Corneal specimens that influence clinical decisions

Refractive surgery
Corneal dystrophies
Microbial infections

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45 year-old man with progressive corneal opacification and extreme pain
Critical Anatomy

- Tear film
  - Oxygen delivery system
  - Passive resistance to infection
    - Mucous
    - Lysozymes
    - Glycocalyx
  - Active resistance to infection
    - Non-nodal lymphocytes
    - Langerhans cells
Stratified squamous epithelium

- Conserved radius of curvature
- Homogeneous cell population
- Lipid barrier to drug delivery
- Sensory nerve plexus
- Able to secrete proteolytic enzymes
The Limbus; junction of cornea and sclera

- Arteriovenous arcades
- Lymphatic channels
- Stem cells; source of corneal epithelial cells
Bowman’s membrane

- Acellular
- No mechanism for repair
Corneal stroma (matrix)

- Type I collagen and proteoglycans (non-renewable)
- Resting keratocyte (fibroblast) population
  - Collagenase
  - Gelatinase
- High tensile strength
- Limited repair capacity
Descemet’s membrane

- Resistant to proteolysis
- Not attached to collagen matrix
- Not attached to endothelium
Corneal “endothelium”

- Not vascular endothelium
- A type of mesothelium
- Not renewable
- Can undergo fibrous metaplasia
Refractive Surgery

- Surgical methods for changing the optic characteristics of the eye
  - Usually performed on the cornea
  - Does not completely eliminate the need for glasses.
Radial Keratotomy

- Obsolete procedure
  - Free-hand incisions of the cornea
    - 4 to 40 incisions

- Compromised architectural integrity
  - Unstable radius of curvature - changing vision through the day
  - Wound rupture with minimal trauma

3/5/2010
LASIK (Laser assisted in situ keratomileusis) [?LAISK]

- Thinning a cornea with an excimer [excited dimer] laser
  - Short wave length superficial penetration
  - Surface pain and scarring
- Superficial flap
- Apply laser energy directly to corneal matrix
LASIK

- Short circuits host defenses; alters repair mechanisms
  - Microbial infection
  - Epithelial ingrowth
- Altered biochemical environment
  - Sands of Sahara syndrome
  - Epithelial ingrowth
Corneal Dystrophy

- Visible, heritable, biochemical abnormality of the cornea
- Classification changing with new genetic mapping
  - Bilateral
  - Progressive, variable clinical expression
Keratoconus

- Progressive weakening of the architectural strength of the cornea
  - (? Proteolysis, matrix metalloproteinases)
- Progressive change of corneal curvature
- Risk of rupture of Descemet's membrane [corneal hydrops]
- Risk of rupture of the full-thickness cornea [corneal perforation]
Keratoconus

- Risk of perforating Descemet’s membrane; corneal hydrops
  - Sudden onset
  - Partially reversible
- Risk of perforating full-thickness cornea
  - A surgical emergency
  - Risk of bacterial endophthalmitis
Recurrence of Corneal Dystrophies

- **Granular dystrophy**
  - Abnormal protein produced by epithelium
  - Graft epithelium is replaced by host epithelium

- **Lattice corneal dystrophy**
  - Amyloid (? keratocytes)
  - Primary localized amyloidosis
  - Graft keratocytes replaced by host keratocytes.
Fuchs’ endothelial dystrophy

- Progressive dysfunction of the corneal endothelium since birth
  - Clinical expression at age 60 to 80 years
  - Progressive corneal edema (endothelial “pump failure”)

- Treatment
  - Penetrating keratoplasty
  - Deep lamellar endothelial keratoplasty (DLEK)
Endothelial Failure (PKP)

- Epithelium
  - Subepithelial bullae
  - Intraepithelial basement membrane formation

- Descemet’s membrane
  - Generalized thickening
  - Localized thickening (guttata)
  - Loss of endothelial cell density
Endothelial Failure (DLEK)

- Anterior cornea remains intact
- Deep lamellar keratoplasty
  - Posterior corneal stroma replaced
  - Descemet’s membrane replaced
- Descemet’s membrane
  - Excrescences of Descemet’s membrane
  - En face sections of guttata
  - Few if any endothelial cells identified.
Microbial Infection of the Cornea

- Host defenses
- Passive
  - Tear film
  - Mucous
  - Glycocalyx
- Lipid epithelial barrier
  - Vulnerable posterior to corneal epithelium
  - Limited density and activity of keratocytes
- Langerhans cells
- Non-nodal lymphocytes
Pseudomonas Keratitis

- Contact lens overwear
  - Oxygen deprivation
  - Loss of passive proteins
- Adhesion of pseudomonas organisms
- Production of collagenase
  - By the organism
  - By PMN
- Progressive corneal proteolysis (‘melting’)
Crystalline Keratopathy

- Prolonged treatment with topical steroids
  - Corneal surgery
  - “allergic” keratitis
- Creation of localized immune deficiency
- Proliferation of bacteria in colonies
- Separated by stromal lamellae, appearance of crystals
- *S. epidermidis*
Herpes Simplex Keratitis (HSV-1)

- Primary cutaneous infection
- Residual organism in ganglia (dormant)
- Reactivation and migration to cornea epithelium via sensory axons
Epithelial Herpes Simplex Keratitis (Dendritic keratitis)

- Intraepithelial proliferation
  - Loss of desmosomes (vesicles)
  - Cell death; linear ulceration “dendritic figures”

- Spontaneous resolution
  - Mechanical removal
  - Antivirals
Stromal Herpes Simplex Keratitis
(Discoid keratitis, Herpes *metaherpetica*)

- Recurrence at unpredictable rate and interval
- Change in antigen status of stromal matrix
  - Giant cell reaction to membranes
  - Proteolysis of matrix
- Corneal perforation
  - Tissue adhesives
  - Penetrating keratoplasty
Acanthamoeba Keratitis

• Acanthamoeba
  – Protozoan commonly found in water
  – Forms
    • Trophozoite
    • Encysted

• Organism neurotrophic
  – Presents with extreme pain
  – Progressive ring opacity followed by ulceration
Acanthamoeba Corneal Risk Factors

- Contact lens overwear
  - Oxygen deprivation
  - Microrupture of corneal epithelium
  - Large areas of epithelial cell loss (corneal abrasion)

- Exposure to high organism concentration in hot tubs

- Exposure to stagnant water (Mississippi River Flood 1993)
Acanthamoeba Infection

- Crosses lipid barrier
- Stimulation of corneal nerves
- Production of proteases and migration through the corneal matrix
- Able to penetrate to and through Descemet’s membrane
- Not associated with endophthalmitis
- Both cornea and conjunctiva are infected
Acanthamoeba Organism

- Clinical confocal microscopy
  - Trophozoites not visible
  - Double wall of encysted organism

- Light microscopy
  - Trophozoites not visible
  - Any stain
  - Thick walled cysts
  - Usually no inflammatory infiltrate

- Treatment: unsatisfactory
Cornea: Review of Important Points (1):

– The corneal epithelium is the principle dynamic element in corneal pathology
  • Protects the structurally essential nature of the collagen matrix from infection
  • Able to contribute to structural damage by producing proteases
  • Biochemical abnormalities of the epithelium may lead to progressive corneal opacity (granular dystrophy)
Cornea: Review of Important Points (2):

– Refractive surgery potentially compromises the function of the cornea
  • Loss of architectural strength (radial keratotomy [RK])
  • Corneal scarring (photorefractive keratectomy [PRK])
  • Circumvent corneal defenses’ (laser-assisted in situ keratomileusis [LASIK])
Cornea: Review of Important Points (3):

– Corneal dystrophies recur following penetrating keratoplasty
  • The biochemical defect is in the cells of the host
  • The donor cells of corneal transplantation are replaced by host cells
Cornea: Review of Important Points (4):

- Corneal infections are often associated with proteolysis leading to corneal ulceration and possible corneal perforation
  - Pseudomonas keratitis
  - Herpes simplex keratitis
  - Acanthamoeba keratitis
Corneal specimens that influence clinical decisions

Refractive surgery
Corneal dystrophies
Microbial infections

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1) Corneal specimens that influence clinical decisions: refractive surgery, corneal dystrophy, microbial infections.

