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Case Report

Acanthamoeba Keratitis, a Semi-Quantitative Study of Encysted Organisms in a Corneal Specimen

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Abstract

Purpose: This report describes semi-quantitation of Acanthamoeba cysts in a corneal specimen.

Observations: A 59-year-old contact lens-using immunocompetent male was treated for suspected right Herpes keratitis for four months beginning 1/3/17. Topical steroids, added to minimize stromal opacities around healing dendrite-like corneal lesions, likely aggravated the Acanthamoeba corneal infection. On referral 5/5/17, a ring ulcer in the right eye and minimal hypopyon prompted confocal microscopy which documented stromal cysts typical of Acanthamoeba. Topical therapy including chlorhexidine, brolene, and polyhexamethylene plus fluconazole for several weeks was not beneficial and penetrating keratoplasty (PK) was performed 6/6/17. Clinical exam and repeat confocal microscopy on the graft 8/2/17 suggested epithelial recurrence prompting a second PK 12/20/17. No organisms were identified histologically in the second specimen. Cataract extraction and IOL were done on the right eye 6/19/18. He continues to do well seven years after the second graft, remaining on topical antibiotics with stable five clock hours of corneal neovascularization. VAs 5/22/20 were 20/30 right and 20/50 left due to nuclear sclerosis.

Results: Histopathological study of the corneal button from the original PK specimen demonstrated numerous typical Acanthamoeba cysts, some clearly double-walled, more frequent in the anterior than posterior stroma as previously reported. Enlarged 200X montage photomicrographs of Grocott methenamine silver (GMS) stains enabled counts of cysts by region across a complete central section of the button and an estimation of the number of organisms present in a 4.5 mm central section of cornea of 60,846 cysts per mm3.

Conclusions & Importance: Consistent with prior reports, Acanthamoeba cysts were more numerous in the central anterior stroma than posterior stroma. Trophozoites, although suspected, could not be definitely identified with light microscopy. CD68 red immunohistochemistry confirmed a histiocytic inflammatory stromal response. Cysts were minimal in stroma deep to the surface ring ulcer suggesting biopsies for diagnosis should avoid sampling the bed of the ulcer. This case illustrates many of the difficulties of treating Acanthamoeba keratitis including delayed diagnosis, confusion with Herpes, and possible recurrence after grafting. GMS staining with a light blue counterstain offers easy recognition of Acanthamoeba cysts in corneal tissue, although routine H&E, PAS, and tissue gram stains also demonstrate the organisms. Fluorescence microscopy after Calcofluor white staining did not alter assessment of completeness of excision by GMS staining and light microscopy.

Keywords: Acanthamoeba; Grocott methenamine silver; Calcofluor white

Case Report

A 59 you male physician with a history of soft contact lens daily wear with good lens hygiene swam and surfed regularly. He had no history of ocular trauma and was immunocompetent. His ocular history included a 3.5-month duration right keratitis with decrease in vision from 20/20 to 20/200. Initial evaluation by a general ophthalmologist noted right epithelial irregularity and dendritiform corneal epithelial lesions. He was diagnosed with HSV keratitis and treated with Zirgan (ganciclovir) topically for two weeks, which initially seemed to promote corneal healing. However, corneal lesions persisted leading to referral to a corneal specialist who added topical steroids plus systemic acyclovir in hopes of reducing stromal haze and scarring. A ProKera (amniotic membrane disc) and bandage lens were applied 2/8/17 after filamentary keratitis developed suggesting a neurotropic etiology. Right eye vision improved thereafter to 20/70. Serum tears and doxycycline added 3/31/17 were without benefit. A hypopyon was noted 5/1/17. Oral prednisone was added (30 mg daily) with decrease of the hypopyon per patient history. A ring infiltrate and recurrence of symptoms after ProKera and bandage contact lens removal prompted referral to the Cornea clinic at UCI 5/5/17 for evaluation, (Figures 1 & 2). A second PKP was performed on 12/20/2017.

