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SHORT COMMUNICATION

Case report

Listeria monocytogenes-induced endogenous endophthalmitis in an otherwise healthy individual: rapid PCR-diagnosis as the basis for effective treatment

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ABSTRACT: Purpose. Listeria monocytogenes *is a rare cause of endogenous endophthalmitis. To date 15 cases have been published in the literature. All eyes showed similar clinical features and profound visual loss mainly due to delayed diagnosis.*
Methods. *An additional case of an otherwise healthy 73 year-old male, who was referred to our hospital because of acute iridocyclitis with secondary glaucoma, is reported. Within a few days the severity of the intraocular infection increased dramatically, resulting in the clinical picture of acute endophthalmitis.*
Results. *In contrast to most published cases, early identification of the causative pathogen in the aqueous humor after anterior chamber puncture using polymerase chain reaction (PCR) and the initiation of a specific, systemic antibiotic medication, resulted in complete recovery of visual acuity.*
Conclusions. *PCR is very useful for the identification of the pathogen in intraocular infections. (Eur J Ophthalmol 1999; 9: 53-7)*

KEY WORDS: Endophthalmitis, Listeria monocytogenes*, PCR*

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INTRODUCTION

Infectious endophthalmitis, although relatively rare, is potentially the most devastating intraocular condition that may be caused by a variety of bacteria or fungi (1-4). In the great majority of cases the pathogen gains access to the eye from the external environment, for example, through a surgical incision or traumatic laceration. In less common cases the pathogen reaches the eye hematogenously. This form of endophthalmitis is termed endogenous endophthalmitis and predominantly affects immuno-compromised individuals.

Listeria monocytogenes is a rare cause of endophthalmitis. To date, only 15 cases have been published (5-18). In all of the cases the pathogen entered the eye hematogenously. The clinical presentation of these reported cases was quite similar. On slit-lamp biomicroscopy the infected eye showed endothelial precipitates, a fibrinous anterior chamber reaction, a dark hypopyon, and elevated intraocular pressure. In all of the cases the IOP was refractory to corticosteroids. Delayed identification of the causative bacteria resulted in severe and persistent loss of visual function. In this paper, an additional, otherwise healthy patient with the clinical signs of an endogenous endophthalmitis is presented. By using polymerase chain reaction (PCR) we were able to identify *Listeria monocytogenes* as the causative pathogen within 7 hours, and subsequently to initiate an effective antibiotic treatment regimen. This resulted in complete recovery of visual function.

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Case Report

A 73 year old Caucasian man was referred for acute iridocyclitis with secondary glaucoma. On slit-lamp biomicroscopy large keratic endothelial precipitates were observed. The anterior chamber contained a fibrinous inflammatory reaction and a dark pigmented hypopyon. Because of the fibrin in the anterior chamber both the vitreous and the fundus could not be visualized. Intraocular pressure was 37 mmHg, visual acuity was hand motion. The contralateral eye was asymptomatic with a visual acuity of 20/30.

The patient's past medical history and a detailed physical examination were unremarkable. An immunological and laboratory workup was performed. This included a chest x-ray, rheumatoid factor, ANA, ANCA, angiotensin converting enzyme, HLA-B5, HLA-B27, C-reactive protein, complete blood count, serum calcium, and sedimentation rate. All these factors were within normal range. Additional studies included negative serology for human immunodeficiency virus, lyme, syphilis, toxoplasmosis, cytomegalovirus, Epstein-Barr virus, and varicella zoster virus. Herpes simplex virus was IgM negative, but IgG positive.

Anterior uveitis was diagnosed based on clinical intraocular signs. The patient was started on oral prednisone at a dose of 80 mg per day and acetaszolamide 250 mg po three times per day. Intensive topical treatment included corticosteroid eye drops (dexamethasone 0.1% hourly), corticosteroid ointment (0.5% hydrocortisone acetate; at night) and cycloplegic eye drops.

Two days later the size of the hypopyon was increased and ultrasound showed a chain of low-amplitude spikes within the vitreous cavity as a sign of intense vitreous inflammation. At this time the patient was considered as possibly having infectious endogenous endophthalmitis. An aggressive, broad-spectrum intravenous antibiotic regimen, consisting of a combination of two antibiotics (vancomycin and ceftriaxon), was initiated. However, no improvement was observed. In fact, within 24 hours the vitreous inflammation increased in severity and the posterior vitreous was just as affected as the anterior. At this time visual acuity decreased to light perception.

For microbiological diagnosis 200 µl aqueous humor was collected by paralimbal paracentesis. The specimen was then divided into two equal portions. One portion was used to prepare slides for Gram and Giemsa staining and to inoculate petri dishes with brain heart infusion broth, thioglycolate bouillon, blood agar (5% CO_2), Columbia agar (5% CO_2), MacConkey agar, Sabouraud glucose agar (2%) for culturing at 37°C. Microscopic examination of the Gram- and Giemsa-stained slides solely revealed detritus and leukocytes, but there were no bacterial or fungal organisms. After 24 hours of culture the enrichment broth grew Gram-positive rods. For further characterization of the organisms by serological and biochemical procedures they were harvested on blood agar and chocolate agar. A growth of non-pigmented, catalase positive, oxidase negative, b-haemolysing colonies was observed. Gram staining showed Gram-positive rods with a "tumbling" motility. Biochemical identification (Staphaurex+, APIStrep®, Biomerieux GmbH, Nürtingen, Germany) resulted in a typical pattern for *Listeria monocytogenes*. The total time of identification of *Listeria monocytogenes* as the causative pathogen by culture was 72 hours.

