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Evaluation of changes in corneal morphology and sensory functions in patients with keratoconus

PhD thesis

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1.The list of Abbrevations

ALK-Anterior Lamellar Keratoplasty

AS-OCT-Anterior Segment Optical Coherence Tomography

AST-Keratometric Astigmatism

BAD-Belin/Ambrosio Enhanced Ectasia Display

BCVA-Best Corrected Visual Acuity

BFS- Best Fit Sphere

CAST-Calpastatin-Calcium-Dependent Cysteine Protease Inhibitor

COL5A1-Collagen Type V Alpha1

COL4A3-Type IV Collagen Alpha3

COL4A4-Type IV Collagen Alpha4

CTSP-Corneal Thickness Spatial Profile

CXL-Corneal/collagen Cross-Linking,

dDALK-Descemetic Deep ALK

Dk-P = Dk = Diffusion (D) * Oxygen Solubility (k)

DOCK9-Dedicator of Cytokinesis 9

FNDC3B-Fibronectin Type III Domain Containing 3B

FOXO-Forkhead Box O1

HGF-Hepatocyte Growth Factor

ICRS-Intra Corneal Ring Segment

ICL-Implantable Contact Lens

IL1A-Interleukin 1 Alpha

IL1B-Interleukin 1 Beta

IL1RN-Interleukin 1 Receptor Antagonist

IOL-Intraocular Lens

KC-Keratoconus

KCI-Keratoconus Index

KISA- keratometry, I-S, skew percentage, astigmatism

KPI- keratoconus prediction index

KSI-keratoconus severity index

LOX-Lysyl Oxidase

MPDZ-NF1B-Multiple PDZ Domain Crumbs Cell Polarity Complex Component

NI-BUT-Non Invasive- Break Up Time

OSDI-Ocular Surface Disease Index

PK-Penetrating Keratoplasty

PRK-Photorefractive Keratectomy

PTI- Percentage Thickness Increase from Thinnest Point

RAB3GAP1-RAB3 GTPase Activating Protein Catalytic Subunit

RGP-Rigid Gas Permeable Lenses

RSB-Residual Stromal Bed

SLC4A11-Solute Carrier Family 4 Member 11

SOD1-Superoxide Dismutase 1

SRAX- Relative Skewing of the Steepest Radial Axes

TGFBI-TGF Beta-Induced

UCVA-Uncorrected Visual Acuity

USA-United States of America

VSX1-Visual System Homeobox 1

WNT10A-Wingless-type MMTV Integration Site Family Member 10A

ZEB1-Zinc Finger E-box Binding Homeobox 1

ZNF469-Zinc Finger Protein 469

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2. Introduction

Keratoconus has been recognized for more than 150 years by ophthalmologists as part of the group called corneal „thinning disorder" or „corneal ectatic disease". The name keratoconus comes from Greek word (kerato: Cornea; konos: Cone). Exact definition of the disease is not easy, but key findings for diagnosis are bilateral clinical non-inflammatory posterior ectasia with abnormal corneal thickness distribution which involves the central two-thirds of the cornea [1, 2]. Modern and more precise diagnostic tools such as corneal tomography, has increased the ability of ophthalmologist to recognise keratoconus and corneal ectasia at a much earlier stage than previously possible [3]. Regarding the increasing diagnostic potential previously established prevalence of keratoconus 50/100 000 in the general population has changed to a much higher prevalence rate 50-230/100 000 [4, 5, 6].

Global prevalence of refractive errors are increasing. Solely myopia will affect an estimated 4758 million people globally (and moreover 938 million with high myopia) by 2050 [7]. Hyperopia (8.4 % of the USA population of age 40 and older) and corneal astigmatism (1 in 3 people in the USA) also affect a significant population worldwide [8, 9].

In everyday life and during work one have to face a high amount of information. There is a need that people could process and respond to stimuli very fast during our accelerated life pace. Most of the stimuli comes through the visual system. These high standards and the spread of refractive laser procedures generate the need for perfect vision. An estimated 8,4 million people in the USA from 1995 to 2013 had undergone refractive surgery (including all types of refractive procedures) [10, 11]. Only in 2010 in the USA 800 000 refractive surgical procedures were performed [8, 12]. The most feared post-operative complication for laser refractive surgery is corneal ectasia after treatment [13, 14]. The pre-operative risk factors for post treatment corneal ectasia are high myopia, low preoperative corneal thickness, residual stromal bed (RSB) thickness less than 250 μm, younger age and keratoconus (especially forme fruste keratoconus) [15-18]. Despite the reasons detailed above at present time there is no precise and ultimate diagnostic system for early keratoconus [2].

Corneal nerves plays an important role in maintaining the integritiy of the human cornea. The vast majority of corneal nerves are sensory types, and their main function is to protect the ocular surface against harmful impacts. Changes in the keratoconic cornea impact all layers, and also influence the corneal nerves and their functions [1, 2, 3, 6]. Nerve dysfunction is well known for decades in keratoconus, but the exact origin and the correlation with the disease severity is unclear. Weather sensory disfunction is a cause or a consequence is still unknown [1, 2, 6]. Corneal esthesiometry could give exact and comparable information about the different type of sensory nerve functions, and could present additional information during decision making/screening.

Keratoconus screening and early diagnosis is mandatory when laser refractive surgery candidates are selected. The recognition of keratoconus plays an important role in pre- and post-operative surgery candidate management.

2.1 Corneal layers and innervation

The cornea has five definitive layers. The normal cornea is dome shaped, but more precisely it's surface steeper in the center and flatter in the periphery. The average central corneal thickness (CCT) is approximately 550 μm, the thinnest site on the entire cornea is located approximately 0.9 mm from the visual axis, most commonly in the infero-temporal quadrant. The healthy cornea is avascular with oxygen coming mainly from the tear film and metabolic supply from the aqueous humor (**Figure 1**.).

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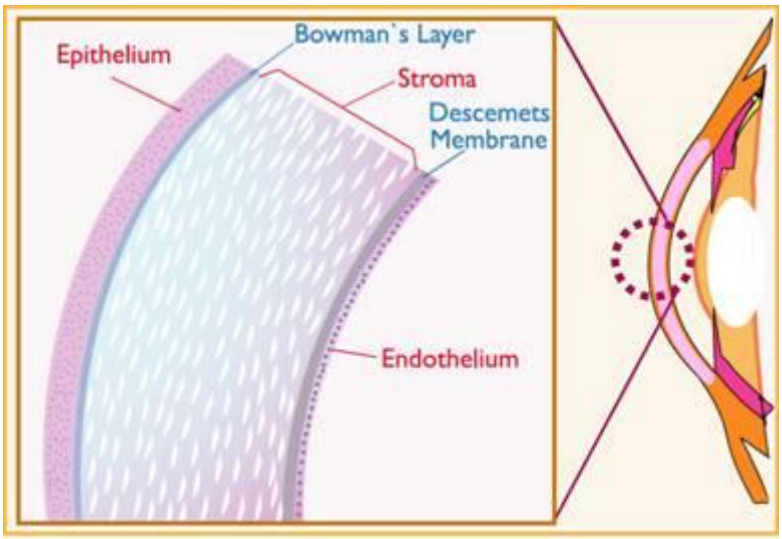

Figure 1.: The structure of the human cornea. (http://www.hybridcornea.org/aboutcornea.htm)

Epithelium: The outermost layer of cells that cover the outer surface of the cornea. It has a thickness of about 50 to 60 μm or 4 to 5 cell layers in thickness. These layers consist of a superficial layer of flattened cells, an intermediate layer of polyhedral cells called wing cells, and a basal germinal layer. The superficial layer cells peel off constantly and are replaced by the cells generated by multiplication in the basal layer, this cycle last about 7 days. The basal layer connected with a collagen-enriched basement membrane to Bowman's layer. The epithelium is filled with thousands of demyelniated nerve endings that make the cornea extremely sensitive to various external (enviromental and noxious) stimuli. The primary functions of the epithelium are to provide a barrier for external materials (dust, water etc.) and bacteria; provide a smooth surface of the eye; to anchor the tear film. An injury at this level can heal without scar formation. [19, 20]

Bowman's Layer: A thin, homogeneous, acellular, non-regenerating and transparent layer. This layer is located between the basal epithelium and the stroma and about 15 μm thick. Composed of compact collagen lamellae, these fibers are tightly connected with the stroma. The primary function is unclear, but acts as a physical barrier to protect the deeper corneal structures, and to orientate the subbasal nerve plexus. If the injury hits the level of this layer, scar formation is present. [21-23]

Stroma: The stroma is the thickest layer of the cornea. It represents 90% of total corneal thickness. It consists primarily of water (78%), collagen (16%), glycosaminoglycans and some keratinocytes between fibrils. Glycosaminoglycans are considered to be the "glue" of the cornea, responsible for providing plasticity and the structural support needed for successful corneal function. Along with other molecules, glycosaminoglycans form the solid portion of the cornea (22%), they provide corneal hydration, structural integrity, transparency and thickness. In normal conditions this layer is avascular, and transparent. About up to 300 regularly arranged (Type I) collagen lamellae and fibrils run parallel and extend across the entire cornea. This strict conformation of collagen lamellae and fibrils is necessary to keep the light-conductivity transparency, as well as the relatively dehydrated state. The stroma is not renewable if injured [24, 26].

Dua's Layer: This newly discovered (in May 2013) sixth layer of the cornea, located just below the stroma. Harminder Dua and his research group were performing experiments with corneal transplants, and during corneal layer air dissection (with air bubbles) some corneas showed other type of dissection than others. Dua's layer is very thin, only 15 microns thick. This layer could play a role in earlier unexplained corneal diseases, and could explain some earlier described pathologies but careful further research is needed. The literature is controversial about the existence of this layer. [27-29].

Descemet's Membrane: It's composed of collagen fibers (Type IV.) and produced by the endothelial cells and is a true basement membrane. The layer is firm and highly elastic, but only about 10 to 12 μm thick. A tough layer, which is resistant to enzymatic degradation by phagocytes and toxins, and serves as a protective barrier against infection and injuries [30-32].

Endothelium: The thin (4 μm), innermost confluent monolayer of the cornea which cells have a polygonal shape. These cells are responsible for keeping the cornea (mainly the stroma) clear by dehydrating it, and serve as a barrier to fluid movement into the cornea. The corneal endothelium actively transports water from the stroma with active and passive ion exchangers. Critical to this energy-driven process is the role of Na^+/K^+ATPase and carbonic anhydrase. Bicarbonate ions formed by the action of carbonic anhydrase are translocated across the cell membrane, allowing water to

passively follow. The main goal is to keep the stroma ~3.5 mg H_2O/mg dry or less, the stroma is highly-ransparent at these values. If this layer damaged or diseased, these cells will not regenerate and won't multiply, and the stroma becomes edematous and hazy, at the end ultimately opaque. Cell density is about 3.500 cells/mm^2 at birth and decrease gradually throughout life at about 0.6% per year and with about 10% loss per intraocular surgery. To maintain healthy stoma dehydration about of 700 cells/mm^2 is required for endothelial functions and metabolism. This layer also allows nutrients and other molecules to enter the stroma, to feed the avascular corneal tissue inner part [33-39].

The human cornea is one of the most richly innervated tissues in the body and the sensory nerves are derived from the ophthalmic division of the trigeminal nerve [40]. These nerve trunks enter the corneal stroma radially at the periphery next to the limbus. The stromal nerve bundles contain mainly nociceptive Aδ and C fibers. Stromal nerve trunks are comprised of approximately 900–1200 myelinated and unmyelinated axons [41]. One millimeter after the limbus the myelinated fibers lose their myelin sheath, and both types of nerves are surrounded solely by Schwann cells [40, 42]. Stromal nerves are organized paralell to the corneal collagen lamellae network. Nerve density increases while nerve diameter thins as the stormal axons progress anteriorly to the superficial stroma. Some axons terminate as free nerve endings, others directly innervate keratinocytes [43, 44]. Superficial stromal axons penetrate the Bowman layer into the epithelium predominantly at the peripheral cornea, and form the subepithelial plexus. These axons form a whorl-like pattern approximately 1 – 2.5 mm inferonasal to the corneal apex. The subbasal plexus run parallel to the corneal surface, and only beaded unmyelinated C fibers travel for a short before turning upward and terminating perpendicularly just beneath the epithelial surface as free nerve endings [45-48].

2.1.1. Corneal sensory nerves and receptors

As mentioned above, the cornea has rich sensory nerve fiber supply. Autonomic nerve fiber axons are also present, but they represent a minority and the exact function is not well understood. These nerves consist of sympathetic fibers that are derived from the superior cervical ganglion and parasympathetic fibers that originate from the ciliary

ganglion [49-52]. Sensory nerves mainly derived from the ophthalmic division of the trigeminal nerve. They have a variety of sensory and efferent functions, sensations result from the activation of sensory nerve afferents, which are the peripheral branches of various types of trigeminal nociceptive neurons. Corneal nerve stimulation produce predominantly a sensation of pain in humans but it is thought to depend on the modality of stimulus acting on the cornea [53-55]. These axons ensure and maintain the ocular (corneal) surface integrity, perceive irritation and pain, mediate midbrain reflexes, regulate tearing and blinking, corneal nerves are responsible for ocular surface sensations and play an important role in wound healing and tear production and thus, contribute to maintaining ocular surface integrity [40].

The distribution of corneal sensory nerves is as follows, about 70% are polymodal nociceptors, 15-20% are mechano-nociceptors and about 10%-15% are cold-sensitive thermal receptors [56]. The detection of stimuli by corneal receptor terminals are the same as in sensory receptors of other tissues of the body. It depends on membrane signaling proteins which convert the external/internal stimuli into a conformational change, which lead to an alteration in ionic permeability and finally cause an electrical depolarization at the membrane of the nerve endings. The electrical potential change (depolarization) at the peripheral nerve endings generates nerve impulses centripetally to the brain. Most transduction molecules are ion channels that are directly opened by the external stimulus or gated by internal molecules or membrane proteins [53, 55, 56]. The receptors at the sensory nerve endings are part of the TRP (Transient Receptor Potential) channel superfamily. The TRP superfamily is evolutionally conserved from nematodes to mammals [57]. These receptors could be divided into five sub-groups. The common point at the TRP family is the six-transmembrane domain unit with a non-selective cation-permeable pore between domains 5 and 6 [58]. Four of these units could form a TRP channel. The main difference between the channels is the intracellular part and cation selectivity. In human corneal nerve endings TRPV1, TRPV4, TRPA1 and TRPM8 receptors expressed mainly [59, 60, 61].

The activation mechanisms of ion channels are unique in that there are a diverse host of stimuli that can activate TRP channels and exhibit sharp differences in stimulatory modes even within each TRP channel subfamily. This means, that with different kind of excitation one can investigate different ion channels/sensory nerve endings. In other

words different sensation modalities linked to different ion channels and indirectly to different sensory nerve types with some overlap [54, 57, 58].

Polymodal nociceptors: TRPV1 ion channels representing mainly this sensory ending. This receptor type activated by noxious exogen and endogen stimuli, and is likely the origin of unpleasant sensations evoked by near-noxious and injurious chemical, thermal, and mechanical stimuli acting on the cornea [56]. Temperature under 29°C and over 40°C, hyperosmolarity, acidity (pH below 6), near-noxious mechanical energy, proinflammatory cytokines (IL-6, IL-8) are activators for TRPV1 containing nerve endings [62]. TRPV4 receptors seems to be an osmosensor for a hypoosmolar challenge [63] as well. Polymodal nociceptors respond to their natural stimuli with a continuous, irregular discharge of nerve impulses that present as long as the stimulus exists. The firing frequency of the nerves roughly proportional to the intensity of the stimulating noxa. So these sensory endings not only signal the presence of unpleasant noxas, but also encodes its intensity and duration in a certain degree [56, 62-64].

Mechanonociceptors: Stretch-activated receptors were described in corneal nerve endings and in other tissues of the body. These fibers fire with low frequency in response to brief or sustained indentations of the corneal surface and, also when the stimulus is larger. They have a very low threshold force for activation, even far below of in skin of the same kind of receptors. Mechanonociceptor function is to transfer very low mechanical sensations and to protect the corneal surface by starting the blinking reflex. These receptors are probably responsible for the acute, sharp sensation of pain produced by touching the corneal surface. Henceforward presumably polymodal nociceptors (TRPV1, TRPA1) are responsible for sustained chronic pain after mechanical impacts [55, 56, 58].

Cold-sensitive thermal receptors: TRMP8 channels have been described as cold sensors in cold thermoreceptor corneal nerve endings. Thermal sensory nerves at the cornea have an ongoing spontaneous firing activity at normal conditions. The normal corneal surface temperature is about 33 °C. These nerves have an increased firing rate when the normal temperature drop below 33°C, and decreased at warming. They react to different type of cooling modalities, and increase firing rate when evaporation at the corneal surface is present or when cold solution is applied on the cornea, blowing cold

air at the corneal surface is also a stimulating factor. Cold receptor fibers are able to detect small temperature variations of 0.1 °C or less. They also encode cold stimuli by changing in impulse frequency, and by this method the perception of non-noxious temperature drop could be a conscious sensation. TRMP8 receptors are probably the main modulators of basal tearing rate by percepting changes in the corneal surface temperature due to evaporation of the tear film [56, 59-61, 65, 66,].

2.2. Corneal degenerations and ectasia

Corneal degenerations are defined progressive deterioration of a tissue or an organ that was previously normal. This deterioration often accompanied by loss of functional activity. Degenerations usually characterized by the deposition of material, vascularization and tissue thinning [2, 67, 68].

The definition of „Ectasia" strictly means as a dilation or distention of a tubular structure. But in ophthalmology this term refer to conditions associated with changes in corneal shape [2].

Under the definition of „corneal ectatic disease" several entities should be characterized including keratoconus, pellucid marginal degeneration (PMD), keratoglobus, and postrefractive surgery progressive corneal ectasia. These conditions could be distinguished by the thinning location and pattern [2]. Corneal ectasias are associated with decreased uncorrected visual acuity (UCVA), an increase in ocular aberrations, and often a loss of best-corrected distance visual acuity (BCVA). To characterize keratoconus as true corneal dystrophy is controversial, because the lack of strict inherited mechanism. Recently there has been find link with some genes, but sporadic cases are also present in a large scale [2, 69, 70].

2.3. Keratoconus

Keratoconus (KC) is a non-inflammatory bilateral corneal ectatic disease, involving all layers of the cornea. In most cases KC present at different stages in each eye of the patient, in other words the disease is asymmetric. Recent findings, and definitions declare that true unilateral keratoconus does not exists [1, 2]. This bilateral disease defined as a progressive thinning of the corneal layers, which involves especially the central two-thirds of the cornea. The progressive thinning causes a decrease in

corneal/stromal biomechanical strength which leads to abnormal posterior ectasia and corneal protrusion causing abnormal corneal thickness distribution (**Figure 2.**) [1, 2]. Changes in the corneal curvature are responsible for myopic shift, irregular corneal astigmatism and visual disturbances at KC patients. The disease in most cases starts at the second decade of life about puberty, and rarely progress after the age of forty [73].

Mandatory findings for keratoconus diagnosis are abnormal posterior elevation, abnormal corneal thickness distribution and characteristic changes in corneal topography [2].

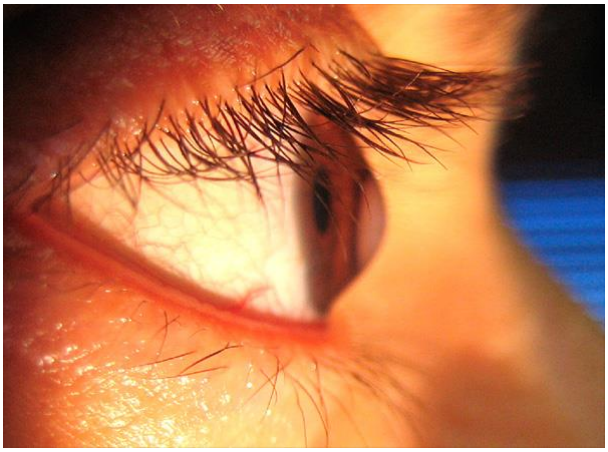

Figure 2.: Keratoconic eye.
(http://eyeworld.org/article-linking-keratoconus-and-floppy-eyelid-syndrome-to-sleep-apnea)

2.3.1. Prevalence

The disease is relatively common, affecting 50-230/100 000 people world wide [4, 5, 6]. It affects only in the United States approximately 300,000 patients [70-72].

