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SPECIFIC PCR AND QUANTITATIVE REAL-TIME PCR IN OCULAR SAMPLES FROM ACUTE AND DELAYED-ONSET POSTOPERATIVE ENDOPHTHALMITIS

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Infectious endophthalmitis is one of the most devastating complications of ophthalmic surgeries.¹ The most common isolated microorganisms are Gram-positive cocci, which constitute up to 90% of all bacterial pathogens.² *S. epidermidis* is the leading cause of endophthalmitis, whereas *S. aureus* and *S. pneumoniae* endophthalmitis are the most severe. The course of infectious endophthalmitis depends upon the organism involved, especially its virulence and antibiotic resistance, the infectious inoculum load, the length of time the infection has been evolving, and the inflammatory and immunological host response. We previously showed³ that species of the genus *Streptococcus* are usually associated with more severe infections. Moreover, *Streptococcus pneumoniae* and *Staphylococcus aureus* (especially methicillin-resistant strains), isolated from 1–13.6%^{4–9} and 1.9–18%^{8,10,11} of cases, respectively, are highly virulent bacteria that often cause loss of vision within 24–96 h post-infection even with adequate treatment.²⁴ We recently showed that virulence is one of the major prognostic independent factors in acute endophthalmitis after cataract surgery.¹² Moreover, PCR techniques may detect genes of antibiotic resistance and virulence.^{13,14} Taken together, these molecular techniques may help to rapidly identify specific pathogens and characterize their potential virulence and antibioresistance. This previous information, if acquired with a short delay only, would be helpful to adapt the therapeutic strategy, such as pars plana vitrectomy, and the administration of appropriate antibiotics and future immunomodulatory agents.¹⁵

Conventional microbiological cultures and panbacterial PCR targeting the bacterial 16S rDNA¹⁶ are considered reference techniques for diagnosis of endophthalmitis. Molecular diagnosis was more recently revolutionized by real-time PCR (qPCR) technology based on real-time detection of fluorescence generated by specific probes during DNA amplification. The major advantage of this technique is that it provides faster results (about 1–2 h compared to 2–3 days for panbacterial PCR).^{16,17} This makes qPCR the investigational method of choice in emergency diagnosis. Moreover, the technology may allow quantification of the bacterial load in clinical samples, which has been advocated to differentiate true infection from exogenous contamination^{17–19} and to evaluate patients' prognosis. These new PCR tools have not been evaluated thoroughly for intraocular samples, especially after intravitreal antibiotic treatment.

In this regard, the present prospective study was designed to evaluate the contribution of the combination of panbacterial PCR, specific qPCR tests targeting *S. aureus* and *S. pneumoniae*, and a qPCR test allowing both detection and quantification of *S. epidermidis* load in intraocular samples, in a large series of acute and delayed-onset postoperative endophthalmitis cases.

MATERIALS AND METHODS

Patients

One hundred fifty-three patients (153 eyes; 284 samples of aqueous humor and/or vitreous) with acute or delayed-onset postoperative endophthalmitis were consecutively included in this prospective study (2008–2015) at three French University Hospitals (Grenoble, Lyon, Saint-Etienne). The study adhered to the Declaration of

Helsinki guidelines for research involving human subjects and was approved by the Institutional Review Board (IRB# 5921, clinical trial number NCT02850653).

The diagnosis of endophthalmitis was made on the basis of clinical signs.⁸ Acute endophthalmitis was defined by an occurrence within the first 6 weeks after surgery. Delayed-onset endophthalmitis was defined by an onset later than 6 weeks after surgery. Chronic endophthalmitis cases (inflammation beginning after 6 weeks after cataract surgery) were excluded.

