



Keratoconus Progression in Patients with Allergy and Elevated Surface Matrix Metalloproteinase 9 Point-of-Care Test Vs Negative Patients

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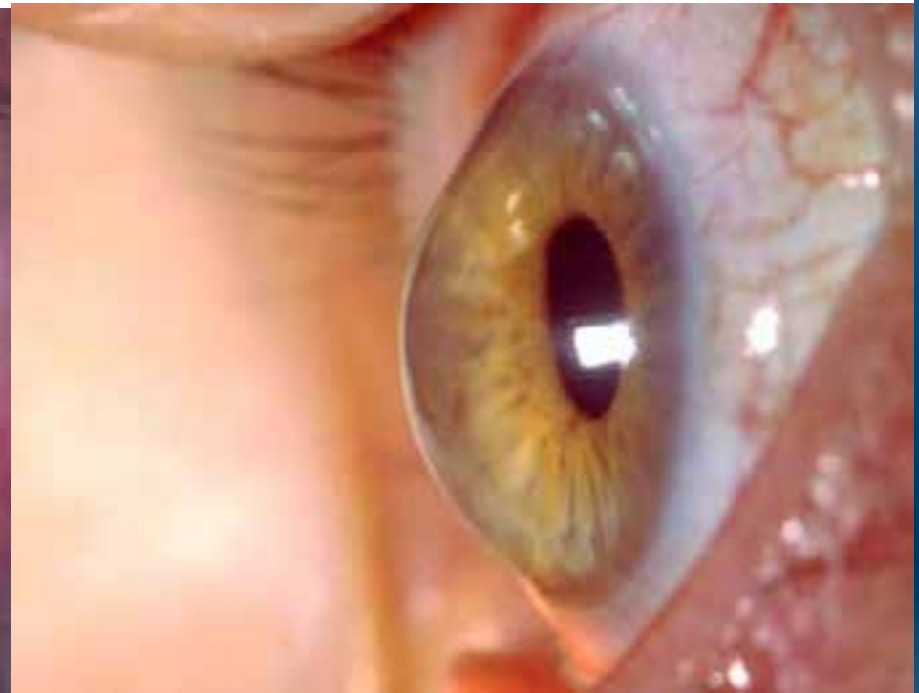
KERATOCONUS: PROGRESSION

- Most frequent in younger patients (< 18 years)
- More rapid in central KC pattern
- Risk of acute KC development (< 16 years)
- More significant and faster refractive and pachimetric worsening in young patients



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Inflammation and Keratoconus



Such results suggest that KC cannot be defined
a noninflammatory disorder and that the pathogenesis of KC
progression may involve chronic inflammatory events

Evidences of Inflammatory processes correlating with KC

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Without contact lens wear	Lema and Duran (2005), ³ Lema et al. (2009), ⁴ Balasubramanian et al. (2012), ⁶ Jun et al. (2011), ⁹ Balasubramanian et al. (2012) ¹²
With contact lens wear	Fodor et al. (2013), ¹¹ Pflugfelder et al. (2007), ¹⁶ Kallinikos and Efron (2004), ¹⁷ Lema et al. (2008), ¹⁸ Edmonds et al. (2004) ¹⁹
With atopic and allergic diseases	Weed et al. (2008), ¹³ Stern et al. (2005), ¹⁴ Contreras-Ruiz et al. (2012) ¹⁵
With ocular rosacea	Dursun et al. (2001), ⁴¹ Jenkins et al. (1979) ⁴³
With rubbing-related corneal trauma	Kallinikos and Efron (2004), ¹⁷ Greiner et al. (1997), ²⁸ Balasubramanian et al. (2013), ²⁹ Korb et al. (1991), ³⁰ McMonnies (2009) ⁵⁹
With degradative enzyme activity	Lema et al. (2009), ⁴ Dogru et al. (2003), ⁸ Jun et al. (2011), ⁹ Zhou et al. (1998), ¹⁰ Fodor et al. (2013), ¹¹ Balasubramanian et al. (2012) ¹²
With wound healing cascades	Wilson et al. (2001), ³¹ Patel and McGhee (2013), ³³ Kuo (2004), ³⁴ Kim et al. (1999) ³⁶

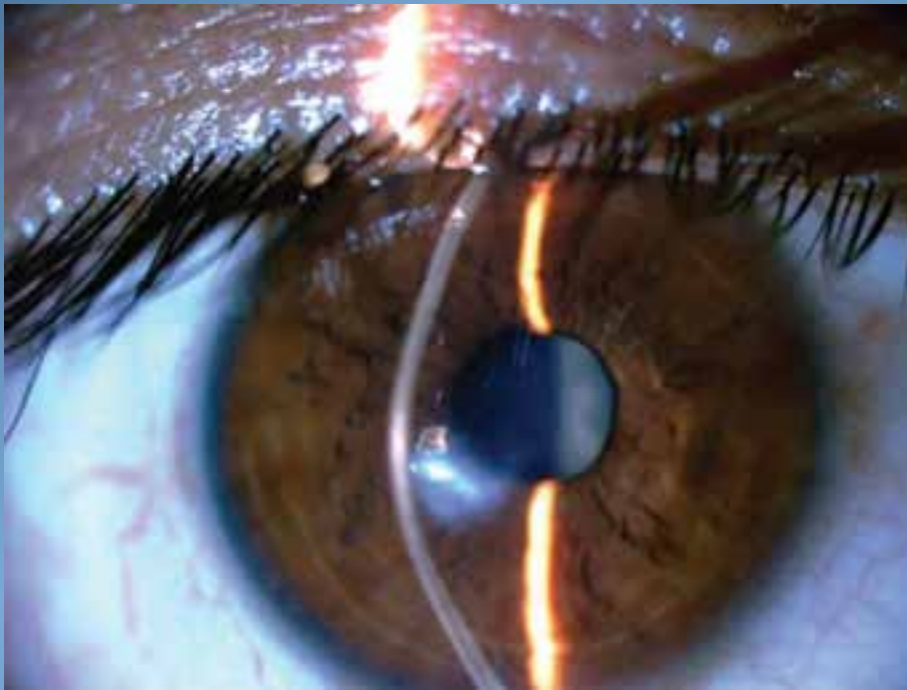
Evidences of Tear Film dysfunction correlating with KC