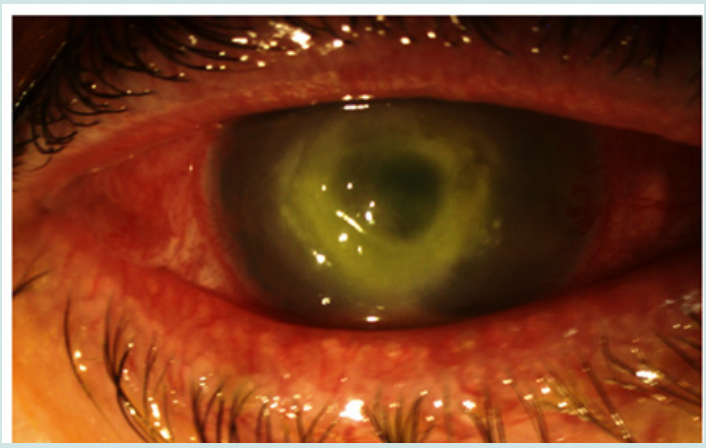


Figure 1: Clinical Photograph 5/5/17 of Ring Ulcer Right Eye. A minimal hyphema was also present, not obvious in the clinical picture.

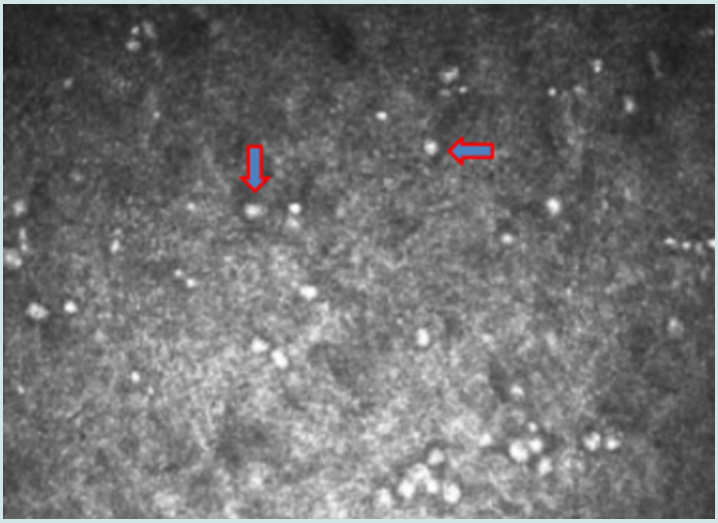


Figure 2: Confocal Microscopy 5/12/17, Double-walls are barely discernable (arrows) around some cysts in corneal stroma.

Histopathology

The first PK specimen was received in Pathology 6/6/17 as a 9 mm button in formalin and was routinely processed in paraffin with staining requests for H&E, PAS, tissue Gram stain, and Grocott methenamine silver (GMS). Additional stains were performed including CD163 and CD68 (red) for macrophages. All stains demonstrated cysts consistent with Acanthamoeba scattered throughout the stroma (Figures 3-5). Necrosis of corneal stroma

was present in focal areas. Mononuclear inflammatory cells were numerous in the posterior stroma. Descemet's was intact. The endothelium was vacuolated where present and absent in many areas. Both CD163 and CD68-red stains were positive, (Figure 6), the latter more prominently and widespread than the former. No cysts were found in corneal stroma deep to the ring ulcers in the epithelium. Repeatable hand counts of cysts present in one central section were accomplished by utilizing color printed enlargements of a 20X montage including the entire section (Figures 3 & 4). The montage had been arbitrarily divided into eight 1.125mm zones extending across the entire original 9mm diameter button. Cysts were easily recognized at the magnification chosen for the montage, (Figures 3 & 4). For purposes of cyst density per unit volume, we utilized cyst counts from the four central zones (299) (Fig. 4) equal to a length of 4 mm, true paraffin section thickness of 0.002 mm and height of 0.546 mm (average CCT in adult males) [1,2].

