Corneal pathology: special approach to corneal disorders and support for clinicians.

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Abstract
Corneal specimens – full thickness corneal buttons and corneal biopsies – form a large part of the tissue material sent to ocular pathology laboratory for histopathological diagnosis. The assessment of these specimens requires considerable effort and specialized knowledge. Pathologic interpretation of corneal tissue should represent the highest level of experience in dealing with sometimes conflicting data based on morphologic and clinical findings. The greater the degree of diagnostic detail and specificity, the better equipped clinicians are to determine the cause of disease, to understand its pathogenesis or to formulate an effective plan of treatment. The aim of this study was to assess the importance of corneal pathology in making a proper clinical diagnosis and to highlight the significance of clinicopathological correlations in corneal cases that require corneal transplants. Histopathological analysis of corneal specimens: full-thickness corneal discs and corneal biopsies, is essential to establish accurate diagnosis, supports clinical diagnosis and makes it possible to introduce proper treatment. Corneal pathology seems to be a guide for solving diagnostic and therapeutic problems and learning the art of differential diagnosis. A good clinician – eye pathologist collaboration helps clinicians to use ocular pathology as a tool to improve care of their patients.

Key words: Corneal pathology – clinicopathological correlation – hydrops – fungal keratitis – immunoglobuline corneal deposits – cystic epithelial ingrowth – Schnyder’s corneal dystrophy.

Introduction
Corneal specimens – full thickness corneal buttons and corneal biopsies – form a large part of the tissue material sent to ocular pathology laboratory for histopathological diagnosis. The assessment of these specimens requires considerable effort and specialized knowledge. Pathologic interpretation of corneal tissue should represent the highest level of experience in dealing with sometimes conflicting data based on morphologic and clinical findings. The greater the degree of diagnostic detail and specificity, the better equipped clinicians are to determine the cause of disease, to understand its pathogenesis or to formulate an effective plan of treatment.

The aim of this study was to assess the importance of corneal pathology in making a proper clinical diagnosis and to highlight the significance of clinicopathological correlations in corneal cases that require corneal transplants.

Case reports
Case Nº 1

Clinical history:
A 60 year-old man presented in the 1st Eye Hospital in Lublin in August 2005 with a sudden decrease of left eye vision 4 days before. His past ocular history was significant for left radial keratotomy performed 11 years before, in April 1994, for anisometropia and myopia –12.0D. His pre–surgical best corrected visual acuity was 20/20 in both eyes with +2.0D in the right eye and –11.0D in the left eye. Radial keratotomy was performed using the Ukrainian method. 12 radial cuts were made leaving 3.5 mm of the clear optical zone. The patient’s best corrected post–surgical visual acuity in the left eye was 20/20 with +2.5D. Seven months after the surgery left eye vision decreased and a marked irregular astigmatism appeared. VA = 20/30 with +3.0 D sph = +2.0 D cyl ax70º. Then the patient was lost for follow–up for 11 years until August 2005. Left eye examination at that time revealed a
central corneal oedema and 12 peripheral radial scars (Fig.1). Visual acuity in the left eye was counting fingers, and in the right eye 20/20. Acute hydrops was diagnosed and treated with hyperosmotics without improvement. In November 2005 a penetrating keratoplasty was performed. An 8.2mm graft was secured with 16 interrupted 10–0 Nylon sutures in an 8.0mm bed. Two additional sutures were placed on dehiscent radial incisions (Fig.2).

**Histopathology:**
Histopathological examination revealed features of keratoconus and acute hydrops (Fig.3A,B) as well as features of healing radial incisions (Fig.4A,B,C,D): defects in Bowman’s layer filled with the epithelium or fibrous proliferation, decreased number of keratocytes, possibly caused by inflammatory cytokines released through Bowman’s layer breaks (this phenomenon is described after PRK and LASIK), stromal edema, Descemet’s break like in acute hydrops, deep scars in the peripheral stroma, epithelial islands within the stroma, fragments of Bowman’s layer within the stroma covered with epi-corneal fibrous membrane.