2.3.2. Etiology and genetics

The exact etiology of KC is still unknown. The recent opinion about KC is that KC is a multifactorial disease caused mainly by environmental factors but it has a strong underlining genetic susceptibility [70]. Keratoconus has a very complex and not well understood nature regarding to its etiology. As described in earlier studies three entities could be distinguished:

I. The majority of KC cases reported by clinicians are isolated KC with no associations with other conditions [70, 72, 74, 75].

II. Increasing evidence show genetic predisposition to KC. Positive family history linked to higher odds ratio in family members for the diagnosis of keratoconus [72, 76]. GWLS (Genome-wide linkage study) and GWAS (Genome-wide association study) have made significant progress in identifying genetic variation that is strongly correlated with keratoconus. SNPs (Single nucleotide polymorphisms) associated with the following genes have been implicated: *LOX*, *CAST*, *DOCK9, IL1RN*, *SLC4A11*, *HGF*, *RAB3GAP1, TGFBI, ZNF469, ZEB1, VSX1*, *COL5A1, COL4A3, COL4A4*, *FNDC3B, FOXO1*, *MPDZ-NF1B, WNT10A, SOD1, IL1B, IL1A*, in addition to the microRNA *MIR184*. Notably, not all analyses of each of these genes completely confirm their role in KC [70].

III. Other conditions could be associated with KC such as Down-syndrome [70, 77], inflammatory bowel disease (IBD) [70, 78], atopic disease including vernal kerato-conjunctivitis, and atopic dermatitis. Higher incidence of KC was also reported in patients with connective tissue disorders (Marfan syndrome, Ehlers-Danlos syndrome) [70, 79, 80]. There is a reverse relationship between diabetes mellitus and KC [70, 81]. Diabetes in some circumstances could play a protective role in the progression of KC by increasing the number of corneal collagen cross-links and by altering the biomechanical properties of the cornea [82, 83].

Briefly most keratoconus cases appear spontaneously, although approximately 14% of cases present with evidence of some genetic transmission [84].

2.3.3. Subjective and clinical signs of keratoconus

Patients with KC often report eye itching, photophobia, distorted vision, glares and halos, progressive visual blur and distortion. Multiple unsatisfactory attempts to obtain optimum spectacle correction or progression from soft contact lenses to toric or astigmatism correcting contact lenses are also common warning signs. The progressively poor vision hardly corrected with spectacles is the most common complaint. These symptoms are secondary to the progressive myopia and irregular astigmatism [1, 2] due to the changes in corneal curvature.

Early signs including scissors reflex during retinoscopy, Rizzutti's sign (a conical reflection on the nasal cornea when light is shone temporally), and asymmetric refractive error with high or progressive astigmatism. Keratometry showing high astigmatism and irregularity are also early signs. A Fleischer ring, or iron deposits within the epithelial layer, might be found near the base of the cone. Fine and almost parallel vertical lines seen in the stroma called Vogt strias are secondary to stromal stress. In later stages of KC corneal protrusion may cause angulation of the lower lid on downgaze (Munson's sign). Corneal hydrops could cause spontaneous tears in the Descemet's membrane, and corneal scarring [1, 2].

2.3.4. Patomechanism and pathology of keratoconus

The exact etiology and trigger factors are still unknown for keratoconus. There are several studies and hypothesis exists parallel in the literature. At present time the most plausible is multifactorial etiology, with the interplay of possible genetic predisposition and a second hit by environmental/risk factors. Experts agreed some risk factors are frequent and could be linked to keratoconus.

- *Mechanical factors: Eye rubbing* is a commonly mentioned risk factor. According to several studies eye rubbing could cause direct micro trauma to the cornea, which activates the wound healing signaling pathway in the epithelium. This mechanism accompanied with the activation of keratocytes and increased hydrostatic pressure in the cornea layers. This assumption explains the higher incidence of keratoconus in *atopic patients (ocular allergy)* or in *contact lens wearers*, where eye rubbing and epithelial micro trauma is common. In this group *floppy eyelid syndrome* and *connective tissue disorders (Marfan syndrome etc.), Ehler-Danlos syndrome* are also occur [1, 2, 85].
- *Oxidative stress:* There are studies indicating an abnormal processing of the superoxide radicals in keratoconic corneas. Due to this change corneal self-repair mechanisms are not working properly or lack. Genomic deletion in the *superoxide dismutase 1 (SOD1*) gene is also often present [86]. An increased rate of free radicals (reactive oxygen species-ROS, reactive nitrogen species (RNS) in the corneal tissue causing direct collagen damage, consequence of this

collagen degradation biomechanical weakening and corneal thinning is a logical final result.

- *Hormonal causes*: Keratoconus usually starts with puberty around in the second decade of life, and accelerated progression often seen in keratoconic patients during pregnancy. Both puberty and pregnancy accompanied by fundamental hormonal changes. This theory is controversial and has not been proven [87, 88].
- *Inflammation:* Although keratoconus definition contains the non-inflammatory nature of the disease, recent studies show that some kind of inflammation may play a role in the pathogenesis of KC. According to studies significantly elevated levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and matrix metalloproteinase (MMP)-9 were found in the tear fluid of patients with KC [89, 90, 91]. Although this inflammation does not meet all the classic criteria for an inflammatory disease, the lack of inflammation is questionable.
- *Genetic associations* have been already explained earlier in this work.

Briefly when keratoconus is present, all layers of the cornea are involved. Histopathological findings are as follows: Corneal epithelial cells usually enlarge and elongate. After involvement of the basal epithelial cells disruption of the basement membrane are frequent. In later stages this degradation could be accompanied with epithelial ingrowth and collagen herniation trough the Bowman's layer forming typical Z-shaped interruptions or breaks in Bowman's layer. Bowman's layer and anterior segment scarring are also seen parallel with collagen fragmentation, fibrillation and increased fibroblastic activity. The stromal collagen has normal size, but the decreased number of collagen lamellae causing stromal thinning. Endothelial cells are also involved, and pleomorphism with polymegathism could also be manifested. Nerve fibers are also thickened, this will be explained in detail later (**Figure 10**). The severity of changes increase with disease duration and showing a higher grade at the apex of the cone than at the base [1, 2, 85].

Regarding to the discrepancies in studies it is hard to distinguish between association, cause and effect in keratoconus pathology.

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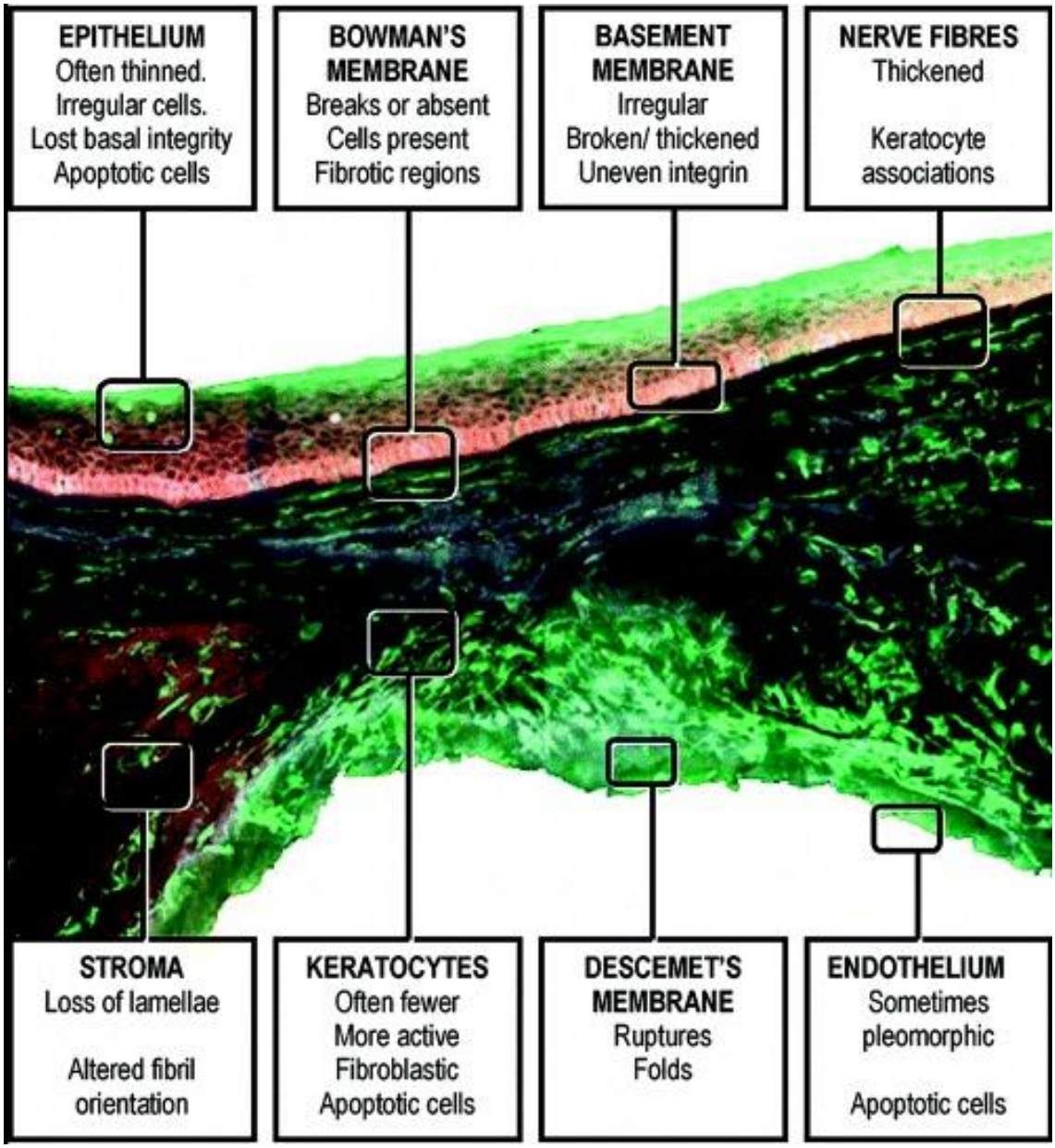

Figure 10.: An anteroposterior section of the central 1 mm of a keratoconic cone from penetrating keratoplasty surgery. The tissue has been labelled with CellTracker Green (Molecular Probes) to mark viable cells and then counter-stained with antibodies to integrin (red) and fibronectin (blue). The cross-section shows some of the classical features of keratoconic pathology. Areas of the cornea are highlighted to show position and type of pathological features in keratoconus. **[**Morphological changes in keratoconus: Pathology or pathogenesis. Available from: https://www.researchgate.net/publication/8634066_Morphological_changes_in_keratoconus_Pathology_or_pathogenesis [accessed Sep 1, 2016]]

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2.3.5. Diagnostics of keratoconus

Diagnosis sometimes could be very difficult, and need a lot of clinical experience in problematic cases. Briefly, diagnosis can be made based on history of changing refraction, poor best spectacle corrected vision, abnormalities in keratometry, corneal topography and tomography findings, in association with abnormal corneal thinning pattern. In advanced cases characteristic slit lamp findings and other signs can support prompt diagnosis of KC. In early stages of keratoconus corneal tomography (Scheimpflug imaging etc.) and the comparison of results to the other eye of the same patient as a reference (rather than artificial numbers or reference curves) are gaining popularity [1, 2, 92, 93, 94].

2.3.5.1. Slit lamp

Slit lamp biomicroscopy is a basic but necessary diagnostic tool. With the evaluation of the anterior segment KC signs could be find, including corneal thinning, Vogt strias, Fleischer ring (more easily with a cobalt blue filter) at the basis of the protrusion, and Descement tears or corneal scarring [1, 2] in more advanced forms.

2.3.5.2. Corneal topography

Most videokeratography systems used in clinical practice are based on placido disk principles. The instrument captures the projected placido disk images reflected from the corneal surface (precorneal tear film). The machine uses a central camera to capture the images from a standard point and digitizing computer software convert data to a color-coded dioptric map of the anterior cornea. The warmer colors (reds, oranges) represent steeper cornea with higher refractive power, the cooler colors (violets and blues) represent flatter cornea with lower dioptric power and greens and yellows represent colors found in normal cornea [95]. Changing the steps in color codes can cause a different look of the same cornea. The smaller steps increase the sensitivity to pick up early keratoconus, but can falsely diagnose a normal cornea as keratoconic, whereas larger steps can miss out on the early changes [95]. Different topographers use different steps of colors, making it difficult to compare two different devices.

Elevation is not measured directly by placido based topographers, but certain assumptions allow the construction of elevation maps for example by Orbscan. Elevation of a point on the corneal surface displays the height of the point (in micron) on the corneal surface relative to a reference surface [95].

For a good quality and reliable scan the patient should have a stable precorneal tear film, and image acquisition requires good patient fixation and compliance to avoid eye lids covering the cornea. Videokeratography is a very useful diagnostic tool for both keratoconus screening, and KC progression follow-up, but it is incapable of capturing early KC changes of the posterior surface (posterior elevation changes).

2.3.5.3. Scheimpflug imaging

The cornea has a conic shape, therefore without using the Scheimpflug principle imaging of this tissue could lead to false results. The name of this imaging method came from Theodore Scheimpflug who worked on correcting ariel distortion in perspective photographs. Briefly, this method could give solution to a problem, when the plane of the prospective image and the plane of the object are not parallel. In this situation it will be impossible to focus all the image on a plane parallel to image plane. Thus this may lead to image distortion. But using the Scheimpflug principle when a planar subject is not parallel to the image plane, an oblique tangent can be drawn from the image, object and lens planes, and the point of intersection is called Schiempflug intersection (**Figure 3**). Careful manipulation of the planes (image and lens) could lead to a sharp and focused image of the non-parallel object [96, 97].

Using rotating Scheimpflug camera (Pentacam HR, Oculus Optikgerate, Wetzlar, Germany) offers significant advantages over placido based curvature analysis. This method allows for the creation of a three-dimensional reconstruction of the anterior segment by measuring not only the both surfaces of the cornea but the lens surfaces as well. Both posterior corneal elevation and corneal thickness map are significantly earlier indicators of KC and ectatic diseases than only anterior curvature and ultrasound pachymetry [98]. With this diagnostic tool ophthalmologist could have the possibility to recognize KC in a far earlier stage with less false positive or negative errors. Scheimpflug imaging also covers significantly more of the cornea than was possible with placido based devices giving the opportunity to a more accurate diagnosis [98]. In

other words devices using Scheimpflug imaging become essential tools in the correct diagnosis and follow up of keratoconus. Placido-based topography analyzes the central anterior corneal surface, whereas tomography (Scheimpflug and/or optical coherence tomography) analyzes the anterior and posterior cornea and produces a near full corneal thickness map [2].

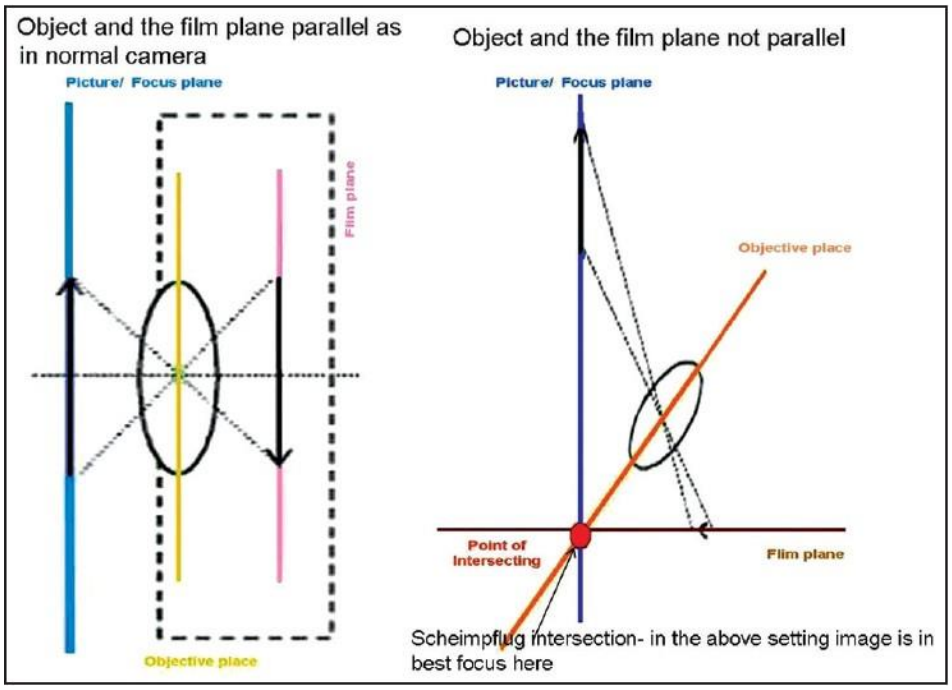

Figure 3.: Scheipflug imaging. Illustration shows Sheimpflug camera working principles, this method of image acquisition enhances the depth of focus (left) [98].

2.3.5.4. Anterior segment optical coherence tomography (AS-OCT)

Anterior segment optical coherence tomography (AS-OCT) is a noncontact imaging modality of the cornea and the anterior segment of the eye with a high resolution which can accurately map corneal thickness. This high resolution cross-sectional imaging modality first used for screening the back of the eye (the retina). [99]. A variety of high speed OCT scanners are now available that can image and measure the corneal thickness. Fourier domain technology provides the advantage of faster scan acquisition with greater axial resolution [99, 100]. This method provide a non-contact corneal pachymetric map (not just spot pachymetric data such as ultrasound pachymeters), with

full coverage of the cornea. AS-OCT has several benefits over placido disc based videokeratography.

Corneal thinning is a key pathologic feature of keratoconus, therefore a KC diagnosis based on corneal thickness measurement may offer additional information not available on topography [101, 102, 103, 104]. Last but not least, AS-OCT imaging provides a fast full view of the corneal surfaces. Recently, epithelial thickness profile maps using Fourier domain OCT have been shown to be useful in detecting subtle epithelial changes, which could be a sign of early keratoconus [105, 106].

2.3.6. Keratoconus staging and classification systems

At present time there is a lack of adequate classification/grading system of keratoconus [2]. Several classification systems co-exist in the literature based on different indicators. These systems usually based on morphology, ocular signs or disease evolution. Index-based systems are also available. Experts agreed that some systems have only historical relevance at this time [1, 2, 98-101].

There is an explosion in the field of ophthalmology devices using different type of diagnostic principles (OCT, Scheimpflug imaging etc.). Currently there is no grading system that could integrate the potential of new imaging modalities into a universal and widely used system.

The prevalence of all kind of ammetropias is rising worldwide, hence the number of corneal refractive procedures is also increasing [7-12]. Before any type of laser refractive surgery, screening the candidates for the presence of KC is one of the most important task to avoid post-operative ectasia [15-18]. Therefore, there is an emerging need for an ultimate and adequate diagnostic system/method for KC.

Experts of the field agreed that new diagnostic systems should take posterior corneal elevation abnormalities into account rather than focusing solely on central pachymetry for diagnosing keratoconus [1, 2].

As I see in the literature functional changes like corneal sensitivity are out of focus in the diagnosis of keratoconus. But probably these functional changes prelude other signs of KC. This become from two important data reported in studies. With corneal in vivo confocal microscopy/imaging several findings shows microstructural alterations of the corneal tissue in KC, briefly derangement in the morphologic and morphometric features of central sub-basal and stromal nerves [107-112]. On the other hand there is a significant correlation reported between central corneal sensation and severity of keratoconus [113, 114]. Investigations on corneal functions (like corneal sensitivity) could forecast KC in an earlier stage than morphometrical changes.

In the followings, I describe some of the popular and widely used KC classification systems.

2.3.6.1. Rabinowitz classification

A) Rabinowitz investigated keratoconus intensively [1, 74, 115]. He described a grading system based on videokeratography findings. During the years as KC diagnostics had an evolution he and his colleagues made some refinement.