Tear dysfunction is associated with inflammation and has been found in KC without contact lens wear and may be exacerbated by contact lens wear	Dogru et al. (2003), ⁸ Pflugfelder et al. (2007), ¹⁶ McMonnies (1996), ³⁷ Stern and Pflugfelder (2004), ³⁹ Luo et al. (2004), ⁴⁰ Pflugfelder (2004) ⁴⁵
Evidence of reduced anti-inflammatory capacity is indicated in KC tears by: Reduced lactoferrin Reduced lipophilins A and C Reduced immunoglobulin A	Lema and Duran (2005), ³ Balasubramanian et al. (2012) ⁶ Acera et al. (2011) ⁷ Balasubramanian et al. (2012) ⁶
Increased serum albumin in KC tears suggests a failure of the blood/ocular barrier in conjunctival vessels	Acera et al. (2011) ⁷

Allergy and Keratoconus

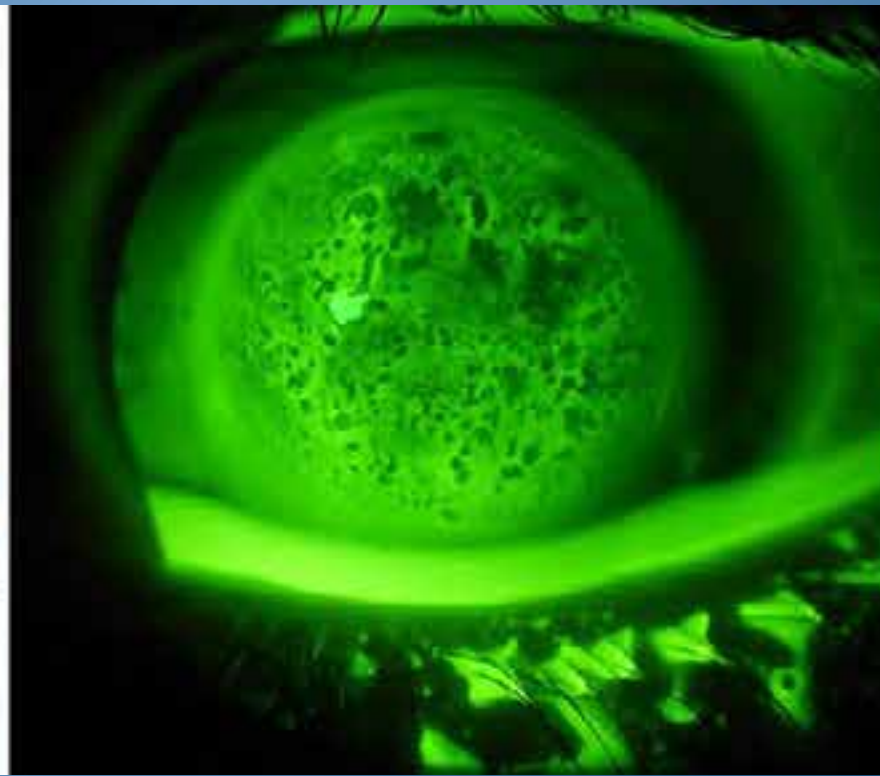
The first reported association between ocular allergy and KC was described by Hilgartner in 1937

Sharma N, Rao K, Maharana PK, Vajpayee RB. Ocular allergy and keratoconus
Indian Journal of Ophthalmology. 2013;61(8):407-409.



Bawazeer AM, Hodge WG, Lorimer B. Atopy and keratoconus: A multivariate analysis.
Br J Ophthalmol.2000;84:834-6

**Acute or Post acute
Corneal Hydrops risk higher
in paediatrics eye-rubbers suffering from allergy**



Laura E. Downie Pediatrics 2014;134:e596-e601

PEDIATRICS[®]

Cytokine Expression in Keratoconus and its Corneal Microenvironment: A Systematic Review

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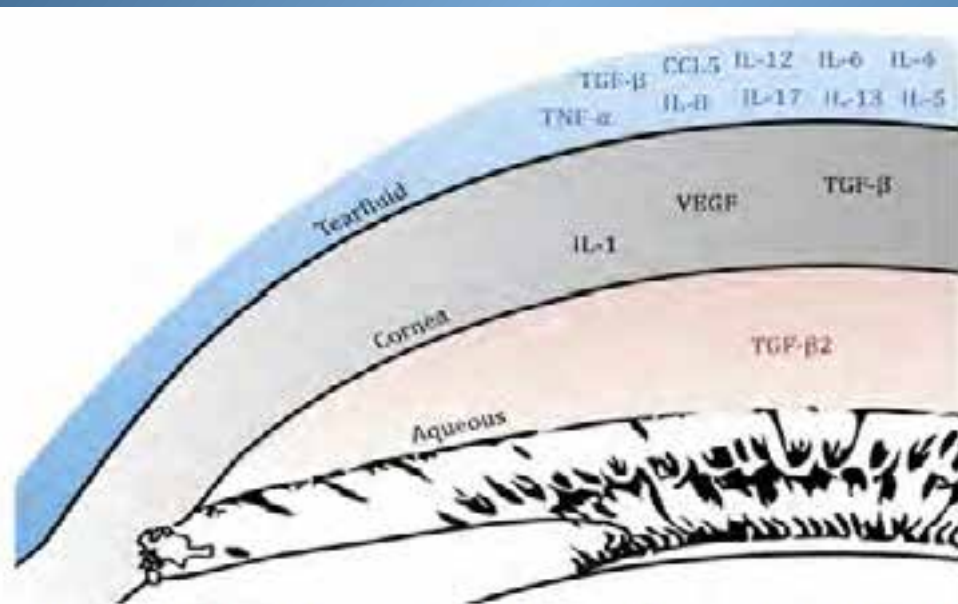
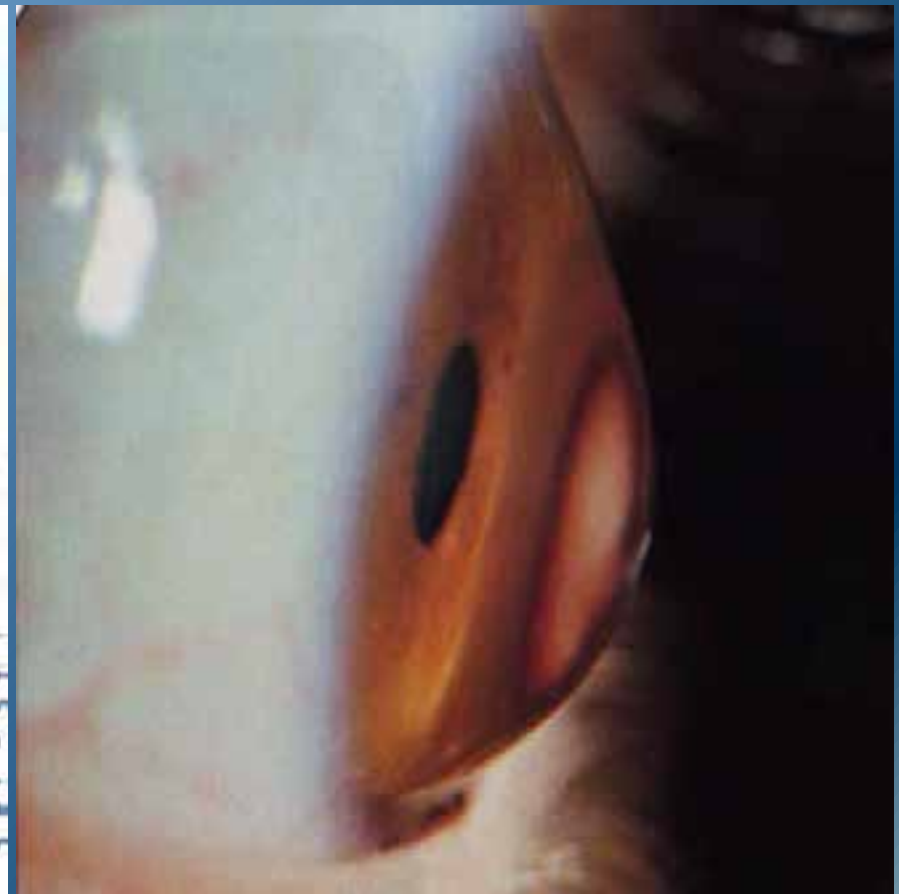


