

# **Ultrasound biomicroscopy as a diagnostic method of corneal degeneration and inflammation**

**Ph.D. Thesis**

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## 1. ABBREVIATIONS

AM	amniotic membrane
AMT	amniotic membrane transplantation
BCVA	best corrected visual acuity
BCCVA	best corrected visual acuity with contact lenses
BCSVA	best corrected visual acuity with spectacles
D	diopter
DMSO	dimethyl sulfoxide
HSV-1	herpes simplex virus 1
IOP	intraocular pressure
KC	keratoconus
MHz	megahertz
OCT	optical coherence tomography
PKP	penetrating keratoplasty
TMS	topographic modeling system
UBM	ultrasound biomicroscopy
UCVA	uncorrected visual acuity

## 2. INTRODUCTION

The demand for noninvasive diagnostic techniques in ophthalmology yielded the development of special noninvasive tools that can function as diagnostic indicators.

Imaging techniques of the anterior segment of the eye provide important information for detecting and managing the pathology, pathophysiology, prognosis and treatment of disorders of the cornea, limbus, anterior chamber, iris, and lens (1).

Anterior segment imaging has significantly altered the diagnosis and evaluation of eye diseases and become a rapidly advancing field of ophthalmology. It could be regarded as more complex method than retinal imaging due to the depth of structures and surfaces of interest being obscured by other anatomical features. Several techniques have been developed over the last years to image objectively the anterior segment of the eye (1-4).

Ultrasonography is an ultrasound-based diagnostic imaging technique, a measurement tool, and a device used for visualizing and characterising ocular tissues. The used ultrasound frequency causes limited resolution (5).

In early 1990's, Pavlin et al. created a new ultrasound instrument for visualising the anterior segment structures (6,7). High-frequency ultrasound biomicroscopy (UBM) makes a more detailed image and more accurate measurement due to the greater resolution than regular ultrasound, but at the expense of decreased tissue penetration (6,7). High-energy sound waves are bounced off the inside of the eye and the echo patterns are shown on the screen of an ultrasound machine. In contradiction to ultrasonography, UBM provides high-resolution in vivo imaging of the anterior segment of the eye in a noninvasive manner (8) and is the most established anterior segment imaging device offering objective, high-resolution images of angle structure. The B-scan mode UBM has a high frequency transducer (35-100 MHz) which limits sound waves through ocular tissues and detects their reflection from tissue interfaces (8). Pathologic changes, involving anterior segment structures can be evaluated qualitatively and quantitatively by this method (8).

UBM has numerous potential uses in clinical situations. Although the anterior segment of the eye is partially visible by direct observation with slit lamp, there are several surfaces not easily accessible by this device, such as the posterior surface of the iris and the region of posterior chamber - ciliary body. Angle depth can be determined by UBM without the



requirement of a clear cornea for gonioscopy. In addition, corneal opacification precluding a view of the anterior chamber would allow an image of the areas behind the opacity with high frequency resolution. Lesions of the iris and the ciliary body are difficult to diagnose with usual ultrasound techniques, but it is easy to determine these alterations with UBM (6-11).

Clinically, it is very important to measure the central and peripheral thickness of the cornea. These parameters could be determined by using a number of examination methods. Optical pachymetry was the gold standard in the past. Later, it has been replaced by ultrasonic pachymetry because of its easy use (1,12-14). Anterior segment imaging with UBM has been shown accurate measuring of the corneal thickness and curvature (8). Optical coherence tomography (OCT) is an emerging technology for performing high-resolution cross-sectional imaging. OCT is analogous to ultrasound imaging, except that it uses light instead of sound. OCT can provide cross-sectional images of tissue structures within the cornea (13,15). UBM is another device that can be used to object and follow the progression of the central and peripheral thickness or thinning of the cornea in different corneal diseases (13,16). Each of the above techniques has certain advantages.

After studying the very advantageous method of UBM, we decided to use this device to find exact diagnosis and to follow the courses of the corneal degenerative and inflammatory diseases. UBM would be more precise than the other methods we used previously, even in following of the accuracy of the treatment we applied. We also carried out the new idea of our group to replace with amniotic membrane (AM) the surface of the artificial corneal defect that was formed when the calcified corneal part was cut out.

### **3. HYPOTHESES AND AIMS**

Based on previous studies, we hypothesized that

- UBM could be useful device in investigating and following the courses of corneal degenerative and inflammatory diseases, and
- AM transplantation might be helpful in the healing process of epithelial defects in corneal degenerative diseases.

In order to get answer for the hypotheses above, patients with Terrien's disease, corneal calcification, and keratoconus were chosen for our investigations.

The aims of this study were:

- to find new information about the diagnosis of corneal diseases with using UBM,
- to estimate the progression of the corneal diseases,
- to replace the surface of the artificial corneal defect with AM,
- to verify and follow the results of the management and treatment of the corneal diseases that were treated by our team,
- to find out whether the histopathological examinations confirm the structural changes diagnosed by UBM.

## 4. BACKGROUND

### 4.1. Terrien's disease

Terrien's disease is a rare form of bilateral asymmetrical corneal degeneration, characterised by a chronic, slow, and progressive thinning of the peripheral part of the cornea (16,17). Its occurrence is confined primarily to men (3:1) mostly between twenty and forty years of age. A variant form of Terrien's disease with prominent inflammatory signs occurring in young population has also been observed (16,18-22).

Because of the slowly progressive and painless property of the disease, when patients visit the hospital the degeneration often has reached an advanced stage at which the risk of spontaneous or traumatic corneal rupture becomes high (16,17,21,22). The first symptom of Terrien's disease is poor visual acuity caused by irregular astigmatism. In the early stages, the upper part of the peripheral cornea is vascularized superficially, producing a semilunar fold. This part of the cornea slowly narrows, then dilates, and becomes ectatic. The narrowed and regular corneal parts are delimited by a sharp yellowish-white border that contains lipid deposits (16,21,23,24).

In the development and progression of the disease, five stages can be distinguished (25-27):

1. Gerontoxon like marginal opacification with peripheral vascularisation.
2. An indentation appears parallel to the limbus indicating the initiation of corneal thinning in addition to the changes noted in the initial stage.
3. Thinning of the cornea progresses, but it does not reach the central part of the cornea. Ectasia of the thinned part of the cornea begins. Spontaneous or traumatic perforation of the thinned cornea and prolapse of the iris may occur.
4. The ectasia reaches the central part of the cornea, its pattern is similar to that of keratoconus.
5. Opacification of the central part of the cornea. The perforation of the cornea usually occurs before their stage.

There have been two main theories concerning the nature of this disease: an inflammatorical and a degenerative theory (28). Many investigators supported the

degenerational theory, partly based on the earlier histopathologic studies which showed little or no signs of inflammation. Other studies dealing with light, electron, and confocal microscopic investigations have reported inflammatory signs as well (25,28).

In the study of Süveges et al. was reported a histochemical and electron microscopic method for finding the pathophysiology of the disease. They demonstrated that in stage 3 of the disease, the phagocytation of the substantia propria by histiocyta-like cells that penetrated the cornea along the vessels led to marked thinning of the cornea (25).

Srinivasan et al. described in their study a nineteen years old female with unilateral Terrien's disease with spontaneous corneal perforation. They followed the patient by Orbscan and suggested that their patient had the inflammatory subtype of Terrien's disease, and the associated inflammation and subclinical epithelial changes may led to spontaneous corneal perforation (29).

Ferrari and coworkers investigated in vivo corneal changes in Terrien's disease with corneal confocal microscopy that allowed real-time visualization of fine corneal structures. They described microstructural abnormalities in a presurgical stage of the progression of the disease, when pathology specimens would not be available. They found an amorphous hyperreflective material corresponding to the lipid deposition and an irregular Bowman layer with direct examination of the peripheral lesion. They have found with anterior OCT examination, that the central cornea may allow initial signs of thinning within the central 10 mm zone close to the peripheral lesion even in cases when the clinical inspections seemed to be normal (30). The group of Ceresara reported an in vivo confocal microscopy study in Terrien's disease. In their studies, they observed inflammatory cell infiltration at the level of paralimbar conjunctiva and in the upper peripheral basal epithelium of both eyes (31).

Penetrating keratoplasty (PKP), crescentic lamellar keratoplasty, and C shaped lamellar keratoplasty have been employed in the treatment of marginal corneal degeneration earlier, but some are difficult to perform and carry high risks for the disease in the advanced stage due to very thin corneal stroma (32-35).

## **4.2. Superficial and deep corneal calcification**

There is a wide spectrum of calcium deposition in the cornea with different types ranging from superficial changes to full thickness calcification. Two types of corneal calcification come into question (36):

- a) The superficial type appears as a band shaped keratopathy and refers to calcium deposition in the Bowman layer and superficial structures (36-38). The causes of superficial calcification of the cornea are unknown.
- b) Deeper calcification of the cornea is described as calcareous degeneration and represents the other type where the calcium deposition founded in the deeper stroma including Descemet membrane. This type may exist with band-shaped keratopathy (36-38).

The etiology of corneal calcification has been classified as metastatic or dystrophic processes, although the mechanism is still unclear (38).

Metastatic calcification occurs, when there is elevation of the calcium phosphate product like in chronic renal failure, sarcoidosis, hyperparathyroidism, hypercalcaemia, hyperphosphataemia, and hypervitaminosis D. In these cases, the calcification is typically mild and restricted to the perilimbal cornea in the interpalpebral zone. The tear pH may be more alkaline as a result of evaporation and desiccation (38,39). Dystrophic calcium deposition can occur in case of superficial inflammation or tissue injury (38-44).

There are several methods to treat corneal calcifications such as mechanical removal of the calcified part of the cornea, the use of freshly prepared chelating agent combined with vigorous and frequent massage of residual deposits to completely remove dense local concentrations of calcium and the use of phototherapeutic keratectomy (45).