The remaining portion of the specimen was examined for the presence of bacterial and fungal pathogens by PCR. Following a 30-min incubation with the enzyme zymolase to ensure a proper lysis of fungal cell walls, total DNA was prepared using the QIAmo Tissue Kit (Quiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The DNA preparation was divided into four aliquots and 10 µl portions of it were subjected to 35 cycles of PCR using digoxigenin-labeled genus-specific primer pairs for the 16S rDNA of eubacteria (19) and the 28S rDNA of fungal organisms (20), respectively. Each clinical specimen was amplified in the presence of negative controls, for the sample agitation and the amplification steps. The third aliquot was spiked with 10^3 molecules of a plasmid serving as an internal positive control to exclude the presence of PCR inhibitors within the sample, and subjected to PCR with a plasmid-specific primer pair. In order to extend the study to several most frequent viral pathogens, the fourth aliquot was subjected to PCR with a set of herpes virus-specific primer pairs. PCR reaction mixtures were separated electrophoretically and analyzed for the presence of specific amplification products in ethidium bromide-stained agarose gels. Nucleotide sequences of the specific amplification products were determined by direct cycle sequencing of both strands of the amplicons using an automated ABI 310A capillary DNA sequencer

(Applied Biosystems GmbH, Weiterstadt, Germany). Computer-assisted comparison of the determined sequences with all GenBank entries revealed the highest level of overall homology with the 16S rDNA of *Listeria monocytogenes.* The time of identification of the causative pathogen by PCR was 7 hours.

Based on the results of PCR intravenous antibiotic treatment was changed to ampicillin (4g/4h), as ampicillin is the treatment of choice for infections with *Listeria monocytogenes*. Within two days after changing antibiotic treatment, the hypopyon was nearly dissolved and the vitreous and fundus could be seen by indirect ophthalmoscopy. At this time visual acuity had improved to 20/100 and intraocular pressure was back in the normal range. Two weeks later visual acuity was 20/30 and, with the exception of a small posterior iris synechiae, the anterior and posterior segment examination was unremarkable. Since then, the patient has remained asymptomatic for the last 12 months.

DISCUSSION

Listeria monocytogenes are aerobic, Gram-positive rods with a widespread distribution in nature (21). The main route of acquisation is foodborne resulting from the ingestion of contaminated food products. *Listeria monocytogenes* crosses the mucosal barrier of the gastrointestinal tract and grows as an intracellular pathogen in macrophages. Within the macrophages *Listeria monocytogenes* invades the blood stream and can be carried to any part of the human body (22, 23). This intracellular process protects listeria from exposure to antibodies and other bactericidal factors.

Infections with *Listeria monocytogenes* are not very common in the general population and restricted to several well-defined populations: neonates and the elderly, pregnant women, and immuno-compromised patients, particularly those with defective cell-mediated immunity. Bacteremic patients usually have an unremarkable history or might present an episode of chills and fever. In most cases a primary infectious site cannot be found. Although any organ can be infected by the pathogen, there is a marked tropism to the CNS with meningitis being the most common disease associated with *Listeria monocytogenes* (24-26).

Ocular involvement with *Listeria monocytogenes* is very rare. Conjunctivitis is the most common ocular presentation, but any extraocular or intraocular structure may be involved (21-24, 27-30). To date, there have been 15 cases of *Listeria monocytogenes* endophthalmitis published and all are presumed to be endogenous (5-18). Of this cohort, 8 patients, like our patient, were otherwise healthy individuals. An additional two patients reported a previous flu-like illness with gastrointestinal symptoms, and another 5 patients were immuno-compromised. In all 15 eyes the clinical appearance of the endophthalmitis was relatively similar, with large keratic precipitates, fibrinous anterior chamber reaction, and a dark hypopyon. Some eyes showed pigment dispersion and all eyes showed elevated intraocular pressure. In 14 of these 15 eyes loss of visual performance was severe and persistent with visual acuity less than 20/200.

A delayed diagnosis was the cause of the unsatisfactory visual outcome. Due to the dark hypopyon and pigment dispersion some investigators have suspected an occult necrotic tumor (5, 7, 11, 15), therefore, the initiation of antibiotic treatment was delayed. However, of greater importance are the problems of microbiological techniques associated with the diagnosis of intraocular infections. Although, classical culture techniques and biochemical identification methods, properly applied, are capable of detecting most bacterial pathogens with reasonable sensitivity, these techniques often take too much time or may fail in the case of intraocular infections. In our case, the culture took 24 hours to detect Gram-positive rods and another 48 hours to identify *Listeria monocytogenes* as the causative pathogen. This time can be too long to protect the eye against irreversible damage. The treatment of *Listeria monocytogenes* infections is difficult and should employ antibiotics that are capable of penetrating the cells and subsequently killing the pathogen. Although *Listeria monocytogenes* is sensitive to vancomycin, this drug cannot penetrate the cells, and therefore is not very effective if structures in the CNS, such as the eye, are involved. In such a situation ampicillin is the antibiotic of choice (23). However, in most clinics ampicillin is not the drug of choice for the treatment of intraocular infection. Therefore, the rapid identification of the causative pathogen is of major importance, so that a sufficient antibiotic treatment regimen may be initiated. To date, a rapid identification of bacteria is possible with the use of new molecular biological techniques, such as polymerase

chain reaction (PCR). Our well established PCR-based detection and species identification technique (31-33) was capable of identifying the causative bacterial pathogen within a period of 7 hours after the collection of aqueous humor. This information allowed us to initiate a specific treatment, and the eye subsequently recovered visual acuity. It is our belief that in the future these new techniques will play an important role in the diagnosis of intraocular infections.

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