Ultrasound biomicroscopy as a diagnostic method of corneal degeneration and inflammation

Ph.D. Thesis

Ákos Skribek M.D.

Department of Ophthalmology
Faculty of Medicine
University of Szeged
Szeged, Hungary
2013

1. 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. 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.

In early 1990's, Pavlin et al. created a new ultrasound instrument for visualising the anterior segment structures. 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. UBM provides high-resolution in vivo imaging of the anterior segment of the eye in a noninvasive manner 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. Pathologic changes, involving anterior segment structures can be evaluated qualitatively and quantitatively by this method.

Clinically, it is very important to measure the central and peripheral thickness of cornea. These parameters could be determined by using a number of examination methods. Anterior segment imaging with UBM has been shown measuring corneal thickness and curvature. UBM is a 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.

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.

2. HYPOTHESISES AND AIMS

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.

3. BACKGROUND

3.1. 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. 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.

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.

3.2. 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.

a) The superficial type appears as a band shaped keratopathy and refers to calcium deposition in the Bowman layer and superficial structures.
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.

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

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 phototherapic keratectomy.

3.3 Amniotic membrane transplantation (AMT)

AM is the innermost layer of the placenta. It contains a high concentration of basic fibroblast growth factor, basement membrane components, and unknown trophic factors. AMT may facilitate epithelization and reduce inflammation, vascularisation, and scarring.

There are several types of AMT using one or more layers of AM as a patch, graft, or sandwich to cover corneal epithelial defects:

- When the corneal diseases with epithelial defects have no or only shallow stromal defects, patch onlay can be used. The local epithelium is expected to grow under the AM and the epithelial defect should close.
- The other type of AM is the single–layer graft inlay that can be used for shallow stromal defects. In this type of AMT, the epithelium is expected to grow over the AM, providing a new basement membrane.
- For deep stromal defects, multilayer–graft inlay can be used. 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.

3.4. Keratoconus (KC)

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.

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

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.

PKP is the most commonly used surgical option for advanced cases of KC which can not be successfully managed with contact lenses.

4. PATIENTS AND METHODS

4.1. Patients

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

Two patients were diagnosed with Terrien's disease during the last ten years at our Department. Patient No.1. was 21 years old. Before her first visit to our Departement, she had conjunctivitis and keratoconjunctivitis several times. Patient No. 2 was a 64-year-old man. At his first visit to our Department, he had an acute keratoconjunctivitis on his right eye.

We treated three patients with persistent, non healing corneal ulcer and calcification. Patient No.1. was a 80-year-old woman. Cataract extraction with phacoemulsification and posterior chamber lens implantation were carried out 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. Patient No.3. was a 54-year old woman with congenital glaucoma and cataract. She had several operations due to previous problems and she used lubricants and β blocker drops for more than 20 years.

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 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. The mean age of the patients in the keratoplasty group was 24 years.

4.2. 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).

4.3. Ultrasound biomicroscopy

The corneal findings of all three investigated goups 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 commercially available unit operates at 50 MHz and provides lateral and axial physical resolution of approximately 50 and 25 μm. 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.

4.4. Human amniotic membrane preparation and preservation

For AM preparation, the human placenta was obtained after elective caesarian delivery. Serological tests were performed to exclude human immunodeficiency virus and hepatitis virus type B and C. 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.

4.5. Surgical intervention

4.5.1. Terrien's disease

In these patients, peripheral full thickness keratectomy using the method of Alberth and Süveges was performed with retrobulbar anesthesia.

4.5.2. Superficial and deep corneal calcification

Before the operation the patients were anesthetized by retrobulbar injection. 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.

4.5.3. Keratoconus

PKP surgeries were performed by retrobulbar injection or general anesthesia. All of the recipient and donor corneas were trephined with hand hold trephines. Graft diameters ranged from 7.0 to 7.1 mm, the host diameter was 7.0 and 7.1 mm.

4.6. 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® (Merck Ltd) and semithin (0.5 μm 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).

4.7. Corneal topography

In this study we used the TMS 1 Topographic Modelling System.

4.8. Statistical analysis

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

4.9. Ethics

This study was conducted in accordance with the Declaration of Helsinki.

5. 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.

5.1. Terrien's disease

5.1.1. 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 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. 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 keratometrical values

were 61.5x143/45.5x53 and 41.75x100/49.5x10 on his right and left eyes, respectively. We performed a full thickness keratectomy.

Two months after his operation the BCVA was 40/50 on his right eye. Fourteen months after the operation, the BCVA was 20/50. The sutures were then removed and his BCVA improved to 40/50.