On admission, an immediate tap of aqueous humor (AH) and/or vitreous fluid (VF) or pars plana vitrectomy (PPV) were performed, followed by intravitreal injection (IVI) of vancomycin (1 mg) and ceftazidime (2.25 mg). The patients also received a broad-spectrum intravenous antibiotic regimen (ciprofloxacin and imipenem or fosfomycin) for 5 days, and topical drugs (corticosteroids, tropicamide). In the first attempt, a vitreous biopsy was required. If VF biopsy was not contributive, an AH tap was performed. PPV was indicated when VA was limited to light perception at baseline or in the case of rapid (within of 48h after admission) anatomical and functional deterioration. PPV was performed in the first 24 hours in 31 cases (20%), including six patients who were vitrectomized immediately before intravitreal injection of antibiotics. In the case of patients with LP, PPV was not performed immediately for 32 of 38 patients owing to unavailability of an operating room or a surgeon or because of a non-fasting patient. Intravitreal injections of antibiotics were performed in the first hour of presentation. A second AH or vitreous sample was collected from the patients when a second IVI or PPV was needed.

An evaluation form^{8,12} was completed at the time of the initial examination and during follow-up until the 12th month visit. Patients were defined as having final good (VA ≤ 0.3 LogMAR, $\geq 20/40$) or poor (VA > 0.7 LogMAR, $< 20/100$) visual function.

In patients treated with one IVI at admission, and who did not require a PPV, a second IVI of antibiotics was administered in most cases 48h after the first injection.

Clinical sample collection

AH samples (150–200 μL) and/or VF samples from tap (200–300 μL) or vitrectomy (500 μL) were collected in a sterile syringe just before the IVI of antibiotics. A total of 151 intraocular samples (69 AHs and 82 VFs, including six VF samples from PPV) were collected at admission before IVI of antibiotics (Figure 1). In seven patients, intraocular samples were only collected at the time of the second IVI of antibiotics, and five patients had both AH and VF sampling at admission. In 133 cases, a second ocular sample (39 AHs, 94 VFs) was obtained at the time of the second IVI of antibiotics or PPV.

If the amount of AH or vitreous specimens was limited, culture was considered as the first-line microbiological technique.

Conventional cultures

In the operating room, intraocular samples were inoculated into pediatric blood culture bottles and rapidly transferred to the bacteriology laboratory for a 14-day incubation in an automated blood culture system (Bact-Alert®, BioMérieux; or Bactec FX®, Becton Dickinson). Positive cultures were plated on agar media, and bacterial identification and antibiogram were performed using phenotypic methods (Vitek II, BioMérieux; or BD Phoenix, Becton Dickinson).

PCR-based techniques (Supplementary Table 1)

Panbacterial PCR (amplification and sequencing of the 16SrRNA gene) was performed as previously reported.²⁰ A fragment of the *femA*-, *lytA* and *tuf* genes were amplified using home-made qPCR techniques for detection of *S. aureus* (qPCR-femA), *S. pneumoniae* (qPCR-lytA) and *S. epidermidis* (qPCR-tuf) strains, respectively (supplementary Table 1). As for *S. epidermidis*, the Qpcr-tuf test also allowed the quantification of bacterial loads in intraocular samples, using a standard curve created by plotting the qPCR cycle threshold (CT) values of ten-fold serial dilutions of a titrated *S. epidermidis* DNA suspension.

Statistical analysis

Quantitative data were expressed as mean \pm standard deviation (SD). The McNemar test was used to compare microbiological techniques for the same sample (PCR versus culture). Matched comparisons of bacterial qPCR results (before and after IVI of antibiotics) were studied using the Wilcoxon test. The correlation between microbiological and clinical data was evaluated using nonparametric tests for qualitative (Mann-Whitney test) or quantitative (Spearman test) data. Using the Statistical Package for the Social Sciences program (SPSS 17.0 for Windows, Chicago, IL, USA), the tests were two-tailed and statistical significance was set at $p < 0.05$.

RESULTS

The demographic and clinical characteristics of the patients are summarized in Table 1. One hundred fifty-three patients, with acute (91%) or delayed-onset (9%) postoperative endophthalmitis were included, mainly after cataract surgery (88%). Other surgeries included glaucoma surgery (trabeculectomy) or pars plana vitrectomy.

