

PRESENTACIÓN DE CASO

Endophthalmitis by *Pseudomonas aeruginosa*. after penetrating keratoplasty, case report with an epidemiological investigation

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Introduction. An endophthalmitis following penetrating keratoplasty by *Pseudomonas aeruginosa* is a devastating case with very poor visual outcomes.

Objective. To determine the origin of an infection after a penetrating keratoplasty.

Materials and methods. After an endophthalmitis an epidemiological study was undertaken with the approval of the ethics committee and support of a medical team comprised of an epidemiologist, infectologist, bacteriologist and ophthalmologists specializing in cornea. Factors that may have contributed to the risk of infection were assessed, for example, the processing and preservation of the cornea in the moment of the extraction, the characteristics of the donor, recipient and infecting bacterium, as well as the details pertaining to the surgical operation.

Results. No risks factors were found in the institution, in the eye bank facilities, in the donor or in the receptor. However, sterile technique could not be guaranteed in the morgue where the corneal extraction occurred, and other isolated cases of endophthalmitis post-keratoplasty had been documented involving tissues from the same morgue that had been processed by two eye banks in the same city. Characteristics of the multi-resistant *Pseudomonas* sp. demonstrated its origin from a hospital environment due to its previous exposure to a variety of antibiotics.

Conclusions. Corneal extraction site must guarantee an antiseptic preparation and aseptic tissue donor recuperation; although in this study it was not feasible to accurately establish the infection source, all of the findings led to suspect a possible contamination at the morgue.

Key words: Endophthalmitis, corneal transplantation, *Pseudomonas*, piperacillin, infection.

Endofthalmitis por *Pseudomonas aeruginosa* posterior a queratoplastia penetrante, reporte de caso con investigación epidemiológica

Introducción. Una endofthalmitis post-queratoplastia penetrante por *Pseudomonas aeruginosa* representa un caso devastador con muy pobre pronóstico visual.

Objetivo. Determinar el origen de una infección luego de una queratoplastia penetrante.

Materiales y métodos. Se efectuó una investigación epidemiológica de un caso de endofthalmitis con el soporte de un equipo humano compuesto por epidemiólogo, infectólogo, bacteriólogo y oftalmólogos especialistas en córnea. Se evaluaron los aspectos en los cuales pudiera existir el riesgo de adquirirse la infección como en el momento de la extracción, el procesamiento y la preservación de la de la córnea, las características del donante, el receptor y la bacteria infectante, además de los detalles relacionados al evento quirúrgico.

Resultados. No se encontraron riesgos en la institución, en las instalaciones del banco de ojos, en el donante ni el receptor. Se encontró que en el sitio de la extracción en la morgue no se podía garantizar una técnica esteril y se documento la presentación de otros casos aislados de endofthalmitis pos queratoplastia penetrante con tejidos de la misma morgue y procesados por dos bancos de ojos de la misma ciudad. Las características de la *Pseudomonas* multirresistente demostraban que venía de un medio hospitalario con exposición previa a múltiples antibióticos.

Conclusiones. El sitio de la extracción de las corneas debe garantizar una preparación antiséptica y una recuperación aséptica del tejido donante pues en este estudio aunque no se pudo establecer con certeza la fuente de la infección, los hallazgos llevaron a sospechar una posible contaminación en la morgue.

Palabras clave: endofthalmitis, trasplante de córnea, *Pseudomonas*, piperacilina, infección.

Post-penetrating keratoplasty infections and particularly endophthalmitis have a prevalence between 0.2% and 0.4% (1,2). These numbers give them an incidence of 3 to 5 times higher than that of endophthalmitis post-cataract surgery (2,3). *Pseudomonas aeruginosa* is rarely involved in this type of infections. However, the literature presents cases of this organism causing endophthalmitis post-penetrating keratoplasty, as described by Panda *et al.* (4). Another article (5) describes the treatment and outcomes of a group of 28 patients with endophthalmitis caused by *P. aeruginosa*. At the end of the study, 18 of the 28 eyes were either eviscerated or enucleated, and the remaining patients had very poor visual outcomes even after intravitreal injections of antibiotics.

Endophthalmitis post-keratoplasty has several risk factors inherent to donors, for example, immunocompromised or hospitalized people who remained in intensive care units with a ventilator before dying (6), or an extended enucleation postmortem time; other factors are related to ocular button such as more than 5 days of storing time and the use of contaminated donor buttons (1,6). Factors associated to the conservation medium refer to its possible contamination or to its composition with insufficient antibiotics or to actual microorganisms resistant, for instance to gentamicin M-K medium.

Other risk factors involved in endophthalmitis are related to corneal tissue processing events, as inadequate quality of surgical preparation at the moment of removing the donor's cornea, high levels of air contamination with bacteria in the atmosphere, electric energy failures that may cause inconsistent refrigeration and finally, if the button is used immediately after extraction from 4 °C. Furthermore, during surgery the tissue may be contaminated as well as the receptor (4).