2) Case presentation: 45 year-old man with progressive corneal opacity and extreme pain
   a) Commonly used clinical terms
      i) Corneal ‘infiltrate’ = corneal opacity
         (1) Most likely due to edema of the cornea and loss of transparency
         (2) Not associated with a cellular inflammatory infiltrate
      ii) Corneal ‘melting’ = loss of corneal tissue
         (1) Most likely due to proteolysis
         (2) Not due to a change in temperature
         (3) Change in temperature would cause shrinking and opacification due to denaturation of collagen

3) Critical anatomy
   a) Tear film: Components produced by the conjunctiva and eyelid
      i) Oxygen delivery system
      ii) Passive resistance to microbial infection
         (1) Mucous
         (2) Lysozymes
         (3) Glycocalyx
      iii) Active resistance to microbial infection
         (1) Non-nodal lymphocytes
         (2) Langerhans cells
   b) Stratified squamous epithelium
      i) Conserved radius of curvature
      ii) Homogeneous cell population (no goblet cells)
      iii) Lipid barrier to drug delivery
      iv) Sensory nerve plexus
      v) Able to secrete proteolytic enzymes
   c) Limbus
      i) Arteriovenous arcades
      ii) Lymphatic channels
      iii) Stem cells, source of epithelial cells
   d) Bowman’s membrane
      i) Acellular collagen
      ii) No mechanism of repair
   e) Stroma
      i) Type I collagen and proteoglycans (non-renewable)
      ii) Resting keratocyte (fibroblast) population
      iii) Keratocytes able to produce collagenase and gelatinase
      iv) High tensile strength
      v) Limited repair capacity
f) Descemet’s membrane
   i) Resistant to proteolysis
   ii) Not attached to collagen matrix
   iii) Not attached to endothelium

1) Endothelium
   i) Not vascular endothelium
   ii) A type of mesothelium
   iii) Not renewable
   iv) Can undergo fibrous metaplasia

4) Refractive surgery
   a) Surgical methods for changing the optical characteristics of the eye
      i) Usually performed on the cornea
      ii) Does not completely eliminate need for glasses

   b) Refractive keratotomy - compromise of architectural strength
      i) Obsolete surgical procedure
         (1) Free-hand incisions of the cornea
         (2) 4 to 40 wounds
         (3) Compromised architectural integrity of the cornea
      ii) Unstable radius of curvature - variable refractive error through the day
      iii) Wound rupture - with minimal trauma

   c) LASIK (Laser-assisted in situ keratomileusis) [mileusis: Greek carving]
      i) Currently used surgical procedure
         (1) Thinning of the cornea with an excimer (excited dimer) laser
            (a) Short wavelength (193 nm)
            (b) Superficial penetration
         (2) Surface application; post-operative pain and corneal scarring
         (3) Superficial stromal application; access via a surgically created “flap”
      ii) Short circuits host defenses, alters repair mechanisms
         (1) Microbial infection - destroys host tissue, no repair mechanism
         (2) Epithelial ingrowth - creates correctable opacity
      iii) Altered biochemical environment - favors inflammation, protein deposits
         (1) Sands of Sahara syndrome
         (2) Acceleration of opacification in granular dystrophy

5) Corneal dystrophy
   a) A visible, heritable, biochemical abnormality of the cornea
      i) Classification changing with new genetic mapping (5q31)
      ii) Bilateral, may be symmetrical
      iii) Progressive, but with variable clinical expression

   b) Keratoconus - most common, generally non-heritable
      i) Weakening of corneal architecture - ?proteolysis [matrix metalloproteinase]
      ii) Progressive change of corneal curvature - non-homogeneous
iii) Risk of perforation of Descemet’s membrane-corneal hydrops
   (1) Sudden onset
   (2) Partially reversible
iv) Risk of perforation of full-thickness cornea
   (1) A surgical emergency
   (2) Risk of bacterial endophthalmitis
c) Recurrence of stromal dystrophies
   i) Granular corneal dystrophy-
      (1) deposition of abnormal protein produced by host epithelium
      (2) donor epithelium replaced by host epithelium
   ii) Lattice corneal dystrophy- deposition of amyloid (primary localized amyloidosis)
d) Fuchs’ endothelial dystrophy
   i) Progressive dysfunction of corneal endothelium since birth
      (1) Clinical expression at age 60 to 80 years
      (2) Progressive corneal edema (endothelial “pump failure”)
   ii) Treatment of Fuchs’ dystrophy
      (1) Penetrating keratoplasty (PKP)
         (a) Epithelium
            (i) Subepithelial bullae
            (ii) Intraepithelial basement membrane
         (b) Descemet’s membrane
            (i) Generalized and localized thickening (guttata)
            (ii) Loss of endothelial cell density
      (2) Deep lamellar endothelial keratoplasty (DLEK)
         (a) Excrescences of Descemet’s membrane
         (b) Few if any endothelial cells
6) Microbial infection
   a) Host defenses
      i) Passive
         (1) Tear film
         (2) Mucous
         (3) Glycocalyx
      ii) Lipid epithelial barrier
         (1) Vulnerable posterior to the epithelium
         (2) Limited density and reactivity of keratocytes
   b) Pseudomonas keratitis
      i) Contact lens overwear; oxygen deprivation
      ii) Adhesion of pseudomonas organisms
      iii) Production of collagenase
         (1) Organism itself
         (2) Polymorphonuclear leukocytes (PMN)
iv) Progressive corneal matrix proteolysis
c) Crystalline keratopathy
   i) Prolonged treatment with topical steroid
      (1) Usually following corneal surgery
      (2) Resistant corneal “allergy”
   ii) Creation of local immune deficiency
   iii) Proliferation of bacterial organism between collagen lamellae (“crystalline” serrated edges)
   iv) Intrastromal colonies of bacteria (*Staphlococcus epidermidis*)
d) Herpes simplex keratitis (*Herpes simplex type I*)
   i) Primary cutaneous infection
   ii) Residual organisms in ganglia, dormant
   iii) Reactivation and migration to corneal epithelium, sensory axons
   iv) Intraepithelial proliferation
      (1) Loss of desmosomes: vesicles
      (2) Cell death: linear ulceration, “dendritic figures”
v) Spontaneous resolution
vi) Recurrence at unpredictable rate and interval
vii) Change in antigen status of stromal matrix; stimulates inflammation
viii) Proteolytic destruction of stroma
ix) Corneal perforation
x) Treatment
   (1) Tissue adhesives
   (2) Penetrating keratoplasty
e) Acanthamoeba keratitis
   i) Protozoan commonly present in water supplies
   ii) Forms
      (1) Trophozoite; mobile (not seen by light microscopy)
      (2) Encysted; unfavorable environment
   iii) Presents with extreme pain
      (1) Organism neurotrophic
      (2) Progressive ring opacity/ulceration of cornea
   iv) Infection
      (1) Cornea compromised by
         (a) Contact lens overwear
            (i) Oxygen deprivation
            (ii) Microrupture of epithelium
            (iii) Large areas of epithelial cell loss (abrasion)
         (b) Exposure to high organism content in hot tubs
         (c) Exposure to stagnant water; e.g. Mississippi River flood 1993
      (2) Neural trophism; associated with extreme pain
      (3) Lipid barrier lost
(a) Trophozoite produces protease  
(b) Migrates through full thickness of cornea  
(c) Does not cause endophthalmitis  

v) Identification of organism  
   (1) Confocal microscopy; method of clinical identification  
   (2) Light microscopy  
      (a) Stains with all routine stains  
      (b) May not be accompanied by an inflammatory reaction.

