

# CORRELATION OF LASIK REFRACTIVE OUTCOMES WITH PRE-SURGICAL TEAR LACTOFERRIN

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## Objectives:

Elective surgery is contraindicated in patients presenting with health-related risk factors. This holds true with laser procedures involving the cornea such as laser-assisted *in situ* keratomileusis (LASIK) and photo-refractive keratectomy (PRK).<sup>1,2</sup> The cornea is avascular tissue that receives much of its required metabolites for normal stasis from the accommodating tear film. The tear film varies widely in response to general and localized health conditions. Physiologic factors, such as these, compromise the integrity of the tear film by altering protein and electrolyte levels. By analyzing these tear components, corneal health assessment can be done prior to surgical procedures.<sup>3</sup> Lactoferrin, a lacrimal protein that is easily quantitated from a small volume of tears, serves as an excellent marker for the biochemical composition of the tear film.

The objective of this study is to assess the corneal health of LASIK patients prior to surgery by measuring Lactoferrin concentrations in their tear film to determine whether the pre-surgical health of the cornea relates to post-LASIK outcomes.

## Background:

Corneal transformation utilizing laser surgery is a well recognized procedure for improving defective vision. This procedure has proven to be highly successful, resulting in legions of satisfied clients. However, like all surgical procedures, LASIK is not totally successful all the time. Initial FDA data from LASIK clinical studies reported a post-surgical success rate of 68% for patients achieving 20/40 or better. Although the reported "adverse event" rate was only 3%, in actuality, 10% to 15% of all LASIK patients require follow-up surgery. It would be highly desirable to keep these enhancement procedures to an absolute minimum. It is an enigma as to why some patients have unsuccessful LASIK results.<sup>4</sup> If these subjects could be identified prior to surgery, many of the enhancement procedures could be obviated. Perhaps there are components in the tear fluid that may help predict which LASIK patients are suitable candidates, and which ones should be deferred. Identifying risk factors prior to surgery would be advantageous to both surgeons and patients.<sup>5, 6, 7</sup>

Lactoferrin was selected as a prime marker for lacrimal proteins. It is a major constituent of the tear film, and has been studied extensively. It is easy to quantitate, and its concentration in tears has been associated with other lacrimal gland proteins such as lysozyme. In addition to Lactoferrin and lysozyme, Lacrimal glands also produce s-IgA, epidermal growth factor and other biochemicals that provide nutrition, infection protection and wound healing factors.<sup>8, 9, 10, 11, 12</sup>

Lactoferrin is an iron binding protein secreted directly by the acinar cells of the Lacrimal glands and is, therefore, considered an ideal marker for assessing Lacrimal gland function.<sup>11, 13</sup> Lactoferrin contributes to modulating inflammatory responses, controlling cell growth, and protecting against infections. Lactoferrin plays a key role in anti-inflammatory responses by the inhibition of classical C pathways and C3 convertase formation.<sup>14, 15, 16</sup> Lactoferrin has been shown to protect the corneal tissue from re-oxygenation injury after extensive hypoxia.<sup>17</sup>

This study examined the effect of pre-surgical tear proteins on post-LASIK refractive outcomes.

### Subjects and Methods:

Patients with no prior LASIK surgery were evaluated and accepted for LASIK surgery. All patients admitted into the study were initially myopic. No attempt was made to control for age, race or sex. Excluded patients were those rejected by normal LASIK protocol, or who had an initial pre-surgical refraction equal to or greater than -7.00D. Patients who had punctal occlusion prior to surgery, or within the study period, were also excluded from the study. Surgery was performed according to standard LASIK protocols. Patients were followed post-surgically according to standard follow-up protocols. Sphero-cylinder refractions were converted to spherical equivalence and expressed in diopters (D).

Tear samples were collected prior to surgery from the inferior marginal tear strip (near the exterior canthus) using a calibrated 0.5 $\mu$ L capillary tube. Each sample was immediately transferred to 25 $\mu$ L of a diluent solution contained in a labeled microvial. Vials were securely closed and mixed gently. Diluted samples were stored refrigerated prior to being assayed.