Figure 1. Representation of the localization of soluble immune mediators in tear fluid, corneal tissue, and aqueous humor of keratoconus patients. Most soluble immune mediator studies have used the relatively more accessible and less invasive tear fluid samples, contributing to a greater variety of reported cytokines such as IL-6, TNF- α and IL-17. Consequently, the number of studies on soluble immune mediators in corneal and aqueous humor samples is much fewer, but they have already revealed altered levels in several factors, including VEGF, IL-1 and TGF- β .



Interaction between soluble inflammatory mediators, keratocytes and stromal collagen

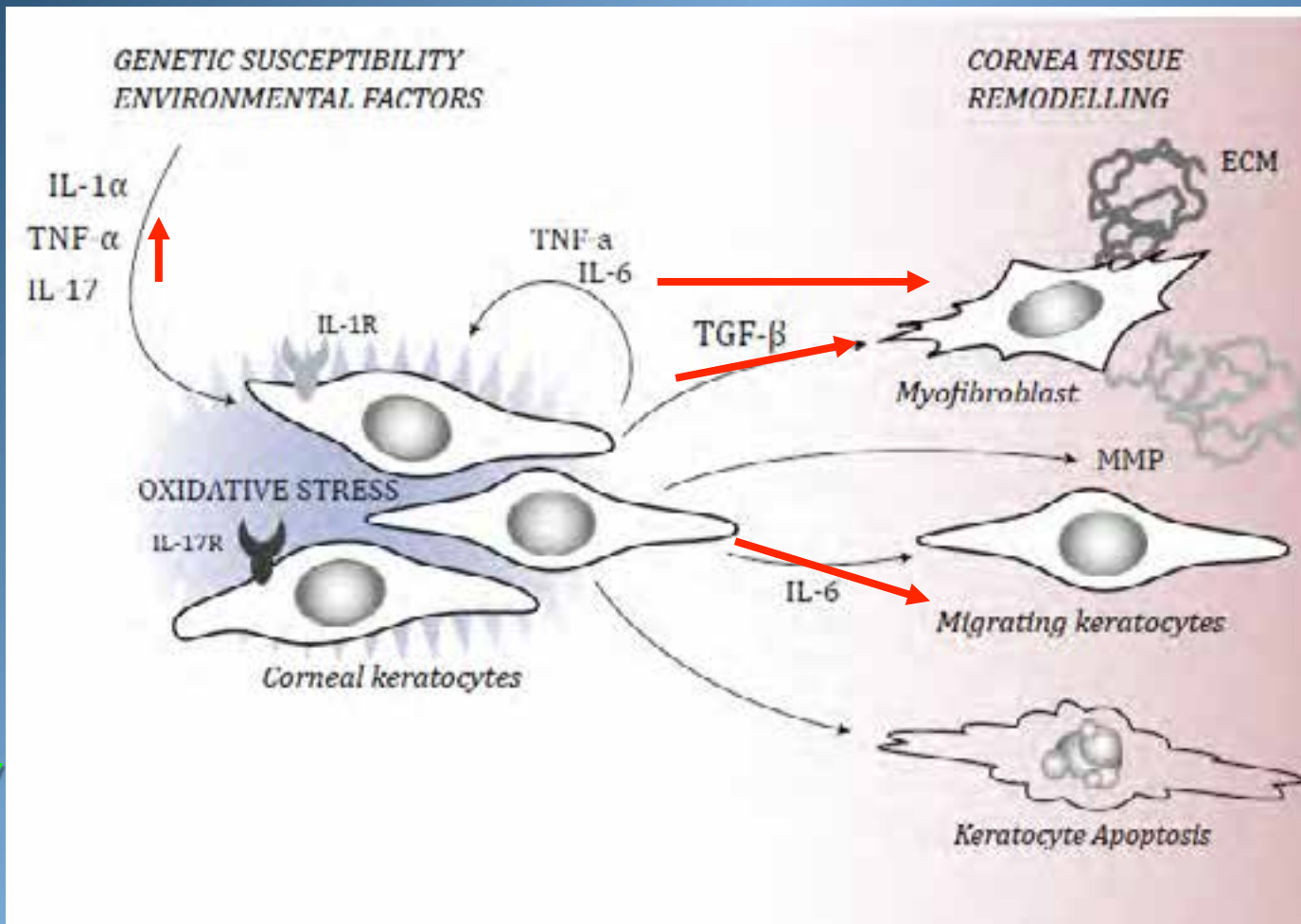


Figure 2. Model of the interaction of several soluble inflammatory mediators with corneal stromal keratocytes, leading to the archetypical corneal tissue remodeling. Various genetic loci, including cytokine genes, in combination with environmental factors such as eye-rubbing and contact lens wear contribute to the increased expression of soluble immune mediators such as IL-1 α , TNF- α and IL-17 that bind to their receptors expressed by keratocytes of the corneal stroma. In combination with numerous other provocative factors (proteases), these keratocytes suffer from oxidative stress that leads to keratocytes apoptosis, IL-6 mediated migration of keratocytes into the corneal epithelium, and TGF- β -induced differentiation of keratocytes into α -SMA expressing myofibroblast that secreted components of extra cellular matrix (ECM). Together these keratocyte changes contribute to corneal tissue remodeling affecting collagen distribution and corneal architecture in keratoconus.

