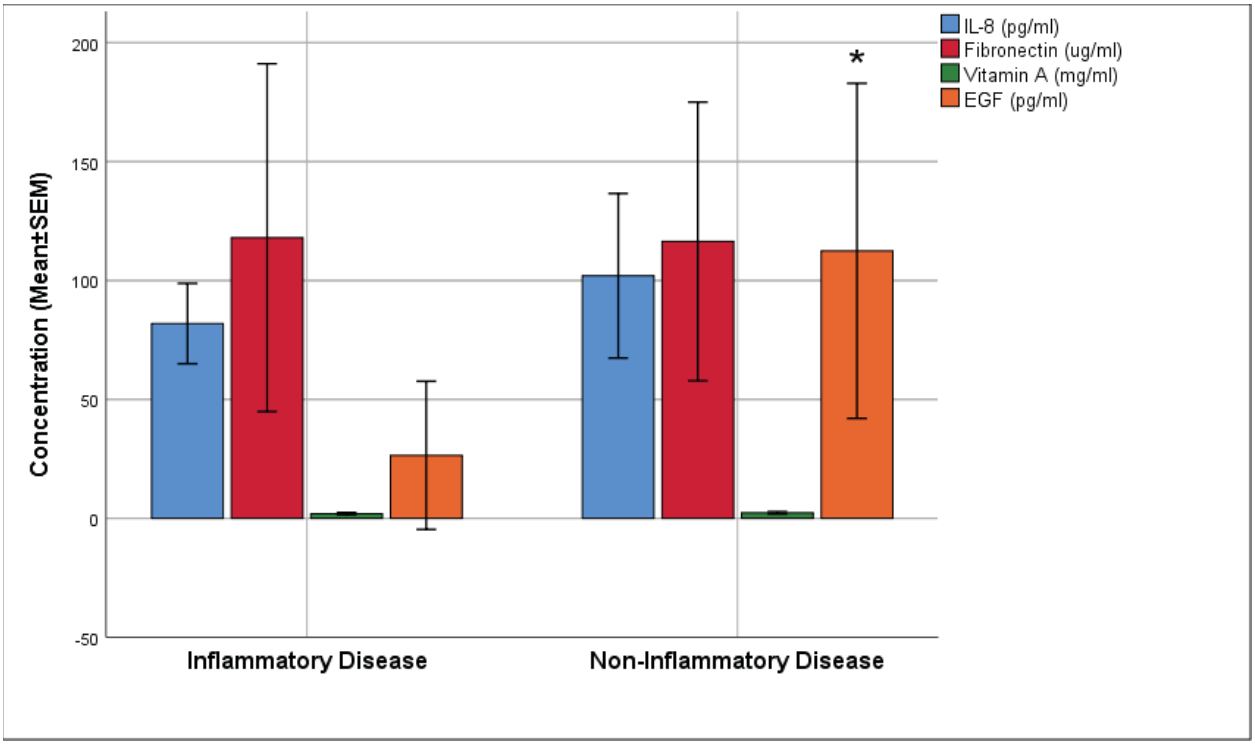


Figure 2: Concentration of ELISA Composition between Localized and Systemic Diseases

| | RA (N=7) | Dry Eye (N=18) | GVHD (N=15) | SJS (N=3) | SS (N=4) | Other[1] (N=5) | p-Value |
|---|---|---|---|---|---|---|---|
| IL-8 (pg/ml) | 131.49±109.17 | 104.54±99.53 | 107.71±105.65 | 284.83±225.74 | 119.01±96.53 | 113.93±136.39 | 0.255 |
| Fibronectin (ug/ml) | 101.49±87.79 | 178.19±172.16 | 107.43±94.29 | 59.01±49.27 | 65.78±45.31 | 131.68±205.67 | 0.47 |
| Vitamin A (mg/ml) | 1.6±0.72 | 2.45±1.04 | 1.52±0.53 | 3.68 | 2.01±0.22 | 2.51±1.02 | 0.122 |
| EGF (pg/ml) | 4.91±12.99 | 89.04±122.81 | 43.37±58.65 | 7.24±12.55 | 51.13±85.84 | 49.21±50.80 | 0.286 |