7) Summary of Important Points  
   a) The corneal epithelium is the principle dynamic element in corneal pathology  
      i) Protects the structurally essential nature of the collagen matrix from infection  
      ii) Able to contribute to structural damage by producing proteases  
      iii) Biochemical abnormalities of the epithelium may lead to progressive corneal opacity (granular dystrophy)  
   b) Refractive surgery potentially compromises the function of the cornea  
      i) Loss of architectural strength (radial keratotomy [RK])  
      ii) Corneal scarring (photorefractive keratectomy [PRK])  
      iii) Circumvent corneal defenses’ (laser-assisted in situ keratomileusis [LASIK])  
   c) Corneal dystrophies recur following penetrating keratoplasty  
      i) The biochemical defect is in the cells of the host  
      ii) The donor cells of corneal transplantation are replaced by host cells  
   d) Corneal infections are often associated with proteolysis leading to corneal ulceration and possible corneal perforation  
      i) Pseudomonas keratitis  
      ii) Herpes simplex keratitis  
      iii) Acanthamoeba keratitis
Advances in Melanocytic Lesions of Conjunctiva

Codrin E. Iacob, New York Eye and Ear Infirmary, New York, NY

American Association of Ophthalmic Pathologists

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Handout

Particularities of conjunctiva as compared to the skin

Conjunctival lamina propria is a thin layer of loosely collagenous tissue rich in vascular supply and in immediate contact to Tenon’s capsule, sclera, ocular adnexae, and to anterior orbit. As a protective barrier conjunctival lamina propria is less efficient than dermis. Constant mechanical abrasion due to blinking favors development of flattened conjunctival lesions.

On the other hand translucency and large mobility of conjunctiva over the underlying tissues permits early detection of lesions and easier assessment of depth of location and attachment to deep structures. But palpebral and fornical conjunctival location of melanocytic lesions are often time related to delayed diagnosis.

Lesions of the conjunctiva not derived from melanocytes that may simulate pigmented lesions

Pigmented squamous lesions (papillomas or conjunctival intraepithelial neoplasia with various degrees of dysplasia up to squamous cell carcinoma, including spindle cell carcinoma)
Pharmaceutical agents (epinephrine plaques, argyrosis, minocyclin deposits)
Metallic (foreign bodies, iron, heavy metals) or industrial products) quinones, aniline dyes) deposition
Mascara deposits
Lymphoid lesions (“salmon patch” lesions)
Mesenchymal proliferative conditions (fibroma, fibrous histiocytoma, elastofibroma, myxoma, nodular fasciitis)
Juvenile xanthogranuloma
Neurofibroma
Pinguecula and pterigium
Pyogenic granuloma
Hemangiopericytoma
Inflammatory conditions (sarcoidosis, other granulomatous or allergic processes)
Pseudopigmentations (blue sclera, staphyloma, scleromalacia perforans)
Endogeneous pigmentations (hemosiderin, bilirubin depositions, Addison disease, Peutz-Jegher syndrome)
Metabolic disorders (ochronosis, Gaucher’s disease)

**Ephelis (freckle)**

Increased local pigmentation in basal epithelial cells without melanocytic hyperplasia

**Complexion-Associated conjunctival pigmentation (racial melanosis)**

Increased bilateral and symmetrical pigmentation in basal epithelial cells without melanocytic hyperplasia is seen. The pigment tends to be most intense at the limbus, fanning out toward the fornices.

**Lentigo simplex**

Increased local pigmentation in basal epithelial cells with basal melanocytic hyperplasia

**Nevi**

Congenital or acquired hamartomatous, well circumscribed, melanocytic lesions on epibulbar conjunctiva, plica, caruncle, or eyelid margins: rarely seen on palpebral conjunctiva.
Specific to conjunctiva are Henle epithelial crypts entrapped in subepithelial nevi forming cystic spaces.

Generally follow the description of their skin counterparts:
- junctional nevi
- subepithelial nevi (analogous to intradermal nevi)
- compound nevi
- blue nevi
- dysplastic nevi (proliferation of cytologically atypical melanocytes attached to a nevus)
- Spitz nevi
- Congenital melanosis oculi and congenital oculodermal melanosis (nevus of Ota): defect of melanocyte migration with formation of diffuse blue nevus in skin, periocular soft tissues, episclera, and sclera in the distribution of the ophthalmic branch of trigeminal nerve; associated with melanocytic proliferation in uveal tract, meninges of optic nerve and orbital periosteum.
Primary acquired melanosis

Specific terminology is used by ophthalmologists and ophthalmic pathologists although the lesions match their skin counterparts. In an effort to reconcile different terms used in the past, the World Health Organizations proposed the currently used term of Primary Acquired Melanosis (PAM) which better characterizes conjunctival melanocytic lesions where application of cutaneous criteria of asymmetry, superficial spreading, and depth of invasion are problematic.

Clinically it presents as an acquired unilateral golden-brown flat pigmentation of the bulbar, fornical, or palpebral conjunctiva in middle aged persons. It displays irregular borders, variable degrees of pigmentation, relentless growth, or “waxing and waning” evolution. Palpebral lesions are sometime continuous with lentigo maligna (Hutchinson freckle) of the eyelid skin. Characteristically PAM is freely movable over sclera.

PAM without atypia (31% of cases)
Some show basal conjunctival epithelial hyperpigmentation without melanocytic hyperplasia (reactive acquired melanosis). Histomorphologic overlap with freckle, but clinically more widely spread.
Some show conjunctival epithelial hyperpigmentation with melanocytic hyperplasia which is uniform and confined to basal layer. Histomorphologic overlap with lentigo simplex but clinically more widely spread.
Both have extremely low potential to evolve to melanoma.

PAM with atypia (68% of cases)
Conjunctival melanocytes harbor enlarged nuclei, palisading of enlarged melanocytes along the basal layer, local pagetoid invasion of melanocytes into the conjunctival epithelium, and nesting of melanocytes. Lesional melanocytes are characterized by cytologic and architectural features which are used to classify PAM with atypia into low and high risk categories for invasive melanoma. The skin counterparts of these two entities could be considered lentigo maligna and lentigo maligna melanoma, respectively.
Low risk cytologic atypia includes small polyhedral, spindle, and large melanocytes with pigmented arborizing dendrites. High risk cytologic atypia includes epithelioid melanocytes (75% risk of evolution to malignant melanoma).
Low risk architectural growth patterns are basilar hyperplasia, basilar nesting, and occasional suprabasilar intraepithelial nesting (all 22% risk of evolution to malignant melanoma). High risk architectural patterns are pagetoid involvement (multiple “buckshot” type melanocytic clusters of various sizes) and nearly complete epithelial replacement (both 90% risk of evolution to malignant melanoma).