Demographic MMP 9 point of care test and KC progression study

BACKGROUND

ear fluid > MMPs levels

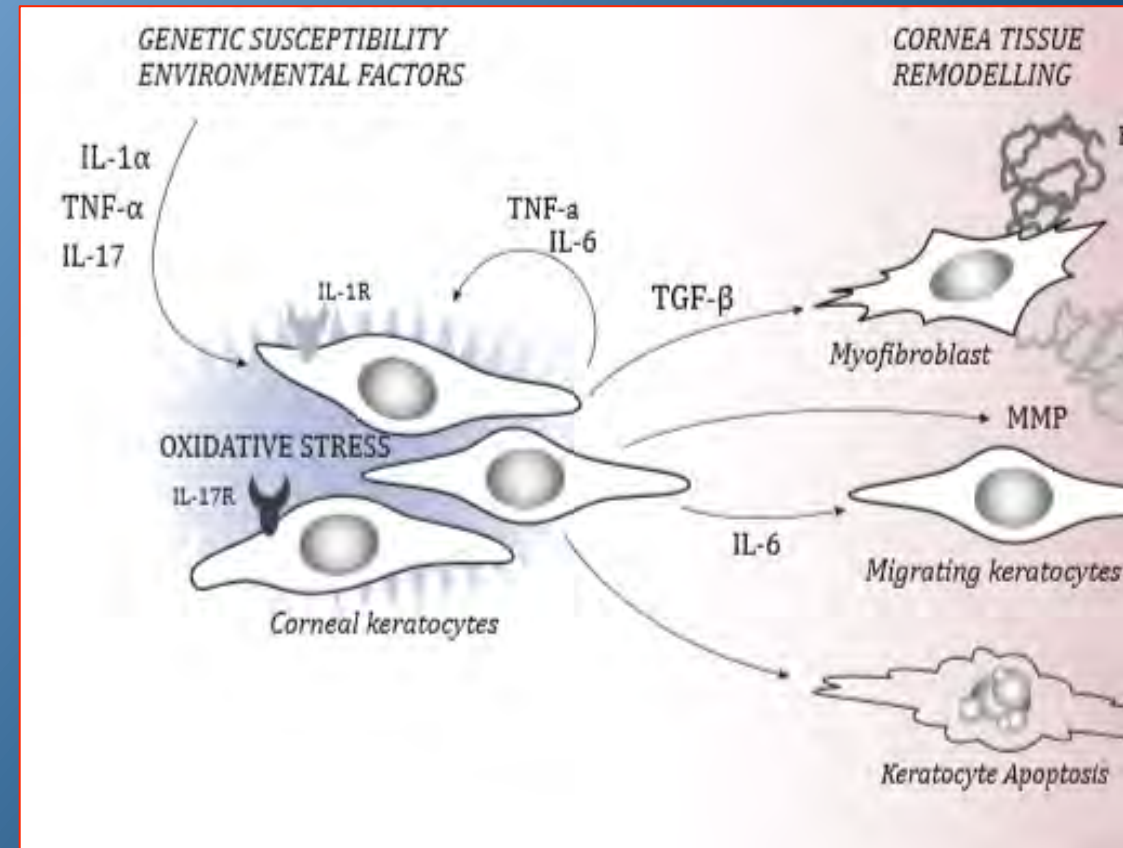
ear fluid < LOX 2 Lysil Oxydase II

-1 α and IL-1 β pro-inflammatory

-1 α and TNF α pro-apoptotic effect

-1 β and IL-6 regulatory activity on MMPs

-1 α , IL-17 and TNF α increased levels of
MMP-9



DECREASED PROTEASES, PROTEASE ACTIVITY, AND INFLAMMATORY MOLECULES IN THE TEARS HAVE BEEN FOUND TO BE RELEVANT IN THE PATHOGENESIS OF KC

- **KERATOCONUS:** stromal thinning is a result of increased expression of lysosomal and proteolytic enzymes, decreased concentration of protease inhibitors, which result in altered collagen configuration and reduced biomechanical resistance
- **ALLERGIC CONJUNCTIVITIS:** allergens are re-introduced into the body through the conjunctiva it reacts with the allergen specific IgE on the surface of mast cells or basophils, releasing vasoactive mediators.
- **MEDIATORS INCLUDE INFLAMMATORY MOLECULES:** histamine, proteases, tumor necrosis factor (TNF)-alpha, and interleukin (IL).
- It has been proven that **EYE RUBBING INCREASES THE LEVEL OF TEAR MATRIX METALLOPROTEINASES (MMP), IL-6 and TNF- alpha even in normal subjects, CONTRIBUTING TO THE DEVELOPMENT AND PROGRESSION OF KC**

asubramanian SA, Mohan S, Pye DC, Willcox MD. Proteases, proteolysis and inflammatory molecules in the tears of people with keratoconus. *Acta Ophthalmol.* 2012;90:e303-308.

asubramanian SA, Pye DC, Willcox MD. Effects of eye rubbing on the levels of protease, protease activity and cytokines in tears: Relevance in keratoconus. *Clin Exp Optom.* 2013;96:214-8.

METHODS

Prospective comparative study including 100 stage I-II keratoconic patients according to Amsler-Rumeich's classification, mean age 5.7 ± 4.6 years.

All patients underwent an anamnestic questionnaire for concomitant allergic diseases and were screened with the MMP-9 point-of-care test and eye-rubbing





Patients were divided into 2 groups: 52 KC patients with allergies (KC AL) and 48 KC patients without allergies (KC NAL).

Allergic diseases	No. of patients (%)
Rhinitis	24 (46,2%)
Allergic conjunctivitis	17 (32,7%)
Asthma	6 (11,5%)
Associations	5 (9,6%)

Dry-eye patients and contact lens wearers excluded

	Group KC AL No. of patients (%)	Group KC NAL No. of patients (%)
Eye-rubbing	52 (100%)	8 (16%)
InflammaDry +	42 (81%)	2 (4%)

Papillary Sub-tarsal Response (PSR) was graded from level 0 to 3 (0: absent, low: 1, moderate: 2, high: 3).

Grade of papillary sub-tarsal response	
	ABSENT (0)
	LOW (1)
	MODERATE (2)
	HIGH (3)

Papillary sub-tarsal grade	Group KC AL	Group KC NAL
Absent (0)	-	38
Low (1)	-	10
Moderate (2)	12	-
High (3)	40	-

KC AL Group patients comprised 40 high and 12 moderate grade PRG cases. KC NAL Group comprised 38 cases with level 0 (absent) and 10 with a low (grade 1) PSR.