In a meta-analysis study by Wilhelmus and Hassan (7) from 17,614 corneal buttons to be transplanted, scleral rims positive cultures to several microorganisms were found in 2,459 (14%), and of these 31 (0.2%) presented endophthalmitis. This medical problem occurred more than 5 times in cornea recipients with scleral rim positive cultures.

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Rim cultures positive to bacteria and fungi should increase endophthalmitis risk from 0.2% to 1%.

Fontana *et al.* (8) became aware of positive cultures of scleral rims in 9.8% of preserved corneas in hypothermic conditions and in 1.3% of preserved corneas in organic cultures, but no endophthalmitis cases were seen; authors attributed this fact to disinfection and cleaning of the annexes and the fornix previous to extraction.

The main infection source is considered to be the donor tissue since microbial contamination incidence of donor's eyes before processing and conservation was found to be 80% to 100% (9,10). In spite of an aggressive treatment endophthalmitis by *P. aeruginosa* is associated with a poor visual prognosis. Eifrig *et al.* (5) reported that 68% of patients ended up with no light perception; it was necessary to enucleate 64%; the best vision was 20/400.

Endophthalmitis by *Pseudomonas* is an emergency requiring immediate care. It is essential to consider the antibiotic resistance and the use of specific antibiotics against *Pseudomonas* in case the infection progresses in spite of empiric treatment to gram-positive and gram-negative bacteria already established.

A precise donor selection as well as adequate practices in handling the corneal button (11) helps to avoid associated graft infections. Furthermore, surgeons must observe the necessary precautions against a possible contamination (6).

Clinical case

The patient was a 63-year-old male. He had a history of vitro-retinal surgery with a secondary bullous pseudophakic keratopathy. A penetrating keratoplasty was programmed since only a chronic obstructive pulmonary disease was the sole pathological antecedent found.

Surgery was done on July 28th 2006, with no complications in 35 minutes of surgical time. Povidone-iodine on the ocular surface was used as a prophylaxis in the surgery room and a sub-conjunctival cephalicidal injection was applied immediately after the surgery. Scleral rim was discarded when the intervention was finished.

The next day in the postoperative control an abundant painless conjunctival secretion was found, the cornea button had edema with no signs of infection. Consequently, it was too difficult to examine the ocular fundus. Because of the edema

and secretions, a postoperative infection was suspected, and an inter-consultation was made with the retinologist who evaluated the case with a B-scan ultrasonography and did not find the typical ocular lesions seen in endophthalmitis. However, because of the signs of infection in the surface and the condition of being a pseudophakic eye, vancomycin and amikacin were suggested and administered; both intra-vitreous and topically, due to the high risk of endophthalmitis.

On the second postoperative day, severe bulbar congestion, abundant corneal button infiltration, dehiscence of sutures and the marked media opacity were very noticeable, suggesting a bacterial infection. According to an ultrasonography, the vitreous cavity was full of infiltrates. A posterior vitrectomy with intravitreal vancomycin plus ceftazidim was performed and it was necessary the cornea button removal. During the surgical act an expulsive hemorrhage appeared. Postoperative evolution was difficult and on August 23rd, it was necessary to carry out an evisceration due to uncontrollable pain; with no visual acuity: no light or color perception.

Cornea and vitreous samples were taken to be cultured in several media as blood, chocolate, sabouraud agars, and thioglycolate. *P. aeruginosa* identification was made with Microscan® (Siemens, Deerfield, Illinois) automated method and its susceptibility was determined by Microscan's micro-dilution technique with commercial panels; the bacterium showed its resistance to gentamicin, amikacin, tobramycin, ciprofloxacin, levofloxacin, piperacillin/tazobactam, cephalosporin, ceftazidim, ceftaxime, cefuroxime, imipenem and meropenem.

Due to the devastating nature of this entity, the institutional committee on infections decided to undertake an epidemiological investigation. With the approbation of the ethics committee an interdisciplinary team including an epidemiologist, infectologist, and ophthalmologists with experience in corneal diseases was conformed for elucidating the origin of this infection in order to prevent the appearance of other cases. There was also collaboration by the eye bank as well as the Forensic Sciences and Legal Medicine Institute.

Discussion

This infection was considered a nosocomial one, since it began 24 hours after surgical intervention and because a bacterium with nosocomial origin was isolated. Infections appearing after a post-

penetrating keratoplasty are categorized as nosocomial when they occur until one year after the surgical procedure (12).

The patient was considered free of a possible colonization with *P. aeruginosa*, because previously to the surgery he had not been hospitalized and there was no immunodepression (13). Samples of stool, urine and sputum were taken in the patient and the cultures were negative for pseudomonas. Furthermore, the patient did not have an acute exacerbation of his chronic obstructive pulmonary disease 2 years prior to the eye surgery.

In accordance with the institutional processes reviewed where the surgical procedure was performed, instrument sterilization controls were negative as well as environment cultures. No other post-surgical infections appeared in the length of time related to the present case. As has been previously informed, the scleral rim was eliminated and there is no available culture. In literature there are papers (7,14) supporting the fact of not cultivating all of the scleral rims. As a matter of fact the institution is now keeping the scleral rim during two weeks and if there is a need, it is sent to laboratory to be cultured.