## **4.3. Amniotic membrane transplantation**

Intact corneal epithelium is one of the most important factors in maintaining ocular surface health. Corneal epithelial defects usually heal without any complications by rapid proliferation of the epithelial cells. Incomplete wound healing would lead to persistent epithelial defect, that can be decreased by several treatments such as artificial tear drops,

lubricants, fibronectin, and growth factors. In cases with persistent defects even after the application of expensive treatments, surgical interventions such as tissue adhesive glue, tarsorrhaphy or conjunctival flap were often tried (46-49).

The newest alternative for the management of the corneal epithelial defect or ulcer is the reconstruction of the surface with using amniotic membrane transplantation (AMT). This method has become well established as a treatment for chronic epithelial defects and conjunctival reconstruction (42).

Amniotic membrane (AM) is the innermost layer of the placenta. It consists of a single layer of ectodermally derived amnion cells, thick basement membrane, and an avascular stromal matrix. AM contains a high concentration of basic fibroblast growth factor, basement membrane components, and unknown trophic factors. These factors might be related to the expression of different anti-inflammatory proteins, inhibition of proteinase activity, exclusion of polymorphonuclear cells by subsequent apoptosis, and decrease of lipid peptidation (50-52). AM can be used as a substrate to replace damaged mucosal surfaces and has recently been used successfully for reconstructing corneal surfaces. AMT may facilitate epithelization and reduce inflammation, vascularisation, and scarring (46-54).

There are several types of AMT using one or more layers of AM as a patch, graft, or sandwich to cover corneal epithelial defects (46,51,52,55-58). In each case the AM is used epithelial side up.

- When the corneal diseases with epithelial defects have no or only shallow stromal defects, patch onlay can be used. The AM is sutured from limbus to limbus over the peripheral epithelial remnants and the centrally denuded stroma. The local epithelium is expected to grow under the AM and the epithelial defect should close (55,59,60).
- The other type of AM is the single-layer graft inlay that can be used for shallow stromal defects. In this case the graft is fixed in this superficial defect with interrupted 10-0 nylon sutures in the border of the corneal ulcer. In this type of AMT, the epithelium is expected to grow over the AM, providing a new basement membrane (55,59,60).
- For deep stromal defects, multilayer-graft inlay can be used. In case of this corneal epithelial defect, smaller portions of the AM could be placed layer by layer into the

ground of the ulcer, which is filled without sutures before a superficial graft is sutured to the periphery of the ulcer. In this case, the epithelium is expected to grow over the uppermost layer of this multilayer graft.

- The sandwich is a special combination of the previous techniques. It consist of one or more grafts and a patch on the top. The epithelium is expected to grow under the patch but over the uppermost graft (55,59,60).

AMT may be beneficial in the treatment of persistent epithelial defects after penetrating keratoplasty, especially when applying the sandwich technique (61,62). AM can be used as a trigger in cases of Mooren' s ulcer which do not heal with intensive immunosuppressive regimens alone (63), for intraoperative conjunctival repair during trabeculectomy (64), in children with symblepharon and pannus (65), and for symptomatic bullous keratopathy as well (66).

#### **4.4. Keratoconus**

Keratoconus (KC) is still an enigmatic disease that remains an era of wide-ranging, dynamic, international research although it was first described more than 150 years ago (67). KC is a noninflammatory, progressive, corneal degeneration. The main characteristic of KC is a bilateral thinning of the cornea without neovascularisation. The development of a corresponding protrusion with an apex located centrally or in an inferior exentric position could be seen (68,69). This disorder impresses both eyes, although only one eye may be affected initially. The high degree of irregular astigmatism, corneal thinning, and scarring that can occur with KC may result in severe visual impairment (70,71). The incidence is approximately 1/2000 in general population (68,71).

The pathology of KC remains unclear. Predisposition to developing KC is related to genetic, constitutional and environmental factors, mechanical and surgical eye rubbing, or other metabolic imbalances (69,72).

The diagnosis of KC is based on the detection of changes in the corneal curvature and corneal thickness, which is thinner than usual (69,73).

Colin (70) and Krumeich and coauthors (74) have proposed a clinical classification of four stages of KC based on astigmatism, corneal power, corneal transparency, and corneal thickness:

1. Excentric corneal steepening, induced myopia and/or astigmatism  $< 5.0\text{D}$ , corneal radii  $\leq 48.0\text{D}$ , Vogt's striae, no scars.
2. Induced myopia and/or astigmatism  $5.0\text{D} - 8.0\text{D}$ , corneal radii  $\leq 53.0\text{D}$ , no central scars, corneal thickness  $\geq 400\mu\text{m}$ .
3. Induced myopia and/or astigmatism  $8.0\text{D} - 10.0\text{D}$ , corneal radii  $> 53.0\text{D}$ , no central scars, corneal thickness  $200\text{-}400\mu\text{m}$ .
4. Refraction not measurable, corneal radii  $> 55.0\text{D}$ , central scars, perforation, corneal thickness  $200\mu\text{m}$ .

The symptoms are widely variable and depend on the stage of the progression of the disorder. In the early stage of the disease there may have been no symptoms at all. In the advanced stage of the disease, the shown contribution of the stromal thinning, conical protrusion, Fleischer's ring, and Vogt's striae may be detectible by slit-lamp. Munson's sign and Rizzuti's sign could be the external signs in a severe form of KC (69,75).

PKP is the most commonly used surgical option for advanced cases of KC which can not be successfully managed with contact lenses (69,73,76).



## 5. PATIENTS AND METHODS

### 5.1. Patients

Three groups of patients were investigated with corneal diseases, like Terrien's disease, corneal calcification, and keratoconus.

Two patients (one female and one male) were diagnosed with Terrien's disease during the last ten years at our Department with a mean age of 43 years. Patient No.1. was 21 years old and she didn't have any systemic diseases. Decreased visual acuity was observed as her first symptom when she was 10 years old. Before her first visit to our Department, she had conjunctivitis and keratoconjunctivitis several times. Patient No. 2 was a 64-year-old man. He was healthy. His ophthalmological problem started with recurrent keratoconjunctivitis in both eyes at the age of 15 years. At his first visit to our Department, he had an acute keratoconjunctivitis on his right eye.

In addition to the above introduced cases, we treated three patients (three females) with persistent, non healing corneal ulcer and calcification with a mean age of 70 years. In this group, patient No.1. was a 80-year-old woman. Cataract extraction with phacoemulsification and posterior chamber lens implantation were carried out on her left eye before development of corneal ulcer that was caused by HSV1. Patient No.2 was a 77-year-old woman with herpes zooster infection causing non healing corneal ulcer. The patient was treated with antibiotic and antiinflammatory eyedrops that contained phosphate buffer for more than a half year.

The keratoconus study consisted of 147 patients with KC. Sixty-six were excluded from the investigation because they were pleased with their spectacle corrections. Among the remaining 81 patients, 65 patients (42 males and 23 females) received contact lenses for their 95 eyes and 16 patients had penetrating keratoplasty (PKP). Thirty subjects wore contact lenses on both eyes and 35 patients only on one eye. The mean age of the patients in the contact lense group was 29 years. Two patients needed repeated PKP because of complications. The mean age of the patients in the keratoplasty group was 24 years. The mean follow-up time was 38 and 36 months for patients with contact lenses and with KC, respectively.

## **5.2. Ophthalmic examinations**

Patients with Terrien's disease, corneal calcification, and keratoconus underwent basic ophthalmic examinations, including visual acuity tests using Kettesy's decimal visual chart, intraocular pressure with Goldmann applanation tonometry, slit-lamp biomicroscopic examination of the anterior segment of the eye using HOYA H-100 (7900, Japan) and Haag Streit (Liebefeld-Bern, Switzerland) slit lamps, and ophthalmoscopic examination with Welch-Allyn 11620 direct ophthalmoscope (Shamateles Falls, NY, USA) and 90 D aspheric ocular lense (060123, Bellevue, WA, USA).

## **5.3. Ultrasound biomicroscopy**

The corneal findings of all three investigated groups were evaluated with UBM. We used two models in these studies: the Zeiss Humphrey, UBM Model 840 and the Sonomed, VuMax 35-50 MHz.

The technology of UBM is based on the 35 to 100 MHz transducer incorporated into a B-mode clinical scanner (8). Higher frequency transducers provide finer resolution of the more superficial structures, lower frequency transducers provide greater depth of penetration with less resolution. The commercially available unit operates at 50 MHz and provides lateral and axial physical resolution of approximately 50 and 25  $\mu\text{m}$ . Higher frequencies, while potentially offering even finer resolution, are more affected by absorption in ocular tissues. Tissue penetration is at least 5 mm. The scanner produces a 5x5 mm field with 256 vertical image lines at a scan rate of 8 frames /s. The real time image is developed on a video monitor (2,8).

Before the ultrasound examination, the examiner applies topical anesthetic drops to the examined eye and inserts an eye-cup under the eyelid. After that, the eye is covered with an eye-cup filled half with coupling solution. The operator slowly moves the probe from one end of the scleral cup to the other, acquiring longitudinal and transversal scans.

## **5.4. Human amniotic membrane preparation and preservation**

For AM preparation, the human placenta was obtained after elective caesarian delivery. The female patients at the Department of Gynaecology and Obstetrics gave written consent regarding their approval for this procedure.

Serological tests were performed to exclude human immunodeficiency virus and hepatitis virus type B and C. Under laminar air hood, the placenta was cleared of blood clots with sterile Hank's balanced salt solution containing fungison, cefuroxim, cefamandol, clindamycin and vancomycin. The amnion was separated from the rest of the chorion by blunt dissection through the potential spaces between these two tissues and flattened onto a nitrocellulose paper with epithelial membrane surface up. The paper with the amniotic membrane was cut into 5.0 x 5.0 centrimeter size, then put into Hank's solution and dimethyl sulfoxide (DMSO) 1:1 mixture. After this preparation, the amniotic membrane was stored at – 80°C. Before using, we had to wait for 30 minutes to defreeze.