MMP family enzymes mediate degradation of extracellular matrix proteins, typically in response to stress or injury. According to several studies the average levels of MMP-9 (ng/ml) in human tears range from 3 ng/ml to 40 ng/ml.

NORMAL LEVELS OF MMP-9				
Study	Normal Controls	Average MMP-9 Levels (ng/ml)	Standard Deviation (ng/ml)	Upper Range (ng/ml)
Acera et al ¹	18	23.6	17.4	41.0
Chotikavanich et al ²	16	8.4	4.7	13.0
Solomon et al ³	17	7.2	2.1	9.0
Leonardi et al ⁴	10	10.5	0.2	11.0
Lema et al ⁵	20	6.9	1.4	8.0
Honda et al ⁶	28	22.7	14.0	37.0
Markoullie et al ⁷	38	11.6	15.2	N/A
Total/Avg/Range	147	12.9	-	41.0

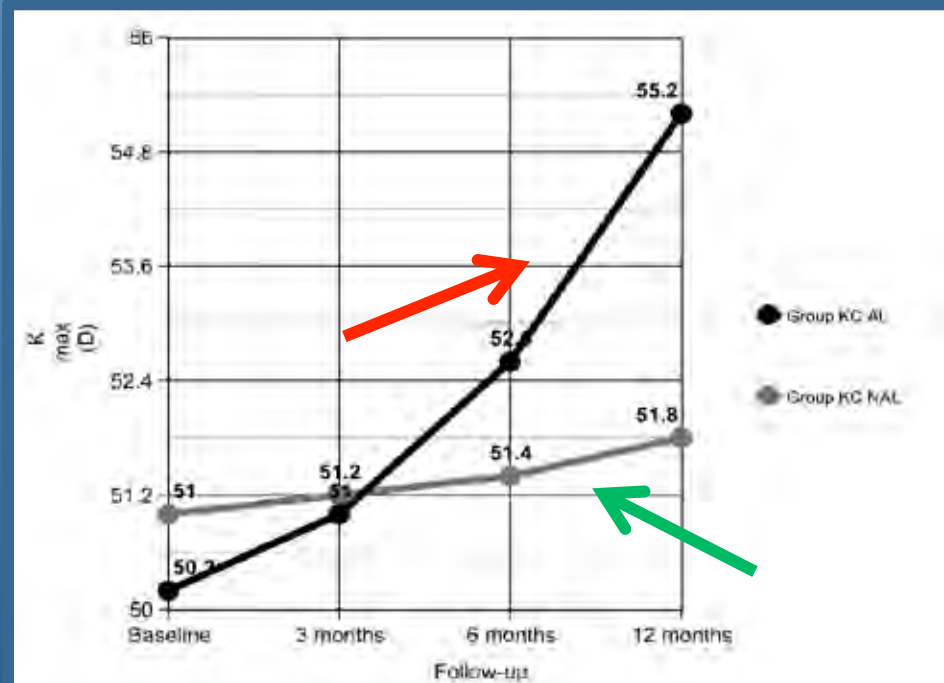
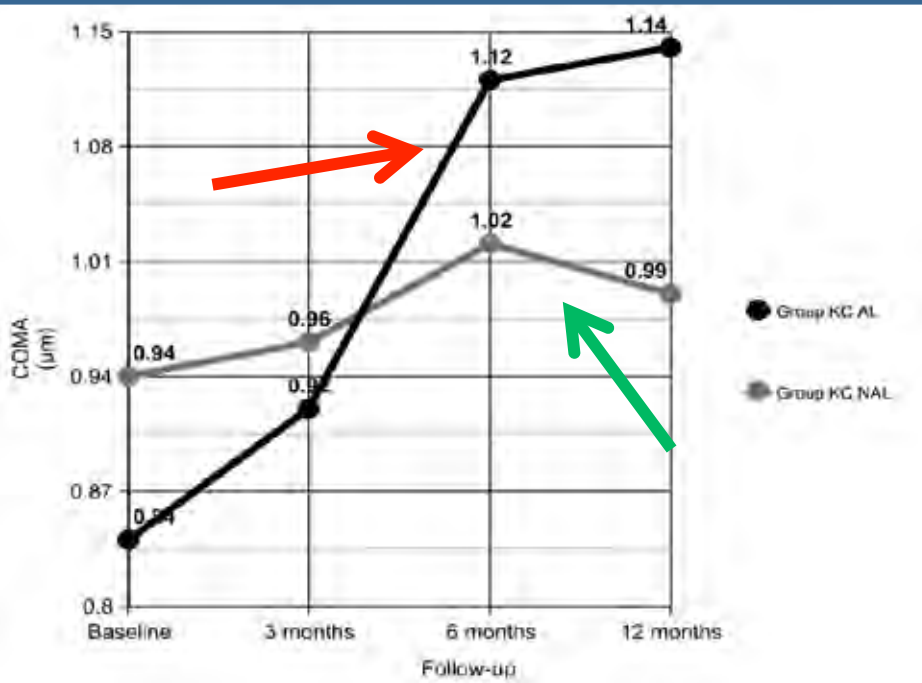
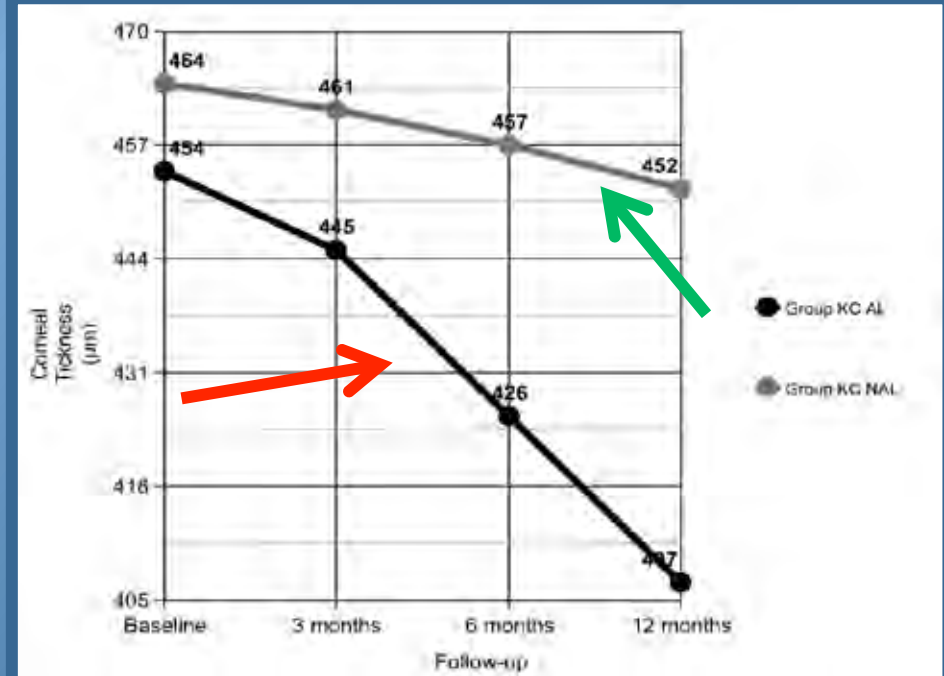
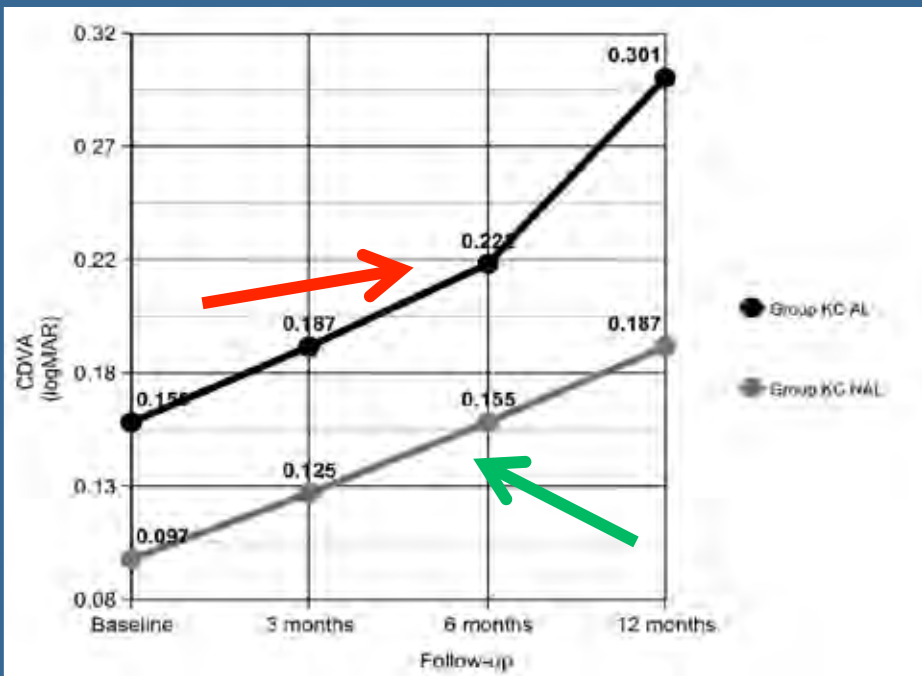
The **MMP-9 point-of-care test** (InflammaDry® test, RPS, Sarasota, FL, USA) detect the presence of MMP-9 (both the proenzyme form and active MMP- 9) on the tear film based on a direct sampling micro-filtration (DSMF) technology.

