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CLINICAL STUDY

Pathological findings in the lens capsules and intraocular lens in chronic pseudophakic endophthalmitis: an electron microscopy study

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Abstract

Purpose **The aim of this study was to describe the pathological findings in lens capsules and intraocular lens (IOL) studied by scanning and/or transmission electron microscopy (SEM and TEM, respectively) in a series of four eyes with chronic pseudophakic endophthalmitis (CPE).**
Patients and methods **We performed a retrospective study of four patients presenting CPE in whom surgical treatment with pars plana vitrectomy, capsulectomy with extraction of the IOL, and intravitreous antibiotic therapy was thereafter performed. The extracted IOL and the capsular remains were studied by SEM and/or TEM and microbiologic analysis of aqueous humour and vitreous aspirate was also carried out in all the cases.**
Results **The presence of microorganisms was observed in the material analysed in all the cases studied. The use of TEM identified bacterial contamination by *Staphylococcus* spp and mixed contamination with microorganisms presenting a bacillar morphology suggestive of infection by *Propionibacterium acnes* in addition to the presence of cocci in the capsular remains. In another two cases, SEM localized colonies of *Staphylococcus* spp on the surface of the IOL in one case and mixed bacterial colonization with cocci plus filamentous bacteria in the other. The presence of macrophages associated with bacteria was observed in the capsular remains.**
Conclusions **Microorganisms were found in the IOL or the capsular material in the four cases studied, thereby explaining the refractoriness and severity of infection. The possible presence of polymicrobial infections, especially in the cases with filamentous bacteria, also explains the recurrence of infection.**
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Introduction

Chronic endophthalmitis following cataract surgery is difficult to treat, as there is generally considerable delay in diagnosis, which thereafter leads to an important problem when implementing therapy due to the peculiar and deceptive evolution of this infection and the slight efficacy of systemic treatment.[1] Although the number of microorganisms causing the development of chronic endophthalmitis following phacoemulsification surgery with intraocular lens (IOL)-bag implantation has increased in recent years, most of these infections are due to anaerobic bacteria of low virulence such as *Propionibacterium acnes* (*P. acnes*) and coagulase-negative *Staphylococcus*.[1] Filamentous fungi such as *Aspergillus* or yeasts such as *Candida albicans* may also, rarely, produce these infections.[1]

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The aim of surgical management of the infection is to completely eliminate, whenever possible, the contaminating foci and thereafter restore sight. The surgical approach, especially in cases produced by *P. acnes*, involves performing a pars plana vitrectomy (PPV) and may include two types of approaches. With the most conservative approach, a selective posterior capsulectomy of the most infiltrated zone of the capsular bag is performed in association with intraocular injection of antibiotics. The second, more radical approach involves total capsulectomy with intravitreous antibiotics as well as extraction of the IOL.[2,3] The aim of this study was to analyse the remains of the lens capsule or the extracted IOL of four cases of chronic pseudophakic endophthalmitis (CPE) with scanning (SEM) and transmission electron microscopy (TEM) and determine the usefulness of these techniques in the orientation of the diagnosis and therapy of this infection. Moreover, the results were compared with those obtained with conventional microbiologic studies.

Patients and methods

Four patients with CPE following phacoemulsification surgery and IOL implantation were included in the study. Preoperative diagnosis was achieved according to the clinical manifestations of the patients and the four underwent surgery by PPV and complete capsulectomy, with extraction of the IOL through a corneal incision together with an intravitreous injection of vancomycin. In the four patients, the IOL and/or the lens material obtained by PPV were studied by electron microscopy with microbiologic study of the aqueous humour and the vitreous also being performed.

Microbiological analysis

Conventional microbiological analysis was carried out for both aerobic and anaerobic conditions. Undiluted aspirates obtained from the aqueous humour and vitreous during PPV surgery were maintained in sterile conditions and cultured in the Department of Microbiology following standardized protocols. In brief, standardized protocol included cultures for aerobic pathogens during 48 h in both sheep's blood and chocolate agar. Sheep's blood agar was also used in anaerobic conditions. Negative cultures were considered after 3 days.

