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Pathology in Focus

Ear involvement in ligneous conjunctivitis: a rarity or an under-diagnosed condition?

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Abstract

Conjunctivitis lignosa, a rare affliction of the conjunctiva, is sometimes associated with other disturbances. We present two children with concurrent conjunctivitis lignosa and ear involvement. In these two cases, there were histopathologically verified ligneous changes of the middle ears.

Routine haematoxylin and eosin, van Gieson, periodic acid-Schiff (PAS) and alcian blue staining of specimens from the eyes and middle ears revealed findings typical for ligneous conjunctivitis. In addition, new histochemical and immunohistochemical studies for glycosaminoglycans on specimens from the eyes and middle ears showed that the accumulations of the amorphous, cell-deficient material stained strongly but heterogeneously for hyaluronic acid and weakly but uniformly for keratin sulphate. The staining for other glycosaminoglycans, e.g. chondroitin-4-sulphate and dermatan sulphate was confined to vessels and areas rich in collagen fibres and fibroblasts.

In patients with conjunctivitis lignosa, the ear involvement may remain undiagnosed due to its resemblance to secretory otitis media with effusion. Since isolated ear involvement may occur, we advocate biopsies for routine haematoxylin and eosin, and specific staining for hyaluronic acid and keratin sulphate, also in children with protracted, refractory otitis media with atypical effusion.

Key words: Child; Conjuntivitis; Ear, Middle; Hyaluronic Acid; Glycosaminoglycans

Introduction

Ligneous conjunctivitis is a rare ophthalmologic condition, mostly affecting children. It is characterized by the development of pseudomembranes adherent to the tarsal conjunctiva. The pseudomembranes consist of amorphous deposits of fibrin, albumin, immunoglobulins and acidic and polyanionic macromolecular material, previously termed mucopolysaccharides.[1–3] Simultaneous involvement of the mucous membranes in the larynx and trachea has been described[4,5] suggesting a more extensive nature of the disease.

Only once before has middle ear and tympanic membrane involvement concurrently with ligneous conjunctivitis been described.[6]

In this paper, we present two cases with ligneous conjunctivitis and ear symptoms. In both of them, there was a histopathologically verified middle-ear and tympanic membrane involvement. In order to characterize the acidic, polyanionic macromolecular material in the lesions, we included a specific histochemical method for the detection of the unsulphated glycosaminoglycan hyaluronic acid (HYA) and five monoclonal antibodies for immunohistochemical identification of other sulphated glycosaminoglycans in our specimens.

Methods

Histological techniques

After fixation by immersion in five per cent formalin, dehydration and embedding in paraffin, all specimens were processed for routine haematoxylin and eosin staining and the van Gieson method. PAS and alcian blue stainings were used to verify the presence of neutral and acid macromolecular polysaccharides in the lesions.

In addition, a biotinylated protein-probe specific for free HYA was used for an accurate localization of this unsulphated glycosaminoglycan. The isolation and biotin labelling of the probe for HYA and the histochemical method have been decribed in detail elsewhere.[7,8]

Furthermore, three anti-chondroitin sulphate monoclonal antibodies (1-B-5, 2-B-6 and 3-B-3) raised against chondroitinase ABC-digested cartilage proteoglycans were used.[9] These recognize chondroitin sulphate oligosaccharide stubs attached to a proteoglycan core protein after chondroitinase digestion of the proteoglycan. The monoclonal antibody 1-B-5 recognizes unsulphated chondroitin and 2-B-6 both chondroitin-4-sulphate and dermatan sulphate after chondroitinase ABC digestion. After digestion with chondroitinase ACII, the monoclonal antibody

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FIG. 1

Thick ligneous plaque adherent to the upper tarsal conjunctiva in *Case 1*. (×5.4).

2-B-6 detects chondroitin-4-sulphate only. The monoclonal antibody 3-B-3 recognizes chronditin-6-sulphate after chondroitinase ABC digestion.[10]

Another monoclonal antibody (7-D-4), was produced in mice immunized with high buoyant density embryonic chick bone marrow proteoglycans as antigen.[11] This recognizes epitopes present in native chondroitin sulphate glycosaminoglycan chains without preceding enzyme treatment and is specific for native chondroitin and dermatan sulphate glycosaminoglycans.

