INTRODUCTION

Keratoconus (KC)

Keratoconus is a progressive, noninflammatory, bilateral (but usually asymmetrical) ectatic corneal disease, characterized by paraxial stromal thinning and weakening that leads to corneal surface distortion (figure: 1). Visual loss occurs primarily from irregular astigmatism and myopia, and secondarily from corneal scarring. Protrusion usually but not exclusively affects the axial and inferonasal cornea. [1] There does not appear to be a significant difference in the incidence of keratoconus between left and right eyes nor between males and females. [2]

Causes

The cause of KC is unknown, although metabolic/chemical changes in the corneal tissue have been documented. [3] However, the disease has been associated with atopy, [4,5] connective tissue disorders, [6,7] eye rubbing, [8] contact lens wear, [9,10] and inheritance. Between 6%-18% of patients with keratoconus have a history of familial disease. In fact, several studies suggest that KC is a complex genetic disease. [11]

Incidence and Prevalence

The prevalence of KC is stated to be 1/2000 persons. Higher incidence was suggestive of a genetic factor being significant in the etiology. [11]

Progression

The rate of progression for a particular patient is impossible to predict. Some patients advance rapidly for six months to a year and then stop progressing, with no further change. Often there are periods of several months with significant changes followed by months or years of no change; this may then be followed by another period of rapid change. [12]

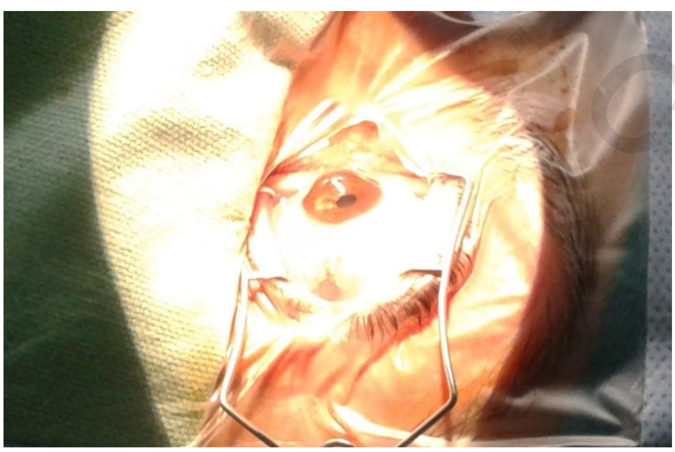

Figure 1: keratoconus

The normal cornea is composed of 5layers: epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium (figure: 2).

1- The corneal epithelium is nonkeratinized, stratified squamous, ranging between 5 and7 cell layers in thickness resting on epithelial basement membrane.

2- The Bowman's layer (Bowman's membrane) is more properly regarded as the most anterior layer of the stroma. The Bowman's layer is acellular and is composed of irregularly arranged collagen fibrils. It is not restored after injury but is replaced by fibroconnective scar tissue.

3- The corneal stroma makes up 90% of the total corneal thickness. It consists of collagen producing keratocytes, collagenous lamellae, and proteoglycan ground substance. The elongated collagenous lamellae are regularly arranged in a precise orientation to yield transparency, allowing for the orderly passage of light through the cornea.

4- The Descemet's membrane is the basement membrane elaborated by the corneal endothelium. The production of Descemet's membrane begins during fetal development and continues throughout adulthood. Descemet's membrane is composed primarily of type IV collagen, and is strongly PAS-positive.

5- The corneal endothelium is composed of a single layer of cells. The cells appear mostly hexagonal en face, such as on confocal microscopy. In a histologic cross-section of the cornea, the endothelial cells have a cuboidal appearance. The primary function of the endothelium is to maintain corneal clarity by pumping water from the corneal stroma. The number of endothelial cells gradually decreases with age, and endothelial cell loss is accelerated in endothelial disease states. Human endothelial cells cannot regenerate; so as the endothelial cell number declines, the remaining cells flatten and elongate to provide coverage of the posterior corneal surface. [13]

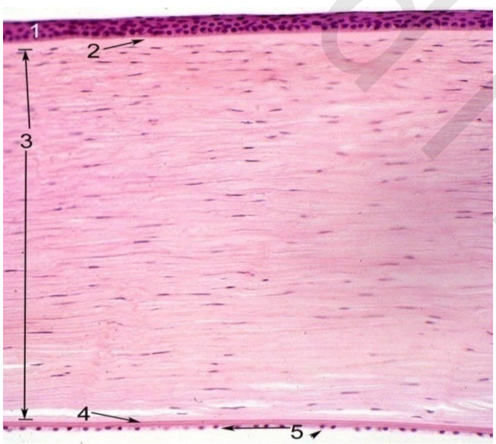

Figure 2: Histology of normal cornea

Pathological and histopathological abnormalities in keratoconus: (figure: 3)

1- Epithelium: Histological analysis of keratoconic corneas has demonstrated significant thinning of the central epithelium [14].confocal microscopy studies of the epithelium demonstrate morphological alterations in the area of the keratoconic corneal apex. Elongated superficial epithelial cells, arranged in a whorl-like fashion, can be observed. Near Bowman's membrane highly reflective changes and fold-like structures are visible. [15]

Apoptotic changes have also been detected in epithelia of keratoconic samples. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive epithelial cells were confined to the superficial epithelium of normal corneas while extending further down in keratoconic corneas.[16]

The keratoconic basement membrane assumes an irregular appearance and breaks in places. [17] It also undergoes a change in composition. [18,19]

Increased visibility of nerve fibres by slit lamp biomicroscopy has been demonstrated in keratoconus. Corneal nerves pass between the stroma and epithelium at sites of early degradative change. [20]

2- Bowman's layer: Scanning electron microscopy has found defects and ruptures in Bowman's layer to varying degrees in all keratoconic corneas examined.[21]

3- Stroma: number of lamellae appears to be significantly reduced compared to normal tissue. [22] reduction in the volume of proteoglycan along the collagen fibrils has been found in keratoconus.[23] Matrix metalloproteinases (MMPs) have long been suspected of mediating the pathological progression of keratoconus. The cornea is 70% collagen by weight and the reduced collagen content of the keratoconic cornea suggests a degraded extracellular matrix. [24]

Keratoconus is also associated with changes in keratocyte morphology as well as loss of keratocyte density especially in the anterior-most part of the stroma. [4,25]

4- Descemet's membrane: Ruptures and folds in Descemet's membrane are common in keratoconus. [17]

5- Endothelium: The endothelium may be normal in keratoconus or may demonstrate intracellular dark structures, pleomorphisms or elongation of cells.[1] Ruptures in Descemet's membrane may directly lead to endothelial cell loss by triggering cell membrane perforation, loss of cell contents and edema.[26]

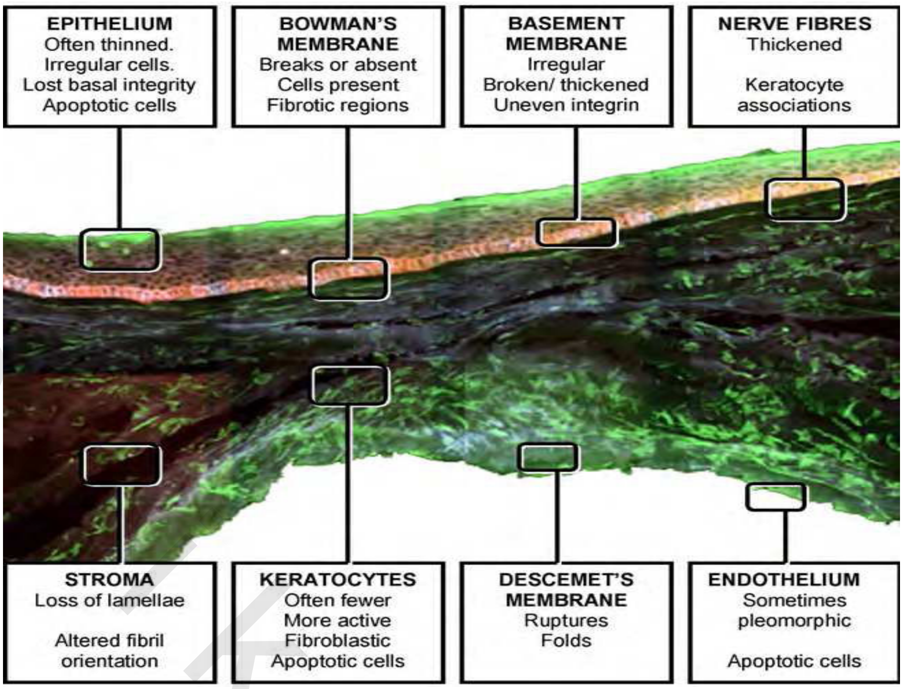

Figure 3: A diverse range of morphological changes have been described in every layer of the keratoconic cornea.