Tear samples were assayed for lactoferrin using an enzyme-linked immunosorbant assay (ELISA). The *in vitro* diagnostic device (Touch Scientific, Inc Raleigh, NC) is FDA approved for the measurement of Lactoferrin in human tears, to assess lacrimal gland function.

Tear samples were transferred to solid phase membranes coated with anti-lactoferrin antibody. Immune complexes form by the interaction of Lactoferrin in the sample with the solid phase antibody. A solution of anti-lactoferrin antibody, conjugated with horseradish peroxidase (HRP), was then added to the solid phase. This conjugated antibody binds to the Lactoferrin previously immobilized on the solid phase. Excess conjugate was removed by using multiple wash steps. A liquid substrate, TMB (tetramethylbenzidine), was then added to the membrane as an indicator of bound Lactoferrin. This enzyme-substrate chromogen produces a blue color on the solid phase membrane which was measured by a reflectance photometer. The intensity of color development is directly proportional to the concentration of Lactoferrin in the tear sample being tested. Values were reported in unit concentrations of mg/mL. The lower the tear Lactoferrin concentration, the more severe the lacrimal gland dysfunction.

### Results and Statistical Analysis

Tear samples were analyzed subsequent to LASIK surgery, and prior to post-LASIK follow-up. This ensured that surgical protocol was not altered by the lactoferrin results, nor were the Lactoferrin results influenced by post-surgical check-ups. Post-LASIK refraction values were obtained at 90 days (minimum) following surgery. Pre-surgical Lactoferrin concentrations and post-surgical refraction were tested for correlation.

The patient population, when analyzed for pre-surgical Lactoferrin concentration, cluster into three qualitative subsets (Fig. 1): low Lactoferrin (n=6), normal Lactoferrin (n=21), and elevated Lactoferrin (n=5).

Figure 1

	Low	Normal	Elevated
Pre-LASIK Lactoferrin concentration	<0.95 mg/mL	0.95 -1.8 mg/mL	>1.8 mg/mL
Post-LASIK refraction (mean)	-0.79D	-0.12D	+0.55D

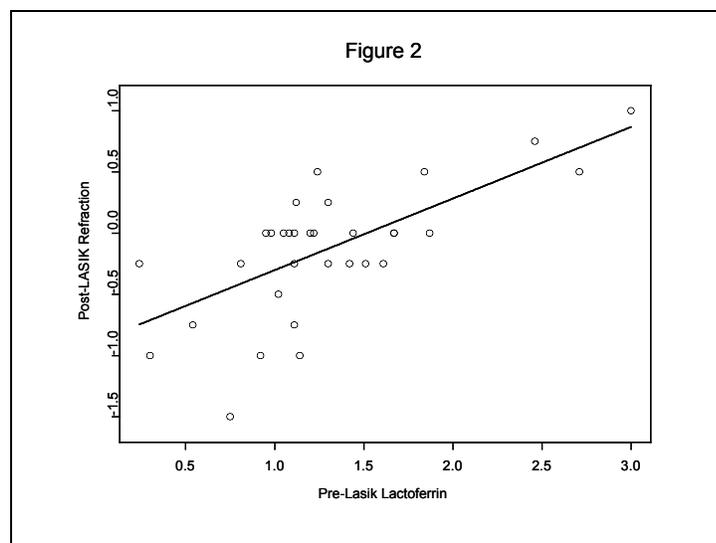
The group with low Lactoferrin had 100% of the subjects with -0.25D to -1.5D post -LASIK refraction. The group with normal Lactoferrin had 19% of the subjects with post-LASIK refraction outside of -0.25D

to +0.25D. The group with elevated Lactoferrin had 80% of the subjects with post-LASIK refraction  $\geq 0.50D$ .

Data were further analyzed using SAS 8.0 (SAS Institute, Inc., Cary, NC) and S-Plus 2000 Professional Edition for Windows, Release 2 (Mathsoft, Inc., Seattle, WA). Linear fixed effect and random effect models were fit using SAS PROCs GLM and MIXED, respectively, and generalized additive models were fit using S-PLUS.