Table 2: ELISA Composition Differences between Diagnoses

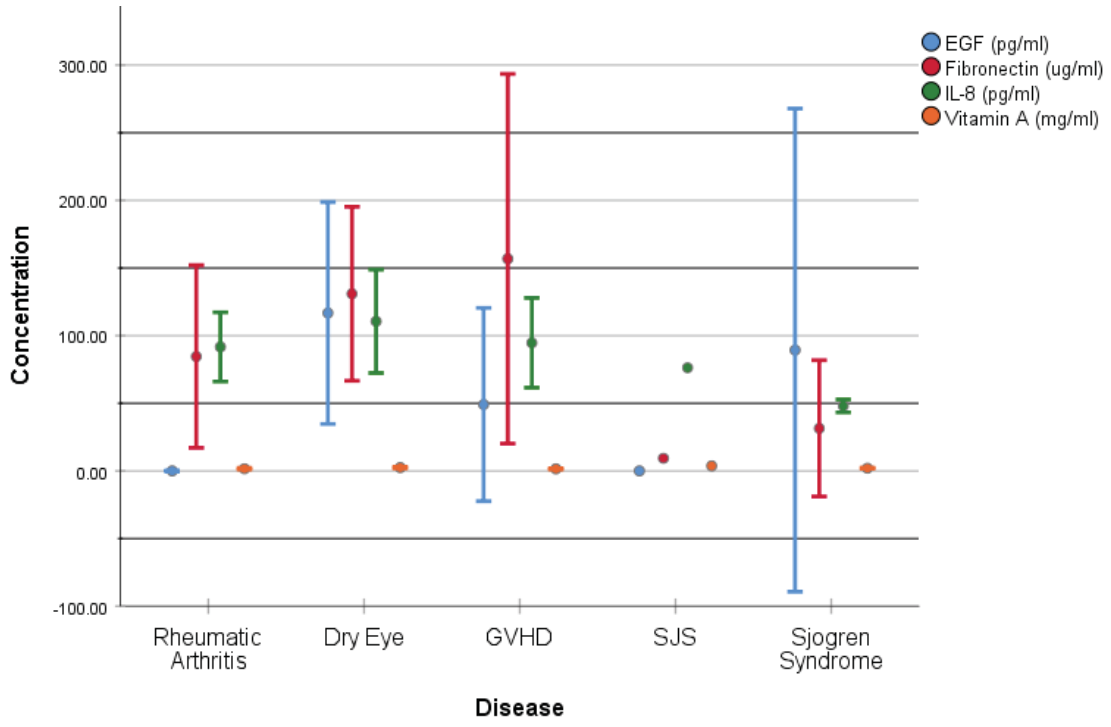


Figure 3: Concentration of ELISA Composition between Disease

| | | With Inflammatory Disease (N=12)* | | | | Without Inflammatory Disease (N=8)* | Total (N=20) | P-value |
|---|---|---|---|---|---|---|---|---|
| | | GvHD (N=7) | Sjogren (N=3) | RA (N=2) | Total (N=12) | | | |
| Age | | 47.57±8.4 | 45.0±13.8 | 53.0±12.7 | 47.83±9.8 | 46.75±9.1 | 47.4±9.28 | 0.806† |
| Gender | Male | 5 (62.5) | 1 (12.5) | 1 (12.5) | 7 (87.5) | 1 (12.5) | 8 (40) | 0.136‡ |
| | Female | 2 (16.7) | 2 (16.7) | 1 (8.3) | 5 (41.7) | 7 (58.3) | 12 (60) | |
| OSDI before AST | | 69.21±10.2 | 58.33±5.2 | 71.1±3.32 | 66.81±9.4 | 41.04±10.1 | 56.5±16.1 | <0.0001† |
| OSDI after AST | | 50.97±7.8 | 41.67±5.7 | 60.42±11.7 | 50.22±9.6 | 25.47±7.9 | 40.32±15.2 | <0.0001† |

* The reduction in OSDI score after using AST in both groups was statistically significant (paired T-Test, $p<0.0001$)
† Independent T-Test
‡ Chi-Square

Table 3: OSDI in Differences between Localized and Systemic Disease