At the beginning four videokeratographic indices were described to help clinicians in discriminating normal corneas from KC:

- *central corneal power >47.2 D*
- *inferior-superior dioptric asymmetry over 1.4 D*
- *Sim-K astigmatism >1.5 D*
- *skewed radial axes >21°*

B) After refinement Rabinowitz et al. made an index called KISA (keratometry, I-S, skew percentage, astigmatism). This index give a % for clinicians to discriminate KC from normal corneas more precisely [1, 74, 115, 116, 117].

The KISA% index is derived from the product of four indices: The K-value, an expression of central corneal steepening; the I-S value, an expression of the inferior-superior dioptric asymmetry; the (corneal astigmatism index), which quantifies the degree of regular corneal astigmatism (Sim K1-Sim K2); the skewed radial axis (SRAX) index, an expression of irregular astigmatism occurring in keratoconus [1, 74, 115, 116, 117]:

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$$KISA\% = (K) \times (I - S) \times (AST) \times (SRAX) \times 1/3$$

KISA% meaning:

-60%-100% *are KC suspects with <0.5% chance of overlap with normal population.*

-100% or higher *without any other ocular pathologies is likely to have clinically detectable KC.*

KISA index could support ophthalmologist in decision making when screening refractive surgery candidates.

C) The Rabinowitz keratoconus percentage index (KISA) and pachymetry/asymmetry index (PA/I-S) combines information from videokeratography and AS-OCT pachymetry measurements. With this refinement ophthalmologist could differ more precisely subclinical KC from normal corneas [114-116]. With this method indices are as follows:

- K value quantifies the central corneal steepening. A value of **47.20 D** or grater is suggestive of keratoconus.
- I-S value quantifies the inferior-superior corneal dioptric asymmetry which is grater in KC corneas than in normal. A value of **1.4 D** or greater is suggestive of keratoconus.
- KISA% incorporates the K and I-S values with a measure quantifying regular and irregular astigmatism into one index. This index is highly sensitive and specific in separating normal from keratoconic corneas. See cut off values above.
- PA/I-S index is the minimum pachymetry value measured with AS- OCT divided by the I-S value. The PA/I-S index allows a more sensitive detection of forme fruste and keratoconic suspects than KISA %.

Grading with this method:

- *Normal:* No clinical signs of KC and no asymmetric bowtie (AB) with a skewed radial axis (SRAX) (ie, AB/SRAX) pattern on videokeratography. *95% of normals have a PA/I-S index of more than 106.*
- *Keratoconus suspect:* The fellow eye of a patient with keratoconus with mild inferior steepening on topography, no clinical signs. *The average K reading is less than 47 D and PA/I-S index would have a value of less than 105.*
- *Forme fruste keratoconus:* The fellow eye of an individual with keratoconus, with AB/SRAX videokeratography pattern, and without clinical signs of keratoconus. *The PA/I-S index would have a value less than 100.*
- *Early keratoconus:* No aberration connected to KC on slit-lamp examination. Scissoring sign on retinoscopy and an AB/SRAX pattern on videokeratography. *Average K reading < 47 D, early keratoconus had a PA/I-S value between 10 and 57.*
- *Keratoconus:* Stromal corneal thinning accompanied by clinical signs of KC on slit lamp biomicroscopy.

D) According to Rabinowitz works Maeda and Klyce also created indexes to help decision making, and to gain accuracy in the diagnosis of KC. They used eight indices from topographic measurements [1, 104, 118]. In this classifier KPI (keratoconus prediction index) derived from eight quantitative videokeratography indexes. KCI% (keratoconus index) is derived from KPI and other four indexes.

-KPI *>0.23 is indicative of keratoconus.*
-KCI% *>0 is indicative of keratoconus*

Briefly several topographic indices have been used for the interpretation of keratoconus. Sedghipour et al. compared the sensitivity and specificity most of the topographic indices used above. They explored that while the K value and AST demonstrated >80% sensitivity and the SRAX demonstrated >90% specificity, SRAX and AST indices had

the lowest sensitivity and specificity, respectively. KISA% was the only index with specificity and sensitivity >90%. Furthermore in their study KISA% was the only index demonstrating positive and negative predictive values >95% [119]. This means that KISA index is very useful detecting early/suspect KC cases, but further research is required to confirm this conclusion [119], in short there is a need for an ultimate index or cut off value to discriminate early KC with great precision.

2.3.6.2. Amsler-Krumelich classification

This grading system was one of the commonly used decision making tool in the past. It used central corneal thickness (CCT) value measured with ultrasonic pachymetry, keratometric readings, and the degree of myopia. The Amsler-Krumeich grading system (**Table 1**) utilized easily measured parameters and the staging followed closely the treatment decision tree [98]:

Table 1.: Amsler-Krumelich classification for keratoconus [98].

Stage I
Eccentric steepening
Myopia and astigmatism <5.00 D
Mean central K readings <48.00 D
Stage II
Myopia and astigmatism 5.00-8.00 D
Mean central K readings <53.00 D
Absence of scarring
Minimum corneal thickness >400 µm
Stage III
Myopia and astigmatism 8.00-10.00 D
Mean central K readings >53.00 D
Absence of scarring
Minimum corneal thickness 300-400 µm
Stage IV
Refraction not measurable
Mean central K reading >55.00 D
Central corneal scarring
Minimum corneal thickness 200 µm

This method has some limitation on videokeratography and on newer devices. Ultrasonic central pachymetry only measured one point on the cornea, which was typically not the thinnest point, and this technic did not reflect to the full thickness profile of the cornea [98]. According to experts central corneal pachymetric value is the least reliable factor in the detecting of KC [1, 2]. This system did not take posterior corneal surface (i.e. posterior elevation) into account, and did not give a picture of the properties of the anterior corneal surface witch is also a key finding in detecting KC [1, 2]. Nowadays this method has only limited value in the era of new imaging technics (videokertography, AS-OCT, Scheipflug imaging etc.).

2.3.6.3. Classification based on corneal topography and tomography imaging

First we have to clear the difference between the two words topography and tomography. Topography means studying of the shape of the corneal surface (like videokeratographers- mentioned earlier in this work).
The emerging number of new corneal investigating devices using different principles (Orbscan, Pentacam, Oculyzer, Galilei, Sirius, AS-OCT-Visante etc.) led the term "corneal tomography" used in the field of ophthalmology. This is because the images generated by new imaging devices are rather a cross section of the cornea (with elevation data analyzed further) than in contrast to enface images of concentric rings from the placido-based devices. Corneal tomography should be used for the examination of the front and back surfaces of the cornea, along with pachymetric mapping producing a three-dimensional cross section of the anterior segment of the eye [120].

Rabinowitz has described KISA % and topography devices became part of the everyday used evaluating methods between ophthalmologists. The magnitude of his work was to give a topography-based index which was derived from easily measurable and calculable topographic parameters from the corneal surface. The index based on these values express corneal surface asymmetry. With this index screening of KC was more precise and gave the opportunity to recognize it in an early stage than before. Since then, new diagnostic techniques for the cornea like corneal tomography, wavefront analysis and biomechanical analyses have been expanded. These technics enable eye care professionals to identify keratoconus earlier than Rabinowitz would

probably have imagined in 1998. [121]. With these new diagnostic methods keratoconus can now be identified on a subclinical level, that is before topographic changes occur. To analyze changes on a subclinical level, it is essential to differentiate properly between ‘normal’ eyes and those with early keratoconus stages. Another important thing is that there are certain fundamental differences in the videokeratoscopes and the Scheimpflug devices, thus the fact that their data is non-interchangeable. These devices work on totally different principles and have different methods of data acquisition, presentation and analysis [122]. Even data from devices using the same principles (placido-disc based, scanning slit beam or Scheimpflug imaging), created by different manufacturers are also not directly comparable [122].

The topographic/tomographic patterns of the two corneas of a healthy individual often show mirror-image symmetry with small variations in patterns are unique for the individual. This phenomenon is called enantiomorphism [123].

A) Classification based on corneal topography (videokeratography):

Normal cornea and corneal pathologies could be characterized by their pattern seen on videokeratographic records. With the several different indexes mentioned above ophthalmologist has the ability to distinguish between healthy and suspicious/non-healthy corneas. The distribution of keratographic patterns in healthy patients includes the following (**Figure 4-5**): round (23%), oval (21%), symmetric bow tie typical for regular astigmatism (18%), asymmetric bow tie (32%), and irregular (7%) [123].

Individuals with keratoconus has different types of pattern seen on topographic maps like (**Figure 6**): global cone, inferior cone, asymmetric bowtie, central cone, temporal cone, oblique bowtie, infero-temporal cone, nasal cone, superior cone [98, 104].

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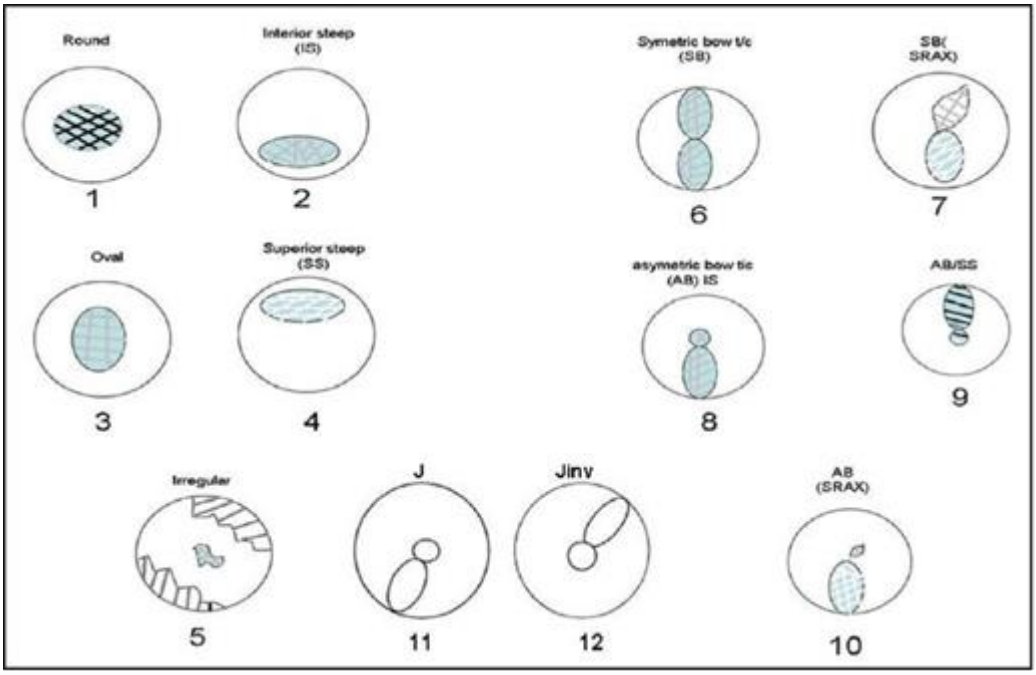

Figure 4.: Normal topgraphy map patterns distibution. Patterns can be classified into circular, oval, steepening (superior or inferior), bowtie (symmetric and asymmetric), and with or without skewing of the radial axes, J and the inverted J as shown in the template. The symmetrical bowtie, round, and the oval are considered normal, the asymmetric bowtie, skewed axes, inferior steepening, and J and inverted J pattern, and their various permutations as suspicious. The Pellucid (crab claw), butterfly, and the keratoconus (D) patterns are examples of abnormal patterns [121].

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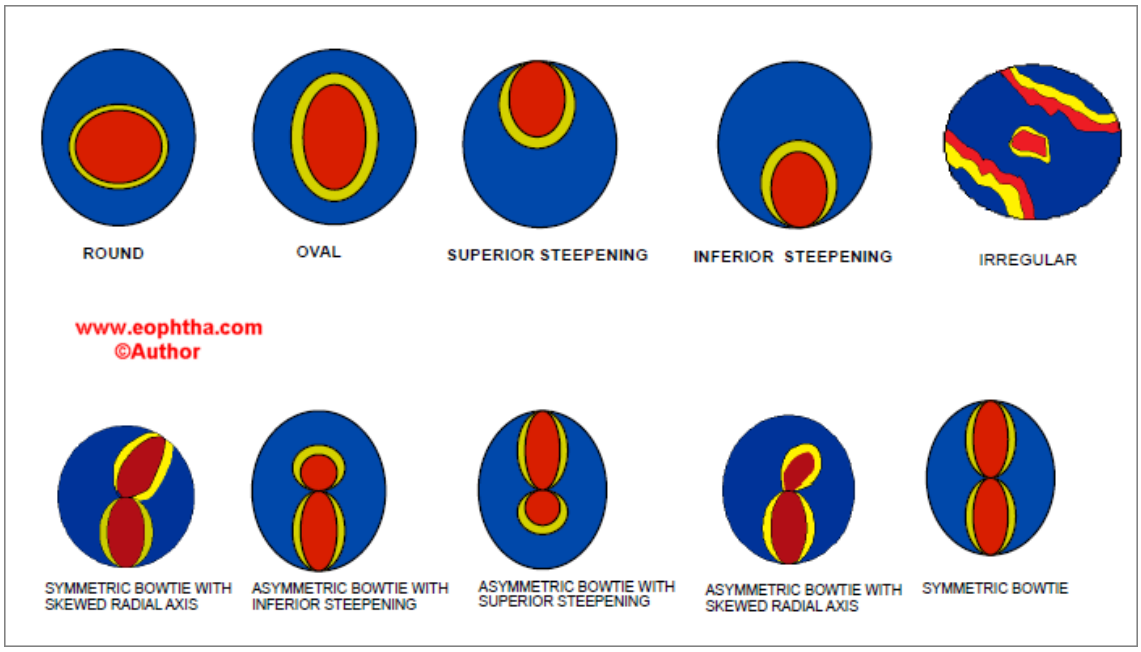

Figure 5.: Patterns of normal eyes seen on videokeratography.
(http://www.ejournalofophthalmology.com/ejo/ejo27c.html. 2016.08.23. 22:10)

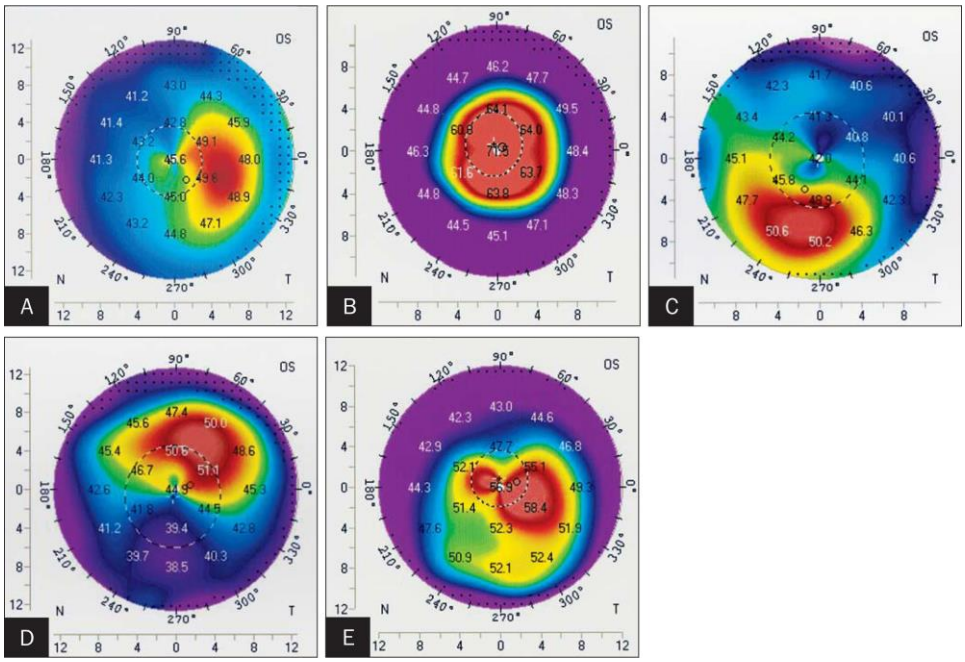

Figure 6.: Types of keratoconus based on topographic patterns A: temporal cone; B: central cone; C: infero-nasal cone; D: superior cone; E: oblique cone (Ertan A, Kamburoglu G, Colin J. Location of Steepest Corneal Area of Cone in Keratoconus Stratified by Age Using Pentacam. *J Refract Surg.* 2009; 25: 1012-1016. doi: 10.3928/1081597X-20091016-07).

B) Classification based on tomography (Scheimpflug imaging):

As mentioned above posterior elevation and the white to white corneal pachymetry map are the precious additions as compared to the videokeratographic (placido-based) devices. Changes in the posterior corneal surface like asymmetry, curvature and elevation differences have been reported in keratoconic eyes by several studies [124, 125, 126, 127]. Briefly these works find greater posterior astigmatism, posterior elevation, and prolacity in suspect eyes when compared to healthy [128]. The consensus about exact values in discriminating KC eyes from normal is lacking. Values extracted from devices using the same imaging principle are also non-comparable, which makes the whole decision making more complicated [2, 122].

Important definitions briefly before reading this part of the work:

-*Best fit surface:* It is that surface that is used for generating elevation maps and can be manually or automatically fitted to the surface in question using different algorithms like float or apex fit.

-*Best fit sphere*: It is a spherical reference surface that best fits the measured surface by the different fitting algorithms.

-*Float:* It is an algorithm to fit the reference surface to the surface in question using minimum square difference.

(Posterior) Elevation and Best Fit Sphere:

To determinate the elevation of a certain point or surface one need to have a reference (surface). Like in terrain topography, the surface elevation is studied in reference to sea level which is fixed. Localized corneal elevations (like in keratoconus) are usually relatively small compared to the whole cornea itself, to uncover these local abnormalities the global corneal curvature must be excluded likewise to pattern standard deviation in computer perimetry. This could be reached by fitting a surface onto the cornea with similar features, this called *reference surface.* This surface has different shapes like: sphere, ellipsoid, toric aspheroid, etc. When calculating elevation map

reference surface selection could affect the final output/image significantly [127]. Although the most commonly used reference surface is spherical, more precise unmasking could be achieved by using toric-ellipsoid as reference in detecting subtle changes in the cornea then with *best fit sphere* (BFS) [127]. Scheimpflug devices as opposed to attempting to generate elevation data from curvature (integral), the calculation of curvature from elevation data provides a unique solution (differential). Floating is the most common method to fit the reference surface to the cornea (**Figure 7**). In short this method basically fits the reference surface to the surface in question with minimum square difference [127, 128]. The fitting type should always be kept in mind while analyzing maps/images captured with different instruments, because it significantly influences the final output.

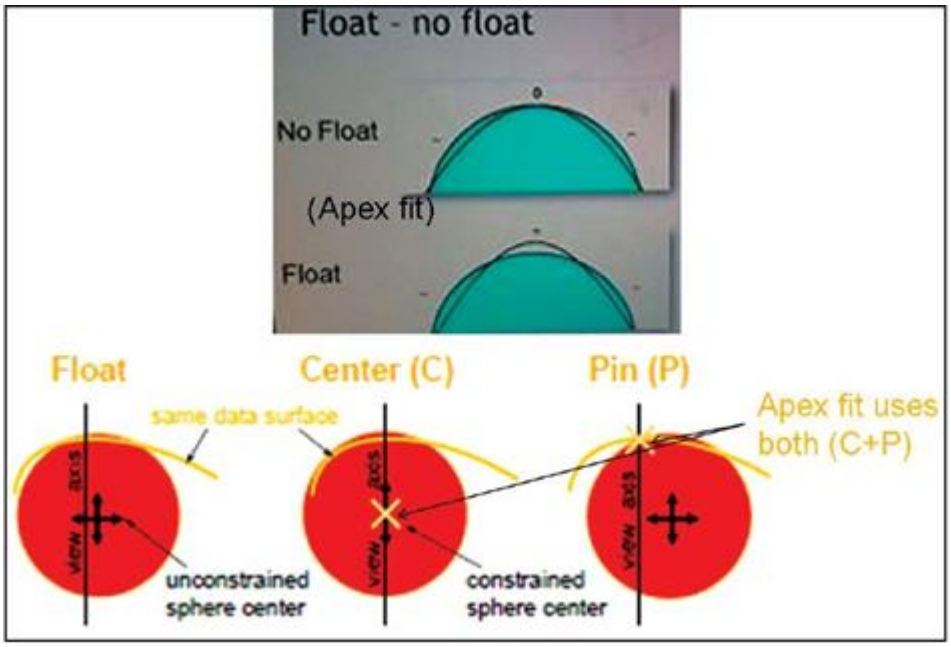

Figure 7.: Fitting methods for a reference surface. Apex fit/center + pinned - Center of reference object is constrained on the view axis and it intersects data surface on the view axis. This flattens the central hill as it centers on it, Float - Center is unconstrained. Reference fits the corneal surface with minimum square difference. Almost all devices use this method as it has the least error [98].