SEM and TEM

Four IOLs and lens capsular material were immediately fixed in 2.5% glutaraldehyde–2% paraformaldehyde in 100 mM phosphate buffer solution (PB pH 7.4) for 2 h and were then contrasted in 1% osmium tetroxide for 1 h. For SEM, the IOLs were serially dehydrated in progressively increased concentrations of ethanol solution followed by critical point drying. The IOLs were thereafter mounted on metal stubs to perform SEM and covered with a layer of thin gold particles using a sputter-coating system. They were then observed with a Hitachi S-23000 SEM (Hitachi Inc., Japan) at 10–20 kV. For TEM, the capsular material was dehydrated in graded acetone series and then embedded in resin (Spurr) for polymerization at 60°C. Ultrathin sections (50–70 nm) were contrasted with uranyl acetate and lead citrate and examined with a Hitachi 800-MT TEM (Hitachi Inc., Japan). The images obtained were digitalized and stored in a tagged image file format in the microscope computer image analysis program (Quartz PCI, Scientific Image Management System, v 5.1, Quartz Image Corp., Canada).

Results

Three IOLs analysed with SEM and TEM were used to study two cases of lens material obtained from PPV surgery in which lens capsules and IOL were extracted. The presence of bacterial biofilm was found to be adhered to the surface of the IOLs in the three cases studied with SEM as well as in the capsular remains of the two cases evaluated by TEM. In one case (Case 4), the findings were suggestive of the presence of microorganisms in the two types of material, the IOL and capsular remains. Three cases showed mixed contamination (bacilli plus cocci or filamentous bacteria plus cocci). In one of these latter cases (bacilli plus cocci), the microbiologic culture was positive for *Staphylococcus epidermidis* (*S. epidermidis*). The culture with simple contamination by cocci was also positive for *S. epidermidis*. In two cases of mixed contamination (Cases 1 and 3), the microorganisms did not grow in bacterial cultures of the ocular fluid. Analysis with SEM was critical for reorientation of systemic clinical treatment in one case of mixed contamination of cocci plus filamentous bacteria (Case 3).

Case reports

Case 1

A 59-year-old male who had undergone uneventful phacoemulsification surgery in the right eye with an IOL-bag implantation 6 months previously developed persistent postoperative inflammation 2 months after the surgery and presented poor response to topical and systemic treatment. Vision was 20/200 and examination with slit-lamp showed whitened capsular infiltration and large keratic endothelial precipitates with 1+ cells in the anterior chamber. The patient was diagnosed of CPE