5.1.2. Slit-lamp and ultrasound biomicroscopy

The thickness of the thinned, ectatic part of the peripheral cornea was 0.221 mm. After one year of the 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.

Abnormal structure of the cornea in the ectatic part could be detected with UBM in case of patient No.2. 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.

5.1.3. Histopathology

The corneal epithelium displayed thick and mosaic-like arrangement of the epithelial cells. The stroma appeared thin and irregularly built. Electron microscopic investigations showed that the epithelial cells and their nuclei were shrunken. The extracellular space was expanded. The epithelial adhesion structures appeared distorted. The Bowman layer could not be detected on the excised parts, and the corneal stroma lost its original and strictly organized structure. The stroma contained irregularly placed fibrocytes and debris phagocytosed by macrophages. The Descemet membrane was also thinner than usual.

5.1.4. Corneal topography

The topographic map of the right eye of patient No.1. showed irregular astigmatism. 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 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. 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.

Three months after the operation, the topographic map showed a rotated symmetrical bow-tie shape. His visual acuity was well corrected with cylindrical lenses.

5.2. Corneal calcification and treatment with amniotic membrane transplantation

5.2.3. Standard ophthalmological examinations

Patient No.1. had a cataract extraction with phacoemulsification and posterior chamber lens implantation, than one month later, she returned with a herpetic ulcer on the same eye caused by herpes simplex virus1 (HSV1), and the visual acuity decreased to 3/50.

Patient No.2. visited our Department with herpes zoster infection of the 1st trigeminal area involving the right cornea. Her best corrected visual acuity was 20/50 on her right, and 50/50 on her left eye. The keratitis progressed into corneal ulcer and was not healed. Since the patient did not agree to have PKP, we performed a single layer AMT.

The AM dissolved shortly after the operation, the visual acuity became 30/50, and the corneal ulcer healed.

The patient returned to our Department in a year with a newly formed, deep corneal ulcer. 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. showed up at our Department with 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.

5.2.2. Slit-lamp and ultrasound biomicroscopy

The central width of the cornea patient's No.1 is 565 μm. 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 μm. The UBM structure of the cornea cannot be recognized in the calcified part, only homogeneous, highly reflective echos can be seen. One month later a non-healing ulcer with supposed calcification. It caused visual impairment, so we cut the calcificated part out and performed a sandwich technique AMT.

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 UBM picture, the transplanted AM was parallel, foliated, and had a rough surface and higher reflectivity than the other part of the cornea.

Two weeks after the reoperation the AM is partly built into the cornea. A deep and shrunken part of the cornea covered by AM could be seen on the UBM picture.

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

The UBM picture of patient No.3. before the operation demonstrates high reflection of the corneal epithelium. This well demarcated central part of the cornea is 176 μm deep, and the extension is 1.53x 0.782 mm.

5.2.3. Histopathology

Most of the removed corneal parts appeared as calcified stroma 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.

5.3. Keratoconus

5.3.1. 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). before and after PKP.

Thirty of the examined 65 patients had contact lenses on both eyes. Those 35

patients, who had contact lense 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.

Measuring the visual acuities before and after PKP a significant improving is shown in UCVA and BCSVA after PKP ($p< 0.05$).

5.1.1. Ultrasound biomicroscopy

The special signs of KC with UBM right after the acute stage are: 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.

The UBM corneal figure of a patient wearing contact lenses because of KC is showed, that 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.

6. 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.

6.1. Terrien's disease

Previously, two studies used UBM as a diagnostic device to define the presence of hydrops in Terrien 's disease. 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.

6.2. Corneal calcification and treatment with amniotic membrane transplantation

We described three patients with different etiology of corneal calcification. We investigated these patients with UBM, and found 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.

Comparing the previous data with our investigations, our patient No. 2. had perfect re-epithelisation two weeks after her AMT.

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.

Several methods are known to treat corneal calcifications, but no one has used AMT to treat corneal calcification so far. We concluded 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.

6.3. Keratoconus

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. 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. 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 lense 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. To reduce the postoperative astigmatism, we performed wedge resection in two cases and relaxed incision in three cases.

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).

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.

7. 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 degenerational corneal diseases. This method could also be helpful in the estimation of the progression of the investigated corneal diseases.

ACKNOWLEDGEMENTS

I greatly aknowledge to **Professor Andrea Facskó**, the head of the Department of Ophthalmology at the University of Szeged, for providing me with the possibility of working at the Department of Ophthalmology and to complete my investigations, as well as for inspiring me to finish my thesis.