Malignant melanoma
Invasion of conjunctival lamina propria and neighboring structures by atypical melanocytes is the harbinger of conjunctival malignant melanoma. In 35-40% of cases malignant melanomas arise from junctional (rare) or compound nevi; in 25-30% of cases they come from primary acquired melanosis; and in 25-30 of cases they arise de novo or
indeterminately. The ones arising from preexisting nevi have a slightly better prognosis than the ones arising in preexisting PAM with atypia or arising de novo. Melanomas in this location can be primary or secondary (from intraocular of distant skin melanomas).

Prognostically important factors are:
- unfavorable locations: palpebral conjunctiva, fornices, plica, caruncle, and lid margins
- favorable location: bulbar conjunctiva
- depth of invasion (measured from the epithelial surface): less than 1.5 mm the prognosis for life is excellent
- some studies consider this threshold at 0.8 mm and consider lesions of 1-4 mm to have a two times higher death rate while those of greater than 4 mm thickness a four times higher death rate.
- more than 5 mitoses/10 high power fields is associated with poor prognosis
- lack of lymphocytic host response is associated with poor prognosis
- lymphatic, vascular, or perineural invasion are also associated with poor prognosis

**Bibliography**

Melanocytic Lesions of Conjunctiva: WT1 and BCl2 As Useful Markers?

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Background

- Melanocytic lesions of the conjunctiva encompass a spectrum that ranges from benign or atypical nevi to malignant melanoma categories.

- Occasionally, definitive diagnosis of malignancy based on morphologic features alone can be extremely difficult.

- The aggressive clinical behavior of malignant melanoma and the increasing practice of multimodality therapy demand additional biological end points to augment conventional histopathologic stratification of patients.
Background (cont.)

- Recent studies indicate that $WT1$ and $Bcl2$ protein are detected in melanocytic lesions of the skin.

- Expression of the Wilms tumor gene ($WT1$) has been documented in several cancer cell types, including cutaneous melanomas.
Background (cont.)

- Bcl2 protein is detected in benign and malignant melanocytic neoplasms of the skin in addition to follicular lymphomas.
Bcl2 is an integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells such as lymphocytes.

Constitutive expression of Bcl2, such as in the case of translocation of Bcl2 to Ig heavy chain locus, is thought to be the cause of follicular lymphoma.

Currently, there are no published reports of Bcl2 studies in melanocytic lesions of the conjunctiva.
Objective

- Evaluation of WT1 and Bcl2 expression in conjunctival melanocytic lesions and comparison the results with other immunohistochemical markers.
Materials and Methods

• Cases (conjunctival melanocytic lesions) (n=123)
  – Benign nevi (n=72)
  – Atypical nevi (n=21)
  – Primary Acquired Melanosis (PAM) (n=11)
  – Malignant Melanomas (MM) (n=20)

• Ages ranged from 2 to 84 years
Immunohistochemistry

- Monoclonal antibodies
  - WT1 (Cell Marque Corp, 1:10 dilution)
  - Bcl2 (Dako, 1:50 dilution)
  - HMB45 (Dako, 1:50 dilution)
  - Melan A (Ventana, prediluted)
- Polyclonal antibody
  - S-100 (Dako, 1:600 dilution)
- Red chromogen was used as the counter stain in heavily pigmented cases
Amelanotic nevus of the nasal conjunctiva.
Malignant melanoma of the conjunctiva and cornea
## Immunoreactivity and Staining Patterns for Bcl2, HMB45, S100, and Melan A in Conjunctival Melanocytic Lesions

<table>
<thead>
<tr>
<th>Specimens, No. (%)</th>
<th>Bcl2</th>
<th>HMB45</th>
<th>S100</th>
<th>Melan A</th>
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<td></td>
<td>D+</td>
<td>F+</td>
<td>D+</td>
<td>F+</td>
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<td>Diagnosis</td>
<td>-</td>
<td>D+</td>
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<tr>
<td>Benign nevi</td>
<td>71</td>
<td>68 (96)</td>
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<td>Atypical nevi</td>
<td>21</td>
<td>20 (95)</td>
<td>5 (24)</td>
<td>13 (62)</td>
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<td>PAM</td>
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<tr>
<td>Without atypia</td>
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<td>3 (100)</td>
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<td>Melanomas</td>
<td>20</td>
<td>17 (85)</td>
<td>3 (15)</td>
<td>14 (70)</td>
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Abbreviations: D, diffuse; F, focal; PAM, primary acquired melanosis; +, positive; -, negative.
### Immunoreactivity and Staining Patterns for WT1 Conjunctival Melanocytic Lesions

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<thead>
<tr>
<th>Diagnosis</th>
<th>Total</th>
<th>D+</th>
<th>F+</th>
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<td>8 (80)</td>
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<td>PAM</td>
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<tr>
<td>Without atypia</td>
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<td>With atypia</td>
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<td>5 (100)</td>
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<tr>
<td>Melanoma</td>
<td>7</td>
<td>6 (86)</td>
<td>1 (14)</td>
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</table>

**Abbreviations:**
- D, diffuse
- F, focal
- PAM, primary acquired melanosis
- +, positive
- –, negative
## Degree of Positivity for Bcl2, HMB45, S100, and Melan A in Conjunctival Melanocytic Lesions

<table>
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<tr>
<th>Antibody</th>
<th>Grade</th>
<th>Benign Nevi (n=71)</th>
<th>Atypical Nevi (n=21)</th>
<th>PAM Without Atypia (n=3)</th>
<th>PAM With Atypia (n=8)</th>
<th>MM (n=20)</th>
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<td>3 (100)</td>
<td>5 (63)</td>
<td>10 (50)</td>
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Abbreviations: MM, malignant melanoma; PAM, primary acquired melanosis; +, weak; ++, moderate; +++, strong; -, negative.
WT1 and HMB45 intensity distributions of nevi and malignant melanomas
Summary

• Bcl-2 expression was positive in all benign and malignant melanocytic lesions (100%) with strong and diffuse reactivity in most cases.

• The expression was more consistent, stronger, and more diffuse than for S100, HMB45, Melan A and WT1.

• HMB 45 and WT1 showed significant differences in staining between benign nevi and malignant melanomas.
Summary (cont.)

- HMB45 and WT1 showed weak or moderate expression in benign nevi, but was strong and diffuse in malignant melanomas.

- Bcl-2, S100 and Melan A did not show meaningful differences compared to HMB45 and WT1.
Conclusions

- Bcl2 is a highly sensitive immunohistochemical marker for melanocytic tumors of the conjunctiva.
- HMB45 and WT1 staining can assist in distinguishing benign from malignant lesions.
Thank you

<table>
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<tr>
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<th>Melanoma</th>
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<td>Bcl2</td>
<td><img src="image1" alt="Bcl2 Nevus" /></td>
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<td><img src="image5" alt="WT1 Nevus" /></td>
<td><img src="image6" alt="WT1 Melanoma" /></td>
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Questions
69 yo female presented with the conjunctival lesion seen above. Which marker is least helpful in confirming the diagnosis?
Answer is E

- This lesion could be either a lymphocytic or amelanotic lesion; therefore, SMA (smooth muscle actin) is not helpful in this setting.
Which markers are helpful in distinguishing between benign from malignant melanocytic lesions of the conjunctiva? (Select two)

- A. MelanA
- B. S100
- C. HMB45
- D. WT1
- E. Bcl2
Answer is C and D. HMB45 and WT1

- **WT1** and HMB45 frequently show diffuse and strong staining in atypical nevi, primary acquired melanosis with atypia, and malignant melanomas compared with benign lesions.