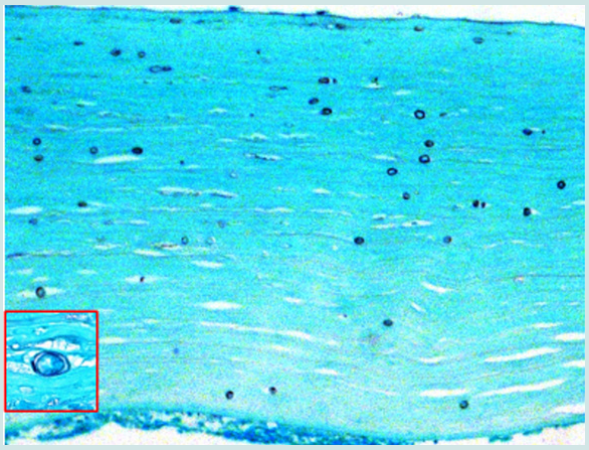
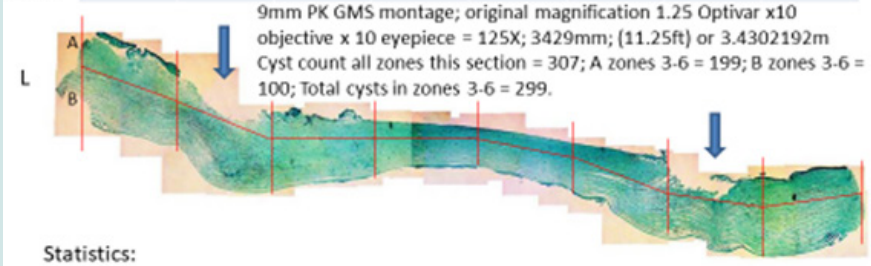


Figure 3: Grocott Methenamine Silver (GMS) demonstrating numerous Acanthamoeba cysts, most numerous in anterior corneal stroma. (Photomicrograph GMS stain original magnification X125). Inset: High magnification microscopy of Acanthamoeba cyst demonstrating typical double-wall of encysted organism. Dimensions of outer cyst wall: 11.42 X 7.05 µm (Original magnification X 787.5 [Oil immersion]).

Hand counts Acanthamoeba cysts/degenerated cysts across PK specimen

zone	1	2	3	4	5	6	7	8
A Ant. stroma	0	5	36	57	62	44	0	0
B Post. stroma	0	2	22	26	22	30	1	0



Statistics:

Student's T-Test Central zones A 3-6 > B 3-6; p-value = 0.0315 * (Statistically significant at 0.05 level).

Volume of central 4 zones in this one section = 4.5 mm length x 0.002 mm (estimated section thickness) x 0.546 mm (estimated CCT in males = 0.546. Approximate number of cysts within central 4 zones of one section = 299.

The volume of the four central zones = 4.5 (length in mm) x 0.002mm (estimated true tissue section thickness)^3 x 0.546mm (CCT in adult males)^4 = 0.004914 cubic mm; or 299/0.004914 = 60,846.56 cysts per cubic mm.

True thickness of 5-micron paraffin tissue section estimated as 5 + 2 microns3

CCT studies in normal males have indicated a mean of 546.11 (+56.9) microns.4

Figure 4: Montage photomicrograph of one entire central corneal button (+ 5µm thick) paraffin sections enlarged from original magnification X125 divided into eight horizontal and anterior/posterior portions. Epithelial surface up. Blue arrows indicate ring ulcer locations.

A companion study of sixteen prior histologically proven cases of Acanthamoeba keratitis (AK) submitted to UCI Pathology was done to compare Grocott methenamine silver (GMS) staining to Calcofluor white (Figure 5) for determining completeness of cyst excision during PK. Tissue blocks from all 16 old cases were retrieved and new sections prepared then stained with Calcofluor white (CFW). CFW is easily applied by simply dropping the dye solution onto unstained slides, followed minutes to hours later by fluorescence microscopy at 400nm. CFW did not alter any prior conclusions based on GMS light microscopic analysis of completeness of cyst excision. In fact, in one prior case GMS revealed a cyst at the margin of excision, not found by CFW. Histopathology on the second graft demonstrated a prominent retro-corneal membrane, stromal vascularization and inflammation including a single granuloma, all negative for organisms (Figures 7A & 7B) .

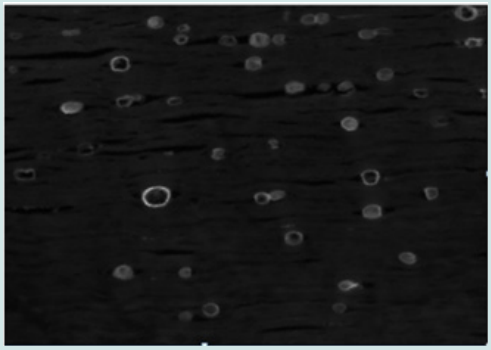


Figure 5: Fluorescence microscopy (400nm) illustrating 46 intact and degenerating Acanthamoeba cysts in corneal stroma. Calcofluor white stain demonstrates chitin within cyst walls as the dye binds to the polysaccharide polymers of amoebic cysts. This dye is able to bind to 1-3 beta and 1-4 beta polysaccharides in chitin and cellulose that is present in cell walls of fungi, plants, and algae. The double wall is not seen as the fluorescence bleeds across the gap between cyst walls.