Raw elevation data alone from normal eyes look very similar to the raw elevation data from abnormal eyes, and makes decision making/screening impossible. So to give a qualitative definition to the elevation data the machine using the above concept of

elevation and float, identifies the dimensions of a selected reference shape that can best fit to the examined surface for each eye tested depending on its individual characteristics. This calculated reference shape varies in dimensions for each eye and its shape and curvatures are indicated on the printout [127, 128]. In the nomenclature of tomography this is called as the *best fit reference surface*. Another characteristic of BFS is the pre-defined "Fit zone" which is 8 mm in diameter in most cases. Different device software has its own specific reference surface setting (e. g., the Belin Ambrosio display (BAD) has the BFS). For further evaluation one can use different reference settings depending on individual preferred practice and experience.

Belin/Ambrosio Enhanced Ectasia Display III (BAD III):

With this method a comprehensive refractive screening display (Belin/Ambrosio Enhanced Ectasia Display III- (BAD III)) is possible, and it is integrated into the Pentacam software. It combines nine different tomographic parameters into a unified screening tool. The display uses the parameters in a regression analysis to aid the ophthalmologists identifying patients with potential risk for corneal ectatic disease [98]:

- *Anterior elevation at the thinnest point*
- *Posterior elevation at the thinnest point*
- *Change in anterior elevation*
- *Change in posterior elevation*
- *Corneal thickness at thinnest point*
- *Location of thinnest point*
- *Pachymetric progression*
- *Ambrósio relational thickness*
- *Kmax*

The BAD III displays each parameter and individually reports them as a standard deviation and then reports a final overall reading that is based on a regression analysis

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to maximize the discrimination of normal corneas from those with keratoconus (**Figure 8**) [98].

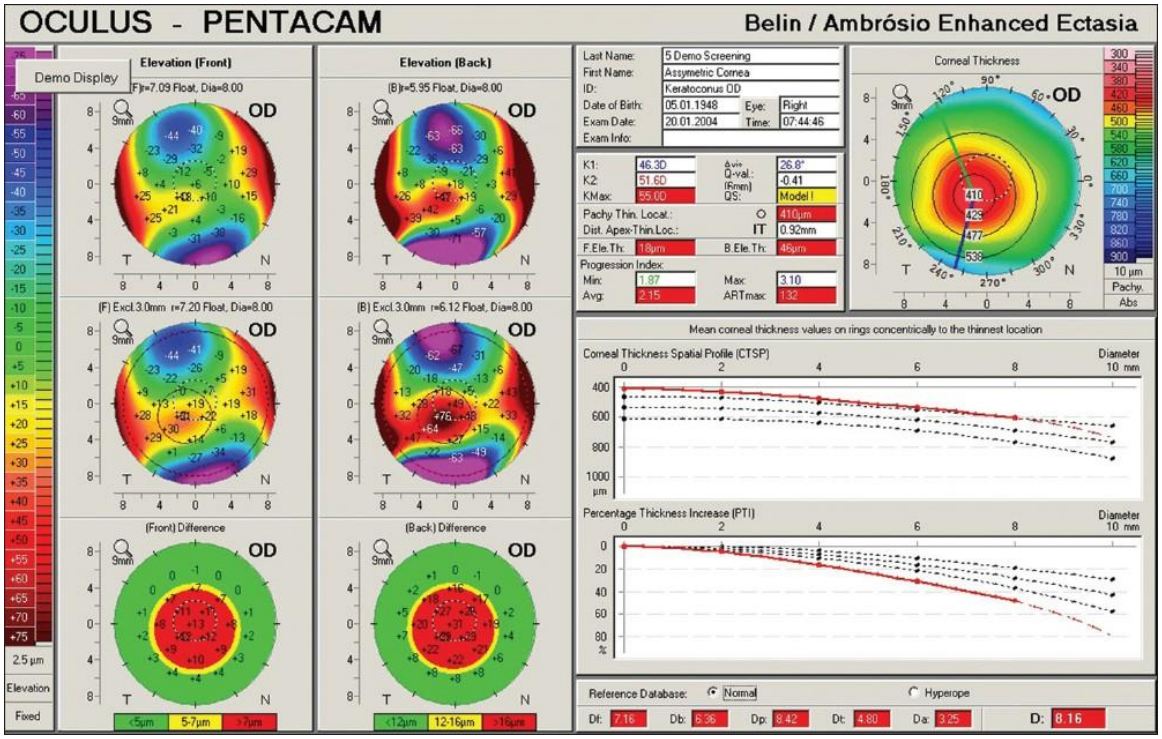

Figure 8.: Keratoconus screening with BAD III. method. This case is a moderately advance keratoconus where all the analyzed parameters measured in the BAD III. analysis are highly abnormal [98].

Keratoconus accompanied with corneal thinning, studies show difference in pachymetric variations between normal and KC corneas regarding to limbus to the thinnest point [129]. BAD III in the Pentacam also incorporates novel parameters as percentage thickness increase (PTI) from thinnest point and the corneal thickness spatial profile (CTSP). The software has the capability to enhance the cone location, by subtracting the 4mm area around the thinnest point and calculating the new BFS for the rest of the cornea (which would be flatter if the cone is located in the excluded area)[129]. As a result when the excluded area is compared with the flatter "new" BFS, it stands out if abnormal in the "enhanced map" that is also shown at the printout for both surfaces. In addition to the features above, the display in its current version (BAD III) incorporates the K max, maximum front, and back elevation in microns, a

pachymetry map, thin point location, displacement of the thin point from apex, and a pachymetry-based classifier the ART max (**Figure 9**). Besides this machine classifier, the main classifier, the "D" value, incorporates 9 parameters for its calculation and has been independently validated in a retest population [129].

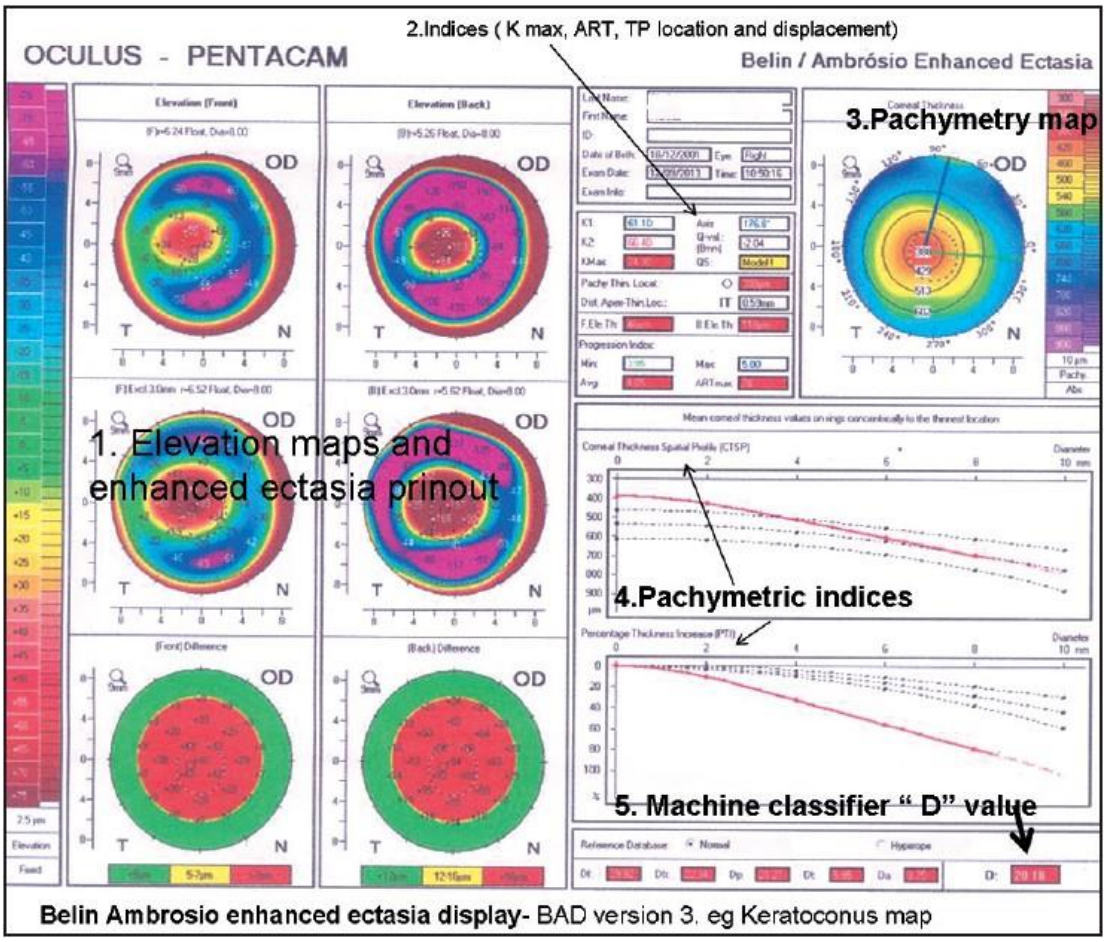

Figure 9.: Belin Ambrosio enhanced ectasia display (BAD) version III. Keratoconus map [98].

To summarize the knowledge, several topographic and tomographic parameters/indices are available to help decision making when corneal ectatic disease screened. It is important to know the advantages and limitations of the method/device being used. Parallel to the careful evaluation of the cornea with such device mentioned above, proper slit lamp examination and clinical findings/signs should also take into account when decision is made. Briefly the findings below made patients suspect for keratoconus [128]:

Axial map abnormalities [128]*:*

1. K greater than 48 D.
2. SRAX greater than 21 degrees.
3. I-S greater than 1.42D.
4. Corneal astigmatism on anterior or posterior surface greater than 6 D.
5. Against the rule astigmatism.
6. S-I difference at the 5-mm zone >2.5 D.

On elevation map [128]:

1. Isolated island or tongue-like extension on either surface (BFS mode).
2. Elevation values greater than 12 microns on the anterior elevation map in the central 5 mm (BFTE mode).
3. Elevation values greater than 15 microns on the posterior elevation map (BFTE mode).
4.

Pachymetry/corneal thickness map (Scheimpflug devices) [121]*:*

1. Thinnest location less than 470 microns.
2. Displacement of the thinnest point >500 microns from the center.
3. Pachymetry difference asymmetry in two eyes at thinnest point >30 microns.
4. S-I difference at the 5 mm circle >30 microns.
5. Cone-like pattern on the thickness map.

2.3.7. Treatment options of keratoconus

Several methods have been used to help patients with keratoconus since the discovery of the disease. Treatment options have a wide spectrum from the correction of refractive errors to surgical procedures. The procedures must be adjusted first to the patient (age, disease severity etc.), than to the doctor's experience in the treatment modalities (**Figure 11**). The two main goals are visual rehabilitation and to halt disease progression [2]. All stages of the disease especially in earlier stages verbal guidance is the most important thing. To explain patients the risk factors, like the importance of not rubbing one's eyes. Therefore the use of topical antiallergic medication in patients with allergy,

and use of topical lubricants (in case of ocular irritation) to decrease the impulse to rub one's eyes is one of the first steps in disease management beside the others. Treatment modalities can be divided into *surgical* and *non-surgical* options [1, 2].

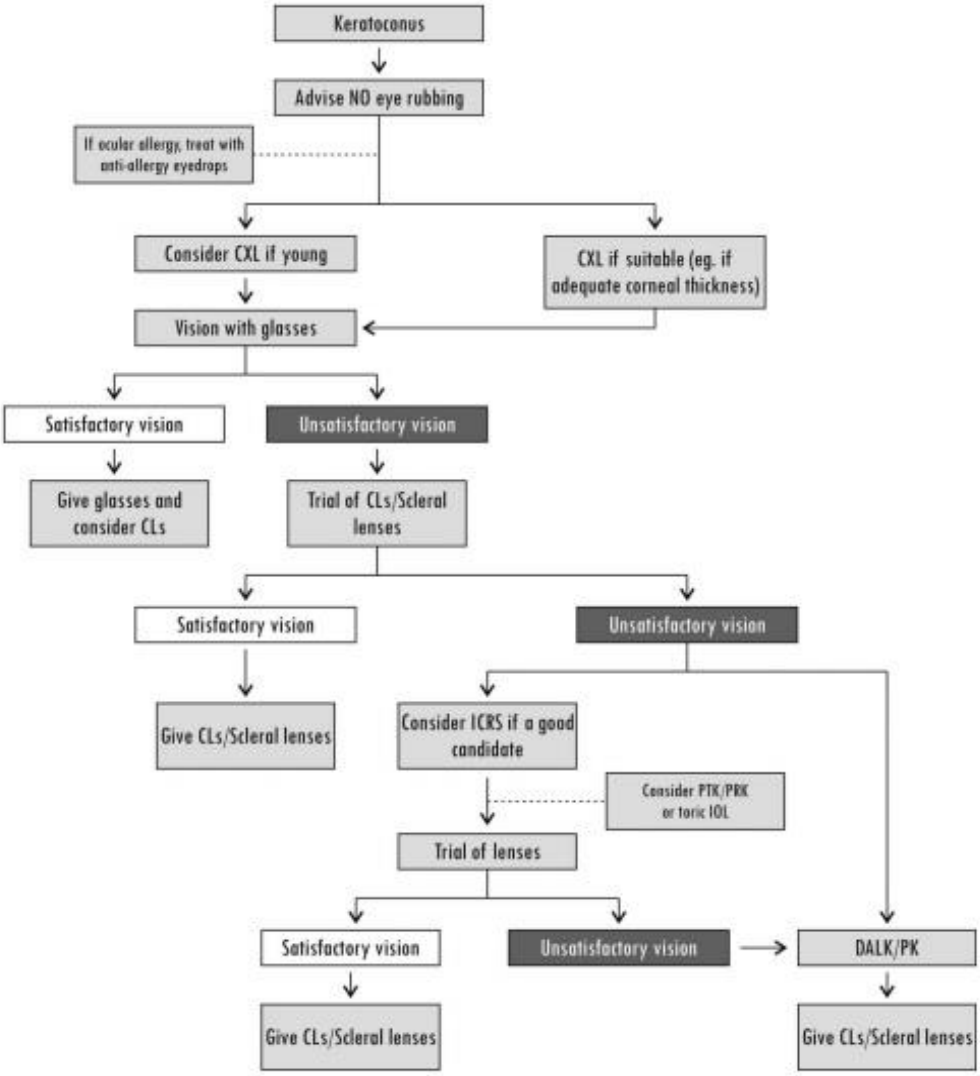

Figure 11.: Keratoconus treatment flowchart. CLs- contact lenses; CXL-corneal cross-linking; PTK-phototherapeutic keratectomy [2].

2.3.7.1. Spectacles

Impaired visual acuity in consequence of keratoconus is initially managed with spectacles. Progressive addition glasses are not contraindicated during the disease, but

they are rarely successful**,** and often very expensive. Hence the vast majority of practicing ophthalmologists does not prescribe multifocal glasses in KC [1, 2].

2.3.7.2. Contact lenses

When doctors/optometrists failed to correct visual disturbances in patients with KC, the next step is the use of contact lenses. Contact lenses usually provide better vision than glasses by masking irregular astigmatism (higher-order aberrations). In mild cases the use of *soft contact lenses* are often enough for vision correction. More advanced cases may require the use of *soft toric* or *custom soft toric contact lenses*. The further step in correcting severe corneal irregularities are *rigid gas permeable lenses (RGP).* They mask higher-order aberrations with higher success rate. Special contact lenses designed for KC patients are exist on the market, such as Super Cone, and Rose K etc.. These special lenses has high oxygen permeability and a more comfortable fit by having a steep central posterior curve to arc over the cone and flatter peripheral curves to approach the more normal peripheral curvature. An alternative to *RGP* is a *hybrid contact lens* (containing: rigid center, soft skirt). This type of lens could provide stable vision by preventing toric rotation-with the soft skirt- accompanied with each blink. One of the widely used lens is SynergEyes-KC (SynergEyes Inc., Carlsbad, CA, USA). The last option for highly irregular corneas is the *piggyback contact lens.* This name means a *soft contact lens* which is fitted to the cornea and an *RGP* lens is placed on top of it [1, 2, 130]. When all other contact lenses fail newly designed scleral lenses made of material with high Dk (Oxygen permeability of a contact lens material; P = Dk = diffusion (D) * oxygen solubility (k)) are available. These lenses could be divided to corneo-scleral, mini-scleral and semi-scleral lenses regarding to the size and coverage of the bulbus [130].

2.3.7.3. Radial keratotomy

When non-surgical therapies fail the next step are invasive methods.

The procedure was first described by Sato (Sato et al., 1953) and popularized by Fyodorov (Fyodorov and Durnev, 1979) in 1974. During this surgery the surgeon place four to 16 tiny incision in the mid-periphery (out of the visual axis) of the cornea with a diamond-edged knife at 95% depth of the corneal thickness [131]. This method could

correct myopia and/or keratoconus. The theory is that keratotomy produces a hyperopic effect due to steepening of central cornea. In keratoconus management this surgery was found to be a reasonable option for the rehabilitation of a selected group of keratoconus patients in the early or moderate stages according to some studies [132-134]. To perform operation, KC patient should have 400 micron or greater central corneal thickness without apical scarring [131-134]. Nowadays this method has only historical meaning. Practicing ophthalmologist could meet patients treated earlier with radial keratotomy, but present time manual radial keratotomy is a rarely performed procedure.

2.3.7.4. Intra stromal corneal ring segments

This is another option for correcting myopia and irregular astigmatism due to keratoconus. This method also needs a clear central cornea. ICRS (Intra Corneal Ring Segment) segments are made of polymethyl methacrylate and have a crescent-shaped arc length of 150°. The inner diameter is 6.8 mm and the outer diameter is 8.1 mm when placed in the cornea. Intacs thickness ranges from 0.25 to 0.45 mm, in 0.05 mm increments. Practitioners insert the segments into corneal stromal tunnels. The tunnels could be made by *mechanical* and *femtosecond laser-assisted* [1].

Briefly when tunnels made mechanically, the surgeon perform radial incisions about 1.8 mm in length with a diamond edged knife approximately 70% of the mean corneal thickness depth. Special pocketing hooks are used to create corneal pockets on each side of the incision. Then the ring segments inserted into the pockets. In the femtosecond laser-assisted way a continuous circular stromal tunnel is created approximately 80% of the corneal thickness with the laser system [1, 134, 135].

Several type and modified Intacs segments exist on the market. For example flexible (sometimes full ring) Intac segment, which could be adjusted after implanted into the corneal pocket is a newly used. ICRS with elliptical cross-section called Intacs SK, Severe Kertaoconus (Addition Technologies Inc.), is also a variant with a smaller 6mm optical zone to provide correction of higher astigmatism/myopia like in keratoconus, and to minimize glare. The Ferrara ring (Keravision Inc., Fremont, CA, USA) is another option in correcting keratoconus. The segments vary in thickness (0.15, 0.20, 0.30 and 0.35 mm) and have a triangular cross-section and the base for every thickness is 0.60

mm wide. The segments could have 160°-210° of arc, and provide an optic zone of 5 mm [1, 135, 136].