Microbiological identification

From the 153 eyes included, before intravitreal antibiotic treatment, the identification rate using panbacterial PCR and culture was 48% (25/52 cases) from AH and 75% (57/76 cases) from VF (Figure 1). After one IVI of antibiotics, 39 AH and 94 VF samples were analyzed, leading to bacterial identification in 3/28 cases (11%) and 56/83 cases (67%), respectively. Finally, a bacterial species was identified in 107 of 153 eyes (70%; Table 2). There was a large majority of Gram-positive cocci (93%) and a predominance of *S. epidermidis* strains (60%, Table 2). A 100% concordance was found for microbiologic organisms identified in the first and second ocular samples, for PCR and cultures.

Evaluation on ocular samples before intravitreal antibiotic treatment

Comparison between culture and panbacterial PCR. In AH samples collected from 69 eyes before the first IIV of antibiotics (Figure 1, Table 3), panbacterial PCR was positive in 18 of 59 samples analyzed (30%) and cultures in 24 of 62 samples analyzed (38%). For the 52 eyes that were analyzed by both methods, the identification rate was 48% (25/52) and the positivity rates of panbacterial PCR and cultures were not significantly different ($P=0.6$). Of the 33 samples with negative cultures, 6/33 cases (18%) had a positive panbacterial PCR test.

In VF from 82 patients, panbacterial PCR was positive in 49 of 78 samples analyzed (63%) and cultures in 53 of 80 samples analyzed (66%). For the 76 eyes that were analyzed using both techniques, the identification rate was 75% (57/76). The rate of positivity of the two techniques was not significantly different ($P=0.6$). Of the 25 samples with negative cultures, 6/25 cases (24%) had a positive panbacterial PCR test.

Contribution of specific qPCR. Analysis was performed in 120 patients, corresponding to 49 AH and 71 VF samples. Among the six eyes infected with *S. aureus*, five AH samples and one VF sample were collected at admission. For AH samples, culture was positive in three of five samples and qPCR-femA in four of five. The two culture-negative samples had a positive Qpcr-tuf test. The panbacterial PCR was positive in two out of three samples analyzed. The qPCR-tuf test did not make additional diagnoses compared to the three cases detected by panbacterial PCR. One VF was positive by culture and qPCR, but negative using the panbacterial PCR.

Among the three eyes infected with *S. pneumoniae*, one AH and two VF samples collected were positive by culture, panbacterial PCR and qPCR-lytA.

Altogether, qPCR tests targeting *S. aureus* or *S. pneumoniae* were not able to make additional diagnoses compared to the combination of culture and panbacterial PCR. qPCR tests had good specificity with no false-positive results for samples from eyes infected by other bacterial species ($n=49$).

Evaluation of panbacterial PCR on ocular samples after intravitreal injection of antibiotics

AH samples were collected from 39 eyes and VF samples from 94 eyes (Table 4). For AH samples, panbacterial PCR was positive in three of 33 samples (9%), and cultures in two of 34 samples (6%). For the 28 eyes that were analyzed by panbacterial PCR and cultures, the identification rate was 10% (3/28) for both techniques ($P=0.99$). For samples analyzed via culture, in AH samples, none second AH samples was positive after IIV whereas culture before IIV was positive in 35% out of the cases and negative in 65%.

For VF samples, panbacterial PCR was positive for 54 of 87 samples (62%), and cultures for 43 of 90 samples (48%). For the 83 eyes that were analyzed by both techniques, the positive rate of panbacterial PCR (60%) was significantly higher than that of cultures (39%; $P=0.05$). For samples analyzed by culture, if the second VF samples were obtained by tap, culture positive results after the first IIV were found in 44% out the cases, and in 4/16 of initially negative cases. If the second VF samples

were obtained by PPV, culture positive results after the first IVI were found in 53.7% out the cases, and in 5/16 of initially negative cases.

