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Acute Postoperative Endophthalmitis Caused by *Staphylococcus lugdunensis*▽

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Acute postoperative endophthalmitis caused by *Staphylococcus lugdunensis* is infrequently reported in clinical studies. Five cases of acute postcataract surgery endophthalmitis caused by *S. lugdunensis* were taken from a multicenter prospective study conducted in four university-affiliated hospitals in France (2004 to 2005). These cases were characterized by severe ocular inflammation occurring with a mean delay of 7.6 days after cataract surgery, severe visual loss (hand motions or less in three cases), and dense infiltration of the vitreous. Each of these patients was initially treated by using a standard protocol with intravitreal (vancomycin and ceftazidime), systemic, and topical antibiotics. Given the severity of the endophthalmitis, even though bacteria were sensitive to intravitreal antibiotics, pars plana vitrectomy was needed in four cases. The final visual prognosis was complicated by severe retinal detachment in three cases. The microbiological diagnosis was reached by using conventional cultures with specific biochemical tests and eubacterial PCR amplification followed by direct sequencing.

Bacterial endophthalmitis, with an estimated incidence following cataract surgery of between 0.07% and 0.3% (5, 12), is among the most feared complications of intraocular surgery and may result in severe vision loss (4, 5, 15, 16). In studies based on conventional culture techniques, coagulase-negative staphylococci (CNS) (e.g., *Staphylococcus epidermidis*) account for nearly 60% of all cases, and *Staphylococcus aureus* accounts for another 20% of the total. *Staphylococcus lugdunensis* is known to be an aggressive coagulase-negative *Staphylococcus* and has been infrequently described as being a cause of endophthalmitis (5.9% of all CNS) (1).

We present five cases of endophthalmitis due to *S. Lugdunensis* among a large series of 126 postoperative endophthalmitis cases included in a multicenter prospective study conducted in four university-affiliated hospitals in France (2004 to 2005). Ocular samplings were obtained before and after intravitreal injection of antibiotics from the aqueous humor and/or the vitreous. The aim of this study was to describe the clinical characteristics of endophthalmitis caused by *S. lugdunensis* more precisely and to report the usefulness of eubacterial PCR in the microbiological diagnosis.

CASE REPORTS

Case 1. An 82-year-old patient underwent cataract extraction (phacoemulsification) and intraocular lens implantation in the anterior segment of his left eye, which was complicated by a capsular lens rupture and vitreous loss. The first symptoms were present 7 days after surgery (loss of visual acuity and red eye), and the patient was admitted to the hospital 2 days later. Slit lamp examination (Table 1) disclosed a severe intraocular inflammation in both the anterior and the posterior segments. The patient was treated with two intravitreal injections of antibiotics (vancomycin [1 mg] and ceftazidime [2.25 mg] as a standardized protocol) and vitrectomy. *S. lugdunensis* was isolated from the culture of the initial aqueous humor sample and was identified using eubacterial PCR on vitreous samples (from pars plana vitrectomy performed after two intravitreal injections of antibiotics) (Table 1). Given these results, the systemic and intravitreal antibiotic therapy was not modified. After 6 months, the anterior chamber and the posterior segment of the eye showed no inflammatory activity, and visual acuity had improved to 20/40. The examination of the fundus showed a tiny macular epiretinal membrane.

Case 2. An 84-year-old patient presented with acute visual loss and intraocular inflammation of the left eye (Table 1) 6 days after cataract surgery complicated by a capsular lens rupture. Before treatment, cultures and PCR performed on aqueous humor and vitreous samples identified *S. lugdunensis* (Table 1). A pars plana vitrectomy was performed 7 days after admission, and cultures were negative (PCR was not performed at this time, since the amount of the vitreous specimen was insufficient for both techniques). The vitrectomy was complicated by a retinal detachment 10 days later, which required an additional vitreoretinal surgery (vitrectomy, cryopexy, and silicone). At the 18-month follow-up visit, visual acuity was limited to hand motions, and there was no inflammatory activity in the eye, although the retina was attached (after removal of silicone oil).