The expected result is that ICRS induces displacement of the nearby anterior corneal surface by adding plus material at the peripheral cornea, hence causing a steepening locally and flattening the central cornea [1]. The implantation also provides biomechanical support for the whole cornea [1, 134, 135]. The method is reversible and could help moderate to severe keratoconus patients. According to studies visual improvement reported in most cases [136,137]. Though there are known and reported side effects like epithelial defects, anterior/posterior perforations, extension of incision toward the visual axis, implant decentration, infectious keratitis, segment superficialization, stromal thinning/ corneal melting etc. [137, 138]. Despite the promising results in corneal ectatic disease (especially keratoconus) after ICRS implantation, the majority of patients require further correction of residual myopia or astigmatism with spectacles/contact lenses.

2.3.7.5. Phakic intraocular lenses

Another surgical technic exists alone or to correct residual ammetropia after ICRS in keratoconic patients. The word "Phakic" refers to those who have their own crystalline lens. During this procedure the crystalline lens is not removed, and an intra ocular lens (IOL) is implanted into the anterior/posterior chamber. There are three main lens designs: The NuVita lens is placed in front of the iris. The Artisan, or iris claw lens is attached on the front of the iris. The Implantable Contact Lens, or ICL, is placed between the iris and crystalline lens [1]. The possibility of remove the IOL form the eye is an advantage over refractive laser procedures. Worsening of keratoconus is a feared problem after implantation of any type of IOL, while progression is leading to refractive change. Hence phakic IOL implantation should be performed when refraction/keratometry is stable. Indications for phakic IOL implantation:

- Clear central cornea.
- BSCVA of 20/50 or better.
- keratometric values ≤52.00 D.
- Stable refraction (cylinder ≤3.00 D) for 2 years.

If these criteria are not met, other option is advised for correcting visual disturbances, like penetrating keratoplasty, corneal/collagen cross-linking (CXL) etc. [1, 139, 140].

2.3.7.6. Photorefractive keratectomy

Excimer laser treatment is available in patients with keratoconus in certain circumstances. In mild to moderate cases where contact lens intolerance is present, and the patient is over age 40 with stable vision, and the cornea is thick enough to perform photorefractive keratectomy (PRK) [1, 2]. Although in the literature there are some discrepancies in the judgement of PRK in keratoconus, but the majority of studies found low disease progression and good results with this method [2, 141]. The possible beneficial effect of PRK is that collagenous internal structure of the cornea is altered [141]. Briefly, topography-guided PRK in keratoconus could be effective in reducing higher order aberrations (high-myopia, irregular astigmatism) and may offer a temporary or permanent alternative to keratoplasty in contact lens-intolerant patients.

2.3.7.7. Anterior lamellar keratoplasty/deep anterior lamellar keratoplasty

Anterior lamellar keratoplasty (ALK) or precisely descemetic deep ALK (dALK) parallel with penetrating keratoplasty (PK) are the most often used surgical treatment options in KC therapy [2]. With this technique the majority of the anterior cornea is removed (epithelium & stroma about 95% thickness of the cornea), and depending the type of the procedure minimal stromal bed remained or just the anterior surface of the Descement membrane. The advantage of this technic in comparison to PK is that host endothelium is preserved avoiding endothelial graft rejection. Another benefit of dALK to keep the eye's structural and immunological integrity in contrast to PK. Indication for any form (dDALK, DALK, ALK) of the procedure: *contact lens intolerance*, *stromal opacities* and *scar*, or *active corneal ulcers*, without concerning the endothelium. Generally, DALK can be considered for all corneal pathologies other than those pathologies affecting the endothelium. The two most important contraindication of this surgery is endothelial dysfunction and deep scars particularly involving Descement's membrane, especially in the optical axis and around (e.g. acute hydrops etc.). Patients with keratoconus are good candidates for this procedure, because they are often young, hence they have good endothelial functions, and in earlier stages Descement's

membrane is frequently intact [2, 142]. Several modified technique exist parallel in the way eye surgeon divide the stroma from Descement's membrane:

- **Layer by layer manual dissection** is the basic technique of this procedure, surgeon manually separate the layers with a crescent knife [2, 142].
- **Air-assisted manual dissection (Archila technique)** is when air is used to aid the manual dissection of the layers [2, 142].
- **Big-bubble technique (Anwar's technique)** is a modified air dissection technique when air is gently injected into the deep stroma until a round, well-demarcated big-bubble is formed extending to the borders of trephination area [2, 142].
- **Hydrodelamination** is a technique where the surgeon inject balanced salt solution into the stroma after some preparation of the anterior cornea. This provide enhanced identification and removal of the deep stromal fibers [2, 142].
- **Viscoelastic dissection** means that after an initial trephination of the corneal stroma, sodium hyaluoronate injected deep into the central corneal lamella near to Descement's membrane to finalize the separation of the posterior deep stroma and Descement's membrane [2, 142].
- **Anterior chamber air (Melles' technique)** method could provide excellent visualization during the surgery. The injected air bubble into the anterior chamber serves as a "mirror", hence helps the surgeon during dissection of the deep stroma. The endothelium-air interface also serves as a landmark to identify the posterior surface of the cornea and helps orientation during the procedure [2, 142].

Briefly regarding to ALK techniques used by the experts in keratoconus dDALK with big bubble technique is the most common technique (more than 51% of the cases) [2].

2.3.7.8. Penetrating keratoplasty

Among patients with keratoconus one of the last options is penetrating keratoplasty (PK). This could be performed on the conventional way or newly femtosecond laser-assisted. The majority of PKs are performed with a standard (nonlaser) technique [1, 2, 143]. Briefly with PK the surgeon change all layers of patient's central cornea in a limited diameter. The disadvantage of this procedure is a mechanically weakened

cornea, and the possibility of endothelial graft rejection. Indications for penetrating keratoplasty as follows: significant corneal scarring (post-hydrops status), contact lens intolerance, fail or contraindication of other surgical strategies (DALK etc.), very thin cornea (≤ 200 μm), when keratoconus considered to be severe with potential risk of acute hydrops/perforation [2, 143]. In short this could be the last hope for correcting visual acuity in severe cases of keratoconus.

2.3.7.9 Collagen cross linking treatment

This technique is one of the most successful option to treat keratoconus. This method was developed in Europe by researchers at the University of Dresden in the late 1990's. In early 2000's it was widely used in Europe, and the procedure received FDA approval on April 18, 2016 [1, 2, 144]. Corneal experts agree that instead the term collagen cross linking one should use the term corneal (collagen) cross linking (CXL) [2].

Briefly the procedure starts with the removal of the corneal epithelium (epi-off technique), than 0.1% of riboflavin (serves as a photosensitizer) applied on the corneal surface. After the diffusion of riboflavin into the corneal stroma, the patient positioned under UV light (usually 365-370um wavelength), typically 1-5cm from the corneal apex for 30 minutes [144]. The main disadvantage of the surgical procedures explained earlier in this work, is that none of them could prevent the progression of keratoconus or reverse it. In our present knowledge CXL is the only method which could halt the underlying biomechanical changes in keratoconus. The three-dimensional configuration of the collagen lamella determines the cornea's resistance, and as it mentioned earlier there is a significant difference in keratoconic eyes than in normal regarding to this finding. Disease progression is mainly due to this fact i.e. weakening of the corneal stroma [1,2 144].

Photo-oxidative CXL technique counteract this progressive corneal thinning and as a consequence halt keratoconus progression. CXL form new covalent bonding between collagen molecules, hence stabilizes collagen frame accompanied with changes in tissue properties [144]. The cross-linking effect concentrated in the anterior 200-300 μm of the cornea, because of the high UV absorption of this area [144]. Otherwise this increasing number of covalent bonding is a normal finding in the aging cornea, or in diabetic

patients (glycation). This could explain why keratoconus progression halts around the age of 40 or in diabetes without any treatment [1].

Indication for CXL are as follows: keratoconus with documented clinical progression; keratoconus with a detected risk of progression (i.e. clinical progression has not been confirmed); keratoconic eyes that previously undergone other type of corneal surgery (ICRS, PRK etc.) or in the case of postrefractive surgery keratectasia [1, 2 144].

Contraindication for CXL are: corneal thickness of ≤ 400 μm; prior herpes infection; severe corneal scarring/opacification; history of poor epithelial wound healing; severe ocular surface disease (dry eye etc.) [1, 2, 144].

In short experts agreed that there is no age below or above which CXL shouldn't be used in keratoconic eyes with evidence of progression. In KC eyes without the evidence of progression CXL is rarely used above the age of 40. At present time corneal cross-linking is the only surgical procedure which halt disease progression and could prevent/reverse biomechanical changes between collagen fibers.

2.3.8. Importance of keratoconus diagnostics before refractive surgery

As mentioned earlier in this work, there is a gaining number of corneal refractive procedures worldwide. There is also an increasing number of all kind of ammetropias (myopia, hypermetropia, astigmatism) - especially myopia due to the changing lifestyle i.e. watching monitors and smart phones etc. – globally [7-9]. Hence further rising among (laser) refractive surgeries could be expected worldwide [8, 10-12]. It is well known from studies that keratoconus is the most common cause of post-surgery ectasia. In other words screening for keratoconus among refractive surgery candidates could prevent the vast majority of post-refractive complications i.e. corneal ectasia [3, 4, 13-18]. So identification of KC is the primary concern when screening these patients. Clinical diagnosis of KC in advanced stages is quite easy with the help of biomicroscopic and keratometric findings. But to rule out subclinical/forme fruste keratoconus is often very difficult. At present time there are several new technologies which could improve keratoconus detection. With the aid of these devices/ screening indexes the accuracy of discriminating normal corneas those from with subclinical keratoconus is increasing. However, many methods have been proposed for screening, there is a lack of defined threshold criteria to define this entity (subclinical

keratoconus), and there is still an equivocality regarding the exact definition of a KC suspect and there are no widely accepted criteria to categorize an eye as subclinical KC. [1, 2, 145, 146]. Recently the evaluation of intereye (corneal) asymmetry in patients came into focus concerning to the diagnostics of keratoconus. The findings namely subjects with keratoconus have significantly greater intereye corneal asymmetry than subjects with normal corneas regarding to values determined by devices (Pentacam, Orbscan, AS-OCT etc.) mentioned above. According to studies this asymmetry between eyes is greater in keratoconus with more severe disease. The basic of these finding is that KC is almost always starts asymmetrical, and affects the two corneas differently. In contrast to this, in the normal population, there is less asymmetry present between tomographic values of the two eyes [147-149]. These studies examined several different aspects of intereye asymmetry, but they solely focused on the presence of this finding [2, 145-149]. Our study group was the first who analysed these finding regarding to enhance subclinical keratoconus detection/recognition. In our opinion this could be a new screening strategy for keratoconus, and could multiply the efficacy of metrical data or indices used till that time. This “personalized” screening method (i.e. the reference is the other eye of the same patient) is far close to the behavior of biological systems (real life), than using strict numbers and pre-defined cut off values. However, with the goal of designing screening protocols that improve the ratio of cost-effectiveness we are led to search for diagnostic criteria that maximize the prevalence of the disease in certain population groups in order to increase the positive predictive value of these diagnostic tests [145, 146].

2.3.9. Keratoconus and corneal nerves

The prominence and visibility of central corneal nerves during biomicroscopical examination have been reported as a clinical sign of keratoconus [6] for decades. Studies which found impaired corneal sensitivity in keratoconus are started in the early 80’s [114]. In healthy subjects corneal innervation is a key player in maintaining the normal corneal structure and function. The involvement of corneal nerves in the pathogenesis of keratoconus has not received attention in the past, and the exact origin is unclear. Whether corneal nerve dysfunction is a cause or a consequence is still a question. New technologies and imaging devices like in vivo corneal confocal microscopy gave as the possibility to see other aspects of this question. Several studies

executed with confocal imaging (in vivo corneal microscopy) found data on the microstructural alteration of corneal nerves in patients with keratoconus. These findings are consistent and showing significant deterioration in the morphologic and morphometric features of nearly all layers. The most important changes including enlargement and irregular arrangement of the basal epithelial cells with reduction in basal epithelial cell density in patients with KC in contrast to normals. There is also a significantly lower anterior and posterior stromal keratocyte density in subjects with keratoconus compared with the controls [108, 111, 113, 150]. Regarding to these findings investigation on corneal sensitivity is also gaining popularity. Examination of central corneal sensitivity with mechanical forces (Cochet-Bonnet esthesiometry) had been used widely, and decreased corneal responses in KC patients in contrast to normals are known [113]. Whatever the keratoconus-related factors might be, scientists found marked changes on the ocular surface that affected not only the corneal, but also the conjunctival epithelium (i.e. lower globlet cell density). The loss or decrease of trophic effects of corneal nerves due to primary or secondary events with the progression of keratoconus may play a role in the pathogenesis of the ocular surface change in keratoconus [151]. In other words investigation on corneal nerve sensitivity in KC patients may help to identify newer screening strategies regarding subclinical keratoconus.

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3. Objectives

Our research group aimed to evaluate and to compare the tomographic and topographic corneal values of normal and early stage keratoconus patient's eyes. Our aim was to find a reliable method to recognize keratoconus as early as possible with high accuracy. Secondly, our purpose was to evaluate corneal sensitivity changes in keratoconus patients, and to assess the relationship between keratoconus grade and corneal sensitivity. The purpose of these investigations was to study keratoconus from functional and morphological aspects. Our focus was on the relation between KC severity, corneal sensory changes and dry eye symptoms connected with tear film dynamics. Weather functional changes like corneal sensory disturbances are a cause or a consequence? The purpose of our research was:

- To assess the relationship between keratoconus severity and intereye asymmetry of corneal tomography values
- Evaluate their combined accuracy in discriminating normal corneas from those with early signs of keratoconus.
- To investigate changes in corneal sensitivity to selective mechanical, chemical, and thermal stimulation in keratoconus
- To asses if there is any correlation present between different stages of keratoconus and changes in corneal sensitivity
- Evaluate the relation between dry eye symptoms and changes in corneal sensitivity in patients with keratoconus.

4. Methods

The clinical studies were performed at the Semmelweis University, Department of Ophthalmology between 2012 and 2015. The studies were conducted in compliance with the Declaration of Helsinki, applicable national and local requirements regarding the ethics committee and institutional review boards. Ethical approval was obtained from the Institutional Review Board (Semmelweis University Regional and Institutional Committee of Sciences and Research Ethics). A written informed consent was obtained before the examination from each patient or from the parent on behalf of the minors/children.

A) Evaluation of intereye corneal asymmetry in patients with keratoconus vs healthy patients, with the guidance of Scheimpflug imaging

The keratoconus group comprised 64 eyes of 32 patients (15 men, 17 women) with a mean age of 36.98±12.34 years. The control group comprised 130 eyes of 65 patients (29 men, 36 women) with a mean age of 39.95±15.44 years.

B) Evaluation of corneal sensitivity and dry eye symptoms in patients with keratoconus vs healthy patients with Belmonte's gas esthesiometer

The keratoconus group (KC group) included one randomized eye in 19 patients (28.9±6.3 years) with bilateral mild or moderate keratoconus and the control group 20 healthy refractive surgery candidates were enrolled (30.2±5.3 years) of both sexes.

4.1.1. Patients

Eyes with severe keratoconus were excluded because of difficulties in topographic map acquisition and potential stromal haze or scar formation, which can alter the optical transparency of the cornea and thus Scheimpflug imaging. Severe keratoconus was defined as having axial topographic pattern consistent with keratoconus, positive slit lamp findings, and an average corneal power higher than 56 D or dense/opaque corneal scarring according to the Keratoconus Severity Score criteria [152]. Both eyes of each patient had a complete ophthalmologic evaluation including slit lamp biomicroscopy, keratometry, retinoscopy, slit lamp indirect ophthalmoscopy, and Placido disk–based videokeratography (TOMEY TMS-4 corneal topographer; TOMEY Corp., Nagoya,

Japan). Diagnosis was based on classic corneal biomicroscopic and topographic findings in accordance with the criteria of Rabinowitz et al. [74]. Inclusion criteria for the control group included a refractive error less than +/– 5.00 diopters (D) sphere and astigmatism less than +/– 3.00 D. None of the control patients had a history of previous ocular disease, surgery or trauma. Rigid contact lenses were not worn for 4 weeks and soft contact lenses for at least 1 week before assessment in either group. Patients were asked whether they rubbed their eyes or experienced previous ocular trauma.

Participants in the control group (esthesiometry study) did not have any clinical signs and/or symptoms of dry eye (ocular surface disease index—OSDI score <10) or significant ocular surface disease and were not using eye drops. Subjects with ophthalmic conditions other than keratoconus including blepharitis, meibomitis, lid abnormalities as well as contact lens wearers were also excluded. Both eyes of each patient had a complete ophthalmologic evaluation including slitlamp biomicroscopy, ophthalmoscopy, Scheimpflug imaging and assessment of tear flow and non-invasive tear film breakup time were performed. Subjects who showed significant corneal staining (>Grade 2, Oxford Scale) [154] were excluded because corneal epitheliopathy could potentially be a confounding factor affecting the ocular surface sensory responses [53, 155, 156].

4.1.2. Scheimpflug imaging in evaluation of intereye corneal asymmetry

All eyes were examined with the Pentacam HR Scheimpflug camera, used by three trained examiners without application of dilating or anaesthetic eye drops or previous tonometry. The readings were taken as recommended in the instruction manual. The measurement results were checked under the quality specification (QS) window, only the correct measurements (‘QS’ reads OK) were accepted; if the comments were marked yellow or red, the examination was repeated. In all cases one reading taken from an eye was saved and processed for further statistical analyses. For local posterior elevation measurements, the reference surface was set to best fit sphere (BFS) with fixed 8- mm-diameter settings. Keratometry at the steep (K_s) and flat (K_f) meridians, central corneal thickness (CCT), pachymetry at the thinnest point (ThCT) and posterior elevation at the thinnest point of the cornea (PE) were measured in both eyes. Intereye asymmetry of pachymetry and elevation data was determined by subtracting the lower

value from the higher value for each variable. The better and worse eyes were designated for each keratoconus patient based on each variable (i.e. the worse eye is with higher K_s, K_f, PE and lower CCT and ThCT).

4.1.3. Statistical analysis in evaluation of intereye corneal asymmetry

Statistical analysis was performed with SPSS software (version 15.0, SPSS, Inc.). The Shapiro-Wilk W test was used to confirm normal distribution of the variables. Paired samples t-test was used to compare means between eyes of the same subject (within-subject variance). Linear regression was used to test significant correlation between parameters of the two eyes of the same subject (within-subject correlation). The repeated measures analysis of variance test (ANOVA) was used to analyze the differences between group means and their associated procedures (within-group and between-group variances). This test allows to compare within-subject parameters (better eye vs. worse eye) in the two study groups by taking into account between-eye correlations by treating data from eyes of patients in statistical analysis as repeated measures. Correlation between keratoconus severity and intereye asymmetry was tested using linear and non-linear regression analysis in each group. In this study keratoconus severity was assessed by corneal thickness values as it was suggested previously [153]. Receiver operator characteristic curves (ROCs) with covariate adjustment were used to compare discriminating ability of posterior elevation and pachymetry data after adjustment for the correlation between keratoconus severity and between-eye asymmetry. In ROC analysis, covariate adjustment is recommended when the accuracy of the test result is dependent on patient characteristic, similarly as adjusting for confounders in multivariable regression. In all analyses, a P value less than 0.05 was considered as statistically significant.