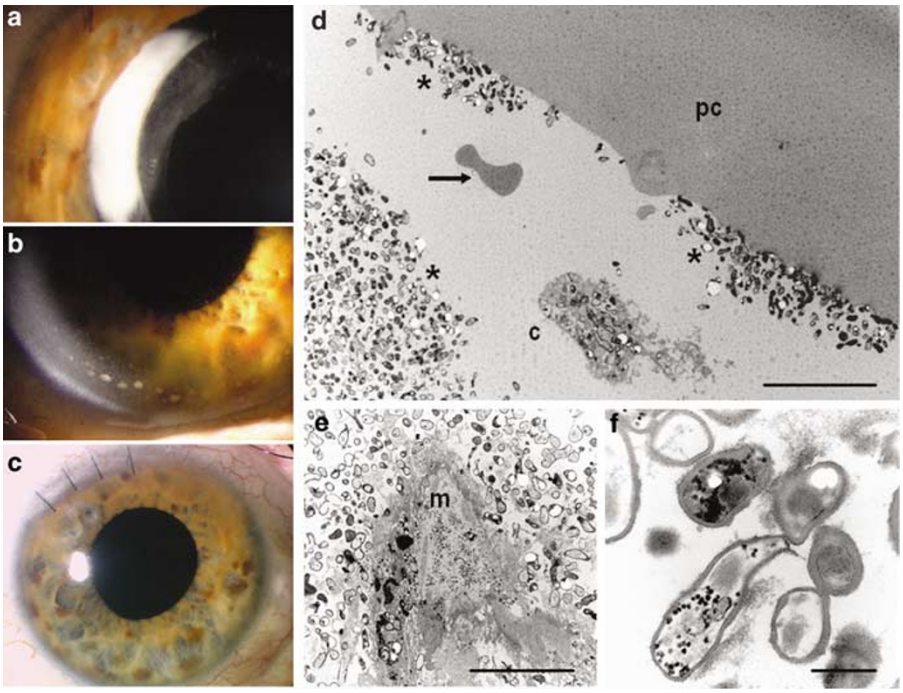

Figure 1 (Case 1) *Slit-lamp examination*. (a) Whitened capsular infiltration is observed. (b) Large keratic endothelial precipitates with 1+ cells in the anterior chamber (c) The postoperative course was correct following PPV and extraction of the IOL with no further inflammatory signs. *TEM analysis of capsular material*. (d) Extensive contamination (asterisk) was observed in association with the remains of the capsular material (PC) with some degenerative cells (c) and erythrocytes (arrow). (e) Active macrophages (m) were also seen in association with colonies of bacteria of sphere-shaped features suggestive of cocci (f) Ultrastructurally, spore-like bacteria were found with the external membrane presenting rods suggesting co-colonization with *P. acnes* or *Bacillus* spp. Bar for (d) 10 μm; (e) 5 μm; and (f) 0.5 μm.

(Figure 1a and b). Surgery was performed, including PPV, partial capsulectomy, and intravitreous injection of 1 mg of vancomycin. Microbiological analysis of both the aqueous humour and the vitreous was negative. The patient received another intravitreous injection of 1 mg of vancomycin 3 months later because of recurrence of anterior segment inflammation. At 6 months, another relapse led to PPV, complete capsulectomy, and explantation of the IOL being performed in addition to another injection of 1 mg of vancomycin. The capsular material was processed for TEM. The postoperative course was uneventful, with the vision being 20/40 6 months later (Figure 1c).

In this case, TEM study of the capsular material presented abundant bacterial colonies associated with the capsular surface (Figure 1d). In addition to scarce erythrocytes, degenerated cellular remains associated with bacteria were observed. Bacterial colonies were found in determined regions of the specimen with the presence of active macrophages presenting secondary lysosomes with degenerative content (Figure 1e). The bacterial colonies demonstrated mixed elements with bacillar-shaped spore-like bacteria suggestive of *P. acnes* or *Bacillus* spp in addition to coccoid structures suggestive of *Staphylococcus* spp (Figure 1f). Microbiological cultures for aerobic and anaerobic bacteria were negative.

Case 2

A 79-year-old woman who had received uneventful cataract surgery by phacoemulsification with an IOL-bag implantation in the left eye 6 months previously was admitted for persistent inflammation 4 months after the surgery. Her vision was 20/400 and examination with slit-lamp showed hypopyon and corneal oedema (Figure 2a) leading to a diagnosis of CPE. Radical surgery including PPV, complete capsulectomy, extraction of IOL, and intravitreous injection of 1 mg of vancomycin was required due to the severity of the presentation. Vision 8 months after surgery was 20/60 with no recurrence of infection (Figure 2b). Microbiological analysis was positive for *S. epidermidis* in both the aqueous humour and vitreous. IOL was processed for SEM.

Analysis of the surface of the IOL with SEM showed the presence of different, disperse, heterogenic bacterial biofilms with apparently low stickiness. These biofilms consisted in bacterial clusters, some of which were abundant (Figure 2c), whereas others were in small colonies of isolated cocci suggestive of *Staphylococcus* spp (Figure 2d). Likewise, areas with a moderate