The first linear models included pre-LASIK refraction and Lactoferrin as covariates; in both models pre-LASIK refraction was not a significant predictor ( $p=0.437$  and  $p=0.167$  for random and fixed effect models, respectively). The next linear models included source of data (Duke vs. Wilmer) and Lactoferrin as covariates. In both models the source of the data was not a significant predictor ( $p=0.777$  and  $p=0.870$  for random and fixed effect models, respectively). A model was then fit using only Lactoferrin as a predictor. Lactoferrin was found to be significant ( $p=0.015$  and  $p=0.001$ , for random and fixed effect models, respectively). A generalized additive model was then fit using a loess smoother for Lactoferrin and treating all observations as independent. The non-parametric effects were not statistically significant ( $p=0.262$ ), implying that a linear fit is adequate for these data (Fig. 2). Pre-LASIK Lactoferrin is a statistically significant predictor of post-LASIK spherical refraction.

**Fig. 2**



## Discussion

This study demonstrates a definite correlation between pre-LASIK Lactoferrin levels in tears and post surgical outcomes. Diminished Lactoferrin prior to LASIK suggests an increased risk for post-surgical regression, while elevated Lactoferrin indicates an increased risk for post-surgical hyperopia.

Lactoferrin should not be inculcated as the lone component effecting post-LASIK refraction. It is only one of many biochemicals responsible for normal corneal health. Lactoferrin should, however, be considered as a prime marker for a complex and dynamic environment.

LASIK candidates with lacrimal dysfunction present with abnormal tear Lactoferrin and, as such, do not have the proper tear constituents required for corneal maintenance or cellular regeneration. It is unreasonable to expect these subjects to respond to corneal surgery as satisfactorily as patients with normal tear components.

Lactoferrin and other secretory proteins are neutrally medicated. Surgical interference with this neurogenic response has been linked to post-surgical induced dry eye.<sup>12,6</sup> It is known that patients with aqueous deficiencies are problematic for contact lens tolerance<sup>14,3</sup> and, as a result, are drawn towards laser vision correction such as LASIK.<sup>4</sup> Many of these patients have sub-acute problems prior to surgery, which are likely to be exacerbated following LASIK.<sup>6</sup> Therefore, dry eye patients are contraindicated for LASIK surgery until punctal occlusion, successful drug intervention or condition that needs to be corrected prior to LASIK.<sup>6</sup>

Since dry eye is a common side-effect of LASIK, it is important to minimize any signs and symptoms of this disease prior to surgery. Testing for Lactoferrin in LASIK candidates would not only improve refractive outcomes, it would also select subjects with aqueous deficiencies for pre-surgical therapy.

### **Conclusions:**

Although this pilot study had a limited sample size, it can be concluded with statistical confidence, that tear Lactoferrin serves as an excellent marker for pre-existing conditions that influence the post-LASIK healing response. Pre-LASIK Lactoferrin levels are a statistically significant predictor of post-LASIK spherical refractions.

Lactoferrin testing should be considered as one tool in assessing the pre-surgical, corneal health of all LASIK candidates. Patients that are diagnosed as ineligible for surgery should be deferred until all aberrant conditions have been rectified. Further studies are recommended to investigate which therapies are the most efficacious in treating pre-LASIK health problems. Such therapies will result in the best possible outcomes for all LASIK patients, while decreasing the need for enhancement procedures.

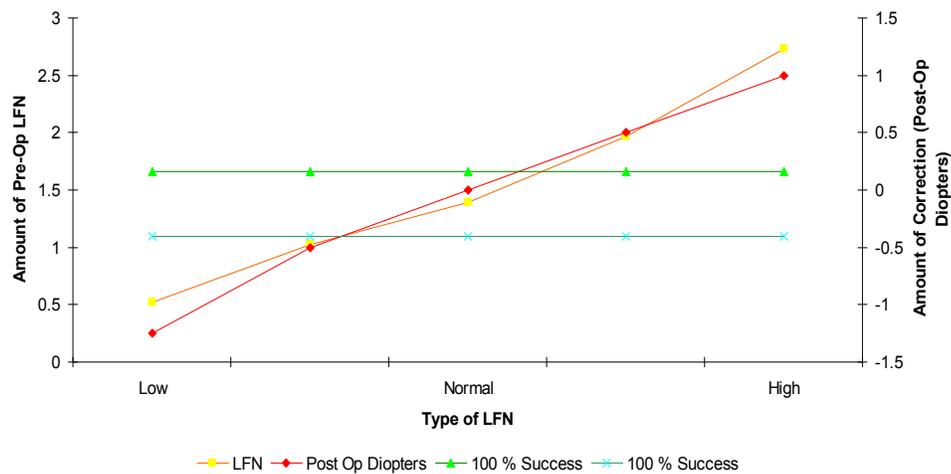
## References:

1. Reinstein DZ, Silverman RH, Sutton HFS and Coleman DJ: *Very high-frequency ultrasound analysis identifies anatomic correlates of optical complications of lamellar refractive surgery*. Ophth 1999; 106(3): 474-482.
2. Liu Z and Plugfelder SC: *Corneal thickness is reduced on dry eye*. Cornea 1999; 4.
3. Lemp MA *Is the dry eye contact lens wearer at risk?* Cornea (Suppl. 1) 1990; 9:S48-S50
4. Special meeting report: *LASIK dry eye link draws attention at AAO-PAAO*. Prime Care Optom News 2000 Jan.
5. Abelson MB and Crampton HJ: *What's new in dry eye treatment*. Review of Ophthalmol 1997; Dec: 68-9.
6. Pickles V: *Unhappy LASIK Patients*. Ophthalmol Manage 2000; 33-40.
7. Lohman CP, Von Mohrenfels CW, Guell JL, Muller A and Reischl U: *Quantitative measurements of mRNA levels in corneal epithelial cells: A screening test to determine patients with strong wound healing response after myopic LASIK*. ARVO 1999.
8. Stuchell RN, Farris RL and Mandel ID: *Basal and reflex human tear analysis. II. Chemical analysis: lactoferrin and lysozyme*. Ophthalmol 1981; 88:858-861.
9. Fullard RJ and Snyder C: *Protein levels in nonstimulated and stimulated tears of normal subjects*. Invest Ophthalmol and Vis Sci 1990; 31(6):1119-26.
10. Mc Gill JI, Liakos GM, Goulding N and Seal DV: *Normal tear protein profiles and age-related changes*. Br J Ophthalmol 1984; 68:316-320.
11. Shimmura S, Suematsu M, Shimoyama M, Tsubota K, Oguchi Y and Ishimura Y: *Subthreshold UV radiation-induced peroxide formation in cultured corneal epithelial cells: the protective effects of lactoferrin*. Exp Eye Res 1996 Nov; 63(5):519-26.
12. Yoshino K, Monroy D and Pflugfelder SC: *Cholinergic stimulation of lactoferrin and epidermal growth factor secretion by the human lacrimal gland*. Cornea 1996 Nov; 15(6):617-21.
13. Lemp MA: *Report of the National Eye Institute/Industry Workshop on clinical trials in dry eyes*. CLAO J 1995; 21(4):221-232.
14. Ballou M, Donshik PC, Rapacz P and Samartino L: *Tear Lactoferrin levels in patients with external inflammatory ocular disease*. Invest Ophthalmol Vis Sci 1987; 28:543-545.
15. Thaler CJ, Labarrere CA, Hunt JS, McIntyre JA and Faulk WP: *Unique epitopes of lactoferrin expressed in human cytotrophoblasts involved in immunologic reactions*. Am J Obstet Gynecol 1999 Aug; 181(2):460-7.
16. Bayeye S, Ellass E, Mazurier J, Spik G and Legrand D: *Lactoferrin: a multifunctional glycoprotein involved in the modulation of the inflammatory process*. Clin Chem Lab Med 1999 Mar; 37(3):281-6.
17. Kijlstra A: *The role of lactoferrin in the nonspecific immune response on the ocular surface*. Reg Immunol 1990-91; 3(4): 193-7.

## Levels of LFN Determine LASIK Correction

Duke University Study September 1998

LFN / LASIK Correction Correlation (Mean Data)



95% of patients that have pre-operative LFN levels, within the normal range of 1.4mg/mL-1.6mg/mL, will have a post-operative refraction of +/- 0.25D (measured 3 months post-operatively)