Finally, a mouse anti-keratan sulphate monoclonal antibody (5-D-4), specific for highly sulphated keratan sulphate sequences was used in a dilution of 1:100 on deparaffinized tissue sections for 30 minutes at room temperature.[12] The first antibody was used with, or without, preceding incubation with chondroitinase ABC. The tissue sections were then incubated with the second antibody – anti-mouse polyvalent immunoglobulins biotin conjugate (Sigma Co., St Louis, MO, USA) in 0.01 M phosphate buffered saline (PBS), pH 7.4 and one per cent bovine serum albumin, 1:250 for 30 minutes at room temperature and rinsed in PBS. The slides were next incubated with an avidin-biotin-peroxidase complex, rinsed in PBS and then incubated with 0.1 per cent diaminobenzidine tetrahydrochloride (Saveen Products for Life Science, Malmö, Sweden), and 0.03 per cent H_2O_2 in 0.05 M Tris-HCl buffer, pH 7.6 at room temperature, producing a water-insoluble brown precipitate.

For the HYA staining procedure, control slides were incubated with *Streptomyces hyaluronidase* (Sigma Co) for four hours at room temperature. All immunohistochemistry control slides were processed under customary conditions but without the primary antibody. Photomicrographs were taken with the aid of a Zeiss Axiophot photomicroscope.

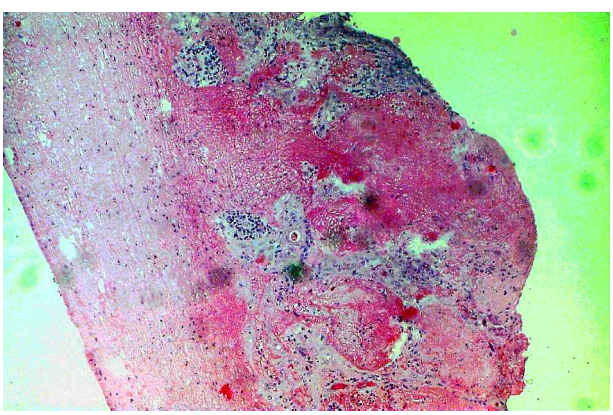

FIG. 2

Colour light micrograph of a histological section of the conjunctival ligneous tissue in *Case 1* (eosin; ×135).

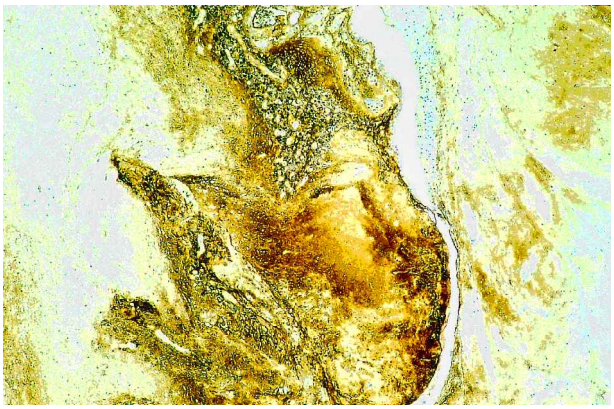

FIG. 3

Colour light micrograph of a histological section of pathological tissue from the conjunctiva in *Case 1*, showing the staining pattern for hyaluronic acid (HYA) in brown. Note the pronounced HYA staining in areas rich in vessels, fibroblasts and inflammatory cells (×135).

Material and results

Case 1

Ophthalmological history. A boy born in 1987 presented at the age of three months with bilateral conjunctivitis with a pseudomembrane of the upper left tarsal conjunctiva. Topical antibiotics as well as excisions had only a limited effect and the boy had relapses of the pseudomembranes during the following year (Figure 1). At the age of 18 months, the diagnosis ligneous conjunctivitis was established.

Microscopical examination of excised ligneous tissue revealed a fibrous connective tissue with numerous inflammatory cells and accumulations of an amorphous and cell deficient material that was eosinophilic on haematoxylin and eosin staining (Figure 2), whereas the HYA staining was irregular but strong in areas with an abundance of cells, vessels and granulation tissue (Figure 3). However, the amorphous and cell-deficient material stained only weakly and unevenly for HYA. Intensive

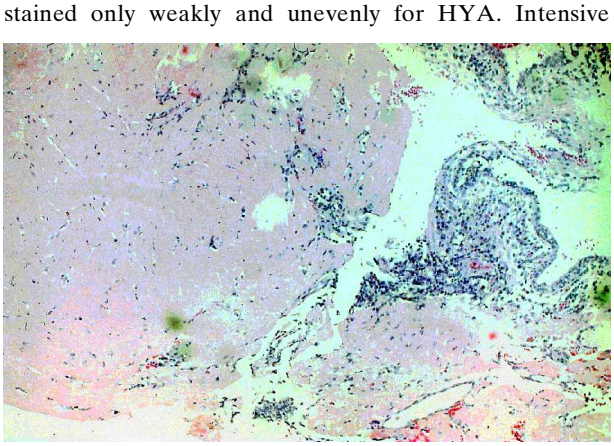

FIG. 4

Colour light micrograph of a histological section of a biopsy specimen from the right middle ear in *Case 1* (eosin; ×135). The staining pattern is similar to the conjunctival pseudomembranes in Figure 2.