Clinical picture:

Symptoms of keratoconus may change as the disease progresses. They include:

- Blurred or distorted vision.
- Increased sensitivity to bright light and glare.
- Problems with night vision.
- Many changes in eyeglass prescriptions.
- Sudden worsening or clouding of vision, caused by hydrops. (27,28)

Slit lamp findings include:

- Fleischer's ring: This partial or complete annular line,which demarcates the peripheral edge of the cone, occurs in about half of keratoconus patients.(figure:4) Histopathologically, it can vary in color from yellow-brown to olive green deposition of iron in the basal epithelial cells. A cobalt blue filter with diffuse illumination can enhance the ring's appearance. (30)
- Vogt's striae: These vertical stress lines in the posterior cornea, near the apex of the cone, appear as a series of sharp, whitish, vertical or oblique lines just anterior to Descemet's membrane (figure:5). You can confirm their presence by applying external pressure on the globe. The transient rise in intraocular pressure causes them to disappear, but rigid lens wear can sometimes highlight them. (30)

- Corneal thinning: In most cases of keratoconus you can actually see the corneal thinning in the inferior central region by using an optic section and high magnification. This thinning will result in the displacement of the corneal apex below a hypothetical line that bisects the pupillary axis. [30]
- Munson's sign. Another common sign, this is the bulging of the lower lid in downgaze (unless the cone is too far inferior)(figure:6). [29]

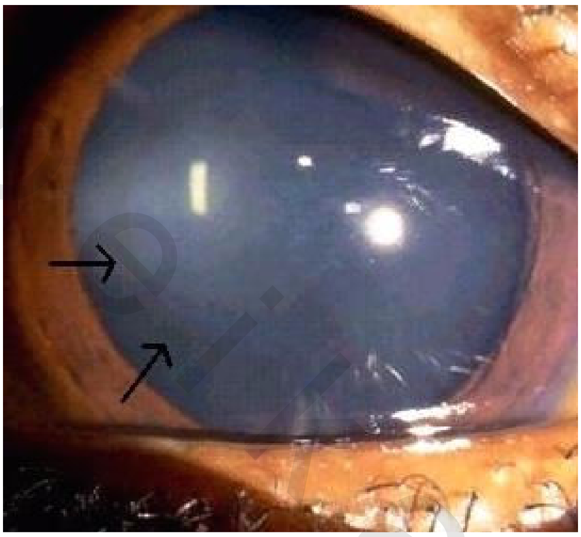

Figure 4: Fleisher's ring

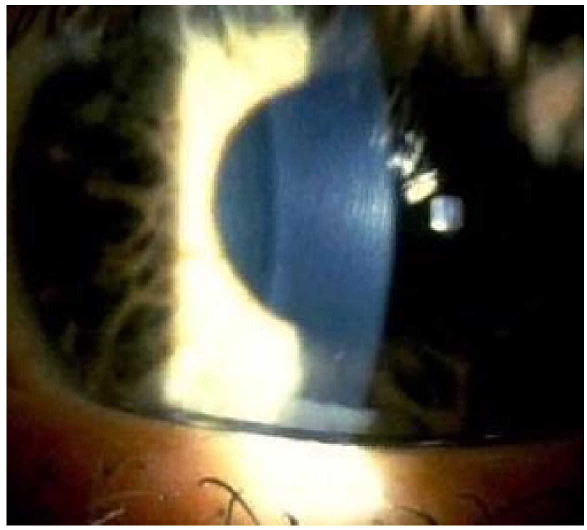

Figure 5: Vogt's striae

Figure 6: Munson's sign

Testing for keratoconus:

Tests include:

1. Retinoscopy: During retinoscopy, the red reflex in a keratoconic eye often demonstrates high amounts of irregular myopic astigmatism with a scissors motion. Sometimes after dilation, keratoconic patients demonstrate a dark annular shadow surrounding the bright reflex at the cone's apex. Total internal reflection of light due to the conical cornea creates this shadow.[19]
2. Keratometry: Keratoconic patients will exhibit several features on keratometry. In many cases, the amount of corneal astigmatism causes the mires to be oval. The irregular corneal surface also distorts mires. The central keratometric rings do not superimpose over one another, suggesting irregular corneal astigmatism, a hallmark of keratoconus. Keratometry also reveals steepening, especially inferiorly. One quick way to check this is to observe the difference in K-readings in primary gaze versus upgaze. Most keratoconic patients demonstrate a dramatic steepening of K's in upgaze. [30]
3. Corneal topography: (also known as computer-assisted videokeratography or corneal mapping) represents a significant advance in the measurement of corneal curvature over keratometry. Most corneal topographers evaluate 8,000 to 10,000 specific points across the entire corneal surface. By contrast, keratometers measure only four data points within the cornea's central 3-4mm; the small size of this area can lead to errors in determining precise toricity.[31]

Topography provides both a qualitative and quantitative evaluation of corneal curvature. It does so by utilizing concentric rings, which project onto the cornea to create a virtual image, it typically measures the deviation of reflected rings and primarily calculates the curvature of the corneal surface points in axial direction. The device compares this image to the target size, and the computer then calculates the corneal curvature giving rise to topographic maps.Every map has a color scale that assigns a particular color to a certain keratometric dioptric range. [31]

Several quantitative indices are available using computer-assisted videokeratography information to screen for keratoconic corneal shape factors. The two most commonly known indices are those of Rabinowitz and Maeda/Klyce.The Rabinowitz diagnostic criteria consists of 3 videokeratography derived indices, which, when abnormal in value, should alert the clinician to consider a diagnosis of keratoconus.[9]

These indices are as follows:

- K value quantifies the central steepening of the cornea that occurs in keratoconus. A value of 47.20 D or greater is suggestive of keratoconus.
- I-S value quantifies the inferior versus superior corneal dioptric asymmetry that occurs in keratoconus. 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. A value of greater than 100% is highly suggestive of frank keratoconus, and the range from 60-100% represents keratoconus suspects.[9]

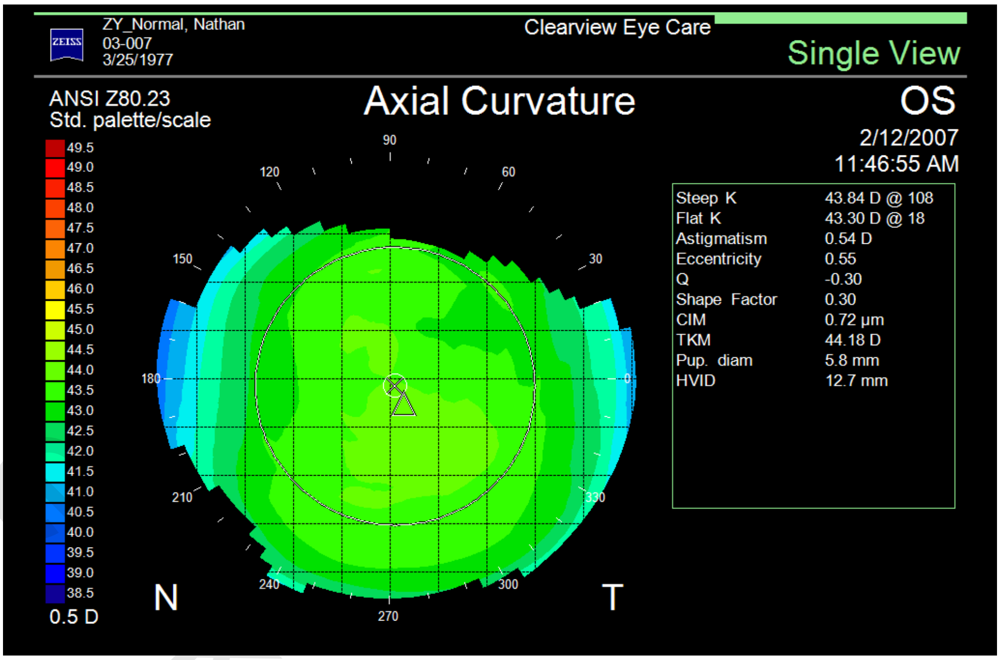

Figure 7: Topography of a normal cornea.