**Follow-up:**
The postoperative course was uneventful, and uncorrected visual acuity five months after the surgery was 20/200.

**Discussion:**
Acute hydrops usually develops in advanced keratoconus due to stretching of Descemet’s membrane associated with continuous stromal thinning within the cone. It is unlikely that subclinical keratoconus was present in the patient’s left eye before surgery. There was no astigmatism and the visual acuity was 20/20 with –11.0D.
Irregular astigmatism developed after surgery, however with no characteristics of cone. Hydrops was found 11 years after radial keratotomy. Microscopical examination revealed features of keratoconus and acute hydrops as well as features of healing radial keratotomy incisions.

**Conclusions:**

It can be speculated that radial keratotomy initiated keratoconus-like changes in the form of disorganisation of the epithelial basement membrane and Bowman’s layer. Scarring and inflammatory processes caused loss of keratocytes, corneal thinning, stretching and finally a rupture of Descemet’s membrane.
Figure 8. A, B, C, D. Photomicrographs of the left cornea. **A and B** H&E stain. A massive corneal infiltrate composed mainly of neutrophils involving focally all corneal layers with focal corneal necrosis. Hypopyon. **C** Numerous fungal hyphae parallel to the corneal surface and to lamellae with vertical fungal hyphae. **D** GMS stain. Fungal microorganisms stain black.

Figure 7. Left eye. 4th May. The corneal infiltrate very dense and much larger with feathery margins and raised borders, involving entire corneal depth. Marked hypopyon in the anterior chamber.

Figure 9. Left eye. Five days after penetrating keratoplasty.
Case N°2

Fungal keratitis remains a diagnostic and therapeutic challenge to the ophthalmologist. Difficulties are related to establishing clinical diagnosis, isolating the etiologic fungal organism in the laboratory, and treating the keratitis effectively with topical antifungal agents. Isolation of fungal microorganisms is often difficult. When corneal smears and cultures are negative and the keratitis in not responding to empiric therapy, then a diagnostic superficial keratectomy or a corneal biopsy is necessary to make diagnosis.

Clinical history:

A 30 year-old healthy man was referred to the 1st Eye Hospital in Lublin with decreased visual acuity, discomfort, foreign body sensation, mild photophobia and tearing of his left eye. He had been wearing soft contact lenses for 15 years and using a particular (Renu with MoistureLoc) contact lens solution for a few years. On his first examination on 22nd April 2006, a grey–white corneal infiltrate was observed, localized paracentrally from 12 to 1 hours position, extending from the epithelium to the superficial stroma (Fig.5). A microbiological smear was done and the treatment with the broad spectrum—antibiotic (Levofloxacin – Oftaquix) was initiated. Two days later, the corneal infiltrate looked more dense with the immunological ring around it, together with a mild inflammatory reaction in the anterior chamber (Fig.6). A corneal scraping was done, but the result of the culture after 48 hours was negative. On 4th May the corneal infiltrate became very dense and much larger with feathery margins and raised borders, involving the entire corneal depth with a 4–5mm hypopyon in the anterior chamber (Fig.7). The anterior chamber tap was submitted for laboratory investigation and the result was positive for fungal microorganisms Acremonium kiliense sp., belonging to the group of filamentous, nonpigmented septated fungi. Despite intensive antifungal treatment the course of disease was progressive and on 10th May a penetrating keratoplasty was performed (Fig.9).

Histopathology:

Microscopical examination revealed a massive corneal infiltrate composed mainly of neutrophils involving focally all corneal layers with focal corneal necrosis. Numerous fungal hyphae parallel to the corneal surface and to lamellae were identified, together with vertical fungal hyphae, suggestive of and usually associated with more aggressive infection. Also a massive infiltrate composed of neutrophils, adjacent to Descemet’s membrane from anterior chamber was observed, consistent with hypopyon (Fig.8A,B,C,D).