- **Reference:**
Squamous and Sebaceous Lesions of Conjunctiva: Advances in Immunohistochemical and Molecular Markers

Charles Eberhart, M.D., Ph.D.
AAOP/USCAP 2010
Washington DC
Overview

• Conjunctival Intraepithelial Neoplasia (CIN) and Squamous Cell Carcinoma
• Conjunctival Spindle Cell Carcinoma
• Sebaceous Carcinoma
Conjunctival Intraepithelial Neoplasia (CIN)

- Older individuals, M>F
- Associated with sun exposure, HIV, HPV
- Generally involve perilimbal conjunctiva
- Sharply demarcated
- Can recur and progress to SCC
Conjunctival Squamous Cell Carcinoma
Conjunctival Intraepithelial Neoplasia (CIN)

• Proposed prognostic markers: p53, Ki67, AgNOR
• Other roles of special stains:
  – Rule out melanocytic lesions (Melan A, MITF, etc.)
  – Rule out sebaceous carcinoma on frozen material (Oil Red O)
  – Look for HPV (in situ)
HPV in Conjunctival CIN and Squamous Cell Carcinoma

• Several studies suggested that “high risk” HPV is often present
• Other studies failed to detect HPV
• It has recently been suggested that the “cutaneous” HPV types 5 and 8 are involved
Human Papillomavirus 16 and 18 Expression in Conjunctival Intraepithelial Neoplasia

Ingrid U. Scott, MD, MPH, Carol L. Karp, MD, Gerard J. Nuovo, MD
Ophthalmology Volume 109, Number 3, March 2002

Human papilloma virus in neoplastic and non-neoplastic conditions of the external eye

Zeynel A Karcioğlu, Tawfik M Issa

HPV 16 or HPV 18 DNA and mRNA detected in 10/10 CIN and no controls

HPV 16 or HPV 18 DNA detected in 25 of 45 (56%) in situ and invasive conjunctival SCC, but also in 32% of normal conjunctival specimens.
HPV in Conjunctival CIN and Squamous Cell Carcinoma

- Several studies suggested that “high risk” HPV is often present
- Other studies failed to detect HPV
- It has recently been suggested that the “cutaneous” HPV types 5 and 8 are involved
No HPV DNA detected in 30 cases of CIN and SCC

No Evidence for a Pathogenic Role of Human Papillomavirus Infection in Ocular Surface Squamous Neoplasia in Germany

24 consecutive cases of CIN and SCC from a single institution examined using IHC and PCR. 15 HPV subtypes analyzed – none detected.
HPV in Conjunctival CIN and Squamous Cell Carcinoma

• Several studies suggested that “high risk” HPV is often present
• Other studies failed to detect HPV
• It has recently been suggested that the “cutaneous” HPV types 5 and 8 are involved
Study in Uganda involving 94 conjunctival SCC, 39 CIN, and 285 controls

- PCR tests for 75 HPV types used
- “Mucosal” HPV rare or absent
- “Cutaneous” HPV (especially types 5 and 8) identified in 45% of SCC and 41% of CIN
- Cutaneous HPV infection and squamous conjunctival lesions seldom seen in the absence of HIV
Conjunctival Spindle Cell Carcinoma
Sebaceous Carcinoma

- Rare (1% to 3% of all malignant eyelid tumors)
- Elderly individuals (F>M)
- Often multicentric
- Prominent intraepithelial spread

Shields and Shields
Intraepithelial Sebaceous Carcinoma
EMA and p16 Immunostains in Sebaceous Carcinoma
p16 Highlights Intraepithelial Spread

EMA

p16
Adipophilin expression in sebaceous tumors and other cutaneous lesions with clear cell histology: an immunohistochemical study of 117 cases

Daniel A Ostler¹, Victor G Prieto²,³, Jon A Reed¹, Michael T Deavers², Alexander J Lazar²,³ and Doina Ivan²,³
Adipophilin can distinguish sebaceous tumors from basal cell carcinoma and squamous lesions.
Mismatch Repair in Sebaceous Carcinoma

- Sebaceous gland tumors and visceral malignancies (most frequently colorectal carcinoma) occur together in Muir-Torre syndrome (MTS)
- Sebaceous adenoma more common than carcinoma
- Mutations in MLH1, MSH2 and other genes associated with DNA mismatch repair
61 year old female with MTS
Our experience with MMR gene IHC and MSI in sporadic sebaceous carcinoma

- 9 sporadic cases stained with antibodies specific for MSH2, MSH6, MLH1 and PMS2
- No loss of staining in any sporadic tumor
- Also no signs of microsatellite instability (MSI) in any sporadic sebaceous carcinoma case tested by PCR analysis of multiple markers
Site and Tumor Type Predicts DNA Mismatch Repair Status in Cutaneous Sebaceous Neoplasia

Rajenda S. Singh, MD,* Wayne Grayson, MBChB/PhD, FCPath(SA),† Mark Redston, MD,‡
A. Hafeez Diwan, MD, PhD,* Carla L. Warneke, MS,§ Phillip H. McKee, MD, FRCPath,‡
Dina Lev, MD,‖ Stephen Lyle, MD, PhD,¶ Eduardo Calonje, MD, Dip RCPath,‖
and Alexander J. F. Lazar, MD, PhD*

FIGURE 2. Anatomic distribution of sebaceous adenomas (A), sebaceous carcinomas (B), or all sebaceous neoplasms outside the head and neck area (C), indicating whether tumors are MMR-intact (MMRI, open circle) or MMR-deficient (MMRD, closed, black circle). A and B, The nonhead and neck lesions are grouped in the lower right aspect.
FHIT may be involved in Muir-Torre cases lacking MSH/MLH changes.
Epidermal growth factor receptor (EGFR) expression in periorcular and extraocular sebaceous carcinoma

Table 1. EGFR expression in periorcular and extraocular sebaceous carcinoma

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<th>Score</th>
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<th>Extraocular</th>
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<td>9 (47.4%)</td>
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<td>1 (5–25%)</td>
<td>7 (36.8%)</td>
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</tr>
<tr>
<td>2 (26–75%)</td>
<td>2 (10.6%)</td>
<td>3 (17.6%)</td>
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<tr>
<td>3 (&gt;75%)</td>
<td>1 (5.2%)</td>
<td>12 (70.6%)</td>
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Score 0: <5% positive cells; score 1: 5–25% positive cells, score 2: 26–75% positive cells and score 3: >75% positive cells.

Fig. 2. EGFR expression (≥75% cells, 3+ intensity) in one of the extraocular sebaceous carcinomas.
Conclusions

- The role of HPV in the pathogenesis of CIN and conjunctival squamous cell carcinoma is not clear
- p16 immunostains can be useful in tracking intraepithelial spread of sebaceous carcinoma
- Immunohistochemical stains suggest an intact mismatch repair system in sporadic periocular sebaceous carcinoma
OUTLINE
I. INTRODUCTION
II. IMMUNOLOGIC DISORDERS OF THE CONJUNCTIVA
III. MEMBRANOUS AND PSEUDOMEMBRANOUS CONJUNCTIVITIS
IV. CONJUNCTIVAL DEGENERATIONS
V. CONJUNCTIVAL DEPOSITS
VI. REFERENCES

I. INTRODUCTION

Significant advances have been made in the understanding of the genetic, molecular and immunologic aspects of various conjunctival disorders. The following conjunctival conditions have been selected because these advances translate into the ophthalmic pathology practice.