Contribution of the qPCR-tuf test

qPCR-tuf tests were performed in patients infected with *S. epidermidis*, on 13 VF samples before antibiotic treatment (84,679 \pm 10,6281 DNA copies/mL, Ct values from 22.97 to 35.49, supplementary Table 2), and 20 VF samples after one IVI of antibiotics (52,084 \pm 99,798 DNA copies/mL, Ct values from 26.89 to 37.25). The bacterial load did not change significantly ($P=0.6$) in the VF of eight patients for whom qPCR-tuf tests were performed before (51,803 \pm 47,161 DNA copies/ml) and after treatment (95,737 \pm 147,431 DNA copies/mL).

Final clinical outcomes

At the 12th month visit, VA was equal to or better than 20/40 in 50% of the cases, between 20/125 and 20/50 in 17.5%, between 20/200 and 20/400 in 2%, and less than 20/400 in 19.5%. Seven (4.6%) cases of phthisis and two retinal detachments (RD, 1.3%) were recorded.

S. aureus infection was associated with one case of RD and two cases of phthisis, and a final VA less than 20/400 in four out the six cases. *S. pneumoniae* infections led to phthisis in one case and RD in one case, and a VA less than 20/400 in two out the three cases.

No significant association was found between the microbiological profile (culture and panbacterial PCR positive; culture negative and panbacterial PCR16S positive; culture positive and panbacterial PCR negative; and culture and panbacterial PCR negative) and final VA, or occurrence of phthisis. The initial vitreous bacterial load for *S. epidermidis* was higher in cases with final VA <20/40 (127,118 \pm 125,848 DNA copies/mL in patients with VA \geq 20/40 vs 350,000 \pm 46,912 with VA <20/40, $P=0.09$).

DISCUSSION

This large prospective multicenter study showed that: (a) the microbiological identification rate was better in VF than in AH before or after antibiotic IVI; (b) panbacterial PCR was more efficient than culture to identify bacteria in AH and VF after antibiotic IVI; (c) qPCR tests targeting *S. aureus* and *S. pneumoniae* were highly specific but their sensitivity was not determined due to the low prevalence of these infections; and if applied to all ocular samples, the cost-effectiveness of these qPCR tests was considered poor; (d) qPCR on vitreous samples infected with *S. epidermidis* showed that high bacterial loads at baseline were more likely associated with poor final visual prognosis; no significant changes in bacterial loads was observed after one antibiotic IVI.

This study aimed to evaluate a combination of different PCR-based techniques in a large cohort of postoperative endophthalmitis, mostly after cataract surgery. This is one of the largest series of postoperative endophthalmitis, especially for the evaluation of microbiological techniques (range in the literature: $n = 5-100$).¹⁶ We acknowledge some limitations such as bias due to the real-life sampling protocol, i.e., the impossibility of carrying out all the techniques evaluated for a few intraocular specimens due to the

limited volume of sample collected and the bias associated with the second ocular sampling (AH or PPV). Cases of delayed-onset endophthalmitis were related to acute bleb-related infection only.

The study confirmed that microbiological investigations are more sensitive for vitreous than AH samples. In the literature, this has been previously reported for culture with a 40–69%^{6,21–25} identification rate in VF versus 22–30% in AH.^{8,21,26,27} These data are confirmed for panbacterial PCR with 56–100% positive results in VF versus 30–88% in AH.^{8,16,18,28} The superiority of VF was even higher in ocular samples collected after IVI of antibiotics: 6% in AH versus 48% in VF using cultures, and 9% versus 62% using panbacterial PCR in the present study. The high rate of positive cultures after one IVI of antibiotics highlights that two or more IVI of antibiotics need to be performed in the management of endophthalmitis patients.^{8,29} The higher rate of positive cultures (48%) in this series, when compared with our previous study (8% positive culture in vitreous from eyes after one IVI⁸) could be partially explained in the change of the culture method, namely the replacement of brain-heart infusion broth with blood culture bottles.