I would like to thank to **Professor Lajos Kolozsvári**, the former head of the Department of Ophthalmology for his invitation to work at University of Szeged and for being my tutor and encourageing me to investigate patients with corneal degeneration and inflammation by ultrasound biomicroscopy.

I am grateful to my co-author **Dr. Nicolette Sohár** for her valuable help and inspiration to write the articles and this study.

I am thankful to **Dr. Antal Nógrádi** for the histological examinations and instructions in our study.

I would like to thank **Mrs Ilona Kovács** and **Mrs Erzsébet Katona** for their technical assistance in the laboratory work, **Ms Emilia Orosz** for the assistance in the ultrasound laboratory.

I am indebted to **Ms Enikő Szabó** for her help in editing the graphs and tables.

I am grateful to **my family** for their endless support and patience during this work and their encourage to accomplish my thesis.

PUBLICATIONS

List of full papers directly related to the subjects of the Thesis:

I. Sohar N, **Skribek A**, Fulop Z, Kolozsvari L. The success of treating keratoconus: visual acuity and follow-up with ultrasound biomicroscopy. Spectrum der Augenheilkunde 2012, 26:(3) pp. 159-164.
IF: 0.274

II. **Skribek A**, Sohar N, Nogradi A, Kolozsvari L. Amniotic membrane transplantation in cases of corneal calcification - follow up with ultrasound biomicroscopy. Spectrum der Augenheilkunde 2011, 25:(3) pp. 210-214.
IF: 0.274

III. **Skribek A**, Sohar N, Gyetvai T, Nogradi A, Kolozsvari L. Role of ultrasound biomicroscopy in diagnosis and treatment of Terrien disease. Cornea 2008, 27:(4) pp. 427-433.
IF: 1.853

IV. **Skribek Á,** Gyetvai T, Kolozsvári L. Ultrahang-biomikroszkópos vizsgálat jelentősége a Terrien-betegség követésében. Szemészet 2006, 143: pp. 50-52.

List of abstracts directly related to the subjects of the Thesis:

1. **Skribek Á**, Sohár N, Facskó A: Elülső szegmentum képalkotó eljárások pellucid marginális degeneráció eseteiben. Magyar Szemorvostársaság Kongresszusa, Siófok, 2012.06.07-2012.06.09. p. 65.
2. **Skribek Á**, Sohár N, Kolozsvári L: Az amnionmembrán-transzplantáció szerepe a szaruhártya mészképződéssel járó eseteiben. Magyar Szemorvostársaság Kongresszusa, Pécs, 2008.05.29-2008.05.31. p. 85.
3. Sohár N, **Skribek A,** Fülöp Zs, Kolozsvári L: Retrospective study of patients with keratoconus. In: The Joint Congress of the European Society of Ophthalmology and American Academy of Ophthalmology: SOE/AAO. Bécs, Ausztria, 2007.06.09-2007.06.12. p. 170.
4. **Skribek Á.**: UH szerepe az uveitisek diagnosztikájában. Kötelező szemészeti szintentartó tanfolyam, Szeged 2007. október 27. 29. 30. 31.
5. **Skribek Á.**: Az amnion szerepe a szaruhártya betegségek kezelésében Kötelező szemészeti szintentartó tanfolyam, Szeged 2007. október 27. 29. 30. 31.
6. **Skribek Á,** Gyetvai T, Hári Kovács A, Sohár N, Kolozsvári L: The role of ultrasound biomicroscopy in the diagnosis and treatment of marginal corneal thinning. In: The Joint Congress of the European Society of Ophthalmology and American Academy of Ophthalmology: SOE/AAO. Bécs, Ausztria, 2007.06.09-2007.06.12. p. 171.
7. **Skribek Á**, Sohár N, Gyetvai T, Kolozsvári L: Keratoconus súlyosságának megítélése ultrahang biomikroszkópos vizsgálattal. In: Magyar Műlencse Implantációs és Refraktív Sebészeti Társaság Kongresszusa Keszthely, Magyarország, 2007.03.30-2007.03.31. p. 71.
8. **Skribek Á**, Sohár N, Gyetvai T, Hári Kovács A, Kolozsvári L: Az ultrahang-biomikroszkóp szerepe a szaruhártya perifériás elvékonyodása diagnosztikájában. In: Magyar Szemorvostársaság 2007. évi Kongresszusa: Szemészet 2007; 144. Suppl., Debrecen, Magyarország, 2007.06.21-2007.06.23. p. 78.
9. Hári Kovács A, **Skribek Á**, Tóth-Molnár E, Kolozsvári L: Three cases of ocular filariasis in Hungary. In: The Joint Congress of the European Society of Ophthalmology and American Academy of Ophthalmology: SOE/AAO. Bécs, Ausztria, 2007.06.09-2007.06.12. p. 213.