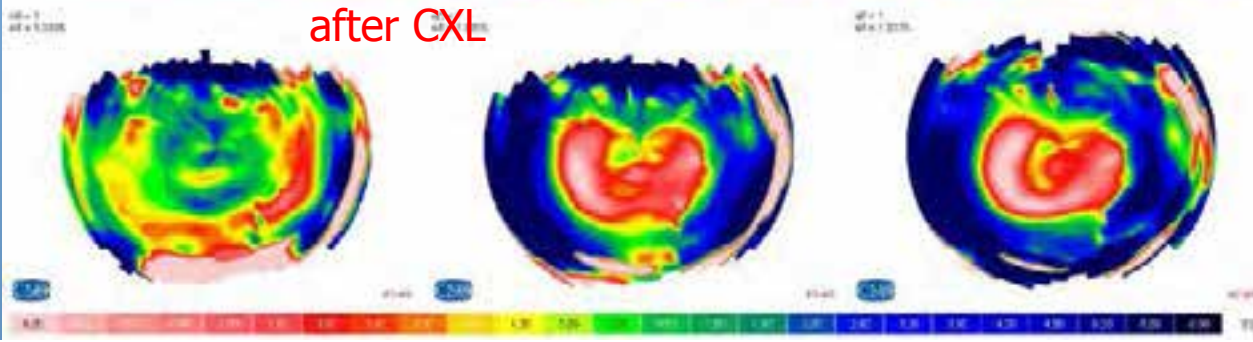
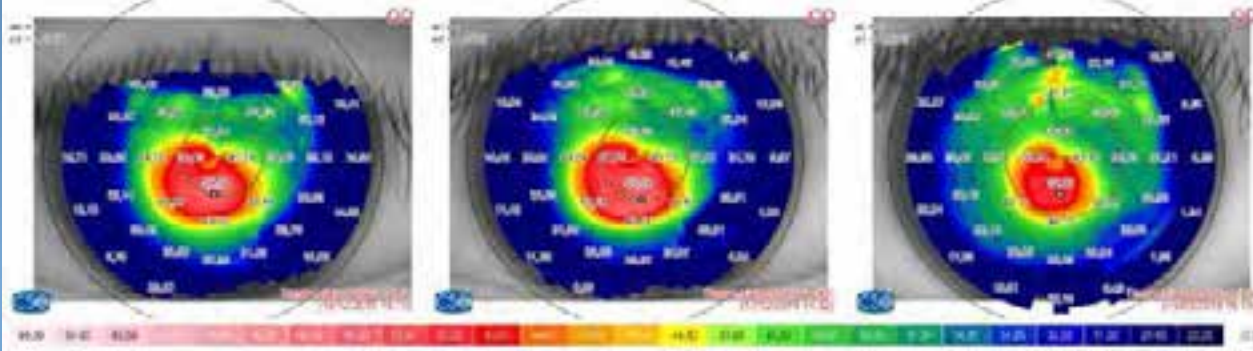
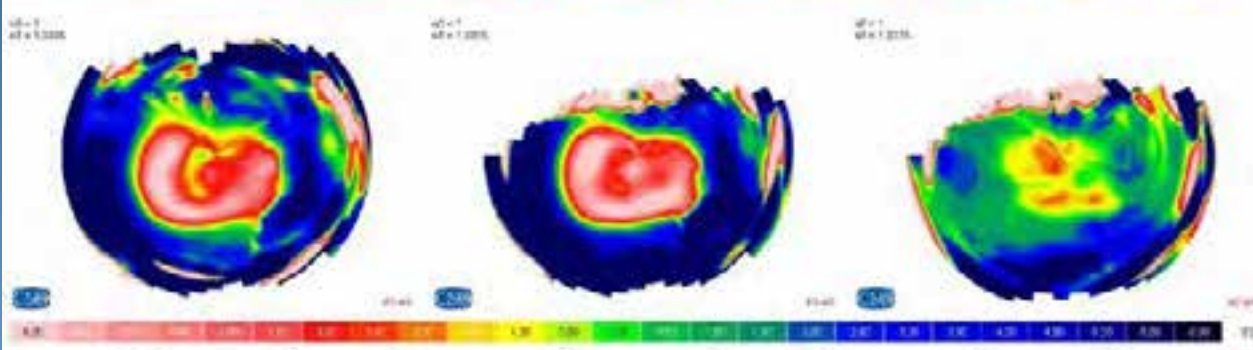
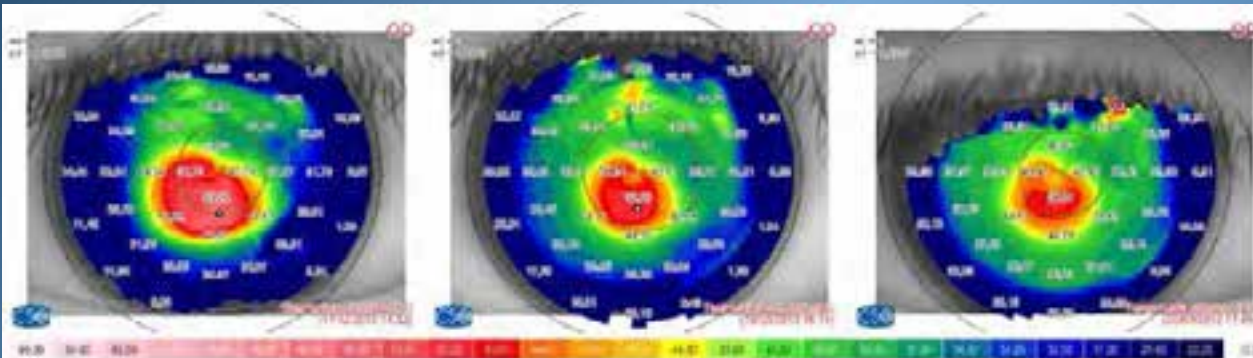