## **5.5. Surgical intervention**

### *5.5.1. Terrien's disease*

In these patients, the peripheral full thickness keratectomy using the method of Alberth and Süveges was performed with retrobulbar anesthesia (21). The upper semilunar thinned and narrowed part of the cornea was cut out, and basal iridectomy was performed. We sutured the corneal wound by 8/0 nylon running sutures.

### *5.5.2. Superficial and deep corneal calcification*

Before the operation the patients were anesthetized by retrobulbar injection of 2 ml of 2% xylocaine and 3 ml of 0.5% bupivacain. The calcified part of the cornea was cut out. In cases of superficial corneal defects, a single layer membrane was used to cover the corneal gap by interrupted 10/0 nylon sutures. In deep calcification, sandwich technique AMT was used (46,48,49). Three pieces of the AM were trimmed to fit the shortage of the cornea, and the final layer was applied to cover the ulcer bed. 10/0 interrupted nylon sutures were placed to anchor the AM grafts to the cornea.

### *5.5.3 Keratoconus*

Penetrating keratoplasty (PKP) surgeries were performed with retrobulbar injection of 2 ml of 2% xylocaine and 3 ml of 0.5% bupivacain or general anesthesia. All of the recipient and donor corneas were trephined with hand hold trephines by one surgeon. Graft diameters ranged from 7.0 to 7.1 mm, the host diameter was 7.0 to 7.1 mm. The graft was sutured by a single line 10/0 nylon running sutures.

## **5.6. Histology**

The affected corneal parts were cut out with diamond knife and immersion-fixed in 2.5% phosphate-buffered glutaraldehyde (pH=7.4). The corneal pieces were embedded in Durcupan<sup>®</sup> (Merck Ltd) and semithin (0.5  $\mu$ m thick) as well as ultrathin sections were cut on a Leica UltraCut-R ultramicrotome (Leica GmbH). The semithin sections were stained with methylene blue and fuchsin or with Alizarin Red after deosmication and removal of resin. Ultrathin sections were stained with lead nitrate, contrasted with uranyl citrate and investigated in a JEOL JEM 1010 electron microscope (JEOL Ltd, Tokyo, Japan).

## **5.7. Corneal topography**

In this study we used the TMS 1 Topographic Modelling System. This projects illuminated concentric rings which provide corneal reflections at approximately 180 micron intervals from a central dot at the apex. The patient should be comfortably positioned on the adjustable chin-rest and asked to look into the cone and fixate at the blinking light. Pressing the joystick button by the operator, we can see a video from the patient's cornea. This image is then analyzed by a program (MSWIN 4.1) which voluntifies the location of circumference points around each ring reflection.

## **5.8. Statistical analysis**

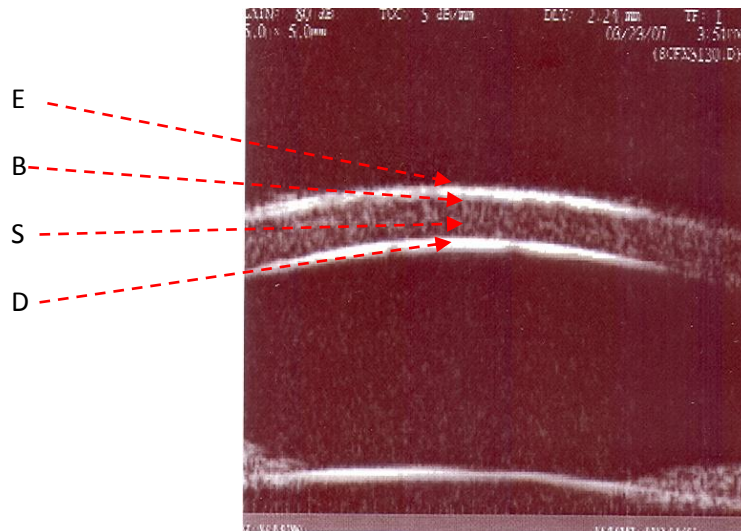
Statistical significance was assessed by the Student *t* test. The results were considered significant, if  $p \leq 0.05$ .

## **5.9. Ethics**

This study was conducted in accordance with the Declaration of Helsinki. This medical research was subject to ethical standards that promote respect for all human beings and protect their health and rights. It conformed to generally accepted scientific principles based on a thorough knowledge of scientific literature, other relevant sources of information. The experimental protocol was approved by the ethical review committee of the University of Szeged. The right of research subjects to safeguard their integrity was always respected.

## 6. RESULTS

We compared the UBM pictures of the eyes of patients with Terrien's disease, corneal calcification, and keratoconus with the UBM picture that represents the regular structure of the cornea (Figure 1). Examining the normal cornea with UBM, epithelial layer, Bowman layer, stroma, and Descemet membrane/endothelium are well resolved (7,8). The epithelium (E) appear as a layer with very slight discontinuation and a regular surface. Below the epithelium is a continuous, highly reflective zone corresponding to the Bowman layer (B). The stroma (S) is a uniform, intermediately reflective region of the cornea. Descemet membrane (D) cannot be individually resolved from the endothelial layer and appears as a line at the posterior extent of the cornea (5,12).



**Figure 1.** Regular structure of the cornea by UBM

E: epithelium; B: Bowman layer;  
S: stroma; D: Descemet membrane

### 6.1. Terrien's disease

#### 6.1.1. Standard ophthalmological examinations

At the time of the first visit of patient No.1. at our Department, her best corrected visual acuities (BCVA) were 2/50 and 50/50 on her right and left eyes, respectively. The intraocular pressure (IOP) was 7 Hgmm, and 13 Hgmm. The keratometrical values were 51.25x38.25 and 44.75x45.5 on her right and left eyes, respectively. One year later, we performed a peripheral full thickness keratectomy in her right eye in order to prevent the

additional thinning of her peripheral cornea and the increase of against-the-rule astigmatism. The postoperative period was without any complications and any signs of inflammation or infiltration. Two months after her operation, the BCVA was 30/50 on her right eye. Her follow up study was not possible because she did not show up at our Department since then.

At the time of the first visit of patient No.2. at our Department, his BCVA was 8/50 and 45/50, his IOP was 13 Hgmm and 14 Hgmm, his keratometical values were 61.5x143/45.5x53 and 41.75x100/49.5x10 on his right and left eyes, respectively. Thinned and ectatic part of the cornea was delimited by a sharp yellowish-white border. Because of the danger of a sudden perforation, we performed a full thickness keratectomy and peripheral iridectomy on his right eye. The postoperative period was without any complications.

Two months after his operation the BCVA was 40/50 on his right eye, and his IOP was 12 mmHg.

Fourteen months after the operation, the BCVA was 20/50. The sutures were then removed and his BCVA improved to 40/50, and his IOP was 11 mmHg. The right eyebulb displayed no alteration by slit-lamp examination.

His last visit was 18 months after his operation. We observed a distinct decrease in transparency towards the central part of the cornea.

#### *6.1.2. Slit-lamp and ultrasound biomicroscopy*

Figure 2 shows the slit-lamp picture of the right eye of patient No.1.. The upper semilunar part of the cornea has thinned with marginal opacification and peripheral vascularisation.



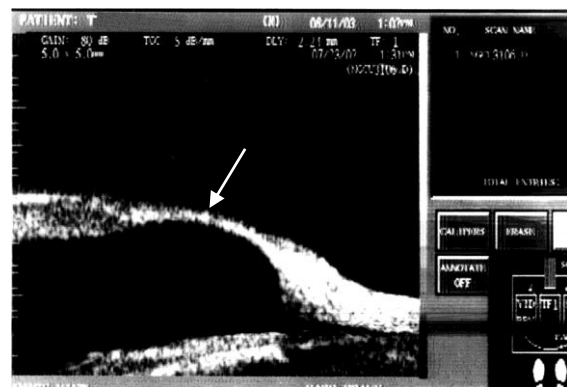
**Figure 2.** Slit-lamp image of patient No.1. right eye at her first visit.

Figure 3A shows an UBM photograph of the cornea of patient No.1. with the characteristic thinned marginal semilunar hollow and peripheral ectatic part at the time of her first visit at our hospital. The thickness of the thinned, ectatic part of the peripheral cornea was 0.221 mm.

Figure 3B shows an UBM picture of the cornea of patient No.1. after one year of her first visit. The thickness of the ectatic part was 0.156 mm in contrast to the intact part, which was 0.678 mm thick. The epithelium of the cornea shows high reflectivity at the ectatic part, the stroma is thinned significantly. The basement membrane and the Bowman layer could not be separated by echography. The width of the ectatic part was 2.163 mm.

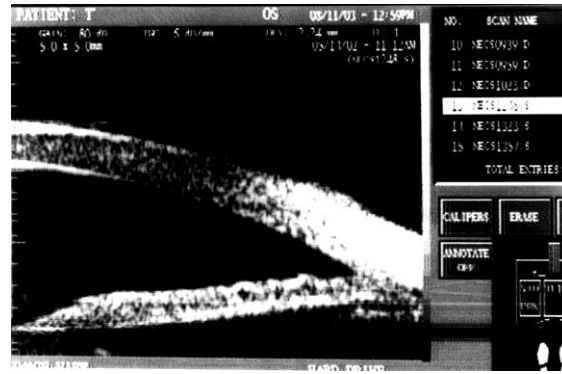


**Figure 3A.** UBM picture of the cornea of the right eye of patient No.1. at the time of her first visit at our hospital.