4.2.1. Corneal esthesiometry

Mechanical, chemical, and thermal (hot and cold) thresholds were determined at the center of the cornea using a Belmonte's gas esthesiometer. This is a safe and reproducible, well documented technic [157, 158]. Traditionally, clinical evaluation of corneal sensitivity has been performed with the Cochet-Bonnet esthesiometer that determines mechanical sensitivity by corneal contact. This widely used procedure has

some crucial disadvantage on Belmonte's gas esthesiometer. First of all this is an invasive method (i.e. corneal contact), and explores only the corneal mechano-nociceptors. Belmonte's esthesiometer cause no alterations of the ocular surface with respect to conjunctival hyperemia and corneal fluorescein staining regarding to studies [157, 158]. Finally as a noncontact instrument, it avoids the risk of producing mechanical damage in hypoesthesic and/or fragile corneas as can occur with contact esthesiometers [157, 158], hence this device is an excellent candidate for investigating corneas with keratoconus. The Belmonte non-contact esthesiometer allows exploration of different types of sensory fibers, such as mechanosensory fibers that respond to mechanical forces; polymodal nociceptive fibers that respond to mechanical forces, irritants, extreme temperatures, and endogenous inflammatory mediators; and cold fibers that are activated mainly by the decrease of temperature [159]. It is known that during mechanical stimulation, when air at increasing flow rates is applied to the corneal surface at a temperature of 34°C, the corneal polymodal nociceptors and mechanoreceptors are predominantly activated. With gas mixtures of increasing CO_2 concentration, a proportional decrease in pH occurs at the corneal surface acting as a specific stimulus for polymodal nociceptors of the cornea with an intensity proportional to the local pH reduction [160]. Likewise, hot air applied to the cornea selectively activates polymodal nociceptors, simultaneously silencing the spontaneously active cold receptors. Finally, moderate cooling exclusively stimulates cold receptors, whereas polymodal nociceptors appear to be weakly recruited by cold air only with corneal temperatures below 29°C [159]. A specific instrument with a rotary potentiometer was built to record intensity rating immediately after stimulation. Subjects were instructed to adjust the potentiometer to the corresponding intensity of the sensations arising during stimulation. A specific computer software written in MatLab program (The MathWorks, Natick, MA) was used to sample the data acquired from the potentiometer and to convert it to numeric values on a 10 unit scale. We measured with the potentiometer the intensity of the irritation sensation evoked by selective mechanical, chemical, and thermal stimuli applied on the central cornea of participants using the gas esthesiometer. Mechanical, chemical (CO_2 in air), and cold stimuli were used during three-second air pulses of adjustable flow rate, composition (CO_2%) and temperature. Mechanical thresholds were determined by using the method

of levels as described previously elsewhere [54]. Mechanical stimulation consisted of variable flows of filtered medicinal air (50 to 200 ml/min). Air was heated at the tip of the probe at 50°C so that it reached the ocular surface at 34°C to prevent a change in corneal temperature caused by the airflow [54]. Thermal stimulation was done by cooling or heating the air to produce the required changes in basal corneal temperature (from -3°C to +3°C) with a flow 10 ml/min below mechanical threshold. For chemical stimulation, a mixture of medicinal air with different concentrations of CO_2 (30 to 50%) was used at 50°C at the tip of the probe and with a flow rate of 10 ml/min below mechanical threshold. After corneal esthesiometry, the Schirmer test was performed.

4.2.2. Assessment of dry eye symptoms with OSDI score

All patients completed a questionnaire to assess dry-eye disease symptoms (ocular surface disease index—OSDI, Allergan Inc., Irvine, CA). In short The Ocular Surface Disease Index is one of the most frequently used instruments to assess dry eye symptoms. This questionnaire is comprised of 12 questions and evaluates the frequency of symptoms over the preceding week. The questionnaire requires approximately 5 minutes for the patient to complete, and the scores range from 0 to 100. Based on the score, the patients' symptoms can be categorized as normal (0–12), mild dry eye (13–22), moderate dry eye (23–32), or severe dry eye (33–100) [161-164]. None of the subjects received any drops at least 6 hours before the measurements.

4.2.3. Measuring non-invasive tear film breakup time (NI-BUT)

The non-invasive tear film breakup time (NI-BUT) was measured using the Keeler Tearscope Plus immediately after a complete blink. The Keeler Tearscope Plus was attached to a slit lamp (Topcon SL-D2, Topcon Medical Systems, Oakland, NJ, USA) in a fixed position to obtain a full coverage of the cornea. The measurement of non-invasive tear film breakup time with Tearscope Plus is based on the projection of a cylindrical source of cool white fluorescent light onto the cornea so that tear film breakup could be observed at any point over the corneal surface. The tear film was recorded by a digital camera (Topcon DV-3, Topcon Medical Systems, Oakland, NJ, USA) attached to the slit lamp, captured videos were exported at a spatial resolution of 1024 × 768 pixels and were analyzed by a masked observer. The non-invasive tear film

breakup time was defined as the time from the last blink when visible deterioration of the projected rings was detectable during the continuous recording. In each subject, NI-BUT was averaged from three consecutive measurements.

4.2.4. Schirmer test

Schirmer I test was performed without anesthesia. Briefly a small strip of filter paper was placed inside the lateral 1/3 of the lower eyelid (inferior fornix). Then the patient was asked to close the eyes for 5 minutes, then the paper was removed, the amount of moisture was measured [165, 166]:

Evaluation of dry eye according to Schirmer I test result

1. Normal:≥15 mm wetting of the paper after 5 minutes.
2. Mild: 14-9 mm wetting of the paper after 5 minutes.
3. Moderate: 8-4 mm wetting of the paper after 5 minutes.
4. Severe. <4 mm wetting of the paper after 5 minutes.

The test was executed soon after the esthesiometry measurement.

4.2.5. Statistical analysis

Statistical analysis was performed with SPSS software (version 21.0, IBM Inc., Chicago, IL, USA). The Shapiro-Wilk W test was used to assess normal distribution of the variables. Due to non-normality of data the Mann–Whitney U test was used for group comparisons. Spearman correlation analysis was used to determine the correlation between corneal sensitivity and age or pachymetric severity of keratoconus. In all analyses a p value less than 0.05 was considered as statistically significant.

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5. Results

5.1. Between eye corneal asymmetry in normal subjects and in keratoconus patients

The keratoconus group comprised 64 eyes of 32 patients (15 men, 17 women) with a mean age of 36.98±12.34 years. The control group comprised 130 eyes of 65 patients (29 men, 36 women) with a mean age of 39.95±15.44 years. There were no statistically significant differences between the keratoconus and the control groups in age or sex distribution ($p>0.05$).Table 2 summarizes mean and standard deviation values of topographic, posterior elevation and pachymetry parameters in the two groups. We have found no significant correlation between self-reported eye rubbing or ocular trauma and the presence of keratoconus in a given eye ($p>0.05$).

Table 2.: Mean ± SD value for each parameter in the Keratoconus and Control Groups.

Parameter	*Keratoconus Group*		*Control Group*		*p*	
	Better eye	*Worse eye*	*Right eye*	*Left eye*	*Between eye*†	*Between group*††
K_f *(D)**	44.90±3.09	47.42±4.58	42.69±1.62	42.92±1.57	*<0.001/>*0.05	*<0.001*
K_s *(D)**	46.84±4.23	51.33±5.56	43.92±1.67	44.32±1.93	*<0.001/>*0.05	*<0.001*
CCT (μm)#	493.73±26.04	463.60±33.53	554.62±26.98	557.31±27.18	*<0.001/>*0.05	*<0.001*
ThCT (μm)#	493.53±47.07	453.83±47.59	546.33±30.91	551.82±28.48	*<0.001/>*0.05	*<0.001*
*PE (μm)**	32.60±29.51	68.00±51.24	6.71±6.42	5.38±6.06	*<0.001/>*0.05	*<0.001*

*Worse eye is the eye with the highest value and #Worse eye is the eye with the lowest value.
†Worse eye vs. better eye in the Keratoconus Group/Right eye vs. left eye in the Control Group; Student's t-test on dependent samples.
††Keratoconus vs. Control groups; Student's t-test on independent samples.
PE: posterior elevation; CCT: central corneal thickness; ThCT: thinnest corneal thickness.
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There was a statistically significant difference in keratometric, CCT, ThCT and PE values between *worse eye* and *better eye* in the keratoconus group (**Table 2**). In contrast, there was no significant difference in these parameters between the *right eye* and the *left eye* of controls (**Table 2**). We found significantly higher values of posterior elevation, flat and steep keratometry ($p<0.001$, for all of the parameters) and significantly decreased central and thinnest pachymetry values in the keratoconus group compared to controls ($p<0.001$, for both parameters, **Table 2**). As Table 3 presents, mean intereye difference was significantly higher for all of the variables when comparing keratoconus eyes with normal eyes ($p<0.001$).

Table 3.: Mean intereye asymmetry of each parameter in the keratoconus and in the control groups.

Parameter	Keratoconus Group		Control Group		p
	Mean intereye asymmetry	Range	Mean intereye asymmetry	Range	
K_f (D)	2.70±3.57	0.3–13.8	0.37±0.39	0–1.5	<0.001
K_s (D)	4.37±5.14	0.1–20.2	0.43±0.44	0–2.3	<0.001
PE (μm)	35.4±37.31	0–161	3.13±3.71	0–21	<0.001
ThCT (μm)	39.70±36.42	0–136	6.57±5.30	0–18	<0.001
CCT (μm)	30.13±35.80	3–113	5.59±4.90	0–18	<0.001

p: Student's t-test for independent samples.
PE: posterior elevation; CCT: central corneal thickness; ThCT: thinnest corneal thickness.
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Correlation analysis showed significant correlation between data from the *worse eye* and data from the *better eye* in the keratoconus group ($p<0.001$, **Table 4**). Data from the *right eye* and data from the *left eye* in the control group also showed strong correlation ($p<0.001$, **Table 4**). The difference between correlation coefficients was significant for each variable (**Table 4**). Intereye asymmetry of pachymetry significantly correlated with decreasing thinnest pachymetry ($r=-0.40$; $p=0.03$) or central pachymetry ($r=-0.72$; $p=0.002$) in the keratoconus group but not in the control group ($p>0.05$). Similarly, correlation was found between intereye asymmetry of PE and increasing posterior elevation ($r=0.82$; $p<0.001$) in the keratoconus group but not in the control group ($p>0.05$). The relationship between intereye asymmetry and keratoconus severity could best be described by an exponential regression model across the two groups with an r value of 0.74 for steep keratometry ($r^2=0.55$, $p<0.001$; **Figure 12A**), with an r value of 0.62 for CCT ($r^2=0.39$, $p<0.001$; **Figure 12B**), an r value of 0.69 for ThCT ($r^2=0.48$, $p<0.001$; **Figure 12C**) and an r value of 0.80 for PE ($r^2=0.64$, $p<0.001$; **Figure 12D**).

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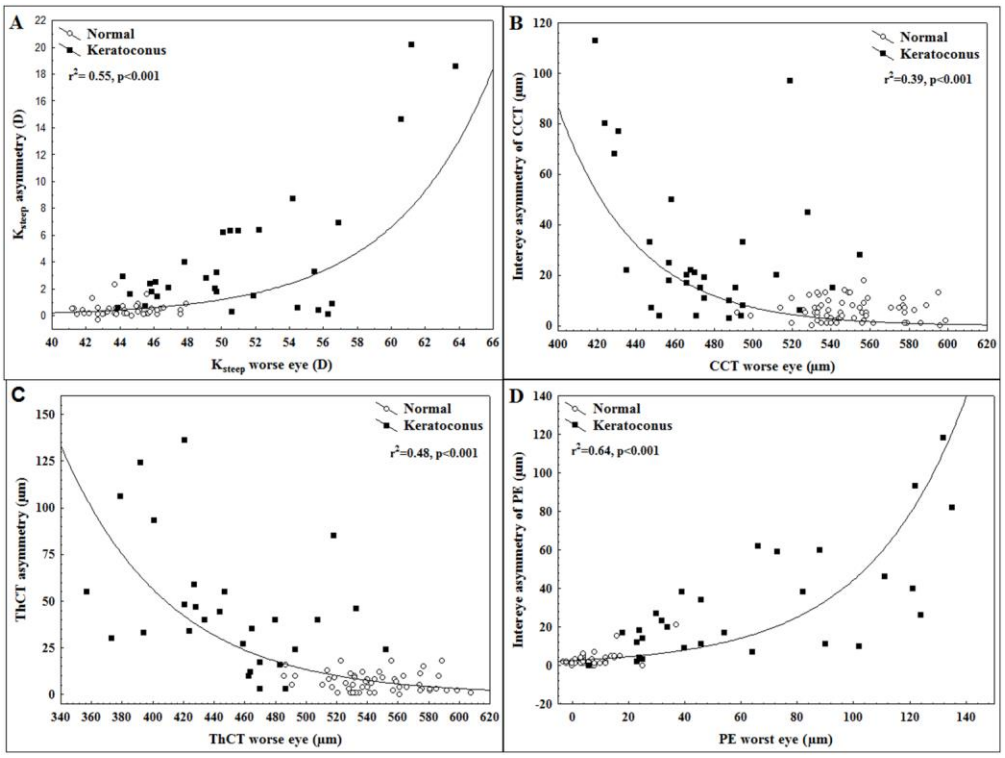

Figure 12.: The relationship between keratoconus severity and intereye asymmetry.

Table 4.: Correlations between data from the two eyes in the keratoconus group, and in the control group.

Parameter	*Keratoconus group*	*Control group*	*p*
Posterior elevation (µm)	*r = 0.70; p<0.001*	*r = 0.87; p<0.001*	*0.003*
Thinnest corneal thickness (µm)	*r = 0.70; p<0.001*	*r = 0.98; p<0.001*	*<0.001*
Central corneal thickness (µm)	*r = 0.68; p<0.001*	*r = 0.98; p<0.001*	*<0.001*

p: difference between r values of the two groups.
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To identify the best parameter to characterize intereye corneal asymmetry in keratoconus, receiver operator characteristic curves with adjustment for keratoconus severity was used. This ROC analysis showed, that asymmetry in thinnest pachymetry had the highest accuracy (AUROC: 0.99) and significantly better discriminating ability for keratoconus than posterior elevation (AUROC: 0.96), ThCT (AUROC: 0.94) or CCT had (AUROC: 0.92; pairwise comparison p<0.05, **Figure 13**, **Table 5**).

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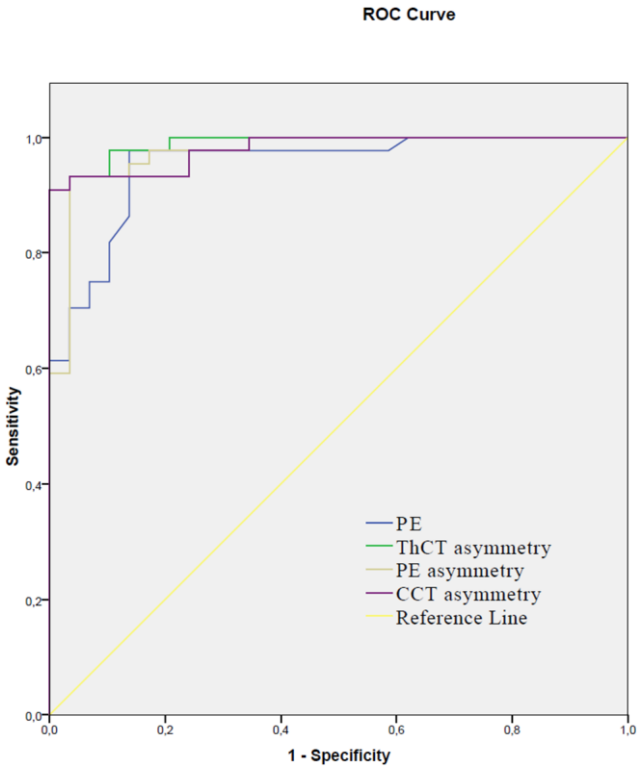

Figure 13.: Receiver operator characteristic curves to plot discriminating ability of the different parameters for keratoconus.

Table 5.: Area under the ROC curve values with 95% confidence limits and pairwise comparisons of different variables for keratoconus vs. normals.

Parameter	*AUROC*	*95% CL*	*Cut off*	*Sensitivity*	*Specificity*	*p*	*p*†
PE	0.96	0.90–0.99	17.5	97	91	*<0.001*	-
CCT	0.92	0.85–0.97	513	91	93	*<0.001*	0.37
ThCT	0.94	0.87–0.97	509	93	89	*<0.001*	0.24
PE asymmetry	0.97	0.90–0.99	7	97	93	*<0.001*	0.33
CCT asymmetry	0.98	0.94–0.99	10	97	94	*<0.001*	0.18
ThCT asymmetry	0.99	0.97–1.00	12	98	95	*<0.001*	*0.03*

†*Pairwise comparison to AUROC value of PE.*
PE: posterior elevation; CCT: central corneal thickness; ThCT: thinnest corneal thickness.
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5.2. Corneal sensitivity esthesiometry and dry eye symptoms in keratoconus patients

There was no significant difference in age and gender between the keratoconus and the control group ($p>0.05$, **Table 6**). Patients with keratoconus had significantly higher steep and flat keratometry values and significantly lower thinnest corneal thickness compared to normals (**Table 6**). Patients with keratoconus had significantly decreased tear secretion and significantly higher OSDI scores compared to controls ($p<0.001$, **Table 6**). There was no significant difference in tear film breakup time between the two groups ($p>0.05$, **Table 6**).

Table 6.: Demographic, topographic and tear film characteristics of the control and the keratoconus groups.

	Control	*Keratoconus*	*P*
Age (years)	30.2 ± 5.3	28.9 ± 6.3	0.55
Gender (male/female)	12 / 8	10 / 9	0.89
Keratometry steep axis (D)	43.9 ± 1.5	49.0 ± 5.7	<0.001
Keratometry flat axis (D)	43.1 ± 1.4	45.8 ± 5.3	<0.001
Thinnest corneal thickness (μm)	551.7 ± 13.9	422.4 ± 77.9	<0.001
Schirmer I test	13.2 ± 2.0	5.3 ± 2.2	<0.001
NI-BUT (s)	10.7 ± 3.8	9.8 ± 4.8	0.31
OSDI score	8.1 ± 2.3	26.8 ± 15.8	<0.001

Data are mean ± SD values in the control (n = 20) and in the keratoconus groups (n = 19). Note: P: Mann–Whitney U test.

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The threshold sensitivity to mechanical stimulation with air pulses of neutral temperature applied to the center of the cornea in the patients with KC was significantly higher than those observed in the control subjects ($p<0.001$; **Table 7**, **Fig 14A**). No correlation was found between mechanical threshold and age in the patients with KC ($r = 0.13$, $p = 0.58$; **Fig 15A**), whereas in the control subjects, mechanical threshold increased proportionally with age ($r = 0.52$, $p = 0.02$; **Fig 15A**).

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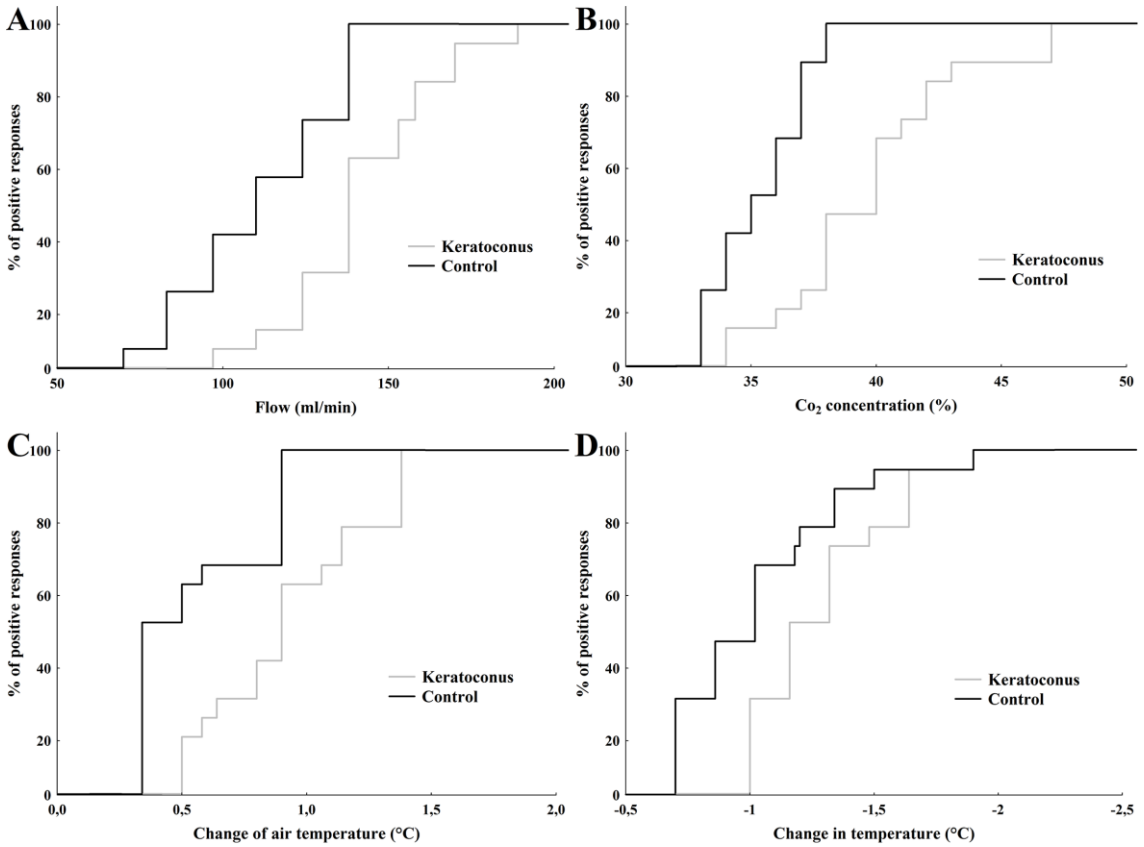

Figure 14.: Cumulative distribution of sensation thresholds to selective stimulation of the central cornea in control subjects and keratoconus patients.