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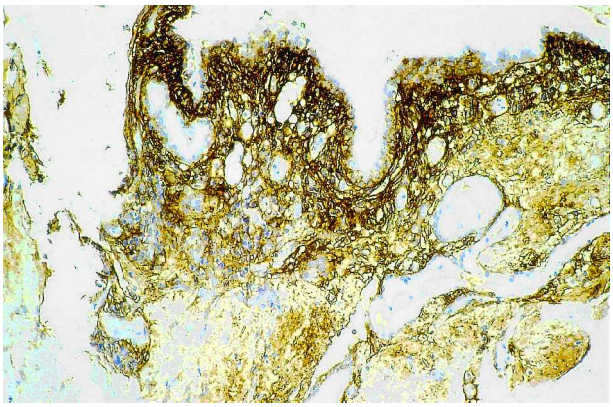

FIG. 5

Colour light micrograph of a histological section of a biopsy specimen from the right middle ear in *Case* 1, showing the staining pattern for HYA in brown. HYA is accumulated close to the mucosa and in connection with the richly vascularized submucous tissue (×270).

topical therapy was initiated with hyaluronidase eye drops (350 IU/ml hyaluronidase in isotonic NaCl at pH 7.0. Penetrase, by Leo-Lövens, Malmö, Sweden, has since been discontinued. Hyaluronidase is now available as Wydase, Wyeth Lederle, Philadelphia, USA). Four months later, the membranes had totally disappeared for the first time. The signs of the chronically inflamed conjunctiva gradually subsided and therapy was discontinued approximately one year after initiation.

At the age of four years, a limited recurrence was observed, but resolved after four months of intensive therapy with hyaluronidase eyedrops. No further eye involvement was subsequently noted.

Otological history. After a period of upper respiratory tract infections at the age of 15 months, a right-sided secretory otitis media was diagnosed. Since the secretory otitis media persisted, a ventilation tube was inserted in the right ear. The clinical picture in the right ear did not differ significantly from what can be seen in children with secretory otitis media, therefore, no biopsy was taken for histopathological examination. At five months after surgery, the ventilation tube was expelled and bilateral secretary otitis media was again present at the following check-ups.

A renewed examination was performed under anaesthesia when the boy was three years old. Because of his

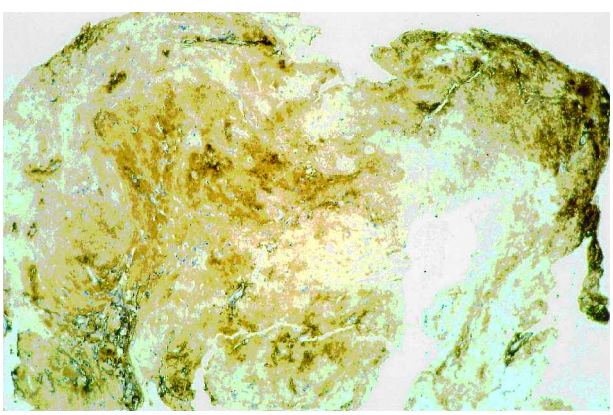

FIG. 6

Colour light micrograph of the same specimen as in Figure 5, showing the heterogeneous and weak HYA staining (brown colour) of the sclerotic amorphous material (×135).

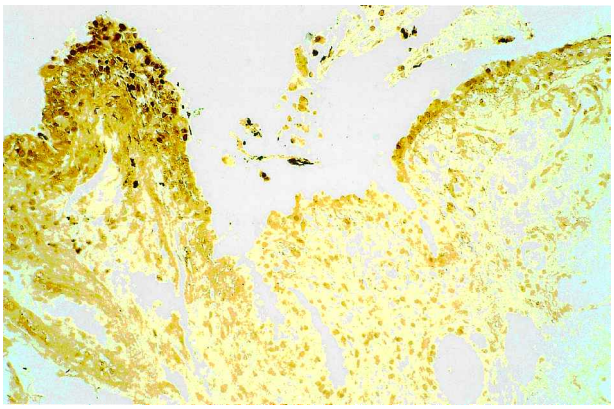

FIG. 7

Colour light micrograph of the same biopsy specimen as in Figure 5, showing the immunoreactivity for chondroitin-4-sulphate and dermatan sulphate (brown colour). Note the strong staining of surface epithelial cells and vessels. A homogeneous but weak staining of the subepithelial connective tissue is related to collagen rich areas with scattered fibroblasts (×270).