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TABLE 1. Clinical features and microbial identification of endophthalmitis caused by *S. lugdunensis*[a]

Patient	Surgery procedure	Delay from surgery (days)	Initial visual acuity	Clinical examination results		Test results		Treatment	Final prognosis
				Anterior chamber	Posterior chamber	Cultures	Eubacterial PCR (% sequence identity with *S. lugdunensis*)		
1	Cataract extraction, capsular lens rupture, anterior IOL	7	Hand motion	Conjunctival hyperemia, Tyndall ++, Hypopyon 1.5 mm	Loss of red reflex, vitreitis +++, retina not visualized	+ in AH	− in AH, + in vitreous from PPV (100) at day 5	3 intravitreal injections and PPV at day 5	VA 20/40 at 6-mo follow-up, epiretinal membrane
2	Cataract extraction Capsular lens rupture, vitreous loss IOL in the bag	6	Light perceptions	Conjunctival hyperemia, lid edema, Tyndall ++, Hypopyon 1 mm	Red reflex +, vitreitis +++, retina not visualized	+ in AH and vitreous (tap), − in vitreous from PPV at day 7	+ in AH and vitreous tap (100) (PPV, sample not done for PCR)	2 intravitreal injections and PPV at day 7 and day 17	Surgery for retinal detachment; retina reattached after silicone removal, hand motions at 18-mo follow-up
3	Cataract extraction, IOL in the bag	5	Light perceptions	Conjunctival hyperemia, corneal edema, Tyndall +++, Hypopyon 1.6 mm, cyclitic membrane	Loss of red reflex, vitreitis +++, retina not visualized	+ in vitreous (tap), − in vitreous from PPV at day 4	+ in vitreous tap (99), + in vitreous from PPV (99) at day 4	2 intravitreal injections and PPV at day 4	Surgery for retinal detachment, no light perception at 18-mo follow-up
4	Cataract extraction, IOL in the bag	12	20/100	Conjunctival hyperemia, Tyndall +++, cyclitic membrane	Red reflex +, vitreitis +++, retinal details not visualized	+ in vitreous (tap)	+ in vitreous tap (99)	2 intravitreal injections, no PPV	20/20 at 18-mo follow-up
5	Cataract extraction IOL in the bag	7	Hand motion	Conjunctival hyperemia, Tyndall +++, Hypopyon 2 mm, cyclitic membrane	Red reflex +, vitreitis +++, retinal details not visualized, choroidal detachment	+ in vitreous (tap), − in vitreous from PPV at day 5	+ in vitreous tap (100), − in vitreous from PPV at day 5	2 intravitreal injections and PPV at day 5	Surgery for retinal detachment; VA "count fingers" at 6-mo follow-up

[a] AH, aqueous humor; IOL, intraocular lens; IOP, intraocular pressure; PPV, pars plana vitrectomy; VA, visual acuity; −, negative; +, present or positive; +++, dense. Tests of aqueous humor and vitreous samples from taps were always performed at admission (day 1), before the first intravitreal injection of antibiotics. The time period of sampling during pars plana vitrectomy is noted, using day 1 as admission.

Case 3. A 78-year-old man underwent uncomplicated cataract surgery and was admitted 5 days later with a clinical picture of acute endophthalmitis in his right eye (Table 1). Ocular examination revealed visual acuity of light perception, evidence of intraocular inflammation, and no fundal view. Ultrasound examination at this stage revealed a dense vitreous without retinal or choroidal detachment. Cultures and eubacterial PCR of these initial vitreous samples were positive for *S. lugdunensis* at this time (Table 1). A pars plana vitrectomy was performed 4 days later, and undiluted vitreous was sterile on cultures, whereas PCR was positive. Endophthalmitis was complicated by retinal detachment 10 days after vitrectomy. The patient underwent an additional vitreoretinal surgery (using silicone oil), but the final prognosis at 6 months was phthisis and absence of vision.