II. IMMUNOLOGIC DISORDERS OF THE CONJUNCTIVA

Numerous immunologic disorders can affect conjunctiva, including Stevens-Johnson syndrome and toxic epidermal necrolysis, ocular cicatricial pemphigoid, drug-induced pemphigoid, linear IgA bullous dermatosis, graft-vs-host disease, dermatitis herpetiformis, epidermolysis bullosa, lichen planus, paraneoplastic pemphigus, pemphigus vulgaris, pemphigus foliaceous, discoid lupus erythematosus, and phlyctenular conjunctivitis.

A. Mucous membrane pemphigoid (ocular cicatricial pemphigoid)

Definition. Mucous membrane pemphigoid (MMP) and its subset, ocular cicatricial pemphigoid (OCP), is a type II immune-mediated hypersensitivity disorder, characterized by deposition of autoantibodies or complement to the components of basement membrane zone (BMZ) at the epithelial-subepithelial junction of mucous membranes and
occasionally at the dermo-epidermal junction of the skin. MMP is associated most frequently with autoantibodies against bullous pemphigoid (BP) antigen 180 (BP180) and less often with autoantibodies against BP230, laminin 5/epiligrin, laminin 6, uncin, type VII collagen and integrin subunits β4 and α6. OCP is associated mainly with autoantibodies against laminin 5 or β4 integrin.

**General features.** Binding of the antibodies to the target antigens at BMZ leads to complement activation, deposition, and inflammatory cell infiltration, manifesting clinically by sub-epithelial bullae formation and eventual cicatrization. Approximately 10 – 30% of patients with OCP demonstrate circulating IgG and IgA autoantibodies and display serologic activity against classical bullous pemphigoid antigens. Environmental triggers, such as topical medications, have been occasionally implicated in pathogenesis of OCP in genetically predisposed individuals (pseudopemphigoid). Rarely, cicatricial pemphigoid with its representative pathologic findings is observed as a sequela of SJS.

Recent research has focused on elucidating the reasons why despite similar immunopathology of BP and MMP, the MMP is associated with disabling scarring while BP is not. Some studies have found high levels of TGF-β in OCP conjunctiva and implicated this cytokine in fibrogenesis. Other studies have suggested that high levels of expression of vascular cellular adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM-1) on endothelial cells in MMP leads to IL-4 mediated stimulation of perivascular fibroblasts to lay down scar tissue.

**Clinical features.** The disease most commonly begins in the sixth or seventh decade of life and has a slight female predominance. The mucous membrane affected are most frequently oral (85%) and conjunctival (60 – 80%), followed by nasal, nasopharyngeal, anogenital, skin (20%), larynx and esophagus. Patients with extraocular MMP have approximately 4% per year risk of developing ocular involvement. The conjunctival lesions begin in one eye, but bilateral involvement eventually occurs. The clinical course of OCP is characterized by slow relapsing-remitting progression from chronic conjunctivitis to sub-epithelial fibrosis, fornix foreshortening, symblepharon and ankyloblepharon formation, corneal ulceration and opacification, and ocular surface keratinization.

**Microscopic findings.** Histopathology of the conjunctiva in acute stage of OCP shows subepithelial bullae with predominantly subepithelial inflammatory infiltrate, composed mostly of T-lymphocytes, and to a lesser extent of macrophages, dendritic cells, and neutrophils. Later in the course of disease, squamous metaplasia and loss of goblet cells are observed within the epithelium. Activation of fibroblasts leads to abnormal deposition of extracellular matrix and collagen in the substantia propria in the cicatrizing stages of OCP.

**Immunohistologic findings.** Direct immunofluorescence, immunoperoxidase technique, and immunoelectron microscopy demonstrate deposition of IgA, IgG, IgM, and C3 along the BMZ, but negative conjunctival biopsy does not exclude the diagnosis of OCP. *Positive predictive value of conjunctival biopsy with direct immunofluorescence is 60 – 80%. Repeat biopsy from extraocular site increases the chance of confirming a diagnosis of MMP.*
An antilaminin 5 ELISA with high specificity and sensitivity has been developed for circulating antilaminin 5 autoantibodies. Its role in pure OCP has yet to be determined.

**Differential diagnosis.**

1. Other causes of cicatrizing conjunctivitis: infectious (adenovirus, streptococcus, chlamydia, diptheria, gonococcus), inflammatory (rosacea blepharoconjunctivitis) atopic keratoconjunctivitis, immune (Stevens-Johnson, toxic epidermal necrolysis, lichen planus, graft-versus-host disease), trauma (chemical, radiation), drug-induced pseudopemphigoid (topical ocular medications, systemic practolol), post-surgical changes, sarcoidosis, lupus erythematosus.

2. Other bullous disorders: subepithelial (linear IgA disease, epidermolysis bullosa) and intraepithelial (paraneoplastic pemphigus, pemphigus vulgaris).

3. Ocular surface neoplasia.

**Treatment and prognosis.** Systemic immunosuppressive therapy is a mainstay of treatment and includes methotrexate, cyclophosphamide, dapsone, azathioprine, mycophenolate, and possibly IVIg. Topical corticosteroids may suppress the inflammatory response during acute exacerbations. Topical Vitamin A has been shown to reverse, to some extent, keratinization. Surgical therapies include correction of eyelid deformities, mucosal grafting for fornix reconstruction, cultivated corneal/limbal epithelial cell transplantation with amniotic membrane grafting, and keratoprosthesis.

**B. Linear IgA bullous disease**

**Definition and general/clinical features.** Linear IgA bullous disease (LABD) is an autoimmune sub-epithelial and sub-epidermal blistering disorder, which typically affects middle age adults, but has also been reported in children. Purely ocular form can occur, which is difficult do distinguish clinically and pathologically from OCP.

**Microscopic/immunohistologic findings.** Histopathology is similar to OCP. Immunofluorescence demonstrates linear deposition of IgA along BMZ. Deposits of IgG/C3 may also be present. Circulating IgA antibodies to BMZ, epidermal (epithelial), and dermal (stromal) sites can occur in some patients and are helpful in establishing the diagnosis of LABD.

**Treatment.** Treatment is similar to OCP.

**III. MEMBRANOUS AND PSEUDOMEMBRANOUS CONJUNCTIVITIS**

Membranous and pseudomembranous conjunctivitis occurs when the inflammatory discharge rich in fibrin coagulates on the conjunctival surface. A pseudomembrane lies superficially on the epithelial surface and can be peeled away without bleeding. In contrast, a true membrane incorporates conjunctival epithelium and/or granulation tissue.
and peels away with bleeding. The formation of pseudomembrane or a membrane often reflects the difference in the intensity of an inflammatory process, and both membranes and pseudomembranes may be present at the same time.

The components of the exudate in membranes and pseudomembranes may point to an etiology of conjunctivitis. For example, acute membranes in immunologic and infectious disorders are composed of fibrinous exudate, neutrophils, and necrotic epithelial cells, while in ligneous conjunctivitis, lymphocytes, plasma cells, mast cells, and immunoglobulins predominate, with the background of extensive fibrin deposition and granulation tissue formation.

**Ligneous conjunctivitis**

**Definition.** Ligneous conjunctivitis is a rare, chronic, pseudomembranous disease, characterized by wood-like (pseudo)membranes developing on the ocular and extraocular mucosa.

*Although the term pseudomembrane is typically applied to ligneous lesion in clinical literature, it behaves as a true membrane by strict clinical and pathologic terminology.*

**General features.** Ligneous conjunctivitis typically presents in childhood as a chronic, bilateral, recurrent conjunctivitis, the hallmark of which is the formation of firm, wood-like, yellowish (pseudo)membranes. These are usually located on the superior tarsal conjunctiva, and less frequently on bulbar and inferior tarsal conjunctiva. Other mucous membranes can be less frequently involved, including oral cavity, respiratory tract, middle ear, and genital tract. Other conditions rarely associated with ligneous conjunctivitis include juvenile colloid milium and congenital occlusive hydrocephalus.