A positive test result indicates the presence of MMP-9 on tear fluid ≥ 40 ng/ml.

RESULTS

Time Follow-up	Baseline		3 months		6 months		12 months	
Groups	Group KC AL	Group KC NAL	Group KC AL	Group KC NAL	Group KC AL	Group KC NAL	Group KC AL	Group KC NAL
Kmax	50.2±2.7 D	51±2.8 D	51±2.0 D	51.2±1.4 D	52.6±1.8 D	51.4±0.8 D	55.2±1.9 D	51.8±2.2 D
Minimum Corneal Thickness	454±22 µm	464±26 µm	445±24 µm	461±25 µm	426±16 µm	457±18 µm	407±13 µm	452±10 µm
COMA	0.84±0.03 µm	0.94±0.02 µm	0.92±0.04 µm	0.96±0.03 µm	1.12±0.04 µm	1.02±0.04 µm	1.14±0.02 µm	0.99±0.02 µm
CDVA	0.155±1 logMAR	0.097±1 logMAR	0.187±0.699 logMAR	0.125±0.69 9 logMAR	0.222±1 logMAR	0.155±1 logMAR	0.301±1 logMAR	0.187±0.699 logMAR





after CXL

Subfile #
 Acquisition #
 Patient: giampetti, giampetti - 05
 Date: 11/20/2014 08:07:14 AM
 Location: 11/20/2014 10:10:10 AM

Study Information
 Study # / Name
 Characteristics (14)
 Characteristics (14)
 Secondary #1 of 14

Subfile #
 Acquisition #
 Patient: giampetti, giampetti - 05
 Date: 11/20/2014 08:07:14 AM
 Location: 11/20/2014 10:10:10 AM

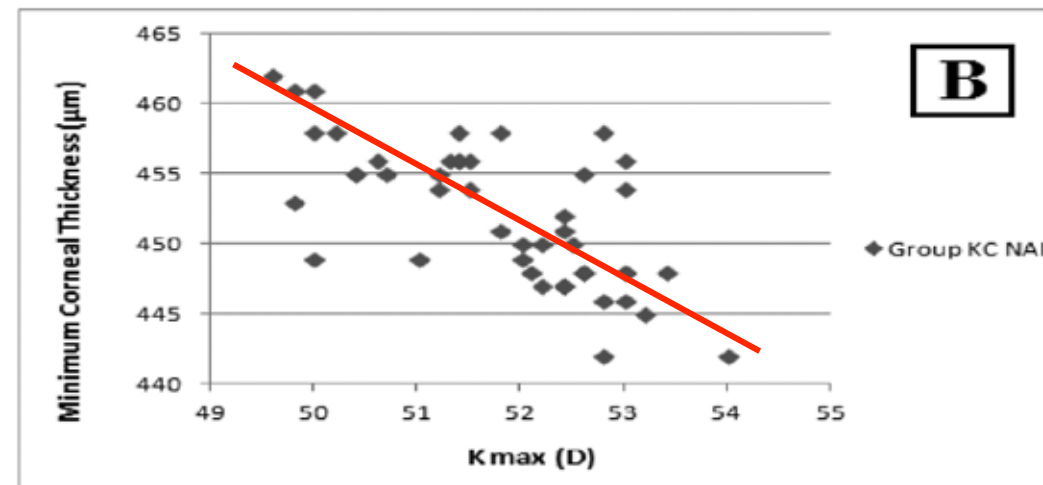
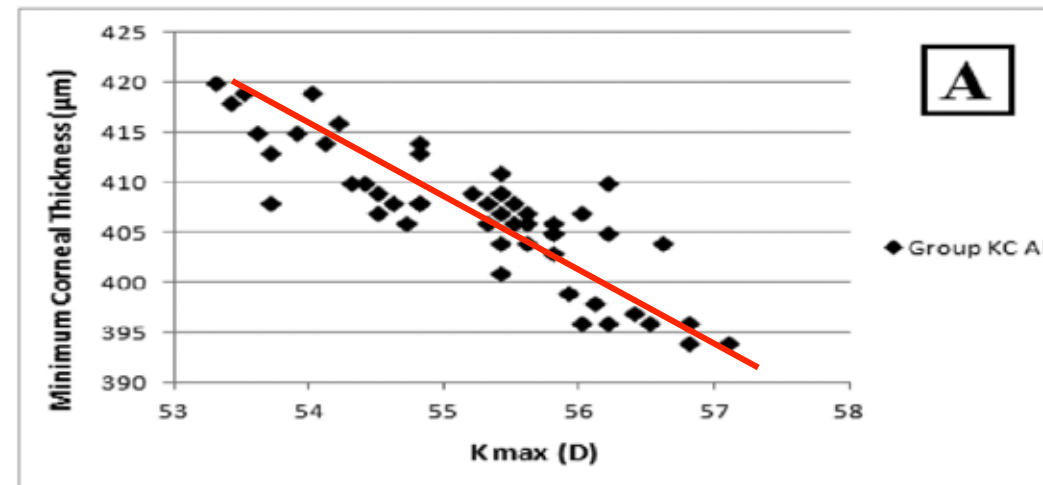
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There is a manifest correlation between KC progression showing a contemporary increase of K max values to the decreasing of Corneal Thickness.