Case 4. A 69-year-old woman suffered from pain, redness, and an acute loss of visual acuity 12 days after uncomplicated cataract surgery on the right eye. This patient had systemic hypertension and cardiac failure. Upon admission, ocular examination revealed visual acuity of 20/100 as well as severe inflammation of the anterior chamber and the vitreous. The patient underwent a vitreous tap at the time of the second intravitreal injection, and both bacterial cultures and eubacterial PCR were positive for *S. lugdunensis*. This ocular sample was taken 2 days after the first intravitreal injection of antibiotics. Since the clinical presentation improved after two intravitreal injections of antibiotics, a pars plana vitrectomy was not necessary. The final prognosis was excellent, with a visual acuity of 20/20 at the 1.5-year follow-up; no anatomical sequelae were noted.

Case 5. A 64-year-old man was operated on for cataract extraction and intraocular lens implantation in the right eye without complications and suffered from acute visual loss without pain 7 days after surgery. The diagnosis of acute postoperative endophthalmitis was evident (Table 1). The patient benefited from two intravitreal injections of antibiotics since the anterior segment was better (clear cornea, absence of Tyndall, and retraction of the cyclitic membrane). However, the vitreitis did not reduce (based on ultrasound imaging). Ocular sampling consisted of two vitreous taps at the time of intravitreal injections of antibiotics. Only the first vitreous tap was positive for *S. lugdunensis* by culture and PCR. Other ocular samples (at the time of the second intravitreal injection and vitrectomy) were negative. The patient was operated on for pars plana vitrectomy. Visualization of the fundus during surgery showed a pale retina of the posterior pole, without retinal hemorrhage. A retinal detachment with a giant retinal tear occurred 15 days after the pars plana vitrectomy and required a second vitreoretinal surgery (peeling of epiretinal membranes, endolaser, and silicone oil). Six months after this surgery, visual acuity was limited to "counting fingers," and the retina remained attached under silicone oil.

For all strains, the minimal inhibitory concentrations were as follows: norfloxacin and ofloxacin, 0.5 mg/liter; fosfomycin, <8 mg/liter; vancomycin, <1 mg/liter; teicoplanin, <0.5 mg/liter; amikacin, <4 mg/liter; cefalotin, <8 mg/liter; and oxacillin, <0.25 mg/liter.

MATERIALS AND METHODS

The five cases of acute postoperative endophthalmitis caused by *S. lugdunensis* were part of a multicenter prospective study conducted in four university-affiliated hospitals in France (2004 to 2005) investigating 126 patients with postoperative endophthalmitis. This prospective study aimed to evaluate eubacterial techniques associated with conventional cultures for the microbiological diagnosis of endophthalmitis. This study adhered to the Declaration of Helsinki for research guidelines involving human subjects. Patients did not have a systemic risk factor for endophthalmitis such as diabetes, steroid medication, or immunosuppression.

At admission, all patients underwent an immediate tap of the aqueous humor followed by intravitreal injection of vancomycin (1 mg) and ceftazidime (2.25 mg). Patients were also initially treated with a broad-spectrum intravenous antibiotic regimen (fluoroquinolone and piperacillin) for 5 days, topical drugs (corticosteroid or tropicamide), and fortified drops (vancomycin or ceftazidime). All eyes were sampled after topical anesthesia and instillation of 5% aqueous povidone iodine solution in the conjunctival sac. After the lid speculum was in place, a new instillation of povidone iodine solution was given. The conjunctival sac was then washed with 20 ml of sterile balanced salt solution before sampling. Aqueous humor samples (200 μl) were collected just before the first intravitreal injection in a sterile syringe immediately after paracentesis in the anterior chamber and then transferred in an aliquot.

When necessary, pars plana vitrectomy was performed, and undiluted vitreous samples (500 μl) were also collected. Pars plana vitrectomy was considered in cases of initial severe clinical presentation (visual acuity less than counting fingers, dense opacities in the vitreous cavity, or other complications such as a retinal detachment or posterior dislocation of the lens) or if there was an anatomic and/or functional aggravation after the first injection of antibiotics. The transfer processing was similar to that of aqueous humor sampling. Aqueous and vitreous specimens were divided in half at the time of sampling under aseptic conditions, with one half (100 to 250 μl) in brain heart infusion broth (10 ml, pH 7.4 ± 0.2) (reference no. ADM 88440; AES Laboratories, Combourg, France) and the other half (100 to 250 μl) in a microcentrifuge tube for PCR.