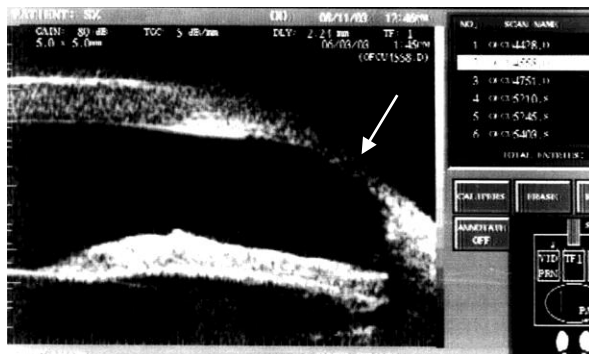
**Figure 3B.** UBM picture of the cornea of the right eye of patient No.1. right before surgery.

Figure 3C shows the UBM picture of the left eye of patient No.1.. Neither the Bowman layer, nor the epithelium with high reflection can be detected with UBM. This picture shows an early stage of Terrien's disease. The thinning and the ectatic process of the cornea cannot be seen, although the superficial and deep arteries of the radial course were visible by slit-lamp. The width of the marginal part was 0.547 mm.

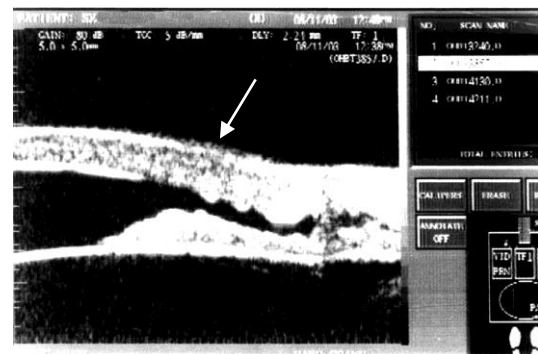


**Figure 3C.** UBM picture of the peripheral cornea of the left eye of patient No.1. at the time of the first visit.

Abnormal structure of the cornea in the ectatic part could be detected with UBM in case of patient No.2. (Figure 4A). The corneal thickness was 0.124 mm at the ectatic and thinned part; the length of the affected area of the cornea was 2.163 mm perpendicular to the limbus. Ten months after the operation, his UBM picture showed regular structure of the epithelium and some irregularity of the endothelial surface due to the surgery (Figure 4B).



**Figure 4A.** UBM picture of the right cornea of patient No.2. before the operation.



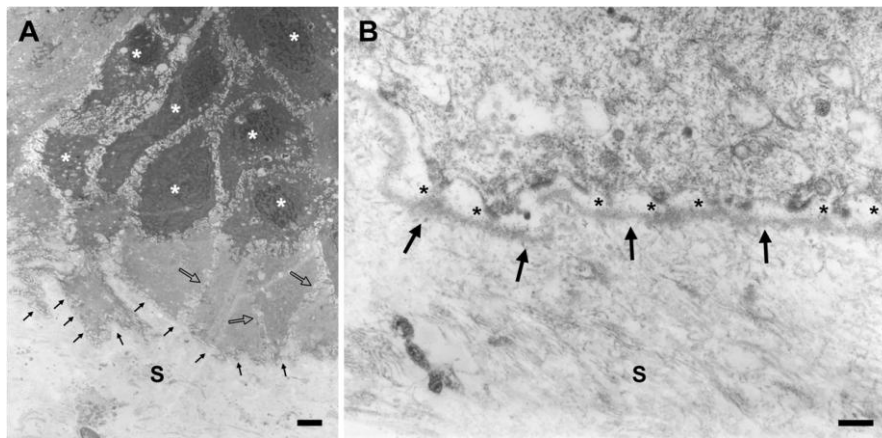
**Figure 4B.** UBM picture of the right cornea of patient No.2. after the operation.

### 6.1.3. Histopathology

During the histological processing of the excised corneal pieces, semithin and ultrathin sections were cut from Durcupan-embedded tissues. The semithin sections of the operated corneal part showed a pathological corneal structure. The corneal epithelium displayed thick and mosaic-like arrangement of the epithelial cells. The stroma appeared thin and irregularly built (not shown). Electron microscopic investigations showed that the epithelial cells and their nuclei were shrunken, some of them being dark (asterisks in Figure 5A), and the



extracellular space was expanded. The epithelial adhesion structures appeared distorted (empty arrows in Figure 5A). The Bowman layer could not be detected on the excised parts, and the corneal stroma lost its original and strictly organized structure (Figure 5B). The stroma contained irregularly placed fibrocytes and debris phagocytosed by macrophages. The Descemet membrane was also thinner than usual (not shown).

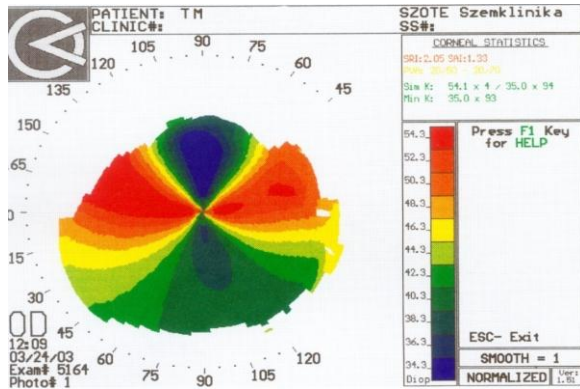


**Figure 5.** Electron microscopic microphotographs of the excised corneal piece.  
 A. Epithelial cells and their nuclei were shrunken; extracellular space was expanded.  
 B. Bowman's layer could not be detected on the excised parts, and the corneal stroma lost its original and strictly organized structure.

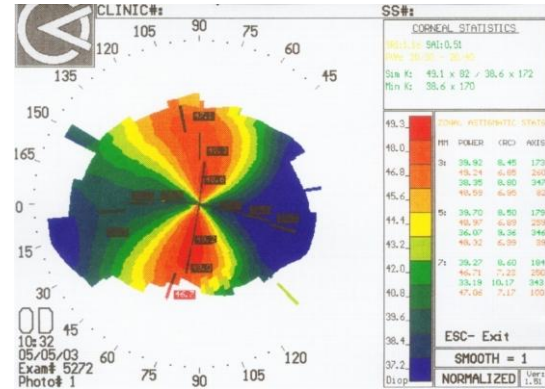
#### 6.1.4. Corneal topography

The topographic map of the right eye of patient No.1. showed irregular astigmatism (Figure 6A). According to the upper cornea, between 11:30 and 1:30 h a marked deepening could be seen, where the refraction power was 34.0-42.0D. This band of the cornea was bordered by a steep corneal part between 1:00 and 3:00 h and 9:00 and 11:00 h. The lower part of the cornea between 4:30 and 7:30 h had regular refraction power (40.0-42.0D).

One month after the operation the topographic map showed regular astigmatism. The difference between the smallest and largest curvature of the cornea was approximately 11.0D that was 19.0D before the operation. This condition manifested as an asymmetrical bow-tie. The nasal, most flat quadrant of the cornea on the map was 37.2D, and the lower, steepest part of the cornea was 49.0D (Figure 6B).



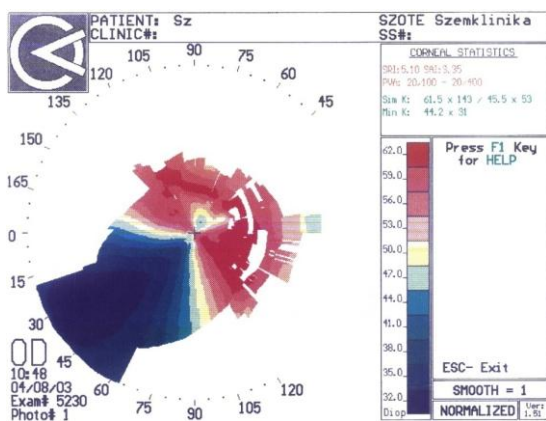
**Figure 6A.** Cornea map of the right eye of patient No.1. before surgery.



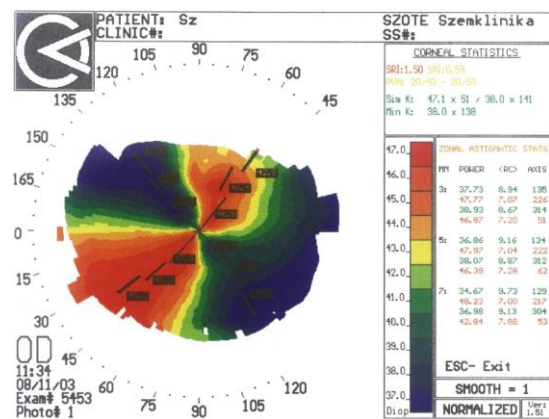
**Figure 6B.** Cornea map of the right eye of patient No.1. after surgery.

The topographic map of the right eye of the patient No.2. could not be evaluated before his operation because of the high degree irregular astigmatism (Figure 7A). It could be seen on the figure that the difference between the steepest and the most flat surface of the cornea was almost 30.0D. The tear film was defected from the irregularity of the cornea's surface.

Three months after the operation, the topographic map showed a rotated symmetrical bow-tie shape. There was no difference between the largest and the smallest refractive power, both were 10.0D, and his visual acuity was well corrected with cylindrical lenses (Figure 7B).



**Figure 7A.** Cornea map of the right eye of patient No.2. before surgery.



**Figure 7B.** Cornea map of the right eye of patient No.2. after surgery.

## **6.2. Corneal calcification and treatment with amniotic membrane transplantation**

### *6.2.1. Standard ophthalmological examinations*

Patient No.1. from the group of corneal calcification had a cataract extraction with phacoemulsification and posterior chamber lens implantation in June, 2007 on her left eye. After the operation she received dexamethasone and ofloxacin drops five times daily and dexpanthenol ointment for some epithelial defects. One month later, she returned to our Department with a herpetic ulcer on the same eye caused by HSV1, and the visual acuity decreased to 3/50. We prescribed fluorometholon drops, acyclovir ointment and 400 mg of acyclovir orally five times daily for one month.