ligneous conjunctivitis and in the light of a literature report of middle-ear involvement in such patients,[6] a biopsy was taken from the right middle-ear mucosa. Histopathologically, the mucous membranes consisted of transitional-like epithelium with oedema, round cells and some polymorphonuclear eosinophilic leucocytes. There was amorphous, hyaline material which stained violet with PAS, blue with alcian blue and red with eosin (Figure 4), indicating the presence of both neutral and acidic macromolecular substances. HYA was abundant sub-epithelially and around vessels (Figure 5). However, the hyaline material stained only weakly and heterogeneously for HYA (Figure 6). The monoclonal antibody for chrondroitin-4 sulphate and dermatan sulphate stained surface epithelial cells, vessel walls, fibroblasts and collagen bundles in the subepithelial tissue (Figure 7). Highly sulphated keratan sulphate (monoclonal antibody 5-D-4) appeared to be present; distributed evenly within the amorphous material, but more specifically in connection with surface epithelia, vessels, fibroblasts and collagen fibres (Figure 8). The staining for chondroitin-6-sulphate, unsulphated chondroitin and native chondroitin was irregular, weak or absent.

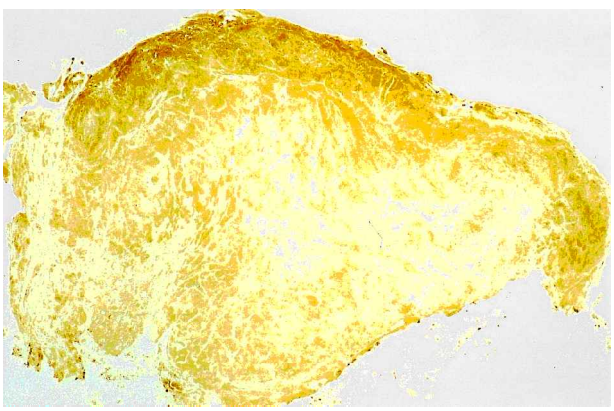

FIG. 8

Colour light micrograph of the same biopsy specimen as in Figure 5, showing the immunostaining for highly sulphated keratan sulphate (brown colour). Note the staining in the epithelial layer and evenly distributed immunoreactivity within the hyaline amorphous material (×135).

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FIG. 9

Colour light micrograph of a histological section of a small cholesteatoma removed during surgery from the right middle ear in *Case 1*. The staining for HYA (brown colour) is concentrated to the sqaumous epithelial matrix enclosing the desquamated keratan (×135).

After consultation with pharmacological authorities, an attempt was made to instill hyaluronidase drops into each middle ear through the ventilation tubes. Since there was no improvement in clinical condition or in hearing, and uncertainty prevailed as to whether the liquid actually passed through the tubes, this treatment was terminated after three weeks. No sign of ototoxic adverse effects appeared during or after the therapy.

Due to a progression of the severely retracted ear-drum on the left side and development of a cholesteatoma, the boy underwent a left-sided tympanoplasty at the age of six years. At surgery, the middle-ear cavity was filled with amorphous material and glue-like secretion. Surgery included resection of the major part of the pathological ear-drum and the middle-ear mucosa. Myringoplasty was performed with temporal muscle fascia and the denuded medial wall was covered with a silicone film (Silastic®). Biopsies from different sites of the middle-ear cavity, including the undersurface of the tympanic membrane, showed a similar histopathological picture as in the biopsies taken from the right middle ear three years earlier.

In the right ear, the retraction pathology progressed with formation of a deep cavity accompanied by cholesteatoma in the anterio-superior quadrant of the tympanic membrane. This necessitated tympanoplasty, performed shortly before seven years of age. Biopsies from the middle ear showed histopathological changes similar to the specimens from previous surgery. In addition, a small cholesteatoma showed strong HYA-positive staining (Figure 9) and some immunoreactivity for chondroitin-4-sulphate and dermatan sulphate in the matrix epithelium. The hyaline sclerotic amorphous material exhibited an irregular, weak or absent staining for chondroitin-6-sulphate, unsulphated chondroitin and native chondroitin.