Pseudomonas aeruginosa is a gram-negative germ, not fermentative, behaving as an opportunist nosocomial bacillus. Related to the explanation of its important and dangerous pathogenic role in hospital-acquired infections, there are several factors such as its very low nutritional requirements, its tolerance to a great variety of physical conditions and its intrinsic resistance to a large number of antibiotics. Although it is known to be part of the human organism's normal flora, rarely does it cause disease in healthy people (13).

Pseudomonas aeruginosa isolated from this patient was multi-resistant to antibiotics, perhaps due to a triple resistance mechanism, a) Amp-C producer by its defense to penicillin derivatives and ceftazidim; b) extended-spectrum beta-lactams (SEBL) because of the barrier against ceftaxime and ceftazidim; and c) with expulsion bombs by the antagonism to penicillin, cephalosporins, imipenem and aminoglycosides. According to Harris *et al.* (15) *P. aeruginosa* resistance to piperacillin/tazobactam may be produced as a consequence of previous exposures to multiple antibacterial drugs.

The cornea donor was a 23-year-old male who died on 23 July at 5:30 a.m. after being shot with a pistol at occipital region. His medical record

(no previous hospitalization, no systemic disease known) and negative laboratory tests (HIV I-II, Hbs-Ag, HTLV I-II, hepatitis C, VDRL) qualified him as a good donor candidate (16). On 23 July at 1:30 p.m., a corneal extraction is performed *in situ* at the morgue general saloon, with asepsis and antiseptics measures through saline solution, prepodine and 5% iodopovidone, the cornea was preserved at 1:40 p.m., in EUSOL-C (Alchemia) with 0.143 mcg/ml gentamicin. This EUSOL-C was packed in a bottle being part of a proportionate technique (aliquots); its lot had been microbiologically analyzed on 6 July with negative results for both aerobic and anaerobic germs. These analyses are performed monthly as routinized procedures.

Until the incident date, the bottles used for EUSOL-C aliquot parts method had been sterilized on February 2006 following a gas conventional sterilization. Now they are submitted to a sterilization process guaranteeing year sterility.

On 28th July after examining the cornea it was considered as apt to be transplanted with an endothelial count of 3,770 cells and five days preservation since its extraction, following international accepted standards (15).

Microbiological controls to equipment and eye bank facilities that intervene in obtaining and processing tissues (as laminar flow camera inside and outside, refrigerator environment, main table environment, storage room and wardrobe environment) that had been taken in June were negative.

From main table surface positive cultures of *Micrococcus* sp., *Bacillus* sp., *Sacharomyces* sp., and *Penicillium* sp., were obtained. On the refrigerator surface a growing of *Bacillus* sp. was found.

These cultures are routinely performed every three months; cleaning activities and decontamination of surfaces and walls are made on a daily basis; a general and thorough cleaning is carried out weekly. In order to prevent bacterial resistance, four cleaning products are presently rotated.

For July (the month in which the event occurred) no data related to refrigerator temperature variations were found, but for the six previous months a regular 2 °C average had been maintained.

In the Forensic Sciences and Legal Medicine Institute we found that general morgue tables are not suitable places for cornea extractions because they do not guarantee an area free off living bacteria and other infective microorganisms.

As Builles *et al.* (17) state that the greatest contamination possibility occurs in the corneal extraction moment.

Cornea is a tissue for grafting that is not 100% sterile; extraction and conservation processes guarantee its usefulness because donor tissue requires the necessary antiseptic preparation and aseptic recuperation (6). At present, the morgue has an exclusive eye bank room with a proper table and couch which receive periodical cleaning and decontamination as well as surface and environment cultures every three months.

In conclusion, the origin of the patient's infection was due to a multi-resistant *Pseudomonas* strain. It was not possible to establish the site for tissue contamination because no risk was found (institution, eye bank facilities, donor or receptor). The bacterial characteristics demonstrated its provenance from a hospital with previous exposition to several antibiotics. Besides other endophthalmitis post-penetrating keratoplasty cases with ocular tissues from the same eye bank on April 2005 (*Enterobacter amnigenus*), on May 2006 (*Pseudomonas* sp.), and another case on June 2006 at another institution with tissues from other local eye bank (*P. aeruginosa*); all of these facts rose suspicion of a possible morgue contamination. Therefore, present investigation suggested a series of actions as follows:

- a) institutional actions such as looking back on antibiotic prophylaxes according to resistance pattern of isolated bacteria;
- b) eye bank actions such as extraction and preservation processes as well as recording procedures were corrected, and finally
- c) Forensic Sciences and Legal Medicine Institute actions such as construction of facilities in order to have an appropriate area for following a sterile technique used in the corneal tissue extraction process. In Cali, during three months no extractions of corneal tissues were permitted until when this area was ready.

Conflict of interest

The authors do not have any commercial interest in products or companies described in this study.

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