Patient No.2. visited our Department with herpes zoster infection of the 1st trigeminal area involving the right cornea in February 2007. At that time, her best corrected visual acuity was 20/50 on her right, and 50/50 on her left eye. This person was treated by us with acyclovir ointment and 400 mg of acyclovir orally both of them five times daily. We also gave neomycin and tubocin drops to prevent bacterial infection. The UBM examination was impossible because of the patient's poor compliance.

The keratitis progressed into corneal ulcer and was not healed by July, 2007. Since the patient did not agree to have PKP, we performed a single layer AMT. The postoperative therapy included ofloxacin, and 800 mg acyclovir orally both of them five times daily for six weeks.

The AM dissolved shortly after the operation, the visual acuity became 30/50, and the corneal ulcer healed. Since she did not have any pain, the antiviral therapy was ceased.

The patient returned to our Department in March, 2008 with a newly formed, deep corneal ulcer. The epithelial defect had a yellowish-white, demarcated plaque in the central-paracentral part of the cornea with deep stromal involvement. Due to the decreased transparency of the cornea the visual acuity worsened, we cut out the central corneal part with decreased transparency and performed AMT using sandwich technique. Complete corneal re-epithelization occurred two weeks after the second operation.

Patient No.3. was born with congenital glaucoma and cataract. Due to these problems, she had several operations at different hospitals. She used  $\beta$  blocker, corticosteroid, and

lubricant drops for more than 20 years. The best corrected visual acuity was 1/50 on her left eye during the last ten years.

Patient No.3. showed up at our Department in December, 2009, as she had decreased transparency of the left cornea. The corneal picture showed a demarcated whitish plaque in the center of the cornea. Since the plaque was very disturbing for her, she wanted to have an operation. We decided to perform AMT using single layer graft inlay after cutting out the demarcated corneal part. The patients' data are summarized in Table 1.

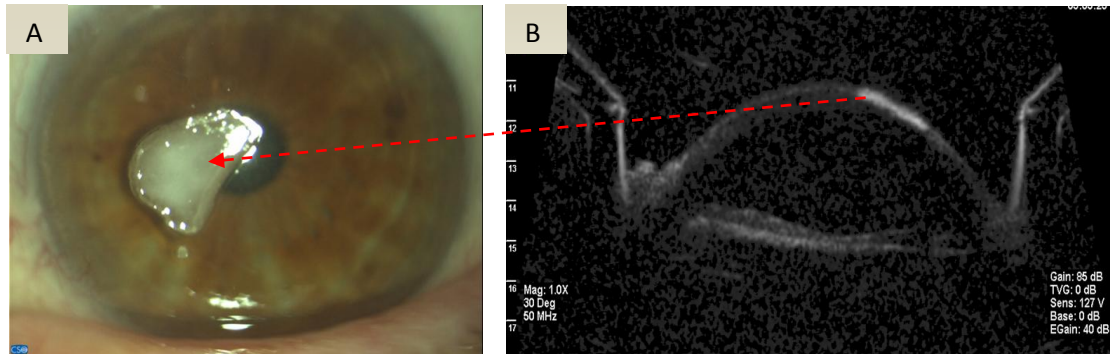
**Table 1.** Clinical data and treatment of patients diagnosed with corneal calcification.

Patients	Age	Sex	Cause	Therapy	Follow-up time (month)	Type of operation	Histology
1.	80	female	cataract and dry eye, herpes simplex keratitis	dexamethasone, ofloxacin, neomycin, acyclovir, tubocin	24	AMT using sandwich technique	stromal calcification
2.	77	female	herpes simplex keratitis	acyclovir, dexamethasone, ofloxacin	40	AMT using sandwich technique	stromal calcification
3.	54	female	congenital glaucoma	timolol, dexamethasone, lubricants	8	AMT single layer graft inlay	stromal calcification

#### 6.2.2. *Slit-lamp and ultrasound biomicroscopy*

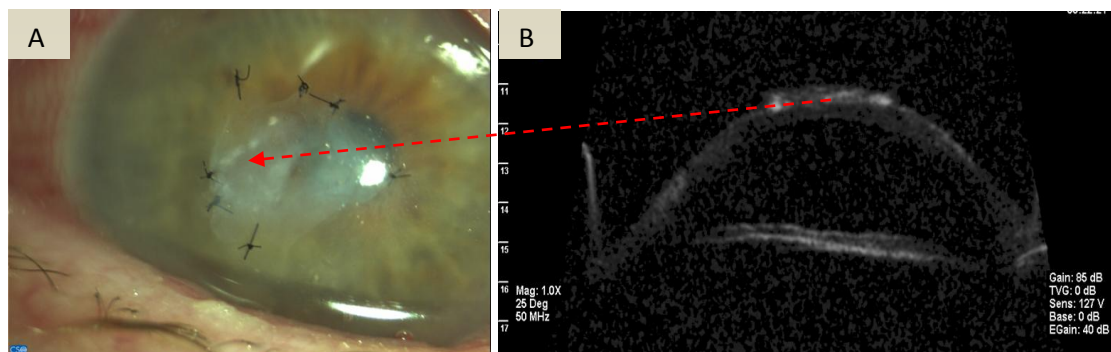
Figure 8 shows the slit-lamp and the UBM pictures of patient No.1. before the operation. The calcified part of the paracentral cornea can be seen in Figure 8A and has yellowish-whitish color on the picture. Figure 8B presents the UBM picture of this state, where the calcified cornea can be seen on the transverse biomicroscopic picture. The central width of the cornea is 565  $\mu\text{m}$ . There is high reflectivity on the paracentral cornea that it is well demarcated from the regular part of the cornea. The extension of this part is 2.68 x 3.45 mm, and the deepness is 248  $\mu\text{m}$ . The UBM structure of the cornea cannot be recognized in the calcified part, only homogeneous, highly reflective echos can be seen. She returned to our Department one month later with a non-healing ulcer with supposed calcification. It caused visual impairment, so we cut the calcified part out and performed a sandwich technique AMT. The patient was followed up daily after the AMT procedure for a week. Thereafter she

did not come to our Department because of her Alzheimer disease. At her last visit, the visual acuity was 10/50 on the left eye.



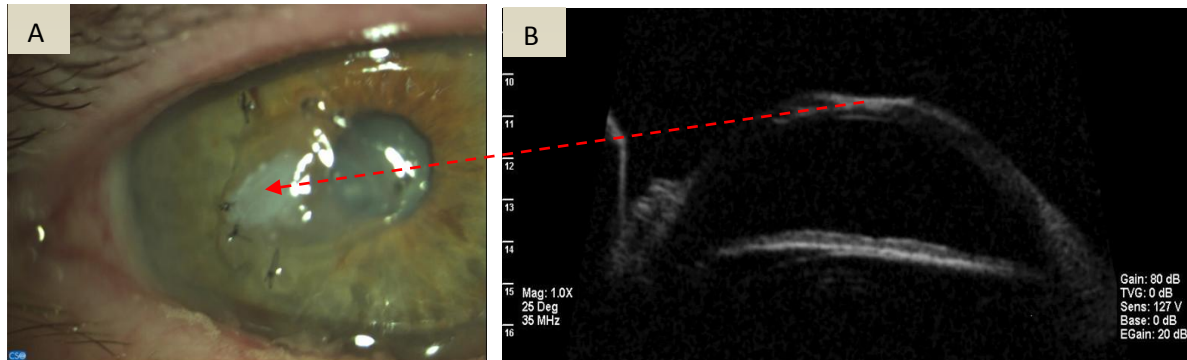
**Figure 8.** Slit-lamp and UBM pictures before surgery of patient No.1. with corneal calcification. **A.** Calcificated part of the cornea by slit- lamp. **B.** UBM picture of the calcificated cornea.

Patient No.2. had pain in her right eye and poor compliance before her first operation, that is why we could not examine her with UBM at that time. One week after her reoperation the AM still could be seen on the top of the corneal excised part on the slit-lamp picture (Figure 9A). On the UBM picture of the transverse segment of the cornea, the transplanted AM was parallel, foliated, and had a rough surface and higher reflectivity than the other part of the cornea (Figure 9B).



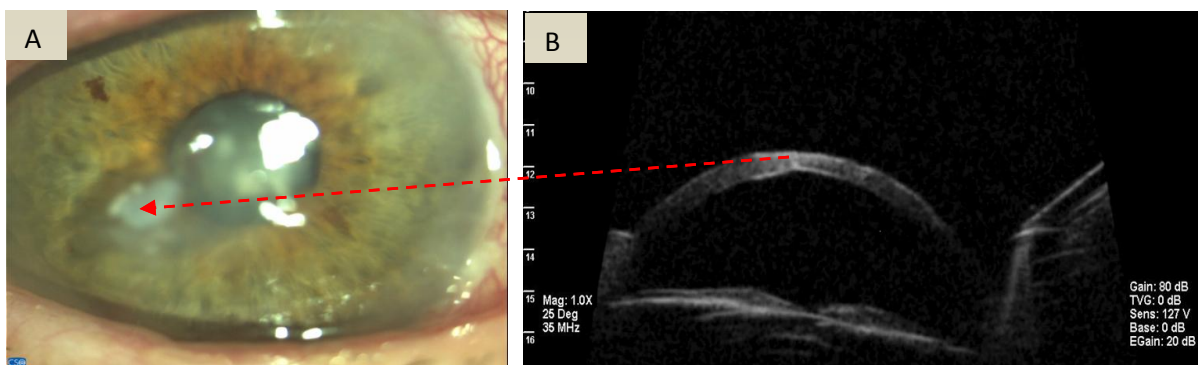
**Figure 9.** Slit-lamp and UBM pictures of patient No.2. with corneal calcification one week after surgery. **A.** Surface of the cornea by slit-lamp. The edges of the AM are attached by 10/0 sutures. **B.** UBM picture of the cornea with AM attached to it.