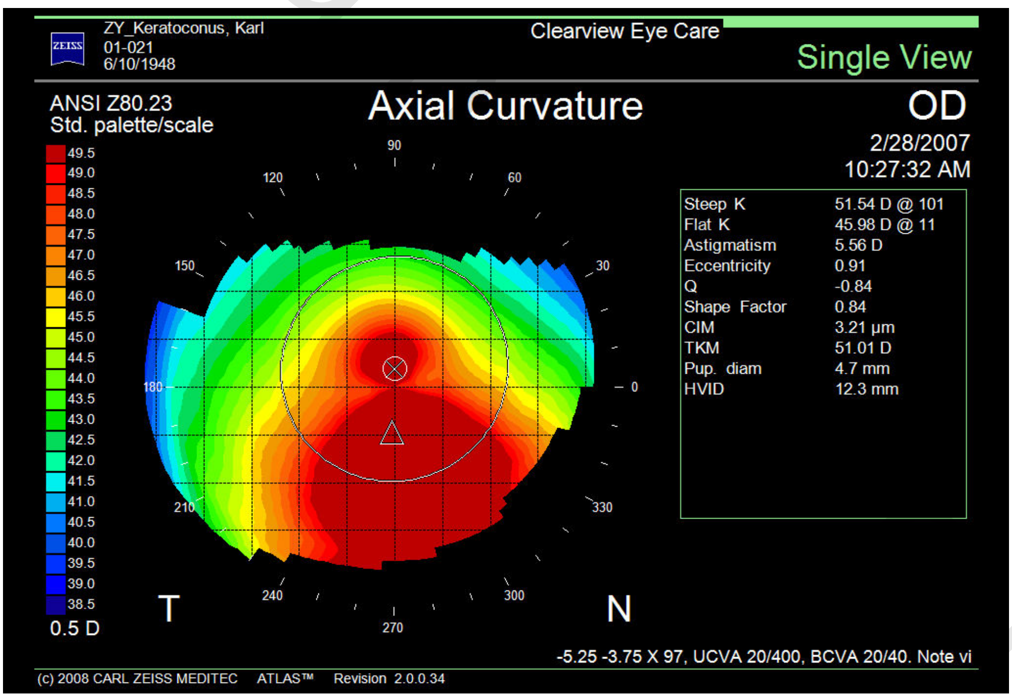

Figure 8: Topography of a patient with keratoconus.

4. Pentacam: Corneal tomography is a new term broader than corneal topography. Recently, the latter is used only to describe the features of the anterior corneal surface, whereas the former is used to describe the features of both corneal surfaces and the matter in between, creating a basic 3-D map of the cornea. It also aids in the diagnosis of ectatic corneal diseases. The Pentacam utilizes a rotating Scheimpflug camera to take up to 100 cross-sectional images of the anterior eye, which produces more than 250,000 true elevation values. These can then be analyzed for information with respect to : [32]

- Anterior and posterior corneal topography.
- Anterior chamber depth and angle.
- Densitometry and location of media opacities (cornea and lens).
- Corneal wavefront aberrometry.
- Lens or IOL and iris movement with accommodation.
- IOL selection following corneal refractive surgery.
- IOP adjustment calculations allowing for corneal curvature and thickness.

The Pentacam consists of a large array of different displays to analyse the collected data. The majority of information is presented as different varieties of colour analysis maps using adjustable colour schemes and scales to represent the selected information.[33]

Most commonly used maps

Corneal tomography produces a graphic representation of corneal features via multiple tomographic maps. The most common tomography maps we use to diagnose and treat ectatic disorders are the axial (sagital) map, the local (tangential) map, the elevation map, and the thickness map.The term topography describes the axial and local maps, and the term tomography describes all of these maps, including topography. [23]

a) Sagital map: This map shows the least amount of detail compared with the others, as it provides a view of the curvature across the entire cornea. Generally speaking, the axial map does not show minor variations in corneal curvature; however, it does use colors to represent the curvature and dioptric values at different spots across the cornea. The hotter colors (reds and oranges) represent steeper areas and the cooler colors (blues and greens) represent flatter areas.[34]

b) Tangential map: This map closely resembles the true corneal shape and depicts curvature and dioptric values with more precision. This map can also pinpoint the position of corneal defects such as cone location in keratoconus. Additionally, the curvature map picks up corneal irregularities or hot spots that have abnormal keratometry (K) readings. These irregularities can be described as round, oval, superior steep, inferior steep, irregular, symmetric bowtie, symmetric bowtie with skewed radial axis (22º or greater difference), inferiorly steep asymmetric bowtie, superiorly steep asymmetric bowtie, or asymmetric bowtie with skewed radial axis ,these patterns shown in figure 9 .With the exception of the symmetric bowtie, these patterns are risk factors for corneal ectatic disorders when they are accompanied by abnormal tomographic parameters.[34]

Within the 5-mm central circle on the corneal curvature map, the symmetrically opposite superior and inferior numbers should be compared. When the superior number is more than 2.50 D greater than the lower number, or when the inferior number is more than 1.50 D greater than the upper number, there is a risk for corneal ectatic diseases. [34]

c) Elevation map: The purpose of this map is to depict the height at which corneal elevations and depressions deviate from a computer-generated reference surface. Shades of red show elevations, and shades of green or blue show depressions. The elevation map of a normal eye has an hourglass-like pattern. If the elevation map shows a tongue-like pattern , this is suspicious and may likely mean that the eye has some type of corneal ectatic disorder. The central circle on the elevation map is 5 mm; using the toric ellipsoid mode, values greater than 12 µm on the anterior elevation map and 15 µm on the posterior elevation map are considered abnormal.[35,36]

d) Thickness map: The most important consideration on this map is the displacement of the thinnest corneal location. In the abnormal cornea, the thinnest point (yellow) is displaced inferiorly or inferotemporally, and in the normal cornea the central area is of a generally uniform thickness (green). For this map, the superior and inferior values,the thinnest location of both eyes, the thickness of the pachymetry apex, the thinnest location, and the Y coordinate of the thinnest location should be compared.The difference between superior and inferior values as well as the difference between the thinnest locations of both eyes should be less than 30 µm. Additionally, pachymetry thickness and the thinnest location should be no more than 10 µm apart. Lastly, if the Y coordinate is less than -500 µm, the cornea is normal; however, if it is between -500 and -1,000 µm, the cornea is suspicious, and if it is greater than -1,000 µm it is abnormal.[27]

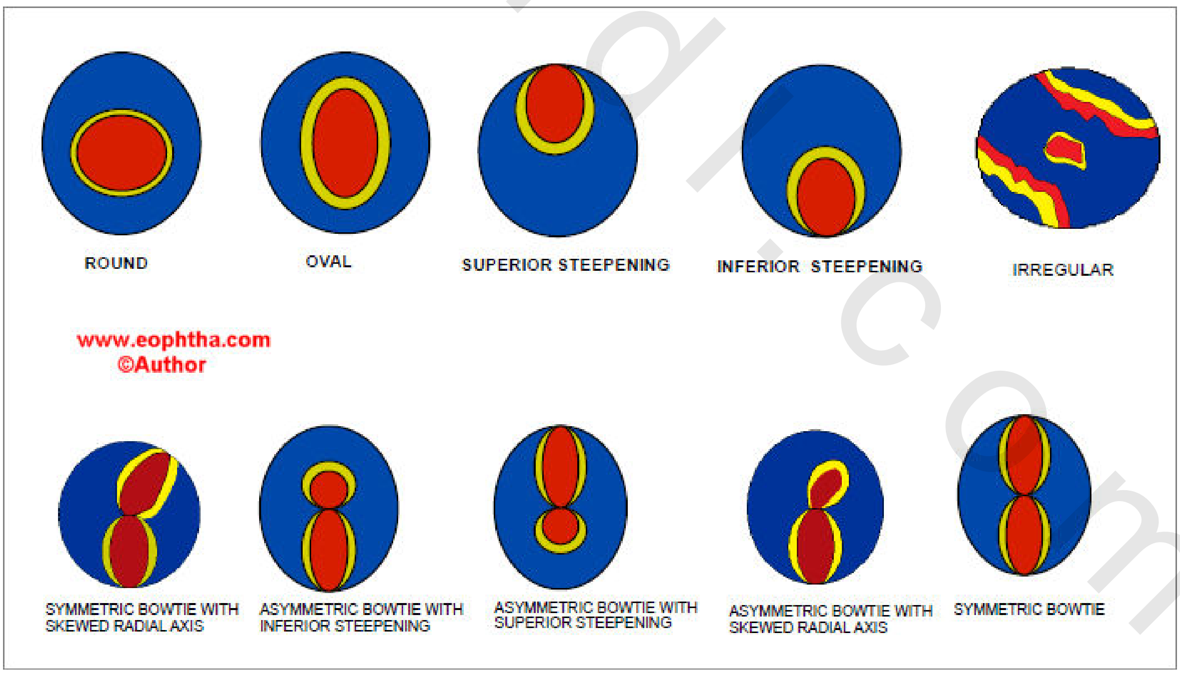

Figure 9: Corneal irregularities as shown by curvature map

The most common presentation of the Pentcam's results is as a four map display (figure: 10) incorporating front and back elevation maps, along with front sagittal curve and pachymetry. These maps are chosen to highlight the inferior decentration of the apex of the cornea on both the front and back corneal surface. This assists in the detection of keratoconus. [35]