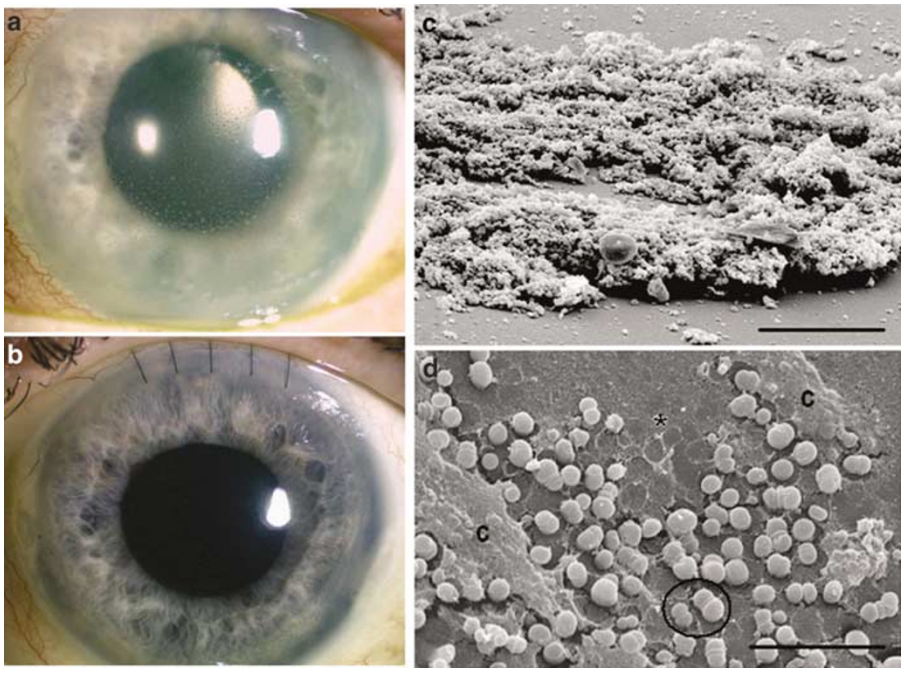

Figure 2 (Case 2) *Slit-lamp examination*. (a) Hypopyon and corneal oedema is observed. (b) Appearance following radical surgery with extraction of IOL and PPV with no inflammatory signs. *SEM analysis of IOL surface*. (c) Disperse, irregular, heterogeneous bacterial biofilm on the surface of the IOL, with apparently low stickiness. (d) Abundant clusters of isolated bacteria with sphere-shaped morphology suggestive of cocci spp were observed associated with cellular remains (c) and deposits of hexapolyssacharide bacterial capsular material (asterisk). *Diplococci* division presenting a divisional plate (circle) was found in different stages of growth. Bar for (c) 20 μm and (d) 5 μm.

accumulation of proteins were found in the bacterial capsule on the surface of the IOL in addition to the presence of cellular remains and scarce erythrocytes.

Case 3

A 74-year-old male who had undergone uneventful cataract surgery by phacoemulsification with an IOL-bag implantation in the left eye 1 year before presented inflammation 2 months after surgery with poor response to treatment leading to the diagnosis of CPE. His vision was 20/200 and examination with slit-lamp showing keratic endothelial precipitates and whitened capsular infiltration (Figure 3a). Surgery was indicated performing PPV, partial capsulectomy, and intravitreous injection of 1 mg of vancomycin. Recurrence occurred at 3 months with hypopyon and infiltrates in the cornea (Figure 3b). The patient was administered treatment with oral clarithromycin (500 mg/12 h) without improvement. In view of the lack of response, another PPV was performed with complete capsulectomy and extraction of the IOL. Histopathological findings by SEM demonstrated filamentous bacteria suggestive of fungi. Systemic treatment with oral fluconazole (200 mg/12 h) was implemented for 8 weeks. In final vision was 20/80 with no evidence of recurrence (Figure 3c).

With SEM the presence of mixed contamination associated with disperse erythrocytes was observed on the surface of the extracted IOL (Figure 3d). Two types of bacterial biofilms were found, one with some coccoid cellular structures suggestive of *Staphylococcus* spp as well as extensive regions with abundant quantities of filamentous bacteria (Figure 3e and f). The second biofilm suggestive of the presence of hyphae was regular and homogeneous with high stickiness. The bacterial cultures performed in aerobic and anaerobic conditions were negative. The finding of contamination by filamentous bacteria in the IOL was important for the reorientation of treatment in this case with the introduction of anti-fungal drugs being required.

Case 4

A 66-year-old woman who had undergone cataract surgery by phacoemulsification and an IOL-bag implantation in the right eye 1 year before presented persistent inflammation since 2 months after the surgery. Her vision was of hand motions and examination with slit-lamp showed capsular infiltration, extensive anterior synechiae and +2 cells in the anterior chamber leading to a diagnosis of CPE (Figure 4a). Surgery with PPV, extraction of the OIL, caspsulectomy, and intravitreous injection of 1 mg of vancomycin was performed. Culture