Two weeks after the reoperation the AM is partly built into the cornea, and some of the sutures are shown intact (Figure 10A). A deep and shrunken part of the cornea covered by AM could be seen on the UBM picture (Figure 10B).



**Figure 10.** Slit-lamp and UBM pictures of patient No.2. with corneal calcification two weeks after surgery. **A.** Surface of the cornea with the AM partly built into the structure of the cornea. **B.** UBM picture of AM shows high reflectivity on the surface of the cornea.

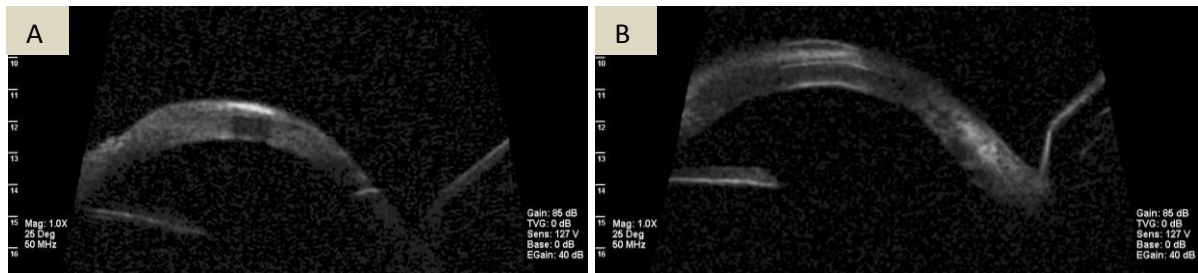
Six month after the operation, the transparency of the cornea is partly decreased, and the AM appears to be integrated (Figure 11A). The cornea is homogenous and has high reflectivity on the UBM picture. The surface is smooth (Figure 11B).



**Figure 11.** Slit-lamp and UBM pictures, six month after surgery of patient No 2. with corneal calcification. **A.** Part of the cornea with decreased transparency. The epithelium is intact, and the AM is not separatable. **B.** UBM picture of the cornea with homogenous structure. High reflectivity can be seen.



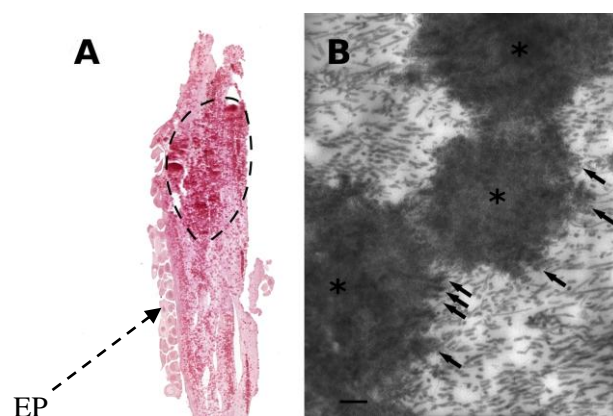
Figure 12 shows the UBM pictures before and two weeks after operation of patient No.3. Figure 12A demonstrates high reflection of the corneal epithelium. This well demarcated central part of the cornea is 176  $\mu\text{m}$  deep, and the extension is 1.53x 0.782 mm. Figure 12B shows the postoperative corneal part with transplanted AM. The surface of the cornea is uneven.



**Figure 12.** UBM picture of patient No.3. with corneal calcification, before and two weeks after the operation. **A.** Shading in the structure of the cornea. The high reflection part of the cornea is well demarcated. **B.** Uneven surface of the cornea after the AMT.

### 6.2.3. Histopathology

Most of the removed corneal parts appeared as calcified stroma (Figure 13A) with an irregular epithelium lining. Some parts showed signs of calcification with less dense. Ultrastructurally, the granular deposits were strongly electron dense with a typical appearance of calcium crystals. At the outer surface of the granular deposits spine-like processes were observed (Figure 13B).

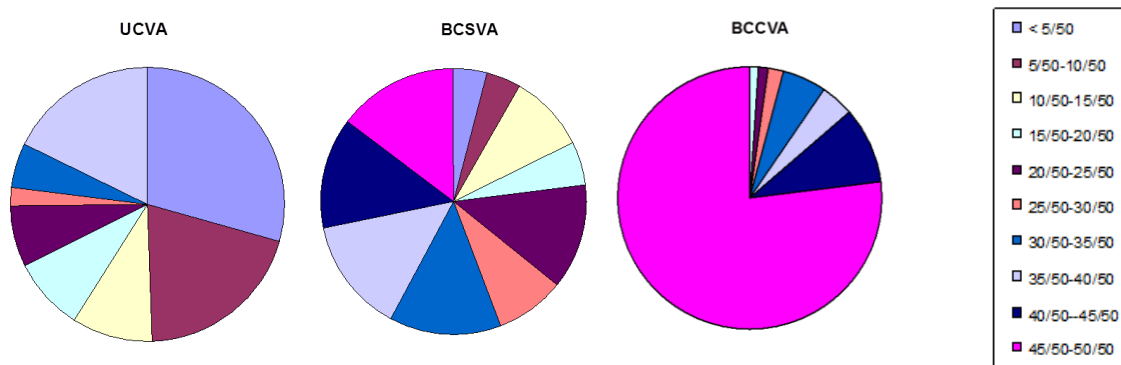


**Figure 13.** Photomicrographs of calcified tissue in the removed corneal stroma (EP=epithelium). **A.** Strong calcification in a smaller tissue fragment in form of dense granular deposits (semithin section, Alizarin Red staining). The area outlined by broken line displays the most dense calcification in the section. **B.** Electron microscopic photograph shows three electron dense granules (asterisks) embedded in a meshwork of irregularly organized collagen fibres. The outer surface of the granules is covered by possibly newly formed spine-like processes (arrows), showing typical appearance of calcium crystals. Scale bars A = 50  $\mu\text{m}$ , B = 200nm.

### 6.3. Keratoconus

#### 6.3.1. Visual acuity

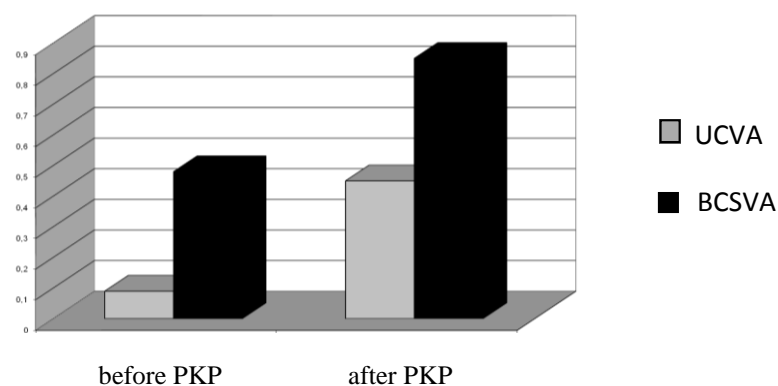
Visual outcome was assessed in terms of uncorrected visual acuity (UCVA) and the best corrected visual acuity with spectacles (BCSVA), and with contact lenses (BCCVA).



**Figure 14.** Visual acuity of the patients with keratoconus in cases without any corrections (UCVA), with spectacles (BCSVA), and with contact lenses (BCCVA). The visual acuities are presented in percentage.

Figure 14 shows the visual acuity in cases of patients without any corrections, and also with spectacles and contact lenses. Thirty of the examined 65 patients had contact lenses on both eyes. Those 35 patients, who had contact lens on only one eye had good enough visual acuity on their other eyes. The visual acuity of 30% and 20% of patients with keratoconus without any corrections were <5/50, and 5/50 - 10/50, respectively. The vision of 4% and 14% of the KC patients with spectacles were <5/50 and 45/50 - 50/50, respectively. The best visual acuity was found with contact lenses: 45/50 - 50/50 in 70 % of the cases.

The visual acuities before and after PKP are displayed on Figure 15. A significant improving is shown in UCVA and BCSVA after PKP ( $p < 0.05$ ).



**Figure 15.** Visual acuities of the keratoconic patients before and after PKP.



Table 2 demonstrates that astigmatism gradually and progressively decreased after PKP. The mean astigmatism was 3.15 D (maximum: 6.0 D) and 3.22 D (maximum: 14.0 D) 12 and 24 months after PKP, respectively. Sutures removal in four cases, wedge resection in two cases, relaxed incision in three cases were performed to reduce the astigmatism. Four patients needed rigid contact lenses after PKP to improve visual acuity.