One year after the tympanoplasty, polypous tissue attached to the anterior part of the right eardrum was excised. Histopathologically, this was in accordance with the ligneous tissue examined earlier. A renewed retraction pocket became visible above the columella. Since this progressed with cholesteatoma retention deep into the middle ear, a revision tympanoplasty was performed when the boy was 10 years old. Ligneous tissue, verified histopathologically, surrounded the retraction pocket, which reached into the mastoid. Hearing tests show a conductive loss of 45 dB for the right ear and it continues to be necessary to use a hearing-aid on that side.

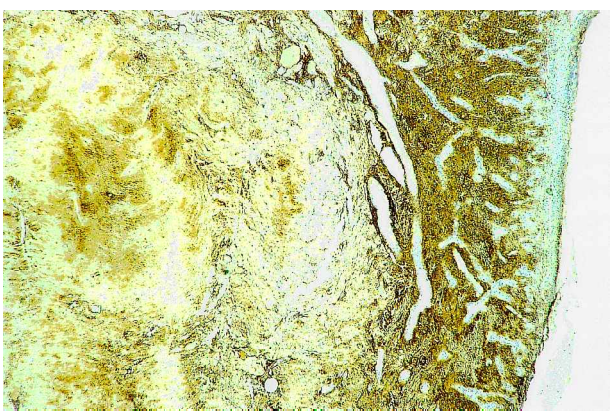

FIG. 10

Colour light micrograph of a histological section of a biopsy specimen of a tumorous mass on the inside of the upper right eyelid in *Case 2*, showing the staining for HYA. Strong HYA staining (brown colour) is seen in the richly vascularized subepithelial connective tissue and a patchy HYA distribution within the hyaline-like amorphous material (×70).

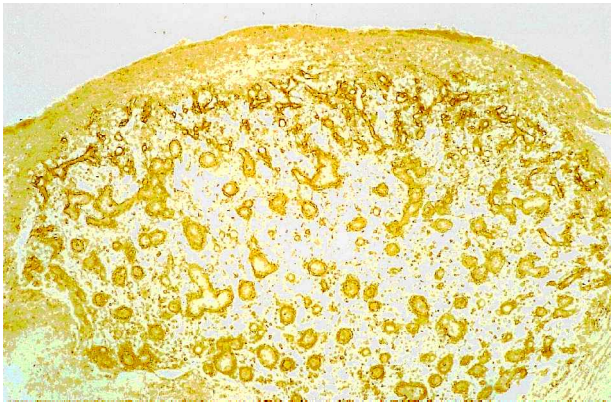

FIG. 11

Colour light micrograph of a histological section of pathological tissue from the conjunctiva in *Case 2*, showing the immunoreactivity for chondroitin-4-sulphate and dermatan sulphate glycosaminoglycans. The immunostaining is specific for the surface epithelium, vessel walls, inflammatory cells around the vessels and along collagen bundles (×135).

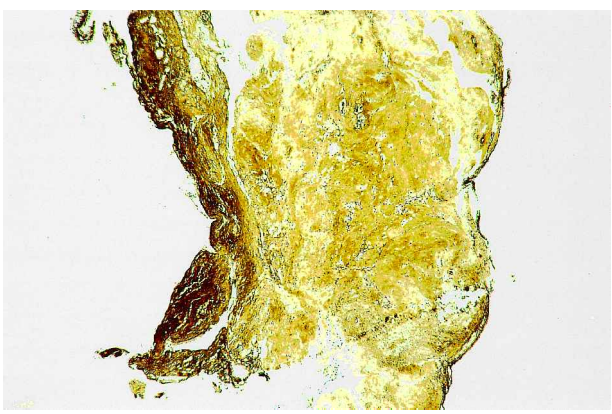

FIG. 12

Colour light micrograph of a histological section of an amorphous mass removed from the right middle ear in *Case 2*. The staining pattern for HYA shows a prominent HYA staining (brown colour) in the subepithelial tissue and a more diffuse localization of HYA within the center of the amorphous mass (×70).

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Case 2

Ophthalmological history. A girl born 1984 presented at the age of 10 years with a tumorous mass on the inside of the right upper eyelid. At a renewed biopsy, about one year after the initial mass appeared, the histopathological diagnosis ligneous conjunctivitis was established by routine methods. The distribution of HYA within the lesions was heterogeneous but consistently confined sub-epithelially within richly vascularized connective tissue and around inflammatory cells, fibroblasts and collagen fibres (Figure 10). The amorphous hyaline-like material stained more diffusely for HYA. Chondroitin-4-sulphate and dermatan sulphate positivity was related to vessel walls, surface epithelia and collagen streaks (Figure 11).