Culture. After culture of the biological sample in brain heart infusion broth, *Staphylococcus* strains were isolated on blood agar plates (bioMérieux, Marcy l'Etoile, France) at 37°C in aerobic conditions for 24 h. Strains were then identified as being *S. lugdunensis* with ID32 STAPH strips (bioMérieux) (13). The ID32 STAPH strips used in this study include 26 tests and, in particular, the detection of ornithine decarboxylase and pyrrolidonyl arylamidase. At least the positivity of these tests are necessary to identify the bacteria as being *S. lugdunensis*. The antibiogram was performed using Vitek II Gram-Positive Susceptibility cards (catalog no. AST P 531; bioMérieux). β-Lactamase production was deduced if MICs were >0.5 mg/liter using the Vitek II Gram-Positive Susceptibility cards, and if the MIC was <0.5 mg/liter, β-lactamase production was studied using the nitrocefin test.

DNA extraction. All DNA extraction procedures were carried out in a class II biological safety cabinet (Faster, Ferrara, Italy) in a room physically separated from the room used to prepare all PCR reagents except DNA and also from the room used to prepare nucleic acid amplification mixes and, finally, from the room used for post-PCR analysis. DNA was extracted from ocular samples (aqueous humor and vitreous) with the High Pure PCR Template Preparation kit (Roche Diagnostics, Meylan, France) according to the manufacturer's recommendations. An extraction negative control composed of all reagents used for DNA extraction minus the ocular sample was processed in parallel with each sample. Amplification of the human beta globulin gene served as an internal positive extraction control (8).

PCR assay. The oligonucleotide primers designed for the 16S rRNA gene PCR were 91E (5′-TCAAA[G,T]GAATTGACGGGGGC-3′) and 13BS (5′-GCCCGGGAACGTATTAC-3′), which produced a 492-bp fragment of the 16S rRNA gene (18). Primers PC04 (5′-CAACTTCATCCACGTTCACC-3′) and GH20 (5′-GAAGAGCCAAGGACAGGTAC-3′) were used to amplify a 268-bp fragment of the human beta globulin gene. The PCR mixture, which was made up to 50 μl with sterile water (Sigma), contained 1× PCR buffer, $MgCl_2$ (2.5 mM), 200 μM each deoxynucleoside triphosphate (including dUTP at a dUTP/dTTP ratio of 1:9), 200 μM of each primer, 2.5 U of *Taq* DNA polymerase (Roche Diagnostics), and 1 U of heat-labile uracil DNA-glycosylase (UNG; Roche Diagnostics) to prevent carryover contamination between PCRs. Five microliters of DNA extract was added to the PCR mixture, which was incubated for 10 min at 20°C for U-DNA cleavage by UNG, followed by UNG inactivation by incubation at 94°C for 10 min. PCR was performed for 32 cycles (denaturation for 30 s at 94°C, annealing for 30 s at 58°C, and extension for 30 s at 72°C) with a Biometra thermocycler, followed by 10 min of incubation at 72°C.

PCR products were analyzed by electrophoresis through a 1.5% agarose gel (Sigma) and sequenced with PCR primer 13BS on an automated sequencer made by Biofidal (Vaulx en Velin, France).

The 16S rRNA gene sequences obtained were compared with those available in the GenBank, EMBL, and DDBJ databases with the BIBI program (Bio Informatic Bacterial Identification; http://pbil.univ-lyon1.fr/bibi/query.php). Identification to the species level was defined as a 16S rRNA gene sequence similarity of 99% or greater with that of the GenBank prototype strain sequence; identification to the genus level was defined as a 16S rRNA gene sequence similarity of 97% or greater with that of the GenBank prototype strain sequence. A failure to identify was defined as a 16S rRNA gene sequence similarity of less than 97% with sequences deposited in GenBank at the time of the analysis (2). Results of PCR were available 3 days after sampling.