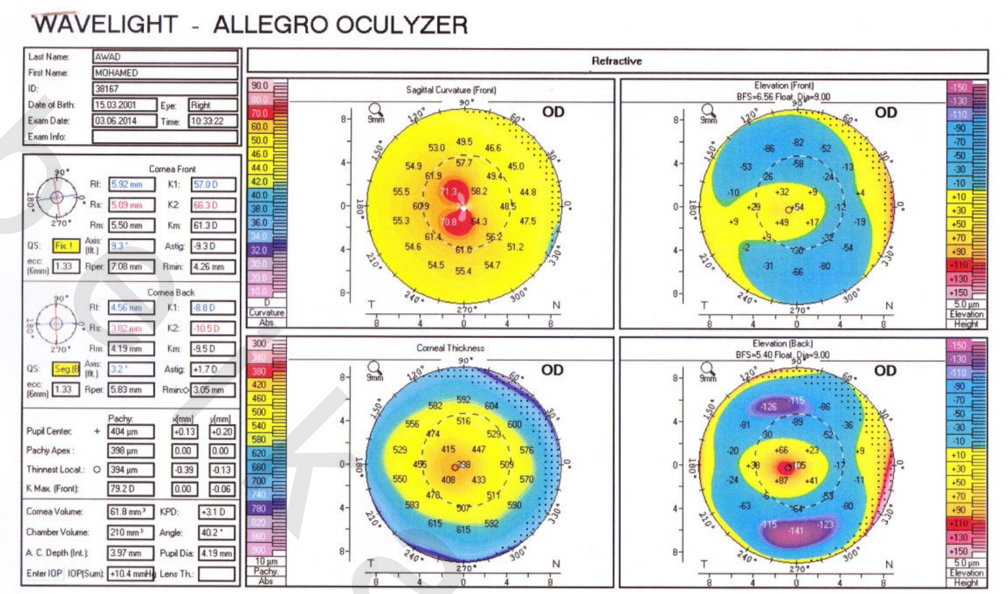

Figure 10: Four map display of keratoconus

Also the Pentacam offers a number of default printouts designed to enhance the detection and monitoring of corneal and anterior eye conditions, in particular keratoconus. One commonly used printout for keratoconus is the Belin-Ambrosio Enhanced Ectasia Map (figure: 11). This is designed to highlight a central ectatic area by displaying standard front and back surface elevation maps, followed by front and back elevation maps compared to a best-fit sphere excluding the central four millimetres of the cornea. The final two maps then show a difference between the previous two. [35]

Additionally, the Belin-Ambrosio display (BAD) performs regression analysis on changes in anterior and posterior elevation, corneal thickness at the thinnest point, thinnest point displacement, and pachymetric progression. It also generates standard deviations (SD) from the mean. Using these values, the BAD creates a new overall map reading, applying colors to represent variations from the mean. Yellow indicates a suspicious cornea (at least 1.6 SD from the mean), and red indicates an abnormal cornea (at least 2.6 SD from the mean). White values indicate a normal cornea. [35]

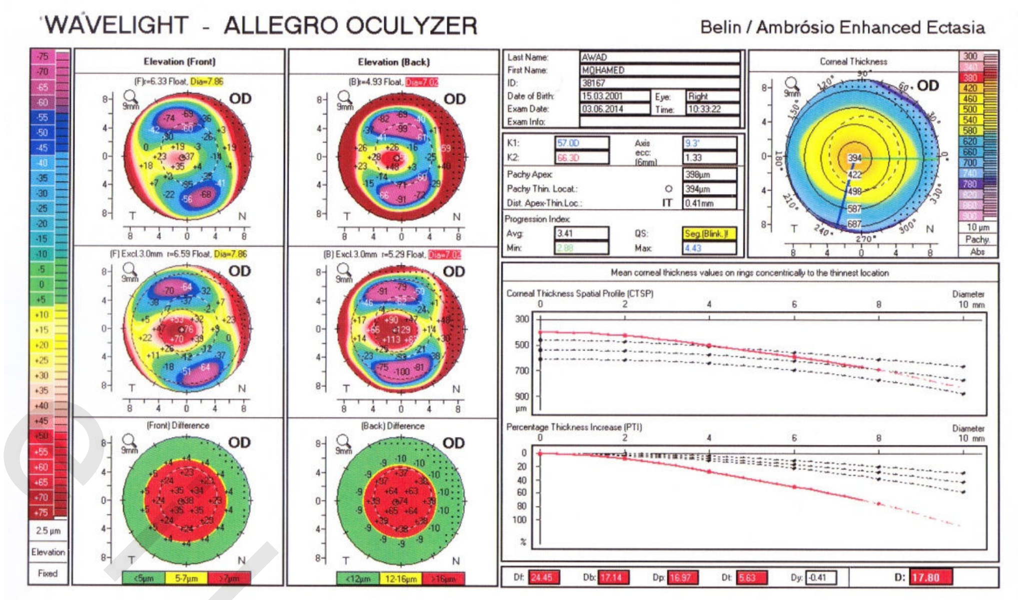

Figure 11: Belin Ambrosio analysis of keratoconus

Clinical classification:

1. Mild keratoconus

- External and corneal signs are often absent or minimal.
- A history of multiple inadequate spectacle corrections of one or both eyes may be noted and may include oblique astigmatism on refraction as well as moderate-to-high myopia.
- Irregularly astigmatic keratometry values (egg-shaped), not necessarily on the steep side of normal (approximately 45 diopters [D]), are consistent with diagnosis.
- Diagnosis can be confirmed with computer-assisted videokeratography, which may reveal corneal inferior steepening (approximately 80% of keratoconus cases), central corneal astigmatic steepening (approximately 15% of keratoconus cases), or even bilateral temporal steepening (extremely rare).
- Diagnosis may also be aided by applying a diagnostic rigid contact lens with its base curve equal to the flat keratometry value. A typical nipple pattern observed by use of sodium fluorescein dye in the underlying tear film. [37]

2. Moderate keratoconus

- One or more corneal signs of keratoconus are often present.
- Enhanced appearance of the corneal nerves is noted.
- Approximately 40% of eyes in patients with moderate keratoconus develop Vogt's striae (fine-stress lines) in the deep stroma.
- Approximately 50% develop Fleischer's ring.

- Approximately 20% develop corneal scarring.
  a) Superficial corneal scarring can be fibular, nebular, or nodular.
  b) Deep stromal scarring may occur, perhaps representing resolved mini-hydrops events.
  c) Some patients show scarring at the level of the Descemet's membrane (posterior limiting lamina), consistent in appearance with posterior polymorphous corneal dystrophy.
- Paraxial (usually inferior to the pupil) stromal thinning may be appreciated.
- Keratometry values typically increase to 45-52 D.
- Distortion of the retinoscopy "scissoring" and direct ophthalmoscope red pupillary reflex may allow observation of oil drop sign.
- The Munson's sign is noted. (37)

3. Advanced keratoconus

- This often results in keratometry values greater than 52 D and enhancement of all corneal signs, symptoms, and visual loss/distortion.
- Vogt's striae are seen in approximately 60% of eyes, and Fleischer's ring and/or scarring are seen in approximately 70% of eyes.
- Acute corneal hydrops can occur.(37)

Amsler-Krumeich keratoconus classification:

Table I: Amsler-Krumeich keratoconus classification

Classification of stages of Keratoconus using Videokeratometry

	VA with glasses	VA with C.Lens	Cor.Indices ISV	KI	Eccentricity in 30°	RMin	Retinoscopy	Cornea
Pre-stage (early signs)	20/20 to 20/15	20/20 to 20/15	<30	1.04 to 1.07	All four values are normal	7.8 to 6.7	No clear light or shadow movement. Hint of scissors effect.	Cornea clear, unobtrusive. Horizontal, oval or round shades central or slightly decentered, when observed under direct ophthalmoscopy.
Level 1	20/25 to 20/15	20/20	30 to 55	1.07 to 1.15	Sometimes one value is abnormal	7.5 to 6.5	Distored retinoscopic reflex. Scissors effect. Severe placido ring distortion.	Clear cornea. Fleischer's ring at apex base. Cone and cone base are clearly visible with direct ophthalmoscopy. Decrease in apex thickness is not visible, but can be measured.
Level 2	20/60 to 20/20	20/30 to 20/20	55 to 90	1.10 to 1.25	Often one value is abnormal	6.9 to 5.3	Clear scissors effect, retinoscopy is difficult to perform.	Often cornea is still clear, apex has become slightly thinner and will eventually decenter. Partial or circular Fleischer's ring. Vogt-Striae (parallel striae) may be visible.
Level 3	20/125 to 20/30	20/40 to 20/20	90 to 150	1.15 to 1.45	At least one value is abnormal	6.6 to 4.8	Distinct scissors effect, retinoscopy is nearly impossible to perform.	Apex has become thinner, decentered, and is often slightly cloudy. Clear and mostly circular Fleischer's ring. Vogt-Striae are clearly visible. Eventually Munson-sign will appear.
Level 4	<20/400 to 20/100	20/100 to 20/40	>150	>1.50	At least one value is abnormal	<5 or not measured	Retinoscopy is impossible to perform.	The cornea is often scarred and opaque in the area of the apex. Munson-sign evident. This is eventual end stage of Keratoconus.