Histopathological diagnosis:

Fungal (Acremonium kiliense sp.) keratitis.
Corneal pathology


Figure 13. Transmission electron microscopy. A) Deposition of cholesterol crystals (clefts) and lipid vacuoles in the central corneal stroma, B) Secondary lipoid degeneration and lipofuscinosis in the anterior corneal stroma: keratocytes with intracellular lipid droplets and lipofuscin granules.
hypercholesterolemia (275 mg/dl, range 120–200) and mild hyperlipidemia (HDL-D 68 mg/dl, range 35–60; LDL-CH 181 mg/dl, range 70–130). Penetrating keratoplasty was performed (Fig.11).

Histopathology:
Microscopical examination revealed decreased epithelial thickness in some areas and localized defects in the basement membrane and Bowman’s layer. Prominent findings were circumscribed nodular collagenous scars at the periphery and the superficial pannus (Fig.12A). Numerous small globular basophilic deposits were observed in the superficial stroma and numerous roundish empty spaces were scattered throughout the central stroma (Fig.12B,C). Ultramicroscopical examination revealed cholesterol crystals and round lipid deposits present in the

Figure 14. A, B. Right eye of the 79 year-old woman. Grayish, crystalline–like central and paracentral opacifications located in the entire stroma.

entire stroma (Fig.13A). Also, features of secondary lipoid degeneration and lipofuscinosis in the anterior stroma were observed: keratocytes with intracellular lipid droplets and lipofuscin granules (Fig.13B).

**Histopathological and ultrastructural diagnosis:**
Schnyder’s central corneal dystrophy.

**Clinical diagnosis:**
Salzmann’s nodular degeneration preceded by unspecific keratopathy since early childhood.

**Comment:**
The patient’s clinical diagnosis was Salzmann’s nodular degeneration preceded by unspecific keratopathy since early childhood. Schnyder’s central corneal dystrophy was the histological and ultrastructural diagnosis. The patient’s family history was negative, but the patient had mild hypercholesterolemia and hyperlipidemia. Analyzing these data, secondary lipid crystalline changes in the eye with Salzmann’s nodular degeneration could be considered.

The case presented could serve as an example of the divergence in the clinical and histopathological diagnosis. However, detailed microscopical and ultrastructural examinations revealed and highlighted some surprising and unexpected features. Therefore, the final diagnosis was the most accurate.

**Case N°4**

**Clinical history:**
A 79 year-old woman was referred in January 2005 to the 1st Eye Hospital in Lublin with the main complaint of bilateral decrease in her vision during the last 3–4 years. Her ocular history was negative and she had no serious general symptoms either. On examination grayish, crystalline-like central and paracentral opacifications located in the entire stroma of the right and left eye were noticed. Her visual acuity was 20/500 in both eyes. Laboratory investigations were significant for high levels of immunoglobulin G. Bone marrow biopsy was done and revealed 15% of matured plasma cells and 20% of non-matured forms. On March 2005 penetrating keratoplasty of the right eye was performed.

**Histopathology:**
Histopathological examination revealed interlamellar deposits of mostly amorphous substance in the mid-stroma, which stain red with Masson trichrome, but are PAS-negative (Fig 14A,B). The amount of deposits increased in deep stromal layers and in pre.Descemet’s area there were cistern-like accumulations of the amorphous substance. Epithelium, Bowman’s layer and superficial stromal layers did not show any significant changes. Descemet’s membrane was intact as well. In the corneal endothelium putative IgG deposits (staining red in Massone trichrome) were seen (Fig.15A,B,C).

Figure 16 A, B, C. Left eye of the 73 year-old woman. Cystic epithelial ingrowth showing very fast growth during the follow-up. A) December 2002 – first examination, B) March 2003 – the cyst filled almost 90% of the anterior chamber, C) May 2003 – one day before surgery.
Clinical diagnosis:
Atypical corneal dystrophy/degeneration.

Histopathological (and final) diagnosis:
Immunoglobulin corneal deposits in the course of monoclonal gammopathy of undetermined significance (MGUS).