To assess the sensitivity of the detection series, 10-fold dilutions were made from 24-h culture colonies of *S. epidermidis*. Equal aliquots were cultured for colony counts and DNA extraction plus PCR amplification. The detection sensitivity was 500 to 1,000 organisms. As a control, aqueous humor samples from eyes that had undergone cataract surgery ($n = 15$) or retinal detachment surgery ($n = 15$) and vitreous samples from eyes that had undergone pars plana vitrectomy (epiretinal membrane, $n = 5$; diagnostic vitrectomy, $n = 5$) were obtained under the same sterile conditions. The control samples were analyzed using the same techniques as those used for infectious specimens, and PCR was negative in all cases.

RESULTS AND DISCUSSION

While CNS are the most common infecting organisms in postoperative endophthalmitis, *S. lugdunensis* is rarely reported as being a causative bacterial agent (1). The five cases described in the present report belong to a large series of postoperative endophthalmitis patients included in a multicenter prospective study in France ($n = 126$) (2004 to 2005, French Institutional Endophthalmitis Study Group). Among 87 cases (69%) bacteriologically documented using eubacterial PCR and/or conventional cultures, *S. lugdunensis* accounted for 5.7% of the bacterial spectrum and 10% of all staphylococci ($n = 50$). Whereas *S. lugdunensis* was first described in 1988 (7), the low frequency of *S. lugdunensis* in endophthalmitis was first reported in the Endophthalmitis Vitrectomy Study in 1997 (1), which observed a 47.7% frequency of CNS (from 250 intraocular bacterial isolates) in aqueous humor and vitreous samples. Among the CNS cases, *S. epidermidis* accounted for 81.9% and *S. lugdunensis* was isolated in 5.9% of the CNS cases ($n = 9$) and 3.6% of all isolates (9/250). *S. lugdunensis* was isolated from the aqueous humor in only one case, from the vitreous in only four cases, and from both specimens in four cases. Furthermore, that previous study showed that for this pathogen, eyelid isolates were found to be indistinguishable from intraocular isolates. Given its isolation from the intraocular compartments of patients with endophthalmitis and the 100% correlation of eyelid versus vitreous association shown by pulsed-field gel electrophoresis (1), *S. lugdunensis* was considered to be a significant opportunistic pathogen. However, the clinical course of these patients was not described in the study reported previously by Bannerman et al., and alternative identification techniques such as eubacterial PCR were not used at that time.

S. lugdunensis has been isolated mostly as a causative agent of skin and soft-tissue infections (6, 7, 10). In recent years, this pathogen has been reported to cause a wide variety of more serious infections including brain abscess, meningitis, sepsis, chronic osteomyelitis, spondylodiscitis, and endocarditis (6, 7, 10, 21). The clinical course of infections caused by *S. lugdunensis* is known to resemble the course of *S. aureus* infections (10, 21). These organisms are also frequently misidentified as *S. aureus* because of their morphological appearance, with yellow pigmentation, complete hemolysis when cultured on blood agar, and positive results in tests for clumping factor (17).

In this report, the pathogen *S. lugdunensis* was identified in all cases from ocular samples by using cultures associated with biochemical tests and eubacterial PCR amplification followed by direct sequencing. In routine laboratory testing, *S. lugdunensis* is most often confused with *S. aureus* or with other CNS (6, 7, 9). Most laboratories use commercial systems that are based on biochemical reactions. However, some commercial identification kits provide unreliable results for CNS, particularly for non-*S. epidermidis* isolates, as a result of the variability of diagnostic reactions within species and the subjective nature of their interpretation (3, 14). The ID32 STAPH strips used in this study include 26 tests, in particular, the ornithine decarboxylase and the pyrrolidonyl arylamidase phenotypic tests. At least the positivity of these tests are necessary to identify the bacteria as *S. lugdunensis*.

As shown in Table 1, the susceptibility data from these cases demonstrate that *S. lugdunensis* is usually susceptible to antibiotics commonly used via the intravitreal route. Only penicillin G was often resistant, with production of β-lactamase (24% β-lactamase positive in a study reported previously by Herchline et al.) (11).