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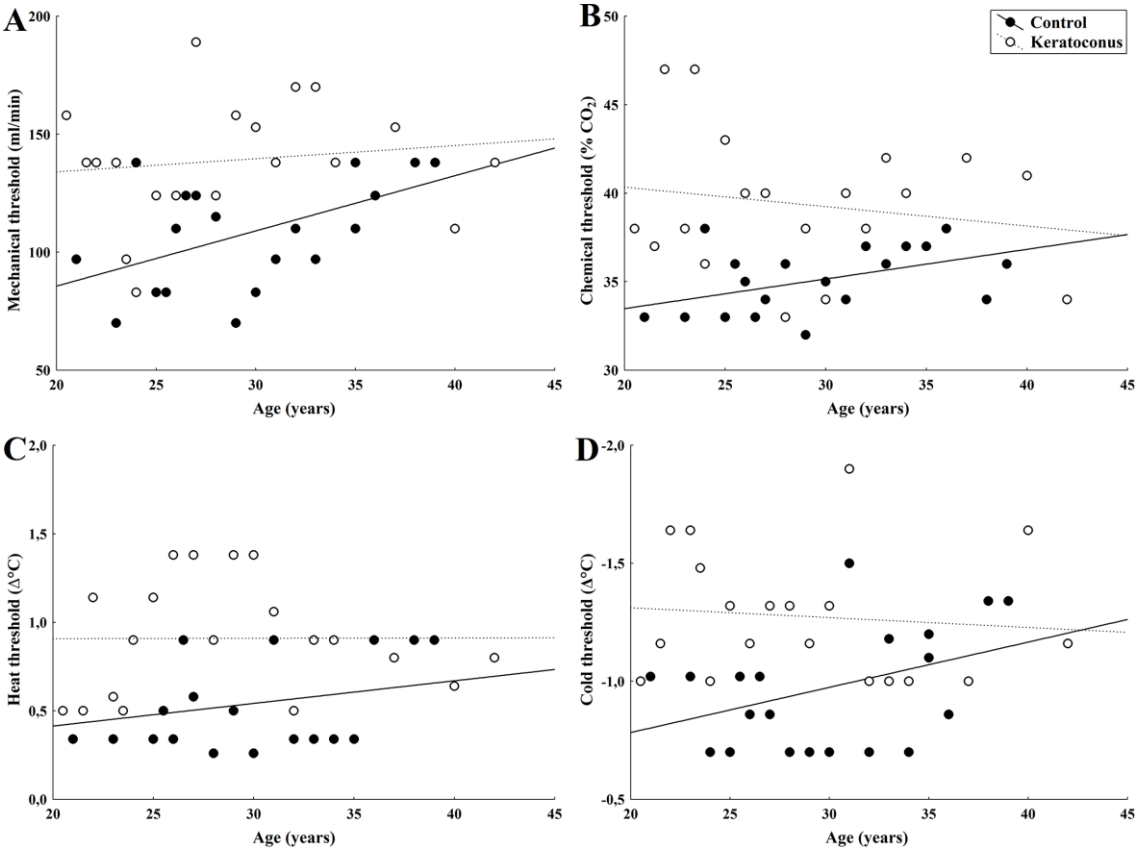

Figure 15.: Relationship between age and corneal sensitivity threshold to mechanical (A), chemical (B), heat (C), and cold (D) stimulation in KC patients and in control subjects.

Table 7.: Sensation thresholds to selective stimulation of the cornea.

Stimulation	*Control*	*Keratoconus*	*P*
Mechanical (ml/min)	109.1 ± 24.0	139.2 ± 25.8	<0.001
Chemical (%CO_2)	35.2 ± 1.9	39.4 ± 3.9	<0.001
Heat (Δ°C)	0.54 ± 0.26	0.91 ± 0.32	<0.001
Cold (Δ°C)	0.98 ± 0.25	1.28 ± 0.27	0.001

Data are mean ± SD values in the control (n = 20) and in the keratoconus groups (n = 19). Note: P: Mann–Whitney U test.

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The mean sensation threshold for selective chemical stimulation was significantly higher in patients with KC than in the control group (p<0.001; **Table 7**, **Fig 14B**). Chemical thresholds did not tend to increase with age in the subjects with KC (r = -0.17, p = 0.46; **Fig 15B**), contrary to the responses of the control subjects (r = 0.47, p = 0.04; **Fig 15B**).

A significantly higher threshold value was obtained with heat stimulation in patients with KC than in the control group ($p<0.001$; **Table 7**, **Fig 14C**), with no correlation between threshold and age ($r = 0.01$, $p = 0.98$; **Fig 15C**) contrary to the responses of the control subjects, in whom threshold and age correlated positively ($r = 0.26$, $p = 0.04$; **Fig 15C**).

Similarly, an elevated threshold value to cold stimulation was observed in patients with KC compared to the control individuals ($p = 0.001$; **Table 7**, **Fig 14D**). Cold threshold responses did not correlate with age in patients with KC ($r = -0.09$, $p = 0.69$; **Fig 15D**), whereas in control subjects the correlation was significant ($r = 0.40$, $p = 0.03$; **Fig 15D**).

In the keratoconus group, corneal thickness did not correlate significantly with threshold values of mechanical, chemical, heat or cold stimulation ($p>0.05$ for all variables, **Figure 16**). Similarly, threshold values of mechanical, chemical, heat or cold stimulation did not correlate to tear flow ($p>0.05$ for all variables, **Figure 17**), NI-BUT ($p>0.05$ for all variables, **Figure 18**) or OSDI score ($p>0.05$ for all variables, **Figure 19**). In the keratoconus group, there was no correlation between thinnest corneal thickness and tear flow, NI-BUT or OSDI values ($p>0.05$ for all variables).

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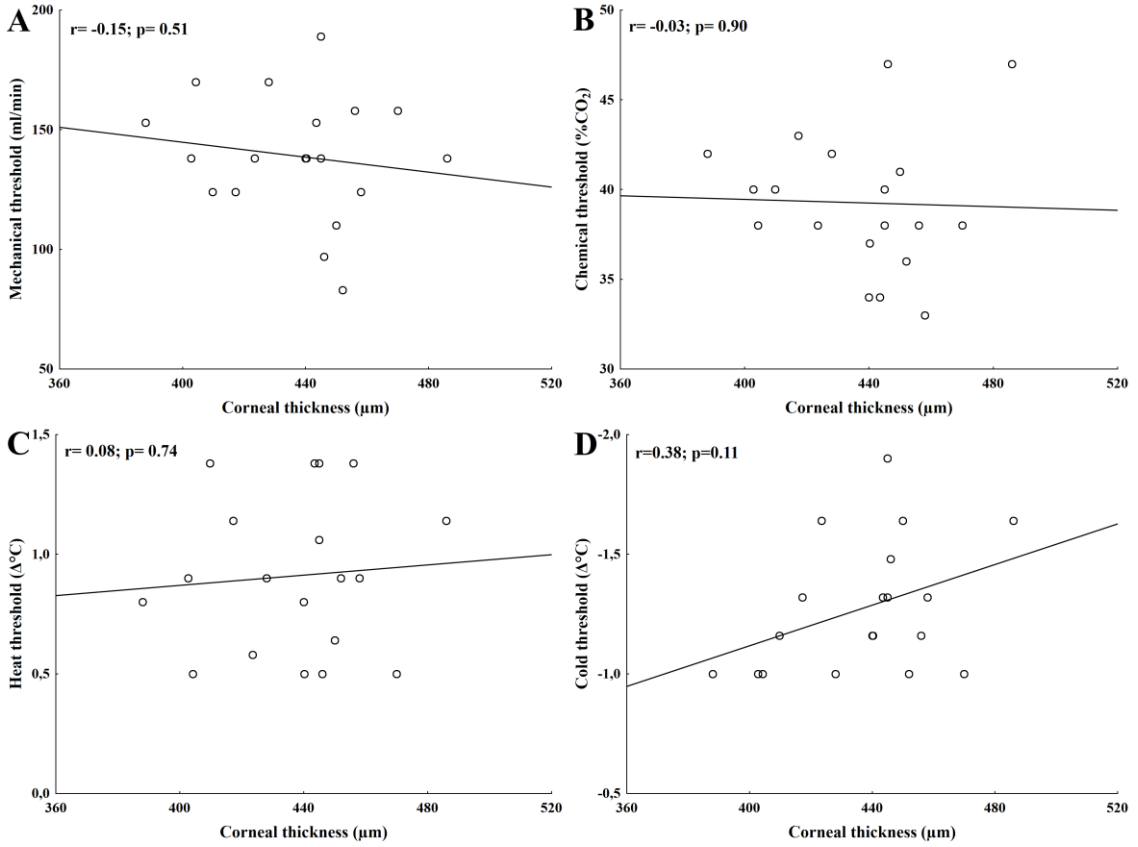

Figure 16.: Statistically not significant relationship between corneal thickness and corneal sensitivity threshold to mechanical (A), chemical (B), heat (C), and cold (D) stimulation in patients with keratoconus.

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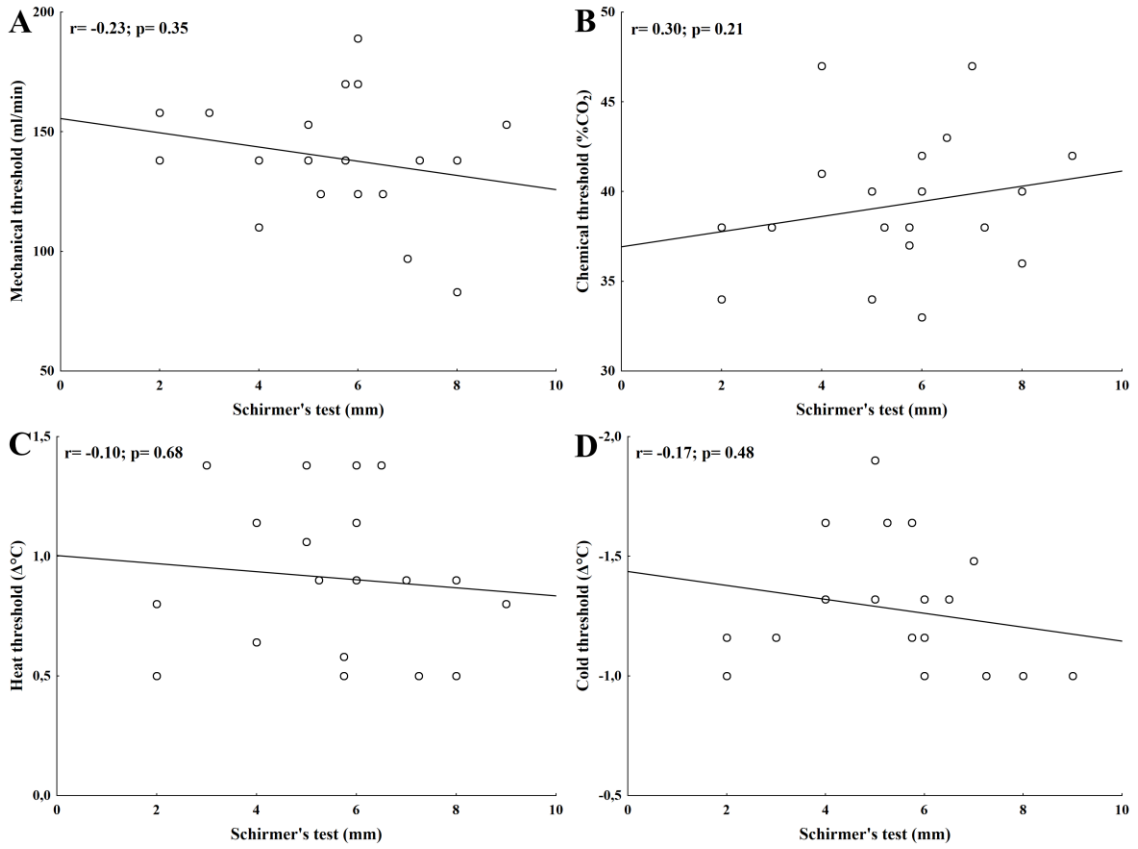

Figure 17.: Statistically not significant relationship between Schirmer's test and corneal sensitivity threshold to mechanical (A), chemical (B), heat (C), and cold (D) stimulation in patients with keratoconus.

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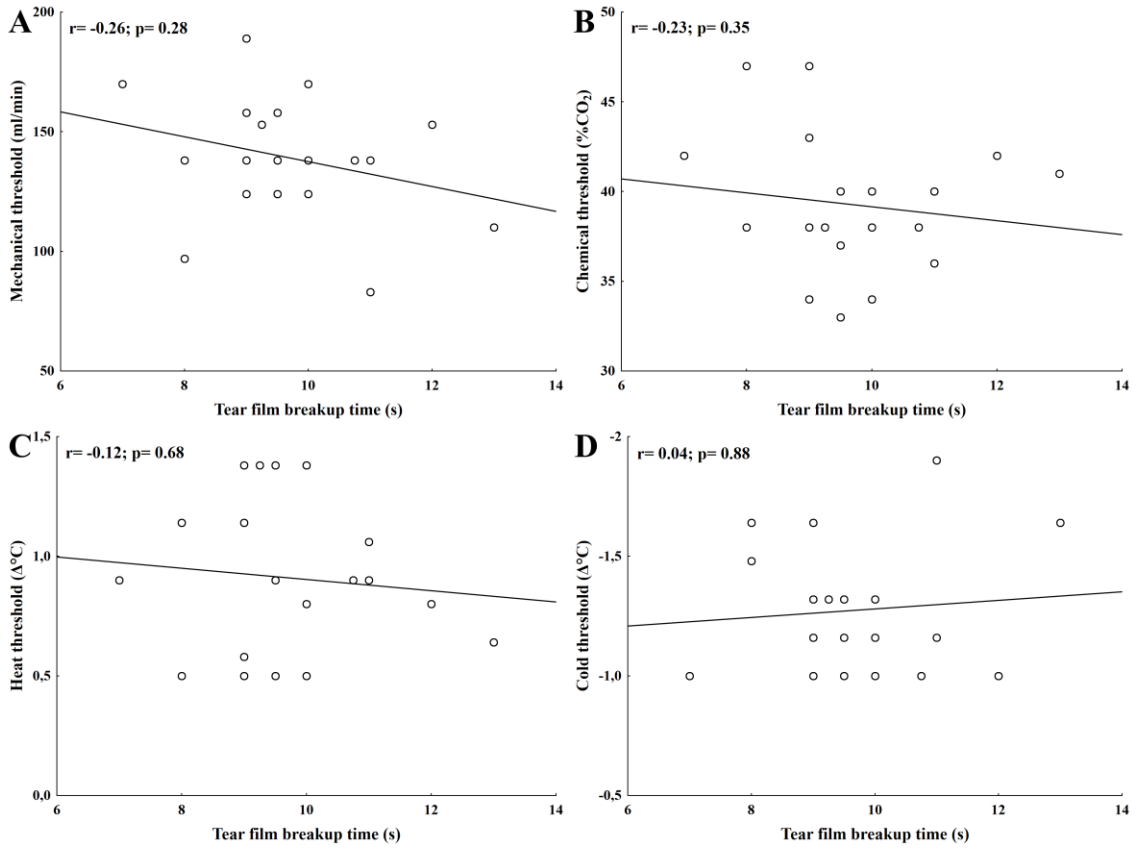

Figure 18.: Statistically not significant relationship between tear film breakup time and corneal sensitivity threshold to mechanical (A), chemical (B), heat (C), and cold (D) stimulation in patients with keratoconus.

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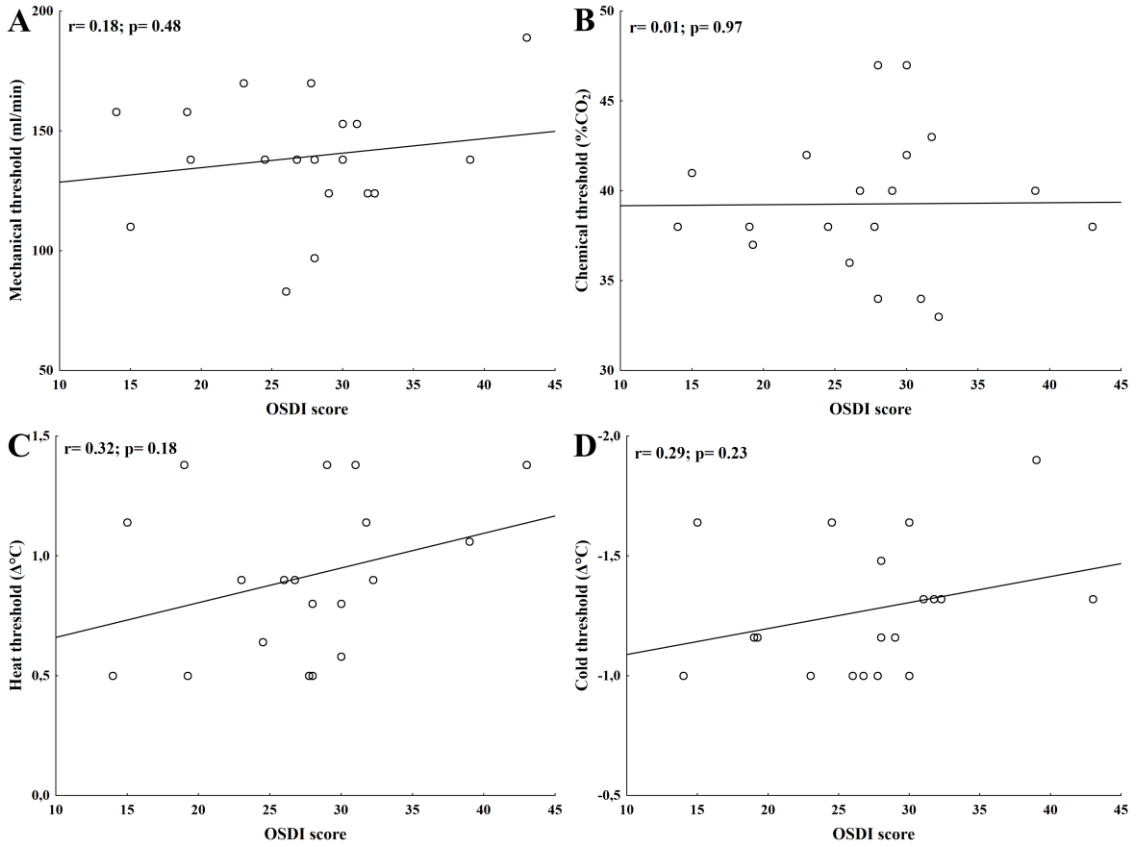

Figure 19.: Statistically not significant relationship between OSDI score and corneal sensitivity threshold to mechanical (A), chemical (B), heat (C), and cold (D) stimulation in patients with keratoconus.