**Table 2.** Improvement in astigmatism after PKP.

Patient No.	Before PKP (D)	Months after PKP (D)				
		1	3	6	12	24
1	10.0	1,00	6,00	4,00	4,00	2,00
2(1)	8.0	3,00	2,00	2,00	2,00	1,00
2(2)	4.0	1,00	3,00	6,00	6,00	4,00
3	8.0	6,00	4,00	4,00	4,00	0,00
4	10.0	6,00	6,00	6,00	6,00	6,00
5	12.0	4,00	4,00	4,00	4,00	4,00
6	8.0	3,00	3,00	6,00	4,00	4,00
7(1)	3.0	4,00	4,00	0,00	0,00	0,00
7(2)	3.0	2,00	2,00	5,00	1,50	6,00
8	6.0	2,00	0,00	1,00	6,00	14.0
9(1)	4.0	3,00	3,00	3,00	1,50	1,50
9(2)	2.0	5,00	5,00	3,00	0,50	0,00
10	5.0	2,00	2,00	5,00	5,00	9,00
11	3,50	2,00	2,00	2,00	3,00	3,00
12	6.0	1,00	2,00	2,00	2,00	5,00
13(1)	3.0	1,00	1,00	2,00	2,00	3,00
13(2)	6.0	1,00	1,00	2,00	2,00	3,00
average		2,76	2,94	3,35	3,15	3,22

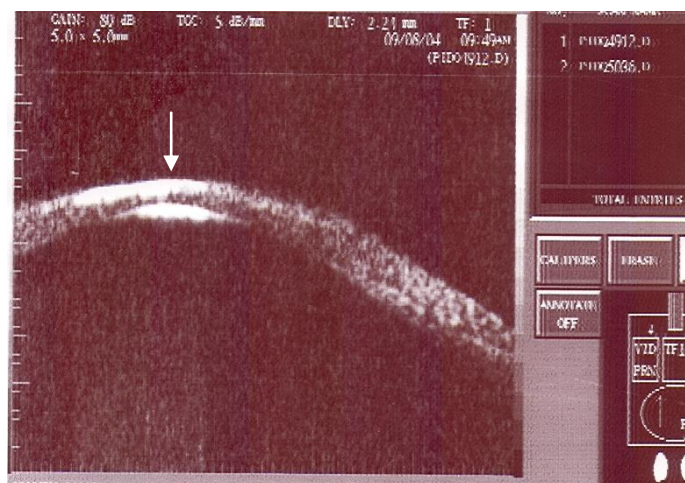
Table 3 shows the complications after PKP. No intraoperative complications occurred. The early problems occurred within one month after PKP. One patient had hypotony because of wound problems, four patients had inflammation of the anterior segment, two patients had anterior synechie, one patient had incongruence between the graft and host cornea, and one patient had deposits on the sutures. The late complications occurred after one month. One patient had uveitis, one had problems with his sutures, and one patient had allograft rejection. Epithelial reaction was defined by subepithelial infiltrates in a graft that had previously been clear.

**Table 3.** Postoperative complications of the keratoconic patients after being treated by PKP.

Appearance of complications after PKP	Signs	Number of patients
Early	hypotony	1
	iridocyclitis	4
	anterior synechia	2
	incongruation of the graft	1
	deposits on the sutures	1
Late	uveitis	1
	rejection	1
	suture problems	1

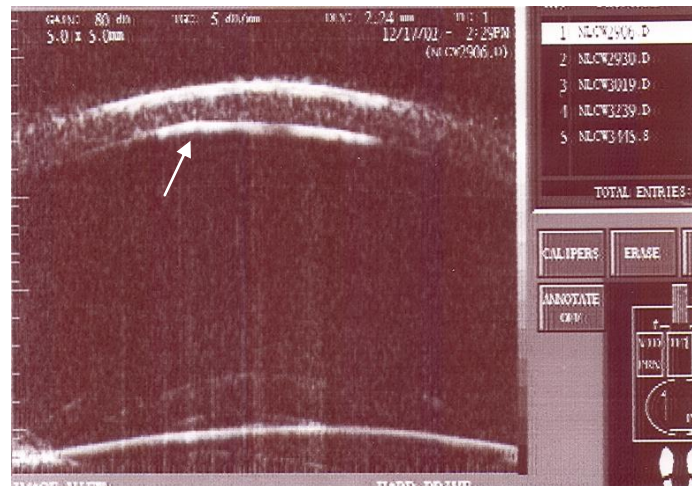
### 6.3.2. *Ultrasound biomicroscopy*

Figure 16 shows the special signs of KC with UBM right after the acute stage. The paracentral coning has a widened highly reflective epithel and the stroma is extremely thinned. The structure of the cornea is unrecognizable at the ectatic part of the cornea. The thickness of the cornea is 0.236 mm. The endothel is thickened.



**Figure 16.** UBM picture of a keratoconic cornea right after the acute stage. Arrow is at the paracentral coning.

The UBM corneal figure of a patient wearing contact lenses because of KC is presented on Figure 17. The thickness of the cornea is 0.464 mm. The epithel is a highly reflective line on the surface of the cornea. The reflective line next to the anterior chamber is the endothel and the Descemet membrane together. The lines of the epithel, the endothel and Descemet membrane are not parallel to each other; the second one shows a little breakage on its curve. The stroma has regular structure.



**Figure 17.** UBM picture of a patient with keratoconus wearing contact lenses. Arrow is at the breakage of the reflective line of the endothel and the Descemet membrane.

Figure 18. demonstrates the picture of KC after PKP. The thickness of the cornea is 0.547 mm. The highly reflective line acrossing the cornea is the suture line (10/0).



**Figure 18.** UBM picture of the keratoconic cornea after penetrating keratoplasty. Arrow is at the suture line.

Figure 19. shows a picture after PKP with complications. The line of the endothel-Descemet membranes is irregularly shaped. The highly reflective echos are due to the inflammatory deposits on the sutures. The width of the cornea is 0.534 mm, the epithel is a smooth line, and the stroma has normal regularity.



**Figure 19.** UBM picture of a cornea after PKP with inflammatory deposits on the sutures. Arrow is at the inflammatory deposits.

## 7. DISCUSSION

High resolution UBM is an excellent echographic method for the examination of the anterior part of the eye. This examination is noninvasive, repeatable, and suitable for diagnostic and morphometric examinations (1,5-7,9,16).

Using slit-lamp we could demonstrate the thinning status of the cornea, but this method is not able to measure the exact corneal thickness. The thickness of the cornea can be evaluated quantitatively by number of methods including ultrasonic pachymetry (11,14) optical slit-lamp pachymetry (12), specular microscopy (12), confocal microscopy, OCT (9,14,77), high frequency UBM (9,16), laser Doppler interferometry, Placido disc based corneal topography, and pentacam (78-81). Dada et al. compared OCT and UBM pictures of the anterior segment of the eye and found, that both methods can be used for the measurements of the anterior segment and yielded comparable results (82).

Earlier studies (71,79) showed the ability of UBM in estimating corneal thickness, both in patients with early stage keratoconus and in normal subjects. The 50 MHz probe is an accurate and reproducible method for determining corneal thickness in clinical practice.

The aims of our studies were to investigate and present the characteristics of several corneal diseases, to measure the corneal thinning in Terrien's disease and KC, to follow the corneal reconstruction after the removal of the calcified part and amniotic membrane transplantation by UBM.

### 7.1. Terrien's disease

Terrien's degeneration is a rare peripheral corneal disorder of unknown etiology, which produces marginal corneal thinning and subsequently a significant amount of astigmatism. Several recent investigations deal with clinical and histological research and treatments of patients with Terrien's disease (29-31,83,84).

Iwamoto and coworkers in their electron microscopic studies found hyperemic blood vessels with increased inflammatory cell infiltrates in the superficial corneal layers in cases of inflammatory Terrien's disease (28). Our electron microscopic investigations of patients with Terrien's disease showed, that the epithelial cells and their nuclei were shrunken in the

thickened epithelium and the extracellular space was expanded. The Bowman membrane could not be detected, and the stroma lost its original structure and contained irregularly placed fibrocytes and debris phagocytosed by macrophages.

Lampé et al. summarized the typical morphological changes in case of Terrien's disease by using corneal topography (85). The postoperative surface was described as bowtie or nipple shaped. The postoperative follow-up examinations showed the decrease of the regular, indirect, and high degree of astigmatism, which can be corrected with cylindrical lenses (85). The topographic map of our patients showed irregular astigmatism before operation. After surgeries, their conditions manifested as an asymmetric-symmetric bowtie.

UBM is a useful tool with high magnification power for examining the anterior part of the eyebulb, finding the right diagnosis, and carrying out morphometry of the anterior segment. Previously, two studies used UBM as a diagnostic device to define the presence of hydrops in Terrien's disease (86,87). In our studies UBM was used to observe the structure and thickness of the degenerative corneal area.

Our study was the first one that presented the echographic changes of the stages in Terrien's disease. We measured the thickness and the width of the thinned, ectatic part of the peripheral cornea. In the second and third stages of the disease, the thickness and the width of the thinned peripheral part of the cornea, the progression of the illness, and the best time for the operation can be determined. The UBM findings correlated with and justified our histologic examinations and corneal topography. The histologic samples showed that the Bowman layer could not be detected on the excised parts; the corneal stroma lost its original and strictly organized structure; and the Descemet membrane was also thinner than the usual ones.

We found that UBM provides reliable information for the diagnosis of Terrien's disease and allows us to follow its course. We proved that it is an excellent diagnostic method for determining the extension, the thickness, and the structure of the disordered, ectatic part of the peripheral cornea. The actual size of the protruded corneal part could also be determined by using UBM which information is important and helpful for planning the operation.

## **7.2. Corneal calcification and treatment with amniotic membrane transplantation**

The causes of deep calcification of the cornea are still not clearly understood. Intact epithelium and absence of Bowman layer in the presence of high level of phosphate ions plus an inflammatory response seem to provide the necessary environment for the formation of calcium salts deep within the stroma of the cornea (37,45). Reports have implicated the role of sodium hyaluronate artificial tears, preservatives of eyedrops with phosphate buffers, timolol, retinoic acid and dexamethasone in calcium salt depositions (36,41,42). Calcium salt deposition can also occur in cases of older patients, decreased tear production, and history with previous herpes virus infection (37,45). The high phosphate concentration leads to a critical increase of the calcium phosphate which together with the breakdown of the tissue barrier, results in the formation of hydroxyapatite deposits (36).

In our studies we described three patients with different etiology of corneal calcification. Although each of our cases had a known risk factor for corneal calcification formation (herpes simplex keratitis, dry eye, persistent epithelial defect, congenital glaucoma), it is possible that corneal calcification is a multifactorial process as was mentioned in the study of Ebrahimi (88). We investigated these patients with UBM. We found that this method provides excess information to establish the diagnosis of corneal calcification, makes possible to determine the extent of the part to be excised, and may be used to estimate the territory that should be operated.