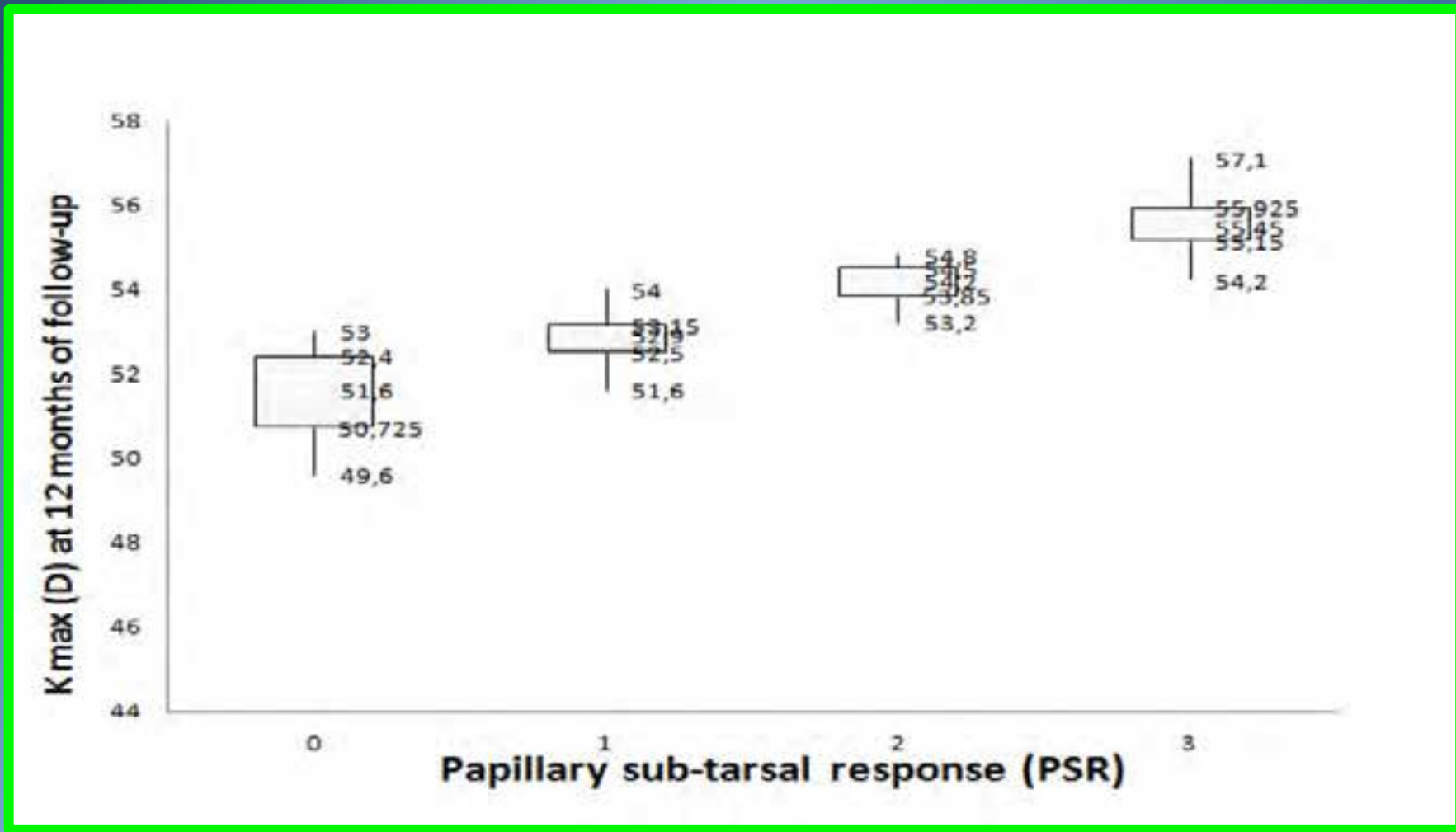
The correlation between K max and Minimum Corneal Thickness at 12 months-follow-up was assessed by the Pearson's Correlation Test.

Figure A shows the highest correlation between K max increasing 12 and Corneal Thickness reduction in the group patients (Pearson value = -0.85)

Figure B shows the lowest correlation between K max and corneal thinnest point redo in the KC group (Pearson value = -0.69).



max increase at 12 months of follow-up according to Papillary Sub-tarsal Response (PSR) in overall patient



Each box includes minimum (min), first quartile (1Q), median (med), maximum (max) and third quartile (3Q). The maximum increase of K max is correlated to highest papillary sub-tarsal response grade recorded in the KC AL group patients. The test shows that the increase of K max is greater in box 3 and 4 corresponding to KC AL group with grade 2 and 3 of PSR.

CONCLUSIONS

The study demonstrated a statistically significant progression of KC in patients with concomitant allergies. A high correlation between severity of allergy, eye-rubbing and keratoconus progression was documented. MMP-9 point-of-care test was positive in 80% of KC AL patients vs 4% of negative being a useful adjunctive information. Patients positive to MMP-9 test in the KC NAL group were both included among the eye-rubbers

Take home message

- **Highlight** the potentiality of ectasia progression in pediatric patients suffering from allergy and eye-rubbing by providing appropriate screening and improved care for atopy and allergy.
- **Stress** the importance of ceasing eye rubbing
- **Do close tomographic corneal monitoring** to allergic/atopic/asthmatic children for higher risk of ectatic disease development or post-acute hydrops and ectasia progression
- **Manage the allergic conjunctivitis** with a topical anti-histamine/mast-cell stabilizer, Steroids and preservative-free lubricants. Cycl A may be used in severe cases with giant papillary response and vernal.
- **Use CXL** as first line therapy of KC, without awaiting progression in young patients especially if **atopy/allergy/rosacea /vkc** comorbidities
- **Alert Pediatricians and Ophthalmologists for KC SCREEN in their allergic patients**