The identification rate in culture using pediatric blood culture bottles presents many advantages. Its efficiency was estimated in noncomparative studies (70–91% positivity in VF)^{30,31} and in three comparative studies.^{32–34} These latter studies showed higher identification rates (69–100%) with this technique compared to conventional cultures using agar media (53–74%).^{10,23,24,35,36} Blood culture bottles have also been used for diluted specimens from vitrectomy cassette, adding 11% microbiological diagnosis (from 73% to 84%).³⁷ Advantages of blood culture bottles include: (a) the simple and time-saving direct and immediate inoculation in the operating room, reducing the risk of contamination associated with multistep processing; and (b) the results are obtained faster because these bottles are managed 24h a day in an automated instrument. In this study, we used the BD BACTEC™ Peds Plus/F broth originally designed for small specimen volumes of less than 3 mL. It also contains resins for antibiotic neutralization and therefore may allow isolation of microorganisms in patients already under antibiotic therapy. The disadvantages of using conventional culture media include: (a) the use of several media; (b) it is less convenient and more time-consuming; and (c) the time between sampling and inoculation of samples on culture media is longer, which may reduce microbial viability.³³ Only one study³⁸ did not find blood culture bottles superior to conventional media (69% versus 72% identification rate, respectively), but adding blood culture bottles to conventional media increased microbiological detection rates from 72% to 81%.

The use of panbacterial PCR (i.e., 16SrDNA PCR amplification with subsequent identification of the amplified product by DNA sequencing)^{8,39–42} has the advantage of covering the entire bacterial spectrum and is particularly useful when a large panel of bacterial species may cause the same disease, such as post-operative endophthalmitis. The rate of identification reported in this study (30% in AH and 63% in untreated VF) is in the lower range reported in a recent review,⁴³ from an analysis of 16 studies showing a 40% identification rate for conventional culture and 82% for PCR. The main limitations of panbacterial PCR⁴⁴ include a lower sensitivity and specificity compared to species-specific qPCR tests, and more importantly a longer turnaround time (2–3 days are required for species identification compared to 2–3h for qPCR). However, false-positive results are considered very rare.⁸

Given the poor visual prognosis associated with *S. pneumoniae*^{4,9,45} and *S. aureus*^{11,45-47} species, there is a need to detect such virulent bacteria as early as possible. As compared to panbacterial PCR, specific qPCR tests allow faster (1–3 h) and more sensitive detection of target bacterial species. Goldschmidt et al.⁴⁸ reported the use of qPCR tests targeting bacterial species belonging to the same bacterial family or genus. Joseph et al.⁴⁹ reported the usefulness of qPCR tests in a series of 64 patients, with identification rates of 66% for qPCR tests and 34% for culture. In our case series, although qPCR tests could yield results within a short time, they did not show better sensitivity than the combination of panbacterial PCR and culture. One limitation of our study is the very low prevalence of *S. aureus* and *S. pneumoniae* infections, which did not allow to draw a definitive conclusion about the contribution of this molecular techniques. The higher value of blood culture bottles in the diagnosis discussed above may also explain the low contribution of specific qPCRs.