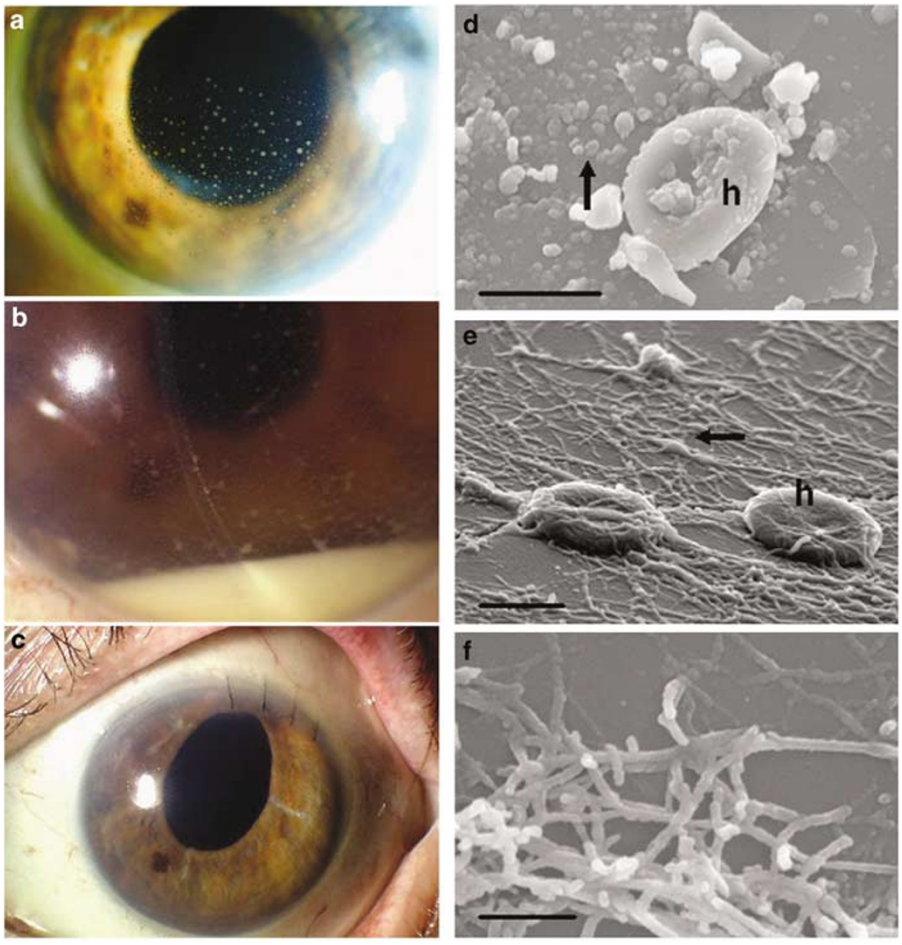

Figure 3 (Case 3) *Slit-lamp examination*. (a) Keratic endothelial precipitates and whitened capsular infiltration were observed. (b) Recurrence developed at 3 months with hypopyon and corneal infiltrates. (c) Postoperative appearance with no evidence of recurrence. *SEM analysis of IOL surface*. (d) Extensive contamination by cocci spp was observed on the surface of the IOL (arrow). Several erythrocytes (h) and cellular remains were also seen. (e) Some areas on the IOL surface demonstrated colonization by abundant filamentous bacteria (arrow) with a biofilm of high stickiness also associated with the erythrocytes (h). (f) Some regions showing important hyphae growth. Bar for (d) and (e) 5 μm and (f) 2.5 μm.

of the vitreous and aqueous humour was positive for *S. epidermidis*. Vision was 20/50 at 6 months with no recurrence of infection (Figure 4b).

Analysis by TEM showed capsular remains with an abundant accumulation of cells in different stages of degeneration (Figure 4c and d). Active macrophages were observed among the cellular remains with degenerative lysosomal material associated with cell clusters with coccoid features (Figure 4d) suggestive of *Staphylococcus* spp. Likewise, SEM showed the presence of possible polymicrobial contamination on the surface of the explanted IOL. In this case, the bacterial biofilm was quite irregular and heterogeneous with characteristics of very low stickiness (Figure 4e). Some proteic clusters with cells with coccoid structures were found, often in zones of co-colonization with cells presenting a bacillar morphology suggestive of *P. acnes* (Figure 4f).