This report highlights the usefulness of molecular methods to quickly detect the presence of bacteria, particularly when a small ocular sample (aqueous humor) is available. In a previous study (unpublished data), we showed that PCR analysis performed on aqueous humor samples (before treatment) could lead to a 65% microbiologic identification rate when used in association with cultures. The effectiveness of aqueous humor samples for both cultures and PCR is of interest since these samples can be easily and rapidly obtained (they are painless and feasible after local anesthesia). Furthermore, PCR techniques are particularly useful when patients have been previously treated with systemic and intravitreal antibiotics, as suggested by the positive PCR observed in the vitreous samples of two patients (patients 1 and 3) taken during vitrectomy after intravitreal injections. In patient 2, the ocular sample was taken after the first intravitreal injection of antibiotics and was positive for *S. lugdunensis* by both culture and PCR. This is consistent with a previous report showing that a single injection of intravitreal antimicrobial agents may be insufficient to eradicate the bacteria from the eye (20). Furthermore, the previous use of intravitreal antibiotics does not seem to affect the ability to PCR amplify DNA in the short term for *S. lugdunensis*. In case 1, the negativity of the PCR associated with positive cultures suggests a lack of sensitivity of the molecular technique. It is likely that a *Staphylococcus*-specific PCR would have been much more sensitive (3, 19); however, for the purpose of clinical diagnosis where a large diversity of causative bacteria can be involved, eubacterial PCR remains the most cost-effective technique. In the second case (patient 5), the negativity of both cultures and PCR in vitreous from vitrectomy suggests that the two previous injections of antibiotics led to an eradication of the bacterial load.

Data from the literature on endocarditis and other tissue infections caused by *S. lugdunensis* emphasize the aggressive nature of the organism and the importance of identifying CNS to the species level. Identification of *S. lugdunensis* in ocular specimens is highly recommended, as the initial presentation was severe in three out of the five cases of this series (Table 1). The clinical course of infections depends on the virulence of the organism, the delay from symptoms to treatment, and the therapeutic protocol. In our cases, pars plana vitrectomy was needed in three out of the four cases since visual acuity was low (light perception) and inflammation of the eye was severe. After testing samples from 100 patients with acute postcataract endophthalmitis by conventional cultures and eubacterial PCR (a part of our prospective and multicenter study from 2004 to 2005), 33 CNS, 5 *S. lugdunensis* strains, and 6 *S. aureus* strains were identified. Compared with patients with other CNS, patients infected with *S. lugdunensis* were characterized by a worse final functional prognosis ($P = 0.07$ at 6 months) and a higher frequency of postvitrectomy retinal detachment (60% versus 3%; $P = 0.05$). The aggressive nature of *S. lugdunensis* may be related to the production of extracellular slime or glycocalyx, which has a role in bacterial colonization and interferes with the phagocytosis-associated activities of neutrophils and the production of enzymes (esterase, fatty acid-modifying enzymes, protease, and lipase) (22). The final prognosis was associated with the occurrence of a retinal detachment in three of five patients after vitrectomy, which is known to be anatomically and functionally severe. The high rate of retinal detachment could be related to the virulence of the *S. lugdunensis* strains causing retinal lesions (such as necrosis) not only on the posterior pole but also on the peripheral retina (causing retinal breaks). These data suggest that prompt and precise identification of the organism *S. lugdunensis* is extremely important so that the appropriate treatment can be administered for a successful outcome.

In conclusion, this case series of endophthalmitis caused by *S. lugdunensis* shows that the microbiological diagnosis can be carried out by conventional microbiological cultures and by eubacterial PCR. The correct identification of this species by molecular methods for only some of the ocular samples emphasizes the clinical benefit of molecular methods. Given the severe presentation of eyes with endophthalmitis caused by *S. lugdunensis*, an appropriate and a rational therapy is of value and necessitates an accurate bacterial identification. Early pars plana vitrectomy is often needed in these cases to allow a useful final visual recovery.

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