The qPCR technology also allows quantification of bacterial loads. A threshold cycle can be determined as the number of amplification cycles required for the fluorescent signal to cross a predefined threshold. In the present study, using a calibration curve, the threshold cycle of the qPCR-tuf assay gave an estimation of the amount of *S. epidermidis* DNA in clinical samples at the time of patients' admission and after IVI of antibiotics. The high bacterial loads that were detected ($1.4 \cdot 10^3$ to $3.9 \cdot 10^5$ copies/mL) suggest that these cases were truly active infections. These results confirmed previous studies^{18,19}, which reported a high number of bacterial genome units in ocular samples (from $1.7 \cdot 10^3$ to $1.7 \cdot 10^9$ genome units/mL). Melo et al.¹⁷ defined a cut-off threshold cycle differentiating infection from contamination, by testing intraocular samples from patients with proven bacterial endophthalmitis and aqueous samples obtained at the end of cataract surgeries taken as controls. Using a broad-range PCR, a threshold cycle value between 19.5 and 34.5 was compatible with bacterial endophthalmitis; while a threshold cycle value of 39 was found for the two contaminated AH samples. In our study, although we used a different qPCR technique, Ct values were less than 39 for all patients, which is consistent with a true infectious process. The results suggest high variability of intraocular bacterial loads between different infected patients. One unexpected finding was the absence of significant reduction in the bacterial load after one IVI of antibiotics. This is consistent with the high rate of positive qPCR tests for vitreous samples after at least one IVI of antibiotics in a previous study.⁸ One possible limitation of qPCR is that detection of bacterial DNA does not imply the presence of viable bacteria, since the amplified DNA might represent remnants of bacteria killed by antibiotics. We also found similar results in the AH of patients with VZV retinitis, with a stable DNA load at the beginning of the antiviral treatment.⁵⁰ One perspective could be the evaluation of bacterial mRNAs as markers for cell viability since these are highly unstable molecules with very short half-lives inside the cell.⁴⁴ The detection of mRNAs would indicate that bacteria are alive and metabolically active, and therefore reverse transcriptase assay targeting the 16SrRNA⁵¹ may be useful to follow the bacterial viability in intraocular specimens.

In conclusion, this real-life study confirms the complementarity of the culture method using pediatric blood culture bottles and panbacterial PCR, especially when antibiotics have been administrated before sampling. This preliminary evaluation of the usefulness of specific qPCR tests for detection of rare but hypervirulent bacterial species such as *S. aureus* and *S. pneumoniae* is not in favor of their systematic use in

postoperative endophthalmitis patients. Optimization of this strategy could be the use of a multiplex qPCR test, a variant of qPCR allowing simultaneous detection of multiple pathogens (DNA targets) in a single reaction.

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Figure 1: Analysis flow chart of aqueous humor samples and vitreous tap.

The calculated percentages of positivity are based on the number of positive samples/the number of samples analyzed using the microbiological technique

Table 1: Demographic and clinical data of 153 patients with postoperative endophthalmitis. Means are expressed with standard deviation (SD).

Table 2: Final microbiological identification

Table 3: Results of PCR and cultures of aqueous humor and vitreous samples, before antibiotic therapy

The rate of positivity between cultures and 16SrDNA PCR was not significantly different in aqueous humor (AH) and vitreous (VF) samples at admission ($P=0.6$). The McNemar test was performed on data in the grey lines (samples that were tested for both cultures and PCR).

AH: aqueous humor, VF: vitreous fluid, ND: not done.

Table 4: Results of PCR and cultures of aqueous humor and vitreous samples from patients with postoperative endophthalmitis, after one intravitreal injection of antibiotics. The McNemar test was done on data in the grey lines (samples that were tested both for cultures and PCR).

AH: aqueous humor, VF: vitreous fluid, ND: not done.

Supplementary Table 1: Techniques of specific *S. aureus* and *S. pneumoniae* rt-PCR and quantitative real-time PCR assay targeting the *tuf* gene of *Staphylococcus* species.

Supplementary Table 2: Real-time quantitative PCR on vitreous samples of 25 patients with postoperative endophthalmitis due to *S. epidermidis*

Figure 1: Analysis flow chart of aqueous humor samples and vitreous tap.