Thereafter, multiple extirpations of ligneous tissue have been performed from the tarsal side of the right upper eyelid. Treatment with dexamethasone and heparin solutions has been used, in addition to cyclosporin eyedrops. At the last ophthalmological check-up, in 1997, the ligneous conjunctivitis was still extant.

Otological history. The girl was examined at the age of four years and bilateral secretory otitis media was diagnosed. In the left ear, a rounded, cystlike structure was found adherent to the ear canal skin close to the ear drum. A histopathological investigation revealed benign granulation tissue, but the same slides, re-examined by another pathologist, revealed typical histopathological signs of ligneous tissue. During the period 1990–1994, the girl was examined for secretory otitis media at another hospital.

Due to longstanding secretions and suspicion of a cholesteatoma formation, the girl was referred at 12 years of age. Bilateral pars tensa cholesteatoma with deep retractions in both the upper anterior and posterior quadrants of the ear drums were found.

At surgery, an amorphous mass was found in the protympanum and sent for histopathological investigation. The routine staining with PAS revealed a precipitate of positive material and haematoxylin and eosin a red eosinophilic reaction, indicating a ligneous manifestation in the middle ear. The staining result was similar to that in *Case* 1, shown in Figure 4. A pronouncd staining for HYA occurred in the subepithelial tissue, whereas the staining reaction was less intense in the amorphous material (Figure 12).

Discussion

In patients with conjunctivitis lignosa, involvement of the ear has been reported only once before,[6] in two children, both with benign courses of the eye and ear affliction. In our cases, the clinical course of the ear disease was more aggressive, with histopathologically proven activity nine years after onset in *Case 1* and eight years after onset in *Case 2*. The diagnosis was not easy to establish. The early clinical picture in *Case 1* did not differ much from what can be seen in patients with secretory otitis media with effusion. In *Case 2*, there was a delay of three years between the onset of the eye symptoms and the time when the ear involvement (which has presumably continued since early childhood) was established.

In this study, the diagnosis of conjunctivitis lignosa has been confirmed histopathologically, using in addition to routine staining with haematoxylin and eosin, van Gieson, PAS and alcian blue, also specific histochemical and immunohistochemical methods in order to establish the true occurrence of polysaccharidic glycosaminoglycans in the lesions. In our cases the histopathological findings from both the ear and the eye biopsies were similar.

Using a specific staining method, we have shown that the pathologic tissue contains HYA. However, the occurrence of this glycosaminoglycan was heterogeneous and specifically related to subepithelial tissue and vascularized connective tissue with an abundance of inflammatory cells and fibroblasts. Our findings of the constant occurrence of HYA in the lesions in this study support the use of hyaluronidase drops in ligneous disease as introduced by Francois and Victoria-Troncosa.[13] Hyaluronidase treatment of the middle ear was attempted for a short period in *Case 1*, but without any obvious benefits or side effects.

The weak but relatively uniform occurrence of keratan sulphate within the hyaline sclerotic amorphous material in ligneous tissue was another interesting finding. Keratan sulphate is known to occur also in cornea, cartilage and brain.[12]

DNA analyses of five patients with ligneous conjunctivitis have shown mutations in the plasminogen gene, indicating a genetic origin of this disease.[14] This finding also opens therapeutic implications illustrated by another study, where a boy with homozygous type I plasminogen deficiency and bilateral conjunctivitis lignosa was treated with repeated intravenous infusions of Lys-plasminogen, which resulted in complete resolution of the pseudomembranes.[15]

It is possible that the ear involvement in some patients with ligneous conjunctivitis can pass undiagnosed due to its resemblance to the common and benign condition in children: secretory otitis media with effusion. We, therefore, suggest increased awareness of this possibility and strongly recommend taking biopsies in such patients, even when they show only mild ear symptoms. The possibility of isolated ear involvement should motivate biopsies also in cases of protracted secretory otitis media with atypical effusion, even if eye symptoms are absent. In patients with atelectatic ear drums showing no obvious cause, the atelectasis could be due to ligneous disease, as illustrated by the course of the ear pathology found in the two children in this study. In all cases, staining with routine haematoxylin and eosin, Van Gieson, PAS and alcian blue should be complemented with specific staining aimed at showing the typical accumulation of HYA.

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