Remarks:

- Pre-stage (early signs): The diagnosis of the pre-stage of Keratoconus is always based on clinical criteria such as a change in power and the axis of the astigmatism, fluctuating refraction values, conspicuous changes in retinoscopy and corneal shadows during observation with the direct ophthalmoskope. Videokeratometry provides supplementary information, but a diagnosis can not be based solely on the results. Corneal tear film irregularities and fixation problems can yield similar images, without the presence of a true Keratoconus.
- This classification was adapted from the classical Amsler- and Muckenhirn standards. It is a topography based graduation and not a clinical one.
- ISV = Index of surface variance KI = Keratoconus index Rmin = minimum value of the curvature of the cornea Eccentricity in 30° refers to the four measuring values nasal, temporal, superior and inferior.
- If visual acuity of 20/25 to 20/20 is achieved with a spectacle correction, contact lenses are not necessarily indicated.
- Munson-sign: The cornea bulges forward. The conical shape is easily recognized in profile, particularly by the acute bulge observed at the lower lid when the patient looks down.

Management:

Depending on the degree of keratoconus, treatment aim at two main entities:

A-Refractive error correction:

1. Optical:

Patients with early keratoconus may successfully use spectacles or spherical/toric soft contact lenses. They may even rarely find that spectacle vision is superior to rigid contact lenses. Rigid contact lenses (CLs) are the mainstay of keratoconus (KC) treatment. When rigid contact lenses are no longer tolerated, some patients can maintain contact lens wear and usable visions with hydrogel contact lenses, piggyback contact lenses, or scleral (haptic) contact lenses but usually at a physiological or visual cost. Figure 12 shows different types of contact lenses used for keratoconus. [38]

Contact lens wear is often complicated by episodes of intolerance, allergic reactions (eg, giant papillary conjunctivitis), corneal abrasions, neovascularization, and other problems, sometimes leading to total intolerance.[38]

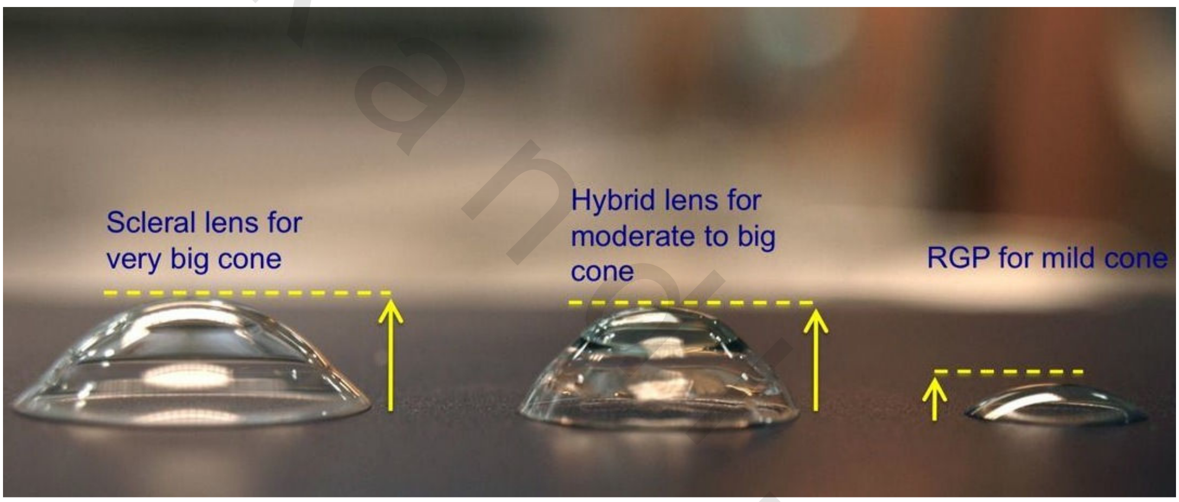

Figure 12: Different types of contact lenses for keratoconus

2. Surgical:

a-Intracorneal ring segment (Intacs) implantation.

Intracorneal rings are PMMA segments that were initially approved by the US FDA and the Communauté Européene for management of myopia and astigmatism. [39,40,41,42] Recent studies have reported their effective use for the treatment of keratoconus and to stabilise ectasia resulting from keratorefractive surgery. Treatment with intrastromal rings (figure: 13) does not eliminate the progression of keratoconus, but it may delay a corneal transplant procedure. [43]

Keratoconus patients with clear central corneas and corneal thickness of ≥400 μm at the point of insertion of intrastromal segments are suitable for this treatment. Colin performed the first implantation of Intacs for keratoconus in 1997.[44] Intacs insertion provided better visual acuity in eyes with keratoconus with relatively low mean K-values (≤53.0 D) and a relatively low spherical equivalent.[45]

The channels for the Intacs can be created mechanically or with the help of femtosecond laser. Previous studies have reported good visual outcomes with the use of mechanical dissection. [46,47] However, the mechanical technique of tunnel creation can cause epithelial defects at the keratotomy site, anterior and posterior perforations, shallow or uneven placement of the segments, introduction of the epithelial cells into the channel, stromal thinning and corneal stromal oedema ,primary spherical aberration, coma and other higher-order aberrations.[48]

The femtosecond laser potentially reduces these complications due to more precise localization of the channel. [49] Besides these complications, infection following implantation of intracorneal rings is a serious complication and can occur many months after the initial procedure. [50,51,52,53]

Recent studies showed that combining riboflavin with Intacs augmented the flattening effects of Intacs. [54]

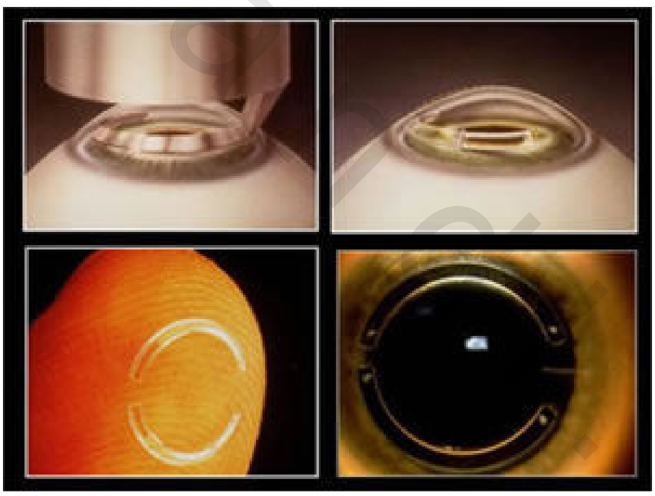

Figure 13 : The creation and placement of the intacs rings

b- Keratoplasty in advanced disease

- **Full-thickness penetrating keratoplasty (PK):**

PK has been a well-accepted surgical treatment for advanced keratoconus (figure:14). However, it can be complicated by allograft endothelial rejection that can lead to endothelial cell loss with subsequent risk of graft failure. Therefore, replacement of a healthy endothelial cell layer with PK with subsequent risk of rejection and rejection-related (either by itself or by treatment with steroids) complications such as glaucoma can be a major problem in patients with keratoconus. In addition, the integrity of the ocular surface would be compromised with the full-thickness PK. [55,56,57]

Recently, advances have been seen and new techniques of keratoplasty have been introduced in the treatment of keratoconus.[58] These are mainly lamellar keratoplasty techniques and the advanced-shaped side-cut techniques, particularly with the use of femtosecond(FS) lasers.[59,60,61,62]

- **Deep anterior lamellar keratoplasty (DALK) :**

DALK technique involve the use of manual dissection, deep stromal injection of air, saline injection, or the use of viscoelastics .[63,64,65,66] In addition, others described careful manual peeling techniques and the use of Trypan blue to aid in visualization of the Descemet's membrane.[67,68]

Studies indicate that DALK has outcomes comparable to the standard penetrating graft, [69,70] and endothelial cell loss is less than or at least equal to that of penetrating grafts. The advantage of deep lamellar keratoplasty is the elimination of a graft–host stromal interface and the associated problems of irregularity and scarring, leading to faster visual rehabilitation and improved visual outcomes. The surgical challenge is to reach the level of the Descemet's membrane and achieve entire removal of the corneal stroma without perforating the Descemet's membrane. [71,72,73,74]

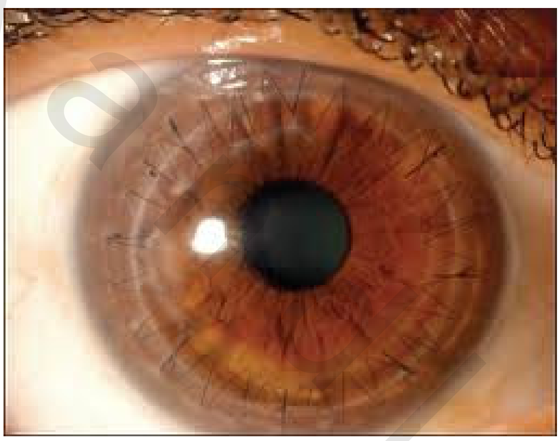

Figure 14: Clear graft after penetrating keratoplasty with eight interrupted sutures and a 24-bite running suture.