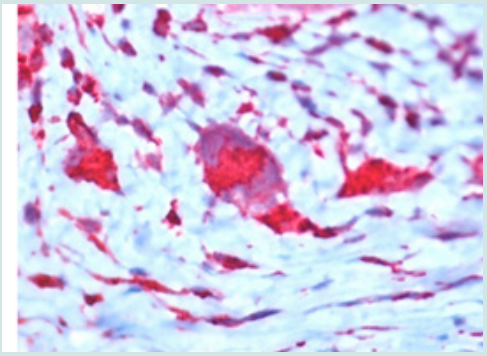


Figure 6A: Photomicrograph of CD68 red stained multinucleated histiocytes in corneal stroma deep to surface ulcer (original magnification X500).

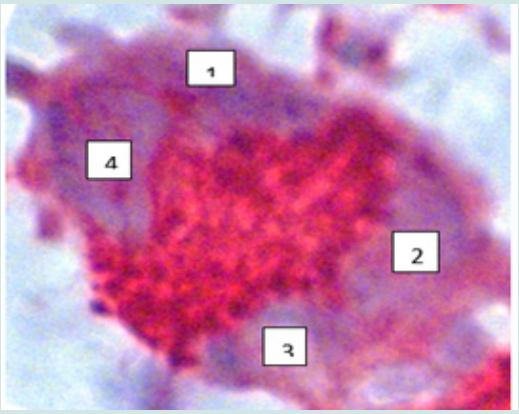


Figure 6B: High magnification photomicrograph of same cell in A; four nuclei (1-4) are visible. (CD 68 red, original magnification 787.5 (Oil immersion).

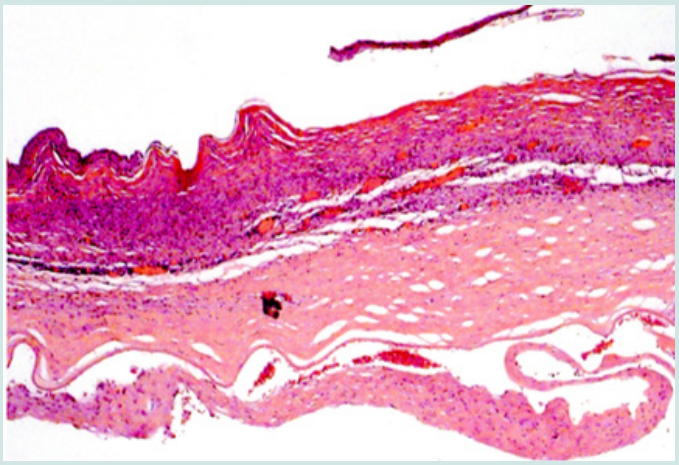


Figure 7A: Histology second corneal graft (Photomicrograph of central stroma from second PK. Neovascularization is prominent along the interface of anterior and posterior stroma. A prominent retro-corneal membrane is present, H&E, original magnification 31.25).

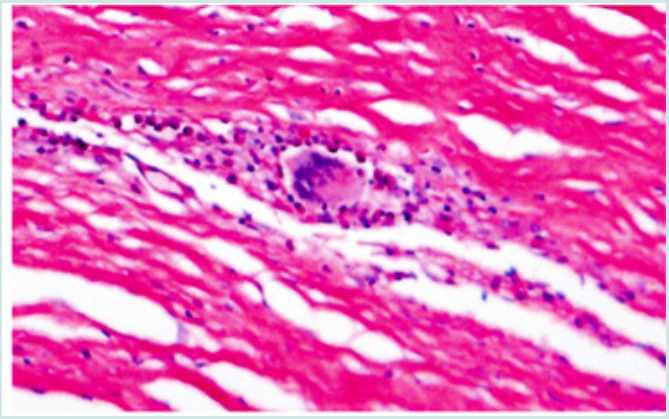


Figure 7B: Solitary granuloma in mid-stroma of second graft. A single multi-nucleated giant cell is surrounded By chronic inflammatory cells.(H&E photomicrograph original magnification X 500).