The calculated percentages of positivity are based on the number of positive samples/the number of samples analyzed using the microbiological technique

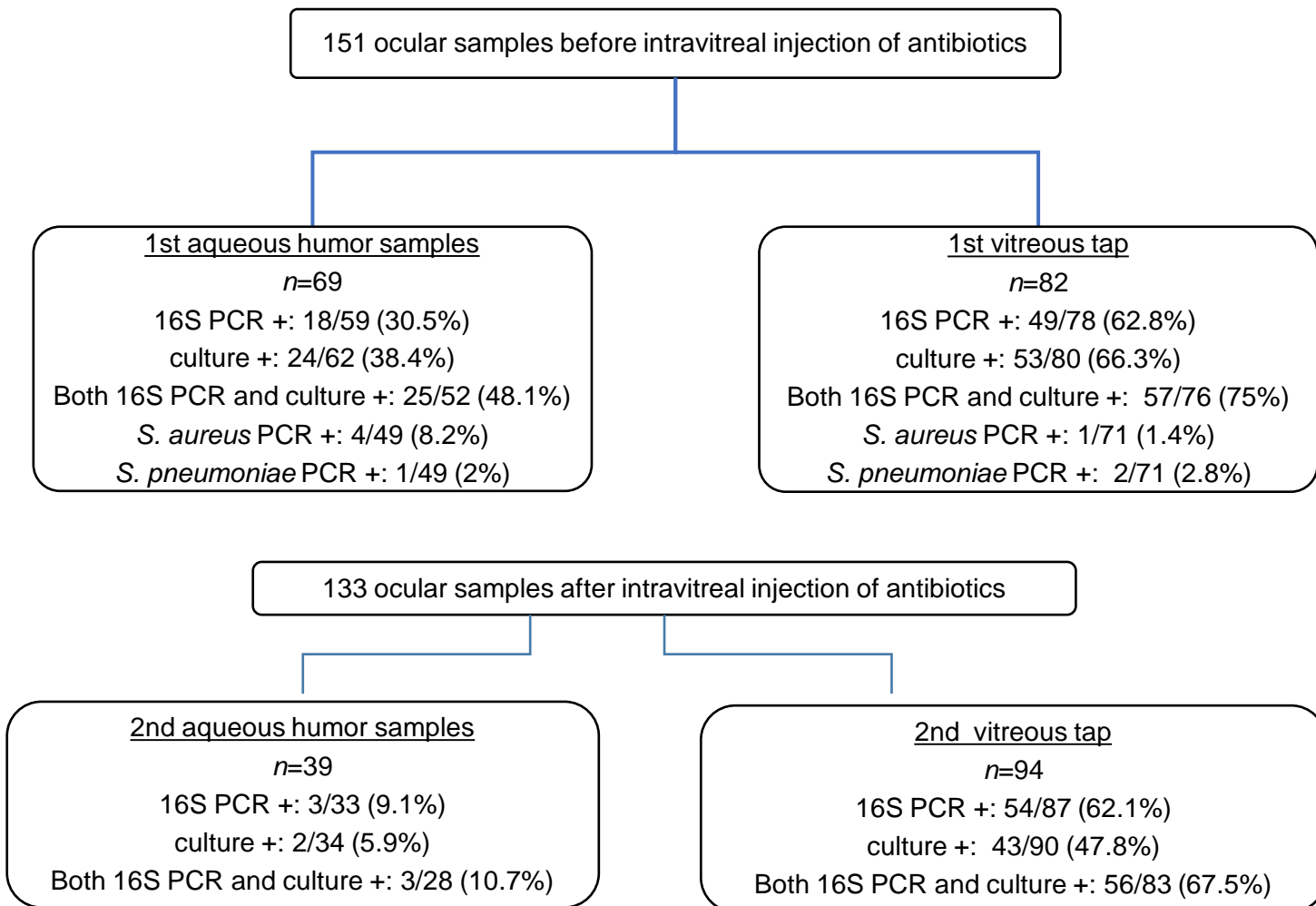


Table 1: Demographic and clinical data of 153 patients with postoperative endophthalmitis. Means are expressed with standard deviation (SD).