Affected patients have homozygous and compound-heterozygous mutations in the type-1 plasminogen gene. Although mostly sporadic, familial cases with autosomal recessive inheritance pattern have been reported. While many reports describe spontaneous onset of disease, ligneous-like conjunctival changes can occasionally occur after ocular surgery. The condition has also been described in patients without plasminogen deficiency, who have been treated with antifibrinolytic medications, such as tranexamic acid.

It is believed that mechanical injury or exposure to external irritants of mucosal tissues is followed by exudation of plasma proteins and immediate coagulation of fibrinogen, the main constituent of (pseudo)membranes. This fibrinogen-rich clot provides local hemostasis at the site of tissue injury, but also provides the substrate for the generation of fibrin matrix, which in turn is replaced by granulation tissue. Normally, fibrinolysis and remodeling of granulation tissue complete wound healing. Plasminogen deficiency results in defective fibrinolysis, thus manifesting in arrest of wound healing at the stage of extensive fibrin deposition and granulation tissue formation.
Microscopic findings.

1. Abundant subepithelial deposits of amorphous, acellular, eosinopohilic, periodic acid-Schiff (PAS)-positive material, which consists of fibrin, immunoglobulin deposit (usually IgG), and mucopolysaccharides.

2. Granulation tissue

3. Inflammatory infiltrate, which is rich in T-lymphocytes, although B-lymphocytes, plasma cells, and mast cells can also be observed.

4. Occasionally, foreign material and bacteria have been demonstrated in the ligneous membranes, possibly providing the inciting stimulus for inflammatory response followed by ligneous membrane formation (debatable).

5. The surface of the ligneous membrane can lack normal epithelium and contains instead a collection of fibrin and inflammatory cells. This superficial layer may be scraped without bleeding, thus imparting a “pseudomembrane” characteristics to the superficial layer of the ligneous membrane.

Treatment and prognosis. Ligneous conjunctivitis is usually a self limited disorder. Serious ocular complications can result, however, including secondary infection and corneal ulceration with perforation. Treatment modalities include surgical excision of the membranes with or without adjunctive cryotherapy and amnionic membrane grafting, although recurrences are frequent. Anecdotal successes with topical corticosteroids, cyclosporine, heparin, purified plasminogen, and with intravenously administered purified plasminogen concentrate have been described.

IV. DEGENERATIONS

A. Conjunctivochalasis (“chalasis” = relaxing, slackening in Greek)

Definition. Isolated bilateral, condition in which redundant, non-edematous bulbar conjunctival tissue interposes between the globe and the lower eyelid, and protrudes over the lid margin.

General features. Conjunctivochalasis typically presents after 5th decade (average age ~70 years). The patients may be asymptomatic, or complain of dry eye, plerolacrima or epiphora. Few reported cases have been associated with nasolacrimal duct obstruction. In severe forms, exposure keratopathy and marginal corneal ulceration may occur.

It is hypothesized that aging and actinic damage induce structural changes in the collagen and elastic fibers leading to conjunctival redundancy. Another theory postulates that inflammatory mediators and matrix metalloproteinases may contribute to the collagen and elastic fiber degradation leading to conjunctivochalasis. Increased levels of
metalloproteinases could be induced by poor tear clearance, particularly in the patients with nasolacrimal duct obstruction.

**Microscopic findings.** Several reports have identified varying histopathologic findings:

1. Fragmentation of elastic fibers and sparsely assembled collagen fibers in substantia propria; lymphangiectasis; no increased inflammation or epithelial changes***
2. Increased chronic inflammatory infiltrate in the substantia propria
3. Actinic elastosis
4. Normal histology

*** The first observation is consistent with our experience with conjunctivas bearing a clinical diagnosis of conjunctivochalasis.

**Treatment and prognosis.** The condition is treated with resection of inferior limbal and bulbar conjunctiva.

**B. Pinguecula and pterygium**

**Definition.** Pingueculae and pterygia are common conjunctival lesions which typically occur in the temporal and nasal bulbar conjunctiva. Pinguecula appears as a yellow-white, often vascularized nodule, while a pterygium presents as a wing-shaped vascularized fold of conjunctival tissue, which has invaded the superficial cornea.

**General features.** Although pingueculae and pterygia traditionally have been viewed as conjunctival degenerations, this concept has been challenged by recent scientific evidence, which suggests that these lesions are non-malignant neoplasms. The pathogenesis of pingueculae and pterygia has been strongly correlated with environmental exposure (sunlight, wind, dust). Ultraviolet (UV) light (actinic exposure), in particular, is believed to be a strong inducer of these lesions. It is hypothesized that the optics of the anterior eye cause focusing of the scattered light at nasal or temporal limbus, accounting for the observed location of pingueculae and pterygia. The mechanisms proposed to explain growth of pterygia include UV-induced alteration of basal stem cells and resultant breakdown of limbal barrier, UV-induced loss of heterozygocity and loss of expression or function of tumor suppressor genes (e.g., P53), overexpression of cytokines (e.g., fibroblast growth factor, transforming growth factor-β, tumor necrosis factor-α, vascular endothelial growth factor) which may lead to upregulation of matrix metalloproteinases, and viral (HSV, HPV) infection.

**Microscopic findings.** Histopathologically, pingueculae and pterygia display actinic elastosis, increased vascularity and inflammatory infiltrate within the substantia propria. Involvement of the superficial cornea and destruction of Bowman’s layer distinguish pterygia from pingueculae. Actinic elastosis is recognized by gray or basophilic amorphous, vermiform, or hyalin degeneration of collagen fibers. These regions stain
with elastin (Verhoeff-van Giesen) stains, but the staining is not abolished by pre-digestion with elastase, supporting the conclusion that the material does not consist of true elastin fibers, but, rather, is elastin-like or elastotic. The conjunctival epithelium overlying pterygia or pingueculae may be normal or hyperplastic. Occasionally, pterygia and pingueculae can harbor ocular squamous surface neoplasia and benign and malignant melanocytic lesions, advocating for careful histopathological examinations of these lesions.

**Treatment and prognosis.** Treatment for pterygia and pingueculae ranges from symptomatic relief with topical lubricants and nonsteroidal anti-inflammatory drugs, to surgical excision with limbal conjunctival autograft and/or amniotic membrane grafts. Adjuvant mitomycin-C, β-irradiation, and anti-vascular endothelial growth factor agents are also occasionally used, particularly in pterygia with high risk of recurrence, and in recurrent pterygia. With sophisticated current surgical techniques the recurrence rate for primary pterygia is <10%.

**V. DEPOSITS**

The conjunctiva can be damaged by the use of topical and parenteral medications. Direct toxicity from active drug ingredients, drug metabolites, preservatives in drug vehicles, and deposition of drugs and their metabolites into the conjunctiva can occur. Compounds known to be actively accumulated in the conjunctiva include silver (basement membrane deposits), gold (all epithelial layers), mercury, amiodarone (epithelium), tetracycline and minocycline (within cystic inclusions), epinephrine (pigmented adrenochrome within cystic inclusions), quinacrine, and chlorpromazine. Pigmentation of the conjunctiva has also been observed after long-term or high-dose therapy with antimalarial drugs and psychotropic medications. Several of drug-induced conjunctival deposits are discussed below.