- **FS-enabled Keratoplasty**

The FS-pulsed laser can cut corneal tissue through the application of numerous adjacent pulses. [75] Compared with conventional blade trephination, the goal of a laser incision for PK is the creation of a more structurally stable and predictable wound configuration. It is also possible to create an infinite variety of nonplanar patterns for corneal transplantation incisions with the use of the FS lasers. Earlier laboratory investigations and recent studies demonstrated that PK incisions created by the FS laser can lead to a sevenfold increase in resistance to wound leakage and possibly less astigmatism. With the FS laser cuts, better alignment of the donor and host anterior surface reduces distortion and the increased surface area of the shaped incision, such as zigzag incisions, may lead to improved tensile strength of the incision as it heals.[51-53,76]

- **FS-enabled DALK**

A technique that can maintain the patient's healthy endothelial layer and replace the entire anterior stromal layer with the addition of superior wound-edge healing and minimal induced astigmatism is ideal. The combination of the big-bubble DALK technique and the FS laser-assisted zigzag cut achieves such a goal. It also preserves the option of a full-thickness PK with the benefits of FS laser incision if the dissection of Descemet's membrane fails. (77)

The FS laser, an infrared laser, has the ability to ablate tissue with less interference from optical haze, and it has been shown to be capable of performing posterior lamellar surgery with preparation of the donor corneal lamella (femto-DALK or FS-enabled DALK). (51, 66) In addition, specific nomograms for depth and energy settings have yet to be worked out for deep ablations. (78,79)

The advancement of the FS laser to produce corneal cuts and the recent lamellar keratoplasty techniques have allowed corneal transplantation in keratoconus patients to become precise, with the goals of faster rehabilitation and higher quality visual acuity. (80)

B-Corneal collagen cross-linking (CXL):

Cross-linking of collagen refers to the ability of collagen fibrils to form strong chemical bonds with adjacent fibrils. In the cornea, collagen cross-linking occurs naturally with aging due to an oxidative deamination reaction that takes place within the end chains of the collagen. It has been hypothesized that this natural cross-linkage of collagen explains why keratoectasia (corneal ectasia) often progresses most rapidly in adolescence or early adulthood but tends to stabilize in patients after middle-age. (81)

Corneal collagen cross-linking (figure: 15) has emerged as a promising technique to slow or halt the progression of keratoconus and postoperative LASIK ectasia. (81)

The beginning

The idea to cross-link the collagen in the cornea was first developed and put into practice by Theo Seiler, MD, PhD, Dresden University, Germany. The suggestion came from previous applications of this chemical-physical process in different fields by dentists and dermatologists. (82)

Preclinical studies on corneal cross-linking began in Dresden in 1995. Several chemical cross-linking substances and light irradiation with different photosensitizing agents were investigated, and in 1998, the first patient was treated with riboflavin and UV light. Because no side effects were reported, a pilot study was started 1 year later. (82)

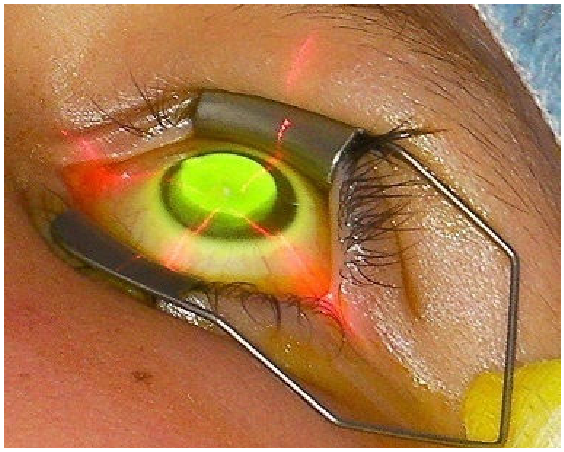

Figure 15: Corneal collagen cross-linking

Effects of CXL:

Keratocytes

In a normal cornea the distribution of keratocytes is denser in the anterior cornea decreasing in density towards the posterior cornea. [13] Removing the epithelial layer results in keratocyte apoptosis to a corneal depth of approximately 300-350 µm one month postoperatively. Repopulation of keratocytes was found to occur in the anterior cornea and mid-stroma in the following few months and was associated with corneal oedema. It has been shown that the repopulation of keratocytes is initiated from activated keratocytes in deeper layers of the stroma.[83,84]

Stroma deeper than approximately 350 µm showed an increased density of keratocytes in the first and third post-operative months. Stromal repopulation was almost complete within six months. Stromal density is increased after CXL is performed because well-structured compact collagen lamellae are produced after repopulation of keratocytes, which is seen as haze. Even though cross-linking does induce cellular apoptosis, the regeneration processes of these cells result in normal tissue histology. [85,86,87]

Corneal transparency and stromal demarcation line:

Faint diffuse haze was observed post-operatively that spontaneously disappeared within one month. [88] The postoperative haze seems to be more prevalent in subjects with more advanced keratoconus. [86] In other studies, [89] hyperactivated keratocyte nuclei in the anterior stroma (to a depth of 80 µm), and reticular patterned dark microstriae (with or without Vogt's striae) were identified as possible risk factors for corneal opacity when observed preoperatively.The haze seemed not to affect patients vision. The risk factors are important as these patients are more likely to not only develop haze but late stromal scarring postoperatively as well. Changes in the corneal microstructure do not occur throughout the entire cornea. The transition between the treated zone and untreated zone of the cornea is called demarcation line and is normally less than 350 µm into the cornea. [86]

Endothelium

Post-operatively few clinical changes morphological changes or changes in cell count have been noted in the endothelium following CXL. [82,90] The most important factor in reducing radiation and thus cytotoxic effects on the endothelium is to ensure a minimal inclusion thickness of 400 µm for all CXL patients. [91]

Stromal thickness

Numerous studies have reported on a decrease in corneal thickness one year after CXL, [88,92,94] while others have found that corneal thickness does not change post-operatively. [93] Optical Coherence Tomography (OCT) has been used to show a significant increase in corneal thickness six months post-operatively. It is believed that this increase is caused by keratocytes producing new proteoglycans. The increase can be seen as an indication that the decrease in corneal thickness after one year is not an indication of progression of keratoconus, as the normal progression of keratoconus is not linked with a period of increased thickness but only thinning. Alternatively the early increase of stromal thickness could be a measurement artifact seen in most patients post-operatively because of haze. This artifact is not present one year post-operatively, because of the disappearance of haze, seen with slit-lamp observation. [88]

Keratometric (K)-readings

After CXL it has been found that K-readings flattened [92] or stabilized [91,93] and that the cornea had a tendency to take on a more symmetric form. [83,90]

In a few rare cases a steepening of K-readings was found. It is important to realize that a flattening effect of a keratoconic cornea has never before been documented and that control groups of keratoconic eyes showed definite progression. This flattening has been called postoperative regression which was found in 70 % of CXL patients. [82]

Refractive status and visual Acuity

A slow but significant improvement in spherical refractive error and cylindrical refractive error was seen in as little as three months postoperatively when compared to mean pre-operative values. [90] Uncorrected visual acuity (UCVA) was statistically significantly improved by approximately 1-1.3 lines,[93, 94] best corrected visual acuity (BCVA) had a statistically significant increase of approximately 1.26 lines[82,85] and best corrected spherical visual acuity (BCSVA) remained stable[90] when comparing pre- and post-operative values.

Interestingly, Doors et al [88] found a significant decrease in BCSVA one month post-operatively but at three, six and 12 months post-operatively the BSCVA was stable compared to pre-operative values. The decrease at one month was attributed to the remodeling process taking place in the cornea. It seems that the improvement in VA happens in the first three to 12 months post-operatively and then remains unchanged. [91,94]

Safety of CXL:

Generally, UV radiation represents a danger to the eye but this is mostly true for UVB radiation (290- 320 nm). UVA radiation (320-400 nm) alone has a damage threshold value of 4 mW/cm^2 at the cornea. When riboflavin is instilled into the cornea the damage threshold drops to 0.35 mW/cm. During cross-linking UVA radiation at 370 nm is used as this wavelength is optimally absorbed by riboflavin thus ensuring that approximately 95% of the UV radiation is absorbed in the cornea. The cytotoxicity level for UVA in combination with riboflavin was found to be 10 times lower than with UVA alone .[83,84]

The cross-linking effect only occurs in the anterior 200-300 µm of the cornea which is due to the high levels of absorption of UVA radiation in this area. [95] This ensures that more posterior structures (for example, the endothelium, lens and retina) are not adversely affected; however, an absolute minimal corneal thickness of 400 µm is required to diminish potential for damage. [94,96]

Damage could also arise when a patient does not maintain fixation on the focus point resulting in the limbus being irradiated. In these low-compliance patients a poly-methyl methacrylate ring can be placed over the limbus to ensure limbal protection. Under normal conditions (adequate fixation) the limbus is not exposed to the effects of UVA radiation. The protection is provided by the presence of the epithelium. [86]

Preoperative swelling of the cornea using hypo-osmolar riboflavin solution safely broadens the spectrum of corneal cross-linking indications to thin corneas that would otherwise not be eligible for treatment. [87,97]

CXL Techniques:

1-Epithelium off CXL:

The standard CXL protocol proposed by Wollensak et al. recommended removal of the epithelium before impregnation of the cornea with a diffusible photosensitizer (riboflavin). Instillation is repeated every few minutes for a total of 30 minutes, and then the cornea is exposed to intense 365-nm UV light irradiation for an additional 30 minutes. The induced photochemical reaction (oxidative deamination), creates new, stable bridges between collagen molecules that reinforce the corneal structure and increase corneal elasticity by a factor of 3 to 5. [82]

Epithelial tissue is mechanically removed with a blunt instrument in a 7-9 mm diameter zone (the epithelium can be debrided with a variety of tools: a Beaver blade, Amoil brush, rotating soft brush,blunt spatula, blunt hockey knife, a blunt crescent knife or a blunt knife). The epithelium is a diffusion barrier to the cornea which needs to be removed to aid the penetration of riboflavin, which is water soluble, into the cornea. [98] A bandage contact lens is usually placed on the cornea post-operatively mostly for three to five days, or until reepithelialization is complete. [90,92,94]

The ideal riboflavin concentration and UVA intensity levels were identified through dose/concentration assays in vitro and in various animal models. The parameters currently used in humans (international multicenter clinical phase 2 study) are 0.1% riboflavin solution and 3 mW/cm2 of UVA. [98,99,100]

Complications of epithelium off CXL:

Unfortunately, there are several major obstacles, at least from the patient's perspective, when epithelial removal is performed during the traditional epithelium off CXL procedure. Patients experience considerable pain for up to 7 days after this procedure. Removing the epithelium also eliminates a natural barrier to infection, and, therefore, the risk of infectious keratitis is increased, also delay of epithelial healing for up to 30 days, hypertrophic epithelial healing, corneal sterile infiltrates, and corneal edema with scarring do occur. During the period of delayed wound healing, most patients are unable to work for a significant length of time. Moreover, contact lenses, which are so vital to a keratoconic patient's visual functioning, may not be worn for up to several weeks. Many of these concerns could be greatly reduced if CXL could be performed without removing the epithelium. [(101)]

2-Transepithelial CXL:

A modification of the technique by keeping the epithelium intact (epithelium-on or transepithelial CXL) is now available, this technique could be used in specific cases, such as ultra-thin corneas with progressive keratoconus; it allows us to maintain the cut-off value of 400 µm in corneas where we would not be able to use the conventional treatment. Children and patients with poor compliance, including Down syndrome patients, are also good candidates. In addition to avoiding potential complications related to epithelium removal, an epithelium-on procedure results in better comfort for the patient, with no pain and a faster visual recovery.[(102)]

Experimental and clinical investigations have shown that the intact epithelium does not block the effect of ultraviolet A (UVA) light, [(103)] but decreases the effectiveness of the treatment by impairing the adequate stromal diffusion of riboflavin. [(104,105,106,107,108,109,110)] However, a limitation of most of the studies that procured the low cross-linking effect or low stromal saturation of riboflavin with the epithelium-on CXL includes the use of the standard—or only slightly modified—Wollensak/Seiler protocol on nondeepithelialized eyes.

CXL enhancers:

Accordingly, various approaches have been tried clinically and in the laboratory to enhance the epithelial permeability of riboflavin.

- Chemical enhancers such as benzalkonium chloride (BAC), ethylenediaminetetraacetic acid (EDTA), gentamycin, tetracaine, 20% ethanol,and d-alpha-tocopheryl poly(ethylene glycol) 1000 succinate (Vitamin E-TPGS).[(105,106,111)]
- Partial grid-like pattern deepithelialization ,excimer laser superficial epithelial removal.[(105)]
- Replacement of the isotonic by hypotonic riboflavin solution [(112,113)]
- Enhanced trans-epithelial Riboflavin absorption using iontophoretic delivery,riboflavin is an effective molecule for iontophoretic transfer as it is small, negatively charged at physiological pH and is easily soluble in water.[(114)]
- Nano-emulsion systems[(115)]

The results varied between the studies, but the majority of the aforementioned methods lead to increased epithelial permeability for riboflavin.

Formulation is the most important factor in facilitating penetration of riboflavin through the epithelium. The standard riboflavin solution (10 mg riboflavin-5-phosphate in 10 mL 20% dextran T-500) has hydrophilic characteristics that prevent full diffusion of this drug through the lipophilic epithelium. Moreover, the electrostatic repulsion between the anionic riboflavin-5-phosphate and the negatively charged surface of the cornea further reduces its epithelial penetration. Thus, an appropriated vehicle should associate both bioadhesive and amphiphilic properties to allow the riboflavin to penetrate the epithelium and diffuse into the corneal hydrophilic stroma. [116]

Accelerated cross-linking:

There is an interest by patient as well as clinicians to reduce the overall CXL treatment time. There are attempts to shorten the illumination time by increasing the illumination intensity. The intensities varied from 3-90 mW/cm ^{2}, with a constant energy dose of 5.4 J/cm ^{2} and corresponding illumination times ranging from 30 to 1 min. This new CXL system proved that higher powers delivered over shorter time periods can provide the same corneal strengthening as lower power over longer time periods.The same total amount of UVA light energy (5.4 J/cm^2) is delivered, with a high irradiance of 30 mW/cm^2 for 3 minutes as compared to 3 mW/cm^2 for 30 minutes. [117]

New methodologies:

While Riboflavin/UVA CXL has been shown to be effective, other methodologies which are potentially more rapid and less invasive, are currently under investigation. Rocha et al. reported a flash-linking process with UVA and polyvinyl pyrrolidone with may have the potential to photo-chemically cross-link the cornea in only 30 seconds.[118] Paik et al. have investigated the topical application of short-chain aliphatic beta-nitro alcohols.[119] and Cherfan et al. demonstrated an almost fourfold increase in corneal stiffness with no reduction in keratocyte viability with rose bengal 0.1% administration and green light application and a less than 5 min total treatment time.[120]Undoubtedly other methodologies and applications will become available over the several next years due to the vast interest in this area of research.

Combined treatment:

Combination of CXL with other surgical procedures in order to correct corneal ectasia has been evaluated by various clinical studies and revealed a significantly greater improvement in vision and K readings, they include:

- Combined Intacs and CXL .[54]
- Topography-guided photorefractive keratectomy followed by CXL on the same day.[121]
- Implantation of ICRS followed by CXL after 6 months.[122]
- CXL followed by wavefront guided ablation afer 6 months.

There are two ways of assessing the effects of cross-linking in the cornea: the demarcation line and biomechanical measurements. Curvature changes, visual acuity and topographic changes are secondary effects to biomechanical effect of increased resistance.[92]Laboratories studies showed that the effective depth of CXL is confined to the anterior 300 µm of the cornea.[123] Recently, the transition zone between the cross-linked and untreated corneal stroma has been visualized using biomicroscopy,[124] confocal microscopy,[86] and anterior segment optical coherence tomography (AS-OCT).The demarcation line detected by AS-OCT showed significant correlation with the transition zone detected by in vivo confocal microscopy, and thus it represents an effective tool to monitor the effective depth of CXL.[88,125]

Anterior segment OCT (AS-OCT):

OCT is a well known and frequently used technology to image the posterior segment. The first report of OCT imaging of the cornea published in 1994. Anterior segment OCT creates cross-sectional images of anterior segment structures. It also `provides measurement tools to document and follow changes in the cornea, angle, and anterior chamber. [126]

OCT utilizes near-infrared light waves to measure distances of anatomical structures. A beam of light is directed onto the structure and the echo time delay of light is then recorded. Employing low-coherence interferometry, the reflected light from the eye is compared to a reference value of a known length. A series of axial scans (A-scans) are combined to form two-dimensional images of the ocular structures in a process similar to ultrasound biomicroscopy; however, light (as opposed to sound waves) is used in OCT. Cross-sectional images are then generated by scanning the incident optical beam. The resultant scans are displayed in a color scale. [127]

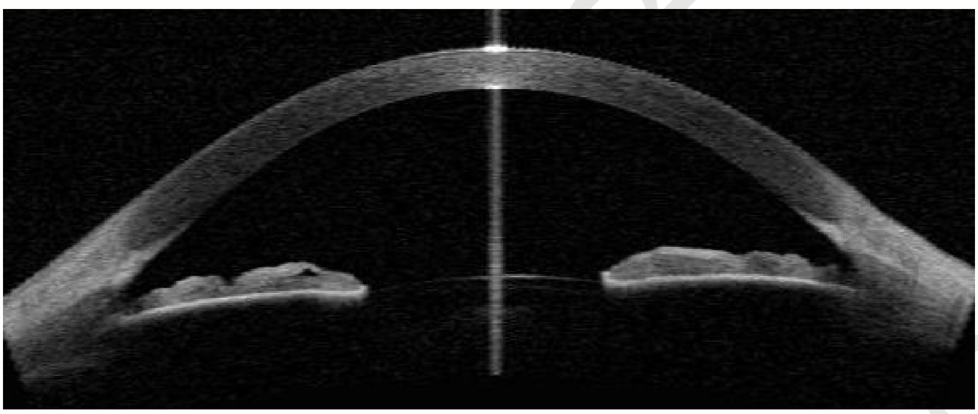

Figure 16: Normal eye imaged on Visante

Using these principles, two OCT platforms have been developed: time domain and spectral (or Fourier) domain. Time domain OCT (TD-OCT) instrumentation utilizes a moveable reference mirror. The mirror moves for each A-scan to determine the ocular structure's depth, thereby limiting the speed at which the image is acquired. Spectral domain OCT (SD-OCT) has a fixed reference mirror to measure the depth information and uses a Fourier transformation algorithm of the spectral interferogram to produce the A-scan, which results in faster acquisition and better image quality. [128,129]