Results

Hand counts of Acanthamoeba cysts present in the central 4.5 mm portions of the corneal stroma when analysed as cyst density (cysts per mm^3 resulted in a staggeringly high number, approximately 60,000 cysts per mm^3 (Figure 4). While hand counts were straightforward and reproducible, precise calculation of the cyst density by tissue volume presents many difficulties. One cannot assume without far more sampling than done here that the density of cysts is uniform across the corneal stroma. Additional issues contributing to inaccuracy include variation in tissue sample thickness after microtome sectioning, and inter-specimen variations in central corneal thickness (CCT).

Discussion

Acanthamoeba, a protist and member of the amoebae genus is a dimorphic free-living amoeba widely distributed in soil and fresh water [3-5]. It can infect the human cornea after contact via contaminated contact lenses or after exposure to fresh or seawater, including hot tubs, and is resistant to standard chlorination. The infective trophozoite can invade the corneal epithelium and stroma. Under stress, including exposure to antibiotics, the organism encysts within a chitin-containing double wall, and becomes relatively impervious to treatment. Cysts may survive with potential reactivation for years. The same organism can cause granulomatous encephalitis, possibly invading the CNS via the nose. IgG antibodies to Acanthamoeba are present in up to 50-100% of healthy humans and tear levels of anti-Acanthamoeba IgA have been found lower in Acanthamoeba keratitis patients than in normals.1 The organism can house bacteria as endosymbionts potentially leading to a mixed amoebic and bacterial keratitis. Mixed viral and amoebic infection is also possible and per one review Acanthamoeba mixed with viral, fungal or bacterial agents account for 23% of total cases [3].

Diagnosis of Acanthamoeba keratitis is typically delayed by confusion with other forms of keratitis. Definitive diagnosis by confocal microscopy achieves 90% or more accuracy when evaluated by an experienced observer. PCR performed on epithelial scrapings detects both living and dead organisms with 84-100% sensitivity [3]. Culture from corneal scrapings or biopsy including the contact

lens case and solutions achieves a 0-77% accuracy. Histopathology of surface scrapings or biopsy including keratoplasty has an overall 31-65% positivity [3]. Clinical signs helpful to diagnosis include dendritiform epitheliopathy, micro erosions and microcysts. The dendritiform lesions do not demonstrate end bulbs typical of herpes simplex lesions.2-4 Multifocal stromal infiltrates are typically present within two weeks and ring infiltrates (Wessely immune rings) may appear during the first month. Perineural infiltrates occur in up to 63% as a manifestation of the neurotropic tendency of the organism [3-4].

Our histopathologic findings illustrate the transition between the active stromal disease with confocally visible and viable amoebic cysts and the chronic stage of infection which is an immune-mediated process characterized by a granulomatous inflammation (deep stromal keratitis or ring ulcer). In the late stages, viable organisms are usually not found, and the clinical signs are likely secondary to the inflammatory reaction, probably to antigens from the amoeba [1,2].

Conclusion

Besides demonstrating that a huge number of organisms may inhabit the central corneal stroma in Acanthamoeba keratitis, to the best of our knowledge, this report offers the first evidence that cysts are less numerous than elsewhere in the corneal stroma deep to the ring ulcer. If true in most cases the biopsies of stroma for diagnosis should avoid the areas beneath the ring ulcer grooves. We found only one published study attempting even semi-quantitation of Acanthamoeba cysts in terms of depth, distribution and density that utilized confocal microscopy [1]. Not surprisingly, the main conclusion was that Acanthamoeba cysts in cases responding to medical treatment are less widely dispersed in the stroma than in those cases undergoing surgery. Our companion study of Calcofluor white (Figure 5) suggests that fluorescence microscopy adds no useful information regarding completeness of excision by PK to that obtained with GMS staining and light microscopy [6-8].

Patient Consent

Written consent to publish this case has not been obtained. This report does not contain any personal identifying information.

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UCI does not require an IRB approval for single clinicopathologic case reports.

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Proprietary Interest

The following authors have no financial disclosures: (DM, MDVE, SK).]

Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

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