**A. Tetracycline and minocycline-induced pigmentation**

**Definition and general features.** Tetracycline and tetracycline derivatives (especially minocycline) have been frequently associated with pigmentation of bones, teeth, skin, thyroid, and less frequently, of oral mucosa. Ocular manifestations of tetracycline and tetracycline derivatives-induced pigmentation are rare, and include scleral, retinal, and conjunctival pigmented deposits. Ocular manifestations usually appear after prolonged antibiotic use, but can be dose and duration independent.

The mechanisms of pigmentation in various tissues differ, but typically include chelation with calcium or iron and oxidation of antibiotic to melanin-like pigment. Conjunctival deposits accumulate in pseudoglands of Henle possibly from degenerated conjunctival cells or their secretions.

**Microscopic findings.** Pigmented and non-pigmented lamellated concretions are observed in pseudoglands of Henle. These concretions demonstrate yellow-green autofluorescence with blue light in unstained sections. Conjunctival concretions do not
bleach or stain with Fontana-Mason stain for melanin, do not stain with Perl’s iron stain, and occasionally stain with Von Kossa stain for calcium

**Differential diagnosis.**

1. Clinical: melanocytic conjunctival lesions and other deposits
2. Histopathologic: adrenochrome deposits

**Treatment and prognosis.** Conjunctival pigmentation is not reversible, but the deposits can be surgically removed. Pigmentation of non-ocular tissues is occasionally reversible after discontinuation of therapy. Screening of patients after 1 year of administration of minocycline is recommended, with discontinuation of therapy at the first sign of tissue pigmentation.

**B. Adrenochrome deposits**

**Definition and general features.** Long-standing administration of epinephrine compounds can lead to black or dark brown deposits in the conjunctiva and cornea composed of oxidized epinephrine, or adrenochrome.

**Microscopic findings.** Histopathologically, an amorphous pink material that stains positively with the Fontana Masson silver stain for melanin and bleaches with potassium permanganate is found within the conjunctival cysts.

**Differential diagnosis.** Melanocytic conjunctival lesions and other pigmented deposits, particularly tetracycline/minocycline.

**Treatment and prognosis.** Conjunctival pigmentation is not reversible by discontinuation of medication, but the deposits can be removed surgically.

**C. Mascara deposits**

**Definition and general features.** Gray subepithelial pigmentation in inferior conjunctival fornices, after use of certain brands of mascara. Deposits consist of ferritin particles, and possibly iron oxide and carbon

**Microscopic findings.** Granular, black, mostly extracellular particles in substantia propria. Demonstrate staining with Perl’s iron stain. Do not bleach or stain with Fontana-Mason for melanin.

**Differential diagnosis.** Melanocytic conjunctival lesions and other pigmented deposits, particularly argyrosis, kohl deposits, or blood pigment.

**Treatment and prognosis.** Deposits are not reversed by discontinuation of mascara application.
D. Silver pigmentation: argyrosis (argyriasis)

**Definition and general features.** Argyrosis results from prolonged environmental exposure to silver compounds in certain industries, ingestion of silver-containing solutions, topical administration of Argyrol eye drops, or after long-term application of eyelash tint. Gray-brown discoloration of the conjunctiva and deep cornea is observed clinically.

**Microscopic findings.** Histopathologically, stippled, granular, dark deposits are noted along the basilar epithelial cell basement membrane and in the superficial substantia propria.

**Differential diagnosis.** Melanocytic conjunctival lesions and other pigmented deposits, particularly mascara deposits, kohl deposits, or blood pigment.

**Treatment and prognosis.** Deposits are not reversed by discontinuation of medication.

E. Alkaptonuria (ochronosis)

**Definition and general features.** Alkaptonuria is an autosomal recessive metabolic disorder caused by the deficiency of homogentisic acid 1,2-dioxygenase enzyme, which results in an increased level of homogentisic acid in serum. Oxidized homogentisic acid gives a characteristic bluish-black color to urine. Oxidized and polymerized homogentisic acid accumulates within the connective tissues (cartilage, skin, nails, and in the interpalpebral sclera, conjunctiva, and cornea), causing bluish-brown discoloration, referred to as ochronosis.

**Microscopic findings.** Ochronotic pigment is seen by light microscopy as variably sized, homogenous, amber globules or fiber-like structures in the cornea, conjunctiva, and sclera combined with degenerated stromal collagen. Similar to melanin, ochronotic deposits are bleached by potassium permanganate, but unlike melanin do not reduce silver substances. The deposits can be stained with elastic (Verhoeff-van Gieson’s), crystal violet, rhodamine B, toluidine blue O, and Luxol fast blue stains.

**Differential diagnosis.** Melanocytic conjunctival lesions and other pigmented deposits, particularly adrenochrome deposits and tetracycline/minocycline-induced pigmentation.

**Treatment and prognosis.** Deposits are irreversible, but can be removed surgically. Treatment of underlying disease.

F. Amyloidosis

**Definition.** Amyloidosis is a group of disorders caused by extracellular deposition of proteinaceous insoluble fibrils with a β-sheet structure derived from aggregation of misfolded proteins.
General features. Conjunctival amyloidosis typically presents in middle-aged and elderly patients as unilateral, diffuse, yellow-pink, vascular mass in palpebral/forniceal conjunctiva, frequently associated with hemorrhage. Bulbar conjunctival location and bilateral involvement are less frequently reported.

Conjunctival involvement by amyloid deposits can be classified into the following broad categories:

1. **Primary localized amyloidosis (localized amyloid light chain amyloidosis, AL)** – most common etiology of conjunctival amyloidosis; no association with systemic disease; caused by local deposition of monoclonal immunoglobulin light chains by usually benign B-cell or plasma-cell clone.

2. **Secondary localized amyloidosis** – less frequent; associated with antecedent local conjunctival trauma or inflammation (such as trachoma), and occasionally with localized conjunctival malignancy (plasmacytoma, B-cell lymphoma)

3. **Primary systemic amyloidosis (systemic amyloid light chain amyloidosis, AL)** – rarely involves conjunctiva – usually associated with light chain producing monoclonal gammopathy

4. **Heredofamilial amyloidosis** – rarely involves conjunctiva, Aβ2M amyloidosis and ATTR amyloidosis

5. **Secondary systemic amyloidosis (reactive systemic amyloid A protein amyloidosis)** – rarely involves conjunctiva; associated with inflammatory/infectious diseases (such as rheumatoid arthritis, syphilis); of note, although systemic association can be found in the patients with conjunctival amyloidosis, no systemic amyloidosis is usually demonstrated

Microscopic and immunohistochemical evaluation. Homogenous, amorphous, eosinophilic material, which stains with Congo red stain and demonstrates apple-green birefringence and dichroism. Immunohistochemical evaluation of localized conjunctival amyloidosis shows monoclonal light chain deposits of IgD, IgA, or IgG. Rarely, a lymphoproliferative process or atypical plasma cell proliferation (such as plasmacytoma) can be observed in the adjacent conjunctival substantia propria and have to be worked-up appropriately.

Differential diagnosis. Clinical misdiagnosis of lymphoid tumor is frequent.

Treatment and prognosis. Systemic work-up to exclude systemic etiologies of amyloidosis is recommended, although it is typically negative. The risk of systemic amyloidosis in patients with conjunctival amyloidosis is approximately 6%. Treatment for primary localized conjunctival amyloidosis includes observation and conservative management with ocular lubricants, and excision of localized lesions or debulking of diffuse lesions in symptomatic patients.
VI. REFERENCES