AS-OCT resolution is based on wavelength and bandwidth. A 1,310nm wavelength captures data at a rate 20 times faster than the original 820nm wavelength utilized in posterior segment examination by TD-OCT.The longer wavelength also allows for better penetration into the sclera, iris and anterior chamber angle.(130)AS-OCT is a non-contact procedure and is more user-friendly when compared to ultrasound biomicroscopy (UBM).(131)

Applications of AS-OCT:

- **Corneal pathologies**: AS-OCT is very useful for assessing corneal pathologies. It can be used to determine the exact depth of foreign body penetration or the existence of a residual defect following removal. AS-OCT helps show the extent and the potential of different contact lens adverse reactions, such as contact lens acute red eye, contact lens peripheral ulcer, infiltrative keratitis, superior epithelial arcuate lesions and corneal erosions. Also this technology permits a more comprehensive view of microbial keratitis and its response to treatment. (132)

- **Corneal dystrophies**: Corneal dystrophies encompass a broad range of disorders that affect one or more of the corneal layers. AS-OCT provides exceptional imagery of the cornea, revealing a clear differentiation of the epithelium, stroma and endothelium. AS-OCT can help diagnose keratoconus with pachymetry mapping as well as demonstrate corneal ectasia through cross-sectional imaging in moderate and advanced cases.Also,this technology is very helpful in postoperative evaluation of keratoplasty and crosslinking used in keratoconus management. In advanced cases, Fuchs' endothelial dystrophy can lead to bullous keratopathy. Corneal guttata associated with Fuchs' dystrophy, as well as findings consistent with corneal edema and bullous keratopathy, can be imaged on AS-OCT. Also, AS-OCT can document corneal topography both before and after a patient undergoes Descemet's stripping automated endothelial keratoplasty (DSAEK) for Fuchs' dystrophy or pseudophakic bullous keratopathy .(133)

- **Corneal degenerations**: Corneal degenerations also can be diagnosed and managed with AS-OCT. for example dellen which are focal areas of corneal thinning that typically are located at the limbus. They are often linked to an adjacent elevation of the conjunctival tissue, such as a pinguecula or episcleritis nodule. Management and progression of dellen can be well documented on anterior segment OCT.(134,135)

- **Refractive surgery:** AS-OCT has dual applications in refractive surgery. Visante OCT, for example, provides global corneal thickness measurements for preoperative planning and offers a difference map feature that illustrates changes in postoperative corneal thickness. Secondly, AS-OCT indicates flap architecture and thickness as well as residual bed thickness, which is particularly helpful in considering re-treatments for patients who were clinically assessed as borderline for LASIK enhancement preoperatively and in diagnosing unexpected postoperative cases of ectasia secondary to thicker-than-expected flaps. (136)AS-OCT can also be useful in helping surgeons determine the proper ablation for phototherapeutic keratectomy (PTK). By establishing the opacity depth and the epithelial thickness, the practitioner can properly remove the opacity and estimate the amount of induced refractive change.(137)

- **Glaucoma:** With the exception of grading pigmentation, angle assessment on AS-OCT has many advantages over gonioscopy—particularly the ability to avoid both light and compression artifacts.[138]

- **Iris evaluation:** AS-OCT allows for observation of the anatomical relationship of the iris, its root, and the angle anatomy at the corneoscleral junction.

- **Phakic IOLs and narrow angle treatment:** Crystalline lens rise (CLR) is a new term that is used in regard to refractive surgery with phakic IOLs. CLR, an indirect measurement of iris convexity, may be used to indicate removal of the crystalline lens vs. laser peripheral iridotomy (LPI) in narrow-angle patients. [139]

Demarcation line:

A corneal stromal demarcation line indicating the transition zone between cross-linked anterior corneal stroma and untreated posterior corneal stroma can be detected in slit-lamp examination as early as 2 weeks after treatment. The line is best identified with a thin slit and high illumination levels by using a slit lamp that provides high levels of white light (figure 17). In the corneal periphery, the line gradually adopts a conical shape because of the increasing total corneal thickness. The presence of this finding over the anterior two-thirds of the stroma confirms that sufficient CXL treatment has occurred. [124]

OCT has seen some recent interest as a tool for investigating CXL effects, such as corneal thickness before and after CXL for KC, and demarcation line depth following CXL.[121,140,141] In fact, the significance of the demarcation line is uncertain. The increased optical density seen on optical coherence tomography (OCT) or confocal microscopy may represent keratocyte apoptosis and subsequent repopulation. [142]

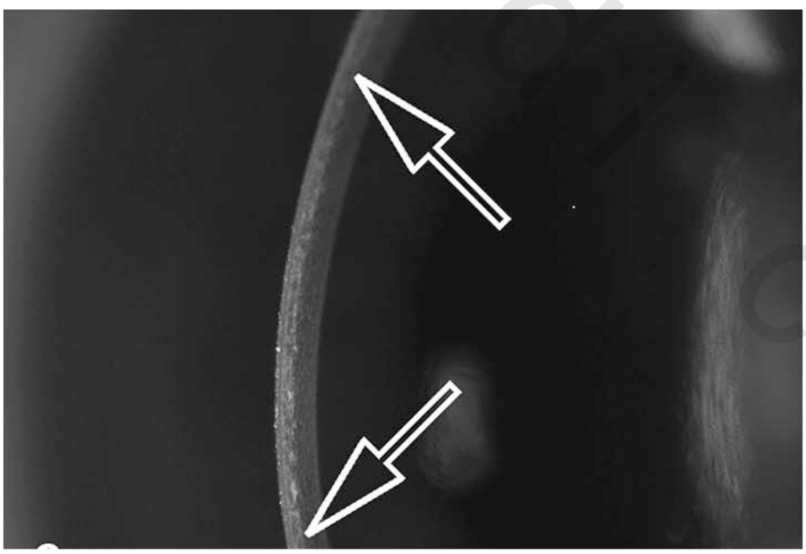

Figure 17: Demarcation line as seen by slit lamp

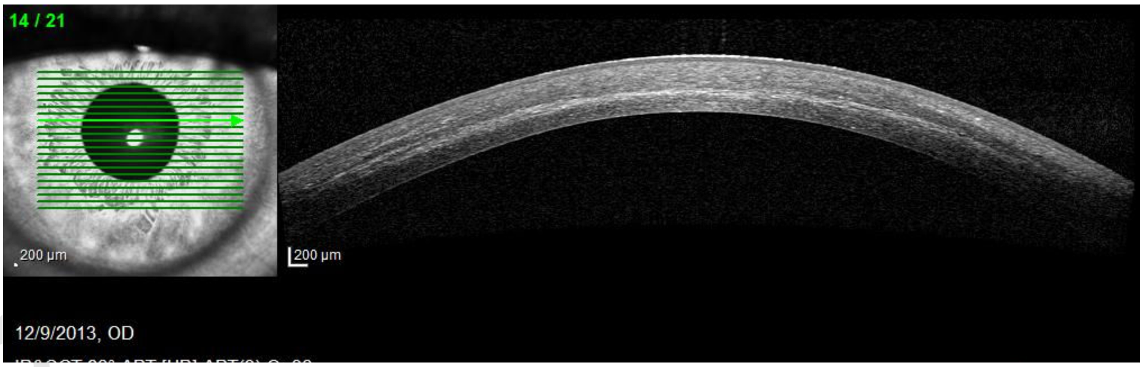

Figure 18: Demarcation line as shown by AS-OCT

The exact cause of demarcation line appearance is still under investigation some studies found that it may result from the difference in refractive indices or reflection properties of cross-linked vs untreated corneal stroma. (124) In a recent study, confocal microscopy showed a transition area where an edematous zone with low cell density merged with a deeper zone with less edema and more keratocytes, at an average depth of 320 µm (84) .The transition can be seen as an opaque/ whitish line when using an OCT for instance, because of the rapid change between the two zones. Generally, the demarcation line disappears within three months post-operatively. The average depth of this line is approximately 313 µm. (88)

From the third to sixth months post-operatively the reflectivity of the anterior area changes from hypo- to hyper-reflectivity because of increased density in this part of the cornea. The line between the normal density cornea and the hyper reflective area is now called the late demarcation line. This late demarcation line can be detected for up to three years post-operatively when using confocal laser scanning microscopy. (86)

Although these lines do not appear to affect vision, as they correspond to changes in stromal density, they appear as brighter (hyper-reflective) areas on cross-sectional corneal OCT scans. However, the depth and extent of stromal changes induced by CXL has been difficult to evaluate quantitatively in the clinic.