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6. Discussion

Regarding the Scheimpflug imaging study we found significantly increased intereye difference in posterior elevation and pachymetry values in keratoconus patients compared to normals, confirming previous reports [94, 149]. We also proved, that there is a strong correlation between the two eyes of the same subject (within-subject correlation) both in healthy persons and those with keratoconus in posterior elevation and pachymetry values. In terms of these parameters the finding in one eye predicts the finding in the fellow eye almost perfectly in healthy persons (called *enantiomorphism*) and moderately in keratoconus patients. The decreased correlation between values measured in the two eyes of the same subject with keratoconus is a consequence of the asymmetrical nature of this disease.

In this study there was no significant difference in posterior elevation and pachymetry parameters comparing right eyes to left eyes ($p>0.05$ for all of the variables) in each group due to the lack of side predilection in keratoconus. In contrast, after categorizing eyes into *"worse eye"* and *"better eye"* we found significant intereye differences for all of the variables in the keratoconus group. The strong correlation of data from the two eyes (between-eye symmetry) together with the small variability of data in the group (between-subject similarity) are characteristic features of the normal group. In the keratoconus group, there were decreased between-eye correlation and increased variability of data as a result of decrease in "between-eye symmetry" and "between-subject similarity" which changes are characteristic features of this progressive, asymmetric disease. An important finding of this study is that keratoconus severity was significantly correlated with intereye asymmetry of keratometric, pachymetric and elevation values with a smooth transition as it was demonstrated with good fit of exponential curves to data. Keratoconus is a progressive disorder ultimately affecting both eyes, although initially only one eye may be affected. It is also known, that atypical, asymmetric topography pattern in normal fellow eyes is associated with higher risk for the development of keratoconus [167]. Previous studies introduced different indices and proposed cut-off values to identify different stages of KC, however, for any quantitative variable there is a significant overlap between KC suspect and normals resulting in lower sensitivity and specificity in detecting mild corneal ectasia compared to discriminating normal corneas from keratoconus. Progression of a chronic disease,

like keratoconus is often depicted in three states: normal, preclinical phase and clinical phase [168] and the screening of the asymptomatic preclinical phase is usually much more difficult than of the symptomatic clinical phase. A clear understanding of progression from the preclinical phase to the clinical phase is therefore important for keratoconus screening. One previous study reported significantly increased keratometric, topometric and elevation parameters in normal fellow eyes of unilateral keratoconus patients compared to normals [169]. According to their results, keratometric asymmetry, topometric indices and anterior/posterior elevation difference may be useful in detecting the earliest form of subclinical keratoconus. In this study, we found exponential correlation of corneal asymmetry with pachymetric severity from healthy to keratoconus. After this correlation with intereye asymmetry of ThCT was taken into account by the ROC analysis, we found significantly better discriminating ability for keratoconus as using posterior elevation or pachymetry data alone (**Figure 13**, **Table 5**). In a previous study, Ambrosio et al. described high AUROC values for ThCT and CCT for discriminating keratoconus (0.955 and 0.909 respectively) [170], however pachymetric asymmetry was not considered in these analyses. In our pachysuvmetry adjusted analysis ThCT asymmetry had significantly better discriminating ability for keratoconus (AUROC: 0.99) than posterior elevation had (AUROC: 0.96, **Table 5**). The pachymetry adjusted ThCT asymmetry utilized all the three significant pachymetric characteristics of keratoconus (lower ThCT, higher variance of ThCT and correlation of ThCT with asymmetry of ThCT) simultaneously for keratoconus prediction. This method showed the best accuracy in discriminating keratoconus cases from normals comparing ROC curves (**Figure 13**) with high sensitivity and specificity (98% and 95%, respectively). All these findings suggest that simultaneous analysis of both intra- and intereye asymmetry (intraeye asymmetry means asymmetry of the tomographic values within one cornea i.e. inferior-superior asymmetry etc.) could be utilized to further improve the diagnostic accuracy of keratoconus. When plotted as a function of the corresponding minimum pachymetry, intereye ThCT asymmetry tended to exponentially increase with decreasing thinnest corneal thickness (**Figure 12**). One clinical relevance of this finding is that increased pachymetric asymmetry can be a warning sign for the presence of keratoconus in subjects with pachymetric values in the subnormal or normal range, often posing

diagnostic problems [171]. According to results of the ROC analysis, asymmetry in corneal pachymetry has good accuracy in predicting keratoconus, when its correlation with disease severity is also taken into account. When controlling for corneal thickness, values of intereye pachymetric asymmetry beyond 10 μm for CCT and 12 μm for ThCT should warn the clinician for a significantly increased risk for the presence of corneal ectasia. These subjects should be processed for further screening for an ectatic disorder and should be assigned for control measurements to detect progressive ectasia. When controlling for the effect of disease severity, the optimal cut-off point for posterior elevation asymmetry was 7 μm and showed 97% sensitivity and 93% specificity in predicting keratoconus. Although these results show, that increased corneal asymmetry predicts keratoconus with good accuracy, the diagnosis of mild cases remains challenging and further studies are needed focusing on simultaneous analysis of within-eye and between-eye asymmetry. Whether this smooth transition in morphological changes during keratoconus progression is accompanied with a parallel deterioration of sensory functions of the cornea, we also evaluated corneal sensory responses in this population.

In previous studies using *in vivo* confocal microscopy, subbasal nerve density has been shown to be lower in corneas with keratoconus and appeared more tortuous in these corneas as compared to controls, with abnormal architecture affecting primarily the region of the cone [108, 111-113, 150-151]. It has also been demonstrated, that the decrease in nerve density is significantly correlated with the loss of corneal sensitivity to contact mechanical stimulation, this correlation being stronger in patients who wore contact lenses [172, 173]. Although there are also some reports on impaired tear secretion in patients with keratoconus [174, 175], the relationship between abnormal ocular surface innervation and tear film dynamics remains unclear.

In our studies we have demonstrated that in keratoconus patients both corneal sensitivity and tear secretion are reduced. Our results show a significantly increased threshold for conscious detection of mechanical, chemical and thermal stimuli applied to the cornea in patients with keratoconus, in comparison with age-matched control subjects. Within the keratoconus group, patients showed the same profile of sensitivity deficiency irrespective of their age, disease severity and tear function, suggesting that sensory deterioration appears early in the development of keratoconus and is

independent of age or ocular surface wetness. Apart from corneal sensitivity threshold values, neither tear secretion, nor unpleasant sensations correlated with keratoconus severity or age demonstrating that in the case of keratoconus corneal hypoesthesia with profound abnormality in sensory input and abnormal tear secretion develops early in the disease and remains unaltered independently of age.

Our finding, that changes in tear flow and tear film breakup time are not related to disease severity or patient's age is in good harmony with previous reports, where lack of correlation was described between topographic severity of keratoconus and dry eye symptoms or tear film parameters [175]. The significantly reduced corneal sensitivity to mechanical stimulation measured with the Cochet-Bonnet esthesiometer has already been described in keratoconus patients, however this device has limited accuracy and only stimulates mechanosensory nerve fibers. Hence, in the present study using the Belmonte's gas esthesiometer we have shown for the first time, that corneal sensory nerve impairment in keratoconus affects all types of corneal sensory nerve endings. The importance of this finding is, that not only sensory nerve input that is responsible for reflex tear secretion (that is, the activity of polymodal nociceptors) but those responsible for maintaining basal tear secretion (that is, the activity of corneal cold thermoreceptors) are also considerably involved in corneal sensitivity loss in KC patients. It has already been shown, that the stimulation of corneal polymodal and mechano- nociceptor fibers results in unpleasant feeling and reflex tearing [55], while the spontaneous activity of corneal cold sensitive nerve fibers is responsible for maintaining basal tear secretion [65]. Cold thermoreceptors are able to detect slight (< 0.5°C) variations in ocular surface temperature and also changes in tear film osmolarity [176], such as those occurring during tear film evaporation, and thus regulating tear flow. Under normal circumstances, the continuous impulse firing from cold thermoreceptors represents a tonic stimulus for basal tear fluid secretion, conceivably activating the lacrimal glands and goblet cells through the parasympathetic fibers from the superior salivary nucleus. During the interblink period, ocular surface temperature falls gradually from approximately 34°C at a rate of 0.3°C/s due to tear film evaporation [177]. Corneal cold receptor endings exhibit a remarkably high sensitivity for dynamic temperature reductions and are thus able to encode into their background firing frequency such small temperature oscillations [178]. In keratoconus patients in whom basal tear secretion is

reduced, the lower number of cold fibers that remain functional presumably fire at higher frequency and evoke dryness sensations even though their summated sensory inflow may be still insufficient to maintain the fraction of the tear flow dependent on cold fiber tonic effects on parasympathetic pathways. In this part of the study we also have demonstrated, that in comparison to healthy controls, in keratoconus patients lower tear secretion and tear film breakup time are associated with the presence of unpleasant ocular surface sensations. Presumably, the altered excitability of corneal cold receptors is the origin of the lowered sensitivity and dry eye sensations and other disaesthesias reported by the patients with keratoconus as the origin of unpleasant sensations in ocular surface dryness is mainly attributed to the abnormal activity of cold receptors secondary to ocular surface desiccation and tear film hyperosmolarity [176, 178]. However, there is a complex relationship between ocular surface sensory function and tear film production, and the lack of correlation between subjective symptoms, tear rate reduction (as measured by the Schirmer test), and ocular surface damage (evaluated with fluorescein and Lissamine green staining) is well known [179]. It has been proposed previously, that changes in the activity of corneal sensory nerves, which are part of the lacrimal functional unit, modify tear secretion and may lead to ocular dryness [180-182]. In the case of keratoconus, it is possible that structural changes of the cornea causes an impairment of sensory nerve activity and a reduction of corneal sensitivity, and as a consequence of their reduced sensory input, tear secretion driven by tonic nerve activity is decreased, thus causing ocular symptoms. Our results demonstrate that there is a significantly decreased tear flow in keratoconus patients with the impairment of both cold- and mechanoreceptor function, and thus both basal and reflex tearing are altered. Taken together these findings it appears reasonable to conclude that in patients with keratoconus the reduced reflex tear secretion is caused by the reduced input to the brain from corneal mechanical and polymodal receptors while the reduction in basal tear secretion is the result of the decreased input from corneal cold receptors secondary to their morphological and functional impairment. The reduced sensory input could be the result of the reduced nerve density [108-110, 112, 150, 172] and/or produced by the reduction of the excitability of sensory nerve endings due to an altered expression of ion channels in trigeminal sensory neurons. However, from our results, it cannot be determined whether this is a direct effect of the disease on sensory nerve endings, or is

secondary to the ocular surface desiccation, as is the case in patients with dry eye of other origins [183]. Whether the abnormal sensory input as a result of impaired function of corneal nerve endings might have a role in the development of abnormal ocular surface sensations and thus evoking eye rubbing is yet unclear but these processes might contribute to the progression of keratoconus. However, the relationship of the corneal nerve deterioration and the progressive corneal thinning in keratoconus needs to be elucidated and further studies are recommended as relationship would be better described when longitudinal data of patients with the entire spectrum of the disease were analyzed. Our future analyses aim to examine whether changes in corneal sensory function precedes corneal thinning or whether early signs of corneal ectasia could be detected before sensory nerve impairment. If further studies shows that functional changes of the cornea in patients with KC are overtake tomographic changes could lead to new screening strategies among refractive surgery candidates. Or this finding could support refractive surgeons in the decision making when subclinical keratoconus is supposed.

7. Conclusions

As a conclusion, in this study we have shown that for corneal topography, pachymetry and elevation outcomes, the degree of intereye asymmetry is associated with disease severity. One might conclude from these results that as keratoconus patients proceed through the disease and becoming more severe, more pronounced intereye asymmetry also occurs. In a previous study analysing clinical outcomes of keratoconus, the degree of asymmetry in keratometry, high contrast, best corrected visual acuity, spherical equivalent, and corneal scarring was related to disease severity [184]. According to our results the relation between intereye asymmetry and severity is pronounced in outcomes relating to local corneal changes measured at the apex of the cone. We found exponential correlation of corneal asymmetry in terms of corneal thickness and posterior elevation with pachymetric severity from healthy to keratoconus. This is an important finding as thinnest corneal thickness is directly related to the clinical care of these patients i.e. the application of corneal crosslinking therapy. Increasing pachymetric asymmetry could be thus considered as a warning sign for disease progression and as therapy indication. In our opinion, the fact that all correlations in this study were in the same direction supports the assumption that disease asymmetry and severity are considerably related in keratoconus. However, further studies are recommended as this relation would be better described when longitudinal data were analyzed. Our future analyses will examine whether the progression of keratoconus proceeds in an asymmetric trend or whether the asymmetry observed at baseline in these patients is simply preserved. This also means a new method with the existing devices (Pentacam, Orbscan, AS-OCT etc.) to screen and recognize KC with high accuracy in an earlier phase, than that was previously possible.

On the other hand our results also demonstrate that there is a significantly decreased tear flow in keratoconus patients with the impairment of both cold- and mechanoreceptor function, and thus both basal and reflex tearing are altered. So decreased corneal sensitivity in all aspects of the sensory functions (cold-, mechano-, nociceptors) with tear flow disturbances could explain the sensations reported by patients with KC. These findings above could help screening KC patients and could strength the diagnosis when problematic cases are present i.e. subclinical keratoconus.

The new findings of our studies:

- When controlling for corneal thickness, values of intereye pachymetric asymmetry beyond 10 μm for CCT and 12 μm for ThCT should warn the clinician for a significantly increased risk of the presence of corneal ectasia.
- When controlling for the effect of disease severity, the optimal cut-off point for posterior elevation asymmetry was 7 μm and showed 97% sensitivity and 93% specificity in predicting keratoconus.
- Using the Belmonte's gas esthesiometer we have shown for the first time, that corneal sensory nerve impairment in keratoconus affects all types of corneal sensory nerve endings. The importance of this finding is, that not only sensory nerve input that is responsible for reflex tear secretion (that is, the activity of polymodal nociceptors) but those responsible for maintaining basal tear secretion (that is, the activity of corneal cold thermoreceptors) are also considerably involved in corneal sensitivity loss in KC patients.
- We have also demonstrated, that in keratoconus patients lower tear secretion and tear film breakup time are associated with the presence of unpleasant ocular surface sensations independently of subject's age or disease severity. However, neither tear secretion, nor unpleasant sensations correlated with keratoconus severity or age demonstrating that in the case of keratoconus corneal hypesthesia with profound abnormality in sensory input and abnormal tear secretion develops early in the disease and remains unaltered independently of age.

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8. Summary

As a summary, in this work we have demonstrated that corneal sensitivity to different types of stimuli is decreased in patients with keratoconus. The other important finding that we found is an exponential correlation of corneal asymmetry in terms of corneal thickness and posterior elevation with pachymetric severity from healthy to keratoconus. The meaning of this observation is that increasing pachymetric asymmetry could be a warning sign for disease recognition (i.e. subclinical KC) or progression, and as therapy indication. In our opinion, the fact that all correlations in this study were in the same direction supports the assumption that disease asymmetry and severity are considerably related in keratoconus. Regarding to this it remained an important question whether the progression of keratoconus proceeds in an asymmetric trend or whether the asymmetry observed at baseline in these patients is simply preserved. The significantly impaired sensitivity suggests that axonal damage and/or altered expression of membrane ion channels involved in this process. Our finding that changes in corneal sensitivity and tear flow are not related to disease severity or patient's age suggests that there is an early development of impaired corneal nerve function in keratoconus. Weather this is a cause or a consequence is still a question and need further investigations. Although the exact mechanism of corneal nerve damage in keratoconus is still unknown, these structural and neural changes may play a role in the impaired tear secretion as well as in the abnormal ocular sensations experienced by keratoconus patients. Our results highlight the need for further studies on the impact of impaired tear secretion and sensory nerve function on anatomical and visual results following corneal collagen cross linking therapy or keratoplasty in eyes with keratoconus. Briefly our examinations concern different aspects of keratoconus which are very important when ophthalmologists meet patients with keratoconus. And the weight of this is to recognize and exclude subclinical keratoconus with high accuracy among the increasing number of refractive surgery candidates.

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8. Összefoglalás

Összefoglalva az eredményeket ebben a tanulmányban demonstráltuk, hogy keratoconusban csökkent a szaruhártya érzékenysége az általunk használt összes ingerléssel szemben. A másik fontos megfigyelésünk, hogy exponenciális korrelációt találtunk a szaruhártya aszimmetriában a szaruhártya vastagság és a hátsó eleváció tekintetében a pachymetriás értékekben az egészségesektől a keratoconusos betegekig. A növekvő aszimmetria a pachymetriás értékekben egy figyelmeztető jel lehet a keratoconus korai felismerése során, segítséget nyújthat a progresszió megítélésében és terápiás indikátorként is szolgálhat. Az tény, hogy minden korreláció a vizsgálataink során azonos irányba mutat, erősíti azt a feltételezést, hogy a betegség aszimmetriája és a súlyossága között jelentős összefüggés van keratoconusban. Fontos kérdés maradt, hogy keratoconusban az aszimmetria a pachymetriás értékekben fokozódik-e a betegség progressziója során vagy a felfedezett aszimmetria megőrződik azon a szinten a betegség előre haladásával. A szignifikánsan csökkent szaruhártya érzékenység alapján feltételezhetjük, hogy axon károsodás és/vagy megváltozott ion csatorna expresszió lehet érintett ebben a folyamatban. Eredményeink nem mutattak összefüggést az életkorral és a betegség súlyosságával a szaruhártya érzékenység és a könnyfilm elégtelenség tekintetében, ezek alapján feltételezhető, hogy már a keratoconus korai szakaszában létrejöhet az idegek érintettsége/károsodása. Az hogy ez a jelenség ok vagy okozat még továbbra is kérdés maradt a számunkra, mely további vizsgálatok szükségességét jelzi. Az ideg károsodás pontos mechanizmusa még nem ismert, ezek a szerkezeti és neurális eltérések feltételezhetően komoly szerepet játszanak az elégtelen könnytermelésben és a keratoconusban szenvedő betegek által tapasztalt szemfelszíni kellemetlen érzetekben. Az általunk észlelt károsodott könnytermelés és érző ideg érintettség nagy valószínűséggel befolyásolja (rontja) a betegség terápiájának eredményességét, azaz a szaruhártya átültetéssel vagy kollagén “crosslinking” terápiával elérhető eredményeket, így ezek további vizsgálata válhat szükségessé. Összefoglalva több vonatkozásból vizsgáltuk a keratoconus betegséget, melyek egyaránt fontosak lehetnek a gyakorló szemorvos számára, amikor ilyen beteggel találkozik. Ezen eredményeink fontosságát az adja, hogy lehetőséget nyújt a szubklinikai keratoconus nagy pontosságú felismerésére illetve kizárására, a világszerte emelkedő számú refraktív sebészeti eljárásra váró emberek közül.

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10. Bibliography of the candidate's publications

List of publications related to the thesis:

Dienes L, Kránitz K, Juhász E, Gyenes A, Takács A, Miháltz K, Nagy ZZ, Kovács I. (2014) Evaluation of intereye corneal asymmetry in patients with keratoconus. A scheimpflug imaging study. *PLoS One,* 8;9(10).

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List of publications not related to the thesis:

Dienes L, Kiss HJ, Perényi K, Szepessy Z, Nagy ZZ, Barsi Á, Acosta MC, Gallar J, Kovács I. (2015) The Effect of Tear Supplementation on Ocular Surface Sensations during the Interblink Interval in Patients with Dry Eye. *PLoS One,* 24;10(8):e0135629.

Kovács I, Miháltz K, Kránitz K, Juhász É, Takács Á, **Dienes L**, Gergely R, Nagy ZZ. (2016) Accuracy of machine learning classifiers using bilateral data from a Scheimpflug camera for identifying eyes with preclinical signs of keratoconus. *J Cataract Refract Surg,* 42(2):275-83.

Kovács I, **Dienes L**, Perényi K, Quirce S, Luna C, Mizerska K, Acosta MC, Belmonte C, Gallar J. (2016) Lacosamide diminishes dryness-induced hyperexcitability of corneal cold sensitive nerve terminals. *Eur J Pharmacol,* 15;787:2-8.

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