Discussion

One of the main problems with PCE is the difficulty in isolating the microorganisms, especially in cases of *P. acnes*.[4,5] Consequently, in most cases, clinical diagnosis is based on the appearance of ocular manifestations and posterior microscopic and microbiologic examination of the intraocular samples obtained. Molecular diagnosis techniques by polymerase chain reaction in the aqueous humour have several advantages, although they are not always available because of their high cost and the infrastructure required for implementation.[6] Although TEM and SEM techniques lack early perioperative diagnostic utility, they may be very useful for improving the understanding of the physiopathology of the infection and, consequently, aid in establishing therapeutic strategies. Our findings are of special

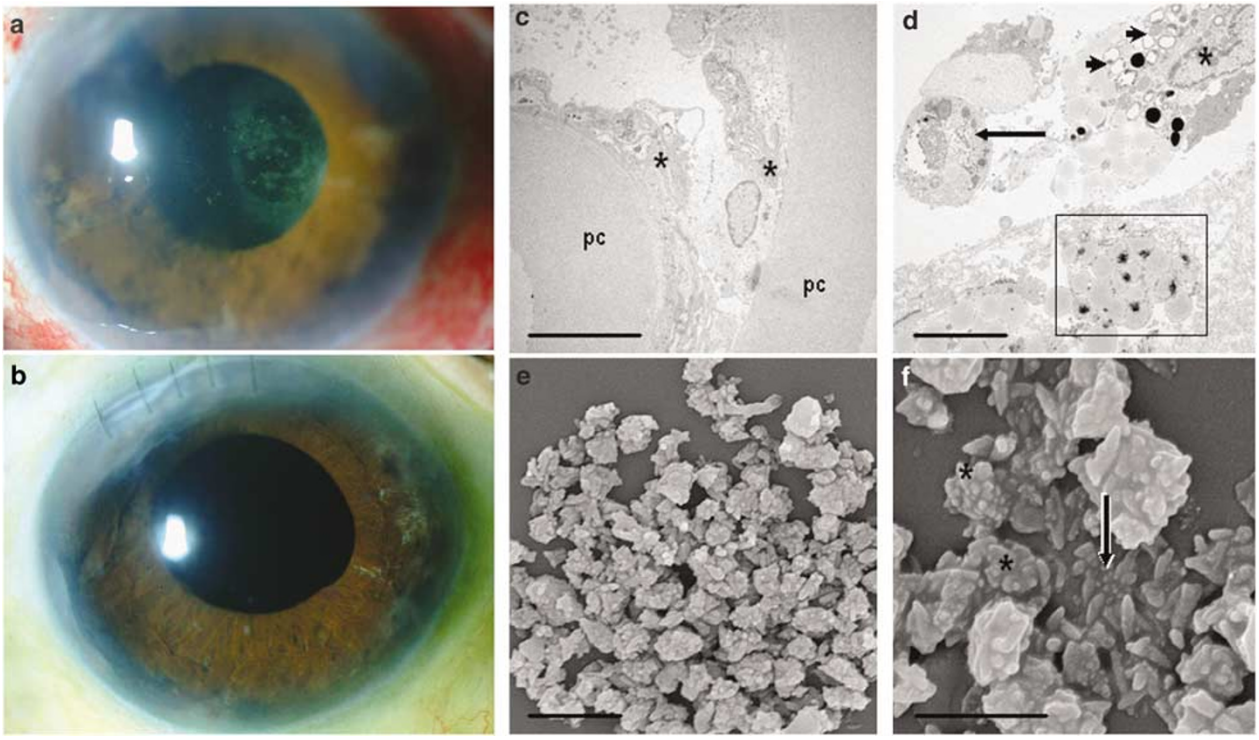

Figure 4 (Case 4) (a) Examination with slit-lamp showed capsular infiltration, extensive anterior synechiae and 2+ cells in the anterior chamber. (b) Postoperative appearance with no reactivation of the infection. *TEM analysis of the capsular material and SEM analysis of the IOL surface*. (c) Cellular remains (asterisk) with different degrees of degeneration were adhered to the capsule lens (pc). (d) Active macrophages with lysosomal content (asterisk) often associated with coccoid cellular structures (arrowhead) were observed in areas presenting several cellular remains (arrow). These regions presented areas of vesicle formation with a lipid content showing, at times, electrodense inclusions (square). (e) SEM detected isolated and very heterogeneous and disperse bacterial biofilms on the surface of the IOL with very low stickiness. (f) These biofilms were made up of colonies morphologically characterized by rod bacteria (arrow) suggestive of *P. acnes* associated with clusters of sphere-shaped elements (asterisk) suggestive of *Staphylococcus* spp Bar for (c) to (f) 5 μm.

interest, as the initial presentation of the four patients in this series was with recurrent or severe forms of CPE requiring radical treatment with complete capsulectomy and explantation of the IOL.