Sex ratio (men/women)	67/86
Age (years)	73 ±10.8
Initial visual acuity:	
· No light perception	6 (3.9%)
· Light perception	52 (34%)
· Hand motions	48 (31.4%)
· Count fingers	26 (17%)
· > 20/400	21 (13.7%)
Risk factors	
· Immunosuppression	12 (7.9%)
· Diabetes	18 (11.8%)
· Capsular rupture	12 (9%)
Affected eye, right/left	86/67
Type of endophthalmitis	
· Acute	140 (91.5%)
· Delayed-onset endophthalmitis	13 (8.5%)
Initial clinical presentation	
· Inflammation of anterior chamber	153 (100%)
· Pupillary membrane	115 (76.2%)
· Hypopyon	119 (78%)
· Fundus visibility	35 (23.3%)
Type of surgery	
· Cataract extraction	135 (88.2%)
· Filtration surgery	13 (8.5%)
· Pars plana vitrectomy	5 (3.3%)

Table 2. Final microbiological identification

Bacteria	Number (% Based on 153 eyes)	
Gram-positive	<i>Staphylococcus epidermidis</i>	65 (42.5%)
	<i>Staphylococcus aureus</i>	6 (3.9%)
	<i>Staphylococcus lugdunensis</i>	4 (2.6%)
	<i>Staphylococcus capitis</i>	2 (1.3%)
	<i>Staphylococcus warneri/ pasteuri</i>	1 (0.65%)
	<i>Staphylococcus saprophyticus</i>	1 (0.65%)
	<i>Staphylococcus haemolyticus</i>	1 (0.65%)
	<i>Staphylococcus caprae</i>	1 (0.65%)
	<i>Streptococcus oralis</i>	5 (3.3%)
	<i>Streptococcus parasanguinis</i>	1 (0.65%)
	<i>Streptococcus salivarius</i>	2 (1.3%)
	<i>Streptococcus mitis</i>	1 (0.65%)
	<i>Streptococcus pneumoniae</i>	3 (2%)
	<i>Streptococcus anginosus</i>	1 (0.65%)
	<i>Granulicatella adiacens</i>	1 (0.65%)
	<i>Enterococcus faecalis</i>	4 (2.6%)
	<i>Corynebacterium spp</i>	1 (0.65%)
Gram-negative	<i>Moraxella lacunata</i>	1 (0.65%)
	<i>Moraxella spp.</i>	1 (0.65%)
	<i>Serratia marcescens</i>	2 (1.3%)
	<i>Rhizobium radiobacter</i>	1 (0.65%)
	<i>Proteus vulgaris</i>	1 (0.65%)
	<i>Pseudomonas aeruginosa</i>	1 (0.65%)
Total	107	

Table 3. Results of PCR and cultures of aqueous humor and vitreous samples, before antibiotic therapy

The rate of positivity between cultures and 16SrDNA PCR was not significantly different in aqueous humor (AH) and vitreous (VF) samples at admission ($P=0.6$). The McNemar test was performed on data in the grey lines (samples that were tested for both cultures and PCR).

AH: aqueous humor, VF: vitreous fluid, ND: not done.

First sample		16S PCR-	16S PCR+	16S PCR ND
AH ($n=69$)	Culture -	27	6	5
	Culture +	9	10	5
	Culture ND	5	2	
Vitreous ($n=82$)	Culture -	19	6	2
	Culture +	9	42	2
	Culture ND	1	1	

Table 4. Results of PCR and cultures of aqueous humor and vitreous samples from patients with postoperative endophthalmitis, after one intravitreal injection of antibiotics. The McNemar test was done on data in the grey lines (samples that were tested both for cultures and PCR).

AH: aqueous humor, VF: vitreous fluid, ND: not done.

		16S PCR-	16S PCR+	16S PCR ND
AH (n=39)	Culture -	25	2	5
	Culture +	1	0	1
	Culture ND	4	1	
Vitreous (n=94)	Culture -	27	16	4
	Culture +	6	34	3
	Culture ND	0	4	