Previous studies showed that AMT promised the restoration of ocular surface integrity, reduction of stromal infiltration, and improvement of vision in ulcerative and necrotising herpetic keratitis. Gabric et al. effectively cured forty patients with persistent corneal epithelial defects (recurrent pterygia, corneal graft, impending perforation) resistant to other treatments covering with AM. There was complete epithelization of the corneal defects of 67% of their patients during the first three postoperative weeks (46). Comparing these data with our investigations, our patient No. 2. had perfect re-epithelisation two weeks after her AMT.

In the management of acute ulcerative herpes simplex keratitis the appropriate antiviral and antimicrobial therapy should be instituted at least several days before AMT. In these cases AMT might be considered and installed after these conservative therapy (47).

We treated our patients before the surgical procedure with acyclovir, neomycin, dexamethason, and lubricant drops, too.

Kim and Spelsberg suggested, that AMT may be an initial surgical option in selected cases with deep ulcers from herpetic viruses (49,89). Shi used the AM in the management of necrotising stromal keratitis of fifteen patients. The adjunctive use of antiviral and steroid therapy with AMT should be recommended in the treatment of patients having corneal classification in order to increase the safety and efficacy of AMT (54).

Seitz and Resch (55), Prabhasawat (56), and Müller (90) published articles dealing with patients treated because of different ocular surface pathologies and histories. Each of these patients required a different technique of AMT. They suggested, that the AM graft served as a new basement membrane for corneal epithelial cells. A differentiated microsurgical technique results in different integration patterns of AM into the human cornea (55,56,88,91). The above studies confirmed, that AM may be integrated fully into the normal corneal surface and preserved there for months, and AM could not notice later.

In our investigations, patient No.1. had phacoemulsification before the formation of the non-healing epithelial defect of the cornea. Corneal calcification was developed after using topical treatment for three months. Deep corneal calcification formed after treating patient No.2. with antiviral, antibiotic, and corticosteroid drops containing phosphate buffer because of her herpes zoster keratitis for more than a half year. Patient No.3. had several operations and used different eyedrops for years in order to treat congenital glaucoma. The anti-glaucomatous eyedrops contained timolol and other phosphate buffers. Using these drugs for ten years, superficial corneal degeneration was formed.

Our results showed, that frequent and long use of several eyedrops containing phosphate buffer may considerably increase the risk of calcium deposition, especially in patients with persistent epithelial defect. The applied eye drops contained phosphate buffers, ofloxacin, neomycin, acyclovir, and dexamethasone. In order to treat these patients, we transplanted AM using either single layer graft inlay or sandwich techniques. Since our histological examinations demonstrated strong calcification in the affected stroma, we had to cut out the calcified part of the cornea before AMT.

In concordance with previous reports, we recommend that predisposed patients who are using eye drops containing phosphate buffer be followed up carefully for the potential



complications of corneal stromal calcification. If possible, nonphosphated corticosteroid eye drops should be used.

Several methods are known to treat corneal calcifications, but no one has used AMT to treat corneal calcification so far. We concluded from our results of imaging studies, that AMT could provide good anatomical and functional information in cases of persistent corneal epithelial defect and ulcer with calcium deposition.

In order to follow and prove the good therapeutic effect of AM, we used UBM. This device was helpful in the follow up of the treatment of patients with AM who had corneal calcification.

### **7.3. Keratoconus**

Keratoconus (KC) is a continuing clinical problem and a leading indication for PKP. Major advances in the treatment of KC include improved technology in rigid gas permeable and hybrid contact lenses and corneal transplantation (72,73,92-94). PKP offers good long-term visual rehabilitation for KC (79,94-98). High postoperative corneal astigmatism, sometimes seen after PKP, could prevent good visual rehabilitation even when a graft is clear and otherwise surgically successful. Some factors influencing postkeratoplasty astigmatism are independent on the surgeon, like wound healing and recipient corneal pathology (99-101). Several surgical techniques like incisional procedures such as astigmatic keratotomy or ablative procedures such as laser in situ keratomileusis are used to treat astigmatism after PKP. In cases of progressive KC, wedge resection is considered. The resection is centered on the negative axis and a wedge of tissue is removed from both the host and the donor, using a nomogram (99). Repeating PKP leads to intraoperative and postoperative complications, therefore this should be only considered when all other options have failed (98, 101).

We compared the visual acuity among patients wearing contact lenses and among those subjects who underwent corneal transplantation. We also investigated the complications of these methods to evaluate recommendations to patients with KC. In our investigations, we used UBM as a proved to be good method to diagnose KC and follow its course. Using UBM we could in vivo diagnose KC, detecting conal shape of the cornea, and corneal thinning.

We found that after PKP, UCVA and BCVA significantly improved and astigmatism gradually and progressively decreased. Wearing contact lenses slows down the formation of KC. The curvature of the endothel showed a little breakage on the UBM figure, but the thickness of the cornea was almost regular (0.464 mm). The different complications of PKP could also be seen in keratoconic patients with UBM that proved to be a safe and reliable device to follow the course of patients with KC.

The visual acuity of the patients with KC without any corrections was mainly <5/50 (30%). The vision of the KC patients with spectacles were <5/50 in 4%, and 45/50-50/50 in 14%. The visual acuity with contact lens was the best, 45/50-50/50 in 70% postoperatively, the average corrected visual acuity was 40/50.

Our investigations showed that astigmatism gradually and progressively improved after PKP. Other studies revealed that factors like sex, donor age, age of the patients when keratoplasty was performed, diameters of host and donor cornea trephinations, suture technique, preoperative astigmatism, and time of suture removal after keratoplasty were not any significant predictors of postgraft astigmatism. Postgraft astigmatism could be caused by the different method of trephination and asymmetry of suture forces (96,97,100). To reduce the postoperative astigmatism, we performed wedge resection in two cases and relaxed incision in three cases.

PKP for KC is associated with a low graft failure rate in most series (79,95,101). In our study, only one patient had rejection amounting to graft failure. One of our patients had incongruation of the sutures and one had hypotony after PKP because of loose sutures. These two patients needed resuturing because these occurred less than 6 months after keratoplasty. Suture problems started at one of our patients later than 6 months. In this case, the allograft rejection episodes were circumvented with prompt removal of the sutures and prophylactic use of topical corticosteroids.

Four of our patients had inflammation after PKP, although prophylactic use of corticosteroids and antibiotics prevented rejection in these cases. Synechiolysis were done with two of our patients who had anterior synechiae.

In conclusion, half of the patients with KC had stable visual acuity with using rigid gas permeable and hybrid contact lenses. We can say that the progression of the KC could be delayed by initiating the wear of contact lenses as soon as possible, even in early stages of

KC. PKP for KC is associated with an excellent graft survival rate (95% success in 36 months) and good visual acuity (the mean BCVA after PKP was 40/50).

Both wearing contact lenses and performing PKP could result in a very good visual acuity for a longer time and good anatomical status is obtained by PKP.

More research remains to be done, and numerous clinical studies must be performed to determine the possible role of UBM in other clinical situations in ophthalmology. However, our results (in cases of Terrien's disease, corneal calcification, and keratoconus) and the unique capabilities of UBM imaging suggest that it has the potential to have significant impact on the diagnosis and clinical management of many eye diseases.

## 8. SUMMARY AND CONCLUSIONS

- In cases of Terrien's disease we found that the thickness of the ectatic part was significantly thinner than the intact part. The epithelium of the cornea showed high reflectivity at the ectatic part, and the stroma was thinned significantly. The basement membrane and the Bowman layer could not be separated by echography.
- UBM provided reliable information for the diagnosis of Terrien's disease and allowed us to follow its course. It proved to be an useful diagnostic tool for determining the extension of the ectatic part, the thickness and the structure of the cornea.
- We published first the echographic changes of the stages of Terrien's disease.
- The UBM pictures of corneal calcification showed that the central width of the cornea was thicker than the normal structure. There was high reflectivity on the paracentral cornea that is well demarcated from the regular part of the cornea.
- UBM was easy to use and provided excess information to establish the diagnosis of corneal calcification, made possible to determine the extent of the calcified part, and might be used to estimate the territory that should be operated. It was also helpful to determine the cornea's pathological processes which could not be seen with slit lamp.
- On the UBM picture of the transverse segment of the cornea, the transplanted AM was built into the cornea. It was parallel, foliated, and had a rough surface and higher reflectivity than the other part of the cornea. The cornea covered by AM was homogenous and had high reflectivity on the UBM picture, and the surface was smooth. AMT could be a proper method to heal epithelial defects in cases of superficial and deep corneal calcification.
- No one has used AMT to treat corneal calcification before.
- We can conclude from our results that UBM is helpful in the follow up and in the improvement of the therapeutic benefits of AMT in cases of corneal calcification. AMT provided good anatomical and functional formation in cases of persistent corneal epithelial defect and ulcer with calcium deposition.

- Examination of patients with keratoconus revealed that the paracentral coning had a widened highly reflective epithel and extremely thinned stroma, and the endothel was thickened. The structure of the cornea was unrecognizable at the ectatic part. The thickness of the cornea was significantly thinner than the normal one. The endothel was thickened.
- Both wearing contact lenses and performing PKP could result in a very good visual acuity for a longer period of time. In addition, good anatomical status has been obtained by PKP.
- UBM was suitable for the examination of the shape, the curvature, and the superficial and innermost irregularities of the cornea.
- The UBM findings correlated with the results of our histologic examinations.
- Since UBM is relatively cheap and easy to use as compared to other examination methods, we suggest the use of UBM in order to get a diagnosis and to follow the course of different inflammatorical and degenerative corneal diseases. This method could also be helpful in the estimation of the progression of the investigated corneal diseases.

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