10. **Skribek Á,** Kolozsvári L: Keratoconus miatt végzett perforáló keratoplasztika műtétek postoperatív gyógyulása és szövődményei. In: Magyar Műlencse Implantációs és Refraktív Sebészeti Társaság Kongresszusa Keszthely, Magyarország, 2006.03.30-2006.04.01. p. 87.
11. **Skribek Á**, Sohár N, Fülöp Zs, Kolozsvári L: Keratoconus miatt gondozott betegeink követése. In: Magyar Szemorvostársaság 2006. évi Kongresszusa, Alpok-Adria Nemzetközi Szemorvostársaság Kongresszusa. Sopron, Magyarország, 2006.06.15-2006.06.17. p. 96.
12. **A Skribek**, T Gyetvai, A, Hári Kovács, L Kolozsvári: The role of ultrasound biomicroscopy (UBM) in the diagnosis and treatment of Terrien's disease. In: János Németh, Béla Csákány, György Barcsay (szerk.) Ophthalmic Echography Konference Budapest, Magyarország: 2006. pp. 13-16.(ISBN:963-85636-5-6).
13. Gyetvai T, **Skribek Á**, Kolozsvári L: A cornea és a sclera vastagságának változása különböző kórképekben. In: Magyar Szemorvostársaság 2006. évi Kongresszusa, Alpok-Adria Nemzetközi Szemorvostársaság Kongresszusa. Sopron, Magyarország, 2006.06.15-2006.06.17. p. 46.
14. T Gyetvai, Z Horóczi, **Á Skribek**, A Hári Kovács, L Kolozsvári: Ultrasound biomicroscopical examination of the corneal incision after cataract surgery. In: János Németh, Béla Csákány, György Barcsay (szerk.) Ophthalmic Echography Budapest, Magyarország, 2006 Budapest:2006. pp. 43-46. (ISBN:963-85636-5-6)
15. **Skribek Á**, Gyetvai T, Hári Kovács A, Kolozsvári L: The role of ultrasound biomicroscope in the diagnosis and treatment of marginal corneal thinning. In: 15th SOE Congress and 103rd DOG Congress. Berlin, Németország, 2005.09.25-2005.09.29. p. 235.
16. Hári Kovács A, Gyetvai T, **Skribek Á**, Kolozsvári L: Ultrasound biomikroscopic findings in corneal astigmatism. In: 15th SOE Congress and 103rd DOG Congress, Berlin, Németország, 2005.09.25-2005.09.29. p. 236.
17. Gyetvai T, Horóczi Z**, Skribek Á**, Hári Kovács A, Kolozsvári L: Ultrasound biomicroscopical examination of the corneal incision after cataract surgery. In: 15th SOE Congress and 103rd DOG Congress. Berlin, Németország, 2005.09.25-2005.09.29. p. 234.
18. Gyetvai T, Horóczi Z, **Skribek Á,** Hári Kovács A, Kolozsvári L: A cornealis sebzés vizsgálata ultrahang biomikroszkóppal katarakta műtét után. In: Magyar Műlencse Implantációs és Refraktív Sebészeti Társaság Kongresszusa Keszthely, Magyarország, 2005.03.31-2005.04.02. p. 92.
19. **Skribek Á**, Gyetvai T, Hári-Kovács A, Kolozsvári L: The role of ultrasound biomicroscopy (UBM) in the diagnosis and treatment of Terrien's disease. In: SIDUO XX Congress of the International Society for Ophthalmic Ultrasound, Budapest, Magyarország, 2004.09.12-2004.09.16. p. 29.
20. T Gyetvai, Z Horóczi, **Á Skribek**, A Hári Kovács, L Kolozsvári: Ultrasound biomicroscopical examination of the corneal incision after cataract surgery. In: SIDUO XX Congress of the International Society for Ophthalmic Ultrasound, Budapest, Magyarország, 2004.09.12-2004.09.16. p. 33.
21. **Skribek Á**, Kolozsvári L: A Terrien-betegségekről három eset kapcsán. In: Magyar Szemorvostársaság Kongresszusa: Szemészet, 140 (Suppl I.). Budapest, Magyarország, 2003.08.28-2003.08.30. p. 73.