Colonization of the IOL by microorganisms is controversial in CPE, as it may signal the possible necessity of explanting the IOL. Saika *et al*[7] published a case of CPE studied by optical and electronic microscopy in which the presence of bacteria on the surface of the IOL could not be detected, despite demonstration of microorganisms in the capsular bag by optical microscopy. In the present series, we found microorganisms on the surface of the extracted IOLs or in the processed remains of the capsulectomy in the four cases studied, although only two of the vitreous cultures were positive for *S. epidermidis* (Cases 2 and 4). Consequently, our histopathological findings demonstrate that a negative culture does not necessarily rule out the presence of microorganisms in cases with clinical suspicion of CPE. These findings also suggest that in cases of greater severity and recurrence, the extraction of the IOL is necessary for definitive eradication of the infection. This is not only valid in the cases of *P. acnes* but also in the secondary infections, which are *Staphylococcus* coagulase negative, although some studies have suggested that IOL explantation is not necessary in these cases.[8] Bacterial biofilm is produced in the IOLs by microorganisms, which have the tendency of adhering to the lens and are related to the concentrations of nutrients between the solid–liquid phases. Bacterial colonization on the surface of the lens in the cases of CPE is initiated by dissemination from the lens capsules.[9,10]

Similar to the findings by Warheker *et al*,[11] the use of TEM in our study identified the association of microorganisms with degenerated cellular remains accompanied by macrophagic activity. This fact emphasizes the need for the use of antibiotics with intracellular action such as clarithromycin.[12] Other antibiotics such as vancomycin, even administered intraocularly, may fail to control the infection because of their low effectiveness to be uptake for the cells.

Another aspect of interest in our findings is the demonstration of polymicrobial infection in two cases,

with only two other cases having been reported previously.[12,13] In one of the patients published,[13] the aqueous and vitreous cultures were negative, although the cultures of the IOL and the capsular bag were positive for *Candida parapsilosis*, *Corynebacterium striatum*, and *S. epidermidis*. In the other case, endophthalmitis was caused by *Alcaligenes xylosoxidans* and *P. acnes*.[14] We believe that the presence of polymicrobial infections is of special relevance when considering filamentous bacteria such as in Case 3 in our study. Fungi may cause postoperative endophthalmitis whether due to *Basidiomyucetus*,[15] *Histoplasma*,[16] or *Nocardia*.[17] Likewise, endophthalmitis by filamentous bacteria may mimic infection produced by *P. acnes*. In the cases of yeasts such as those produced by *Candida* spp, oral fluconazol at doses of 400–600 mg/day are effective.[18] In one of our patients (Case 3), the evolution with treatment was correct and achieved control of the infection. Consequently, the observation that fungi and filamentous bacteria tend to form biofilms with high stickiness in several intraocular tissues including the IOL, in this study as well as others, suggests that biopsy or excision of the capsule should be considered for cultures in pseudophakic patients with strong clinical evidence of refractory endophthalmitis, but previously negative vitreous cultures.[19]

In conclusion, the use of electronic microscopy for the analysis of lens material and the explanted IOL in cases of CPE allows the collection of data related to the physiopathology of the infection. Based on our findings, recurrent cases with greater severity are probably associated with colonization of the IOLs by microorganisms and polymicrobial infections. In these cases, radical surgical treatment by PPV and complete capsulectomy with extraction of the IOL together with intravitreous injection of antibiotics is the treatment of choice to eradicate the infection. Fungal aetiology should also be taken into account in cases that are refractory to conventional surgical treatment with vitrectomy in which the IOL is preserved. Lastly, the use of antibiotics with intracellular penetration such as clarythromycin may favour better resolution of infection.

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