Discussion:
Immunoglobulin deposits in the cornea are uncommon as a manifestation of systemic gammopathies (1–2% of multiple myeloma cases). Ormerod et al. described three patterns of corneal deposits: epithelial/anterior stromal, diffuse stromal, and posterior stromal. In the majority of cases the deposits are located in the anterior and mid-stroma, and have crystal-like appearance.

Conclusion:
Immunoglobulin deposits in the cornea should be suspected in cases of atypical corneal opacities and deposits. Lack of crystalline changes should not exclude gammopathies from differential diagnosis.

In the case presented, it was the histopathological examination, which revealed immunoglobulin deposits in the cornea and was crucial for establishing the final diagnosis.

Clinical history:
A 73 year-old woman presented in December 2002 in the 1st Eye Hospital in Lublin complaining of a gradual deterioration of her left eye vision after 2001. Her past ocular history was significant for the intracapsular cataract extraction (ICCE) in the left eye in 1990 and in the right eye in 1994. In December 2002 visual acuity in the left eye was 20/500, and in the right eye 20/60. A cystic epithelial ingrowth of the left eye was diagnosed, showing very fast growth during the follow-up (Fig.16A). In March 2003 the
cyst filled almost 90% of the left anterior chamber (Fig.16B,C). On 8th May 2003 block excision of the entire cyst combined with the tectonic eccentric sclerokeratoplasty was performed. The surgical procedure involved the block excision 10mm in diameter consisting of upper sclera, ciliary body, iris and cornea, combined with a posterior lamellar resection of the lower cornea and including the entire cyst. The defect was covered with a tectonic graft 10mm in diameter, sutured with interrupted 8–0 Vicryl to the sclera and continuous 10/0 to the cornea (Fig.18).

**Histopathology:**
Microscopical examination revealed atypical growth of an epithelial cyst within the anterior chamber. The cyst walls consisted of the inner corneal stromal surface lined with multilayered epithelium, detached Descemet’s membrane covered with the epithelium inside the cyst and endothelium outside, and scarred iris tissue containing Descemet’s membrane fragments. The epithelium had more layers when growing on the iris tissue and on the vascularized corneal stroma. The detached Descemet’s membrane was continuous with the iris and in this area had folds with fibrous proliferation outside, covered with endothelial cells. A full thickness corneal scar was seen in the area where the epithelial invasion started. The scar had multiple blood vessels, and some vessels could be seen in the deepest corneal layers (Fig.17A,B,C,D).

**Histopathological and clinical diagnosis:**
Cystic epithelial ingrowth.

**Follow–up:**
The transplant remained clear during the two-year follow–up and the integrity of the eyeball was preserved. One year after the surgery the visual acuity of the left eye was 20/100 with +12D.

**Discussion:**
In spite of the progress in ocular microsurgery, epithelial ingrowth can still be observed in clinical practice, and should be considered in the differential diagnosis of post–surgical and post–traumatic wound healing problems. The case presents an atypical growth of an epithelial cyst within the anterior chamber. Usually the epithelium grows on the iris and the inner corneal surface. The cyst was dormant for several years, but started to grow rapidly within several months, gradually detaching the Descemet’s membrane and filling almost the entire anterior chamber. Desquamated epithelium formed a pseudohypopyon.

**Conclusion:**
Block excision seems to be the method of choice in the treatment of both cystic and diffuse forms of epithelial ingrowth. In the case presented the histopathological analysis revealed an atypical and unusual growth pattern of an epithelial cyst within the anterior chamber.

**Summary**
Histopathological analysis of corneal specimens (full–thickness corneal discs and corneal biopsies) is essential to establish accurate diagnosis, supports clinical diagnosis and makes it possible to introduce proper treatment. Corneal pathology seems to be a guide for solving diagnostic and therapeutic problems and learning the art of differential diagnosis. A good clinician – eye pathologist collaboration helps clinicians to use ocular pathology as a tool to improve care of their patients.

**References**
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