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Femtosecond laser-assisted versus phacoemulsification cataract surgery (FEMCAT): a multicentre participant-masked randomised superiority and cost-effectiveness trial

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**FEMTOSECOND LASER-ASSISTED VERSUS PHACOEMULSIFICATION
IN CATARACT SURGERY, A MULTICENTRE PARTICIPANT-BLINDED
RANDOMISED SUPERIORITY AND COST-EFFECTIVENESS TRIAL (FEMCAT)**

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Contribution: CS, ABe & NH conceived and designed the trial; CS was the chief investigator and oversaw the trial throughout. ABe, CG and AM had access to raw data, and planned and performed the statistical analysis. NH and ABe planned and performed the health economy analysis. RS performed the health economy statistical analysis. SR was the trial coordinator. CS, ABr, BC, PJP, PD, DM were principal investigators in each site and participated in recruitment and collection of data. The steering committee was composed of CS (Chief investigator, Head of steering committee), ABe, ABr, BC, PJP, PD, DM, NH and SR. All authors contributed to the interpretation of data, drafting of the report and decided on its content. CS wrote and submitted the final manuscript on behalf of the FEMCAT study group. All authors approved the final version of the manuscript.

Declaration of interests: CS has had advisory roles (compensated) or honoraria for lecturing, outside the submitted work, with Alcon, Allergan, Glaukos, Johnson & Johnson, Novartis, Théa, Zeiss. AB had advisory roles (compensated) or honoraria for lecturing, outside the submitted work, with Alcon. BC is past president of the French Society of Ophthalmology (SFO), past president of the European Society of Cataract and Refractive Surgery (ESCRS), is currently the president of the French Academy of Ophthalmology (AFO), has had advisory roles (compensated) or honoraria for lecturing, outside the submitted work, with Alcon, Cutting Edge, Hoya, Horus, Johnson & Johnson, Novartis, Santen, Shire, Théa, Zeiss. PD is past president of the French Society of Ophthalmology (SFO); is currently the president of the

French Glaucoma Society (SFG). PJP is the past president of the French Society of Ophthalmology (SFO); has had advisory roles (compensated) or honoraria for lecturing, outside the submitted work, with Alcon, Novartis, Santen, Shire, Théa. The other authors declare no competing interests.

Data Sharing: The study protocol, statistical analysis plan and informed consent form are available at <https://nextcloud.chu-bordeaux.fr/index.php/s/a5P57HenXEi7Spq>

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Key words: Cataract, cataract surgery, femtosecond laser, femtosecond laser-assisted cataract surgery, phacoemulsification cataract surgery, cost-effectiveness

Abbreviations/Acronyms:

BCVA: Best Corrected Visual Acuity

CME: Cystoid Macular Oedema

CHU: Centre Hospitalier Universitaire (University Hospital: UH)

FLACS: Femtosecond Laser-Assisted Cataract Surgery

IOL: IntraOcular Lens

OCT: Optical Coherence Tomography

PCR: Posterior Capsule Rupture

PCS: Phacoemulsification Cataract Surgery

RCT: Randomised Clinical Trial

UCVA: UnCorrected Visual Acuity

ABSTRACT

Background: Cataract surgery is one of the most commonly performed operations in healthcare. Femtosecond Laser-Assisted cataract surgery (FLACS) enables more precise ocular incisions and lens fragmentation. We hypothesised FLACS may improve cataract surgery outcomes compared with phacoemulsification cataract surgery (PCS) despite higher costs.

Methods: We conducted a multicentre participant-blinded randomised superiority clinical trial comparing FLACS and PCS in two parallel groups (Permuted-block randomisation stratified on centres, ratio 1:1, web-based application). Five French University Hospitals included consecutive patients eligible for unilateral or bilateral cataract surgery. The primary clinical endpoint was the success rate defined as a composite of four outcomes at 3-month postoperative visit: Absence of severe perioperative complication, best-corrected visual acuity (BCVA) of 0.0 LogMAR, absolute refractive error ≤ 0.75 dioptres (D), postoperative change in corneal astigmatism power (≤ 0.5 D) or axis ($\leq 20^\circ$). The primary economic endpoint was the incremental cost per additional patient presenting a treatment success at 3 months. ClinicalTrials.gov identifier: NCT01982006.

Findings: Of the 909 randomised patients (n=1480 eyes) between October 9, 2013 and October 30, 2015, 870 (n=1389 eyes) were analysed. There was no significant difference in success rate between FLACS and PCS (FLACS:41.1% (n=289), PCS:43.6% (n=299), Odds Ratio:0.85, 95%Confidence Interval:0.64-1.12, p=0.250). The percentage of eyes without complication (94.3 versus 94.0), with BCVA of 0.0 LogMAR (78.7 versus 81.8), with absolute refractive error ≤ 0.75 D (80.1 versus 82.8), or unchanged corneal astigmatism (56.7 versus 58.4) did not differ between FLACS and PCS, respectively. The ICER was 10703.2€ saved per additional patient in treatment success with PCS compared with FLACS.

Interpretation: Despite its advanced technology, femtosecond laser was not superior to phacoemulsification in cataract surgery and, with higher costs, failed to provide an additional benefit for patients or healthcare systems.

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RESEARCH IN CONTEXT

Evidence before this study:

Before the FEMCAT trial, there was evidence femtosecond laser technology applied to cataract surgery provided more precise and reproducible ocular tissue incisions than the current manual phacoemulsification technique and reduced the ultrasound energy delivered within the eye. Yet, few clinical trials have suggested the precise ocular tissue incisions and phacofragmentation provided by the femtosecond laser and the associated real time imaging technology could improve postoperative visual and refractive outcomes or decrease the rate of complication associated with cataract surgery as compared with the current reference standard ultrasound phacoemulsification technique. Thus, despite its higher investment costs, the health benefit of the innovative and advanced technology for patients remained inconclusive. We searched the following databases up to December 2012: PubMed, Embase, Scopus, Cochrane Central Register of Controlled trials, Web of Science and clinicaltrials.gov with the following terms: “cataract surgery”, “femtosecond laser”, “phacoemulsification”, “laser-assisted cataract surgery”, “cost-effectiveness”. We found no adequately powered randomised clinical trial assessing the clinical benefit of femtosecond laser cataract surgery for patients. While the FEMCAT trial was underway, we updated our searches and we identified a Cochrane meta-analysis that could not determine the equivalence or the superiority of femtosecond laser cataract surgery due to a very low certainty of available evidence and unclear risk of bias in included studies. Finally, a recent single centre randomised clinical trial did not find any significant difference between the two surgical techniques for visual or refractive outcomes, but the trial was not adequately powered for the analysis of safety outcomes and did not provide any cost-effectiveness analysis.

Added value of this study:

The FEMCAT trial is the first large scale independent randomised clinical trial comparing the innovative femtosecond laser technology and the standard of care phacoemulsification technique for cataract surgery in two parallel patients groups of similar size. Additionally, the FEMCAT trial is the first to provide a cost-effectiveness analysis with an incremental cost-effectiveness ratio. Our trial shows, in a large sample including a wide range of cataract severity grade, that femtosecond laser cataract surgery is not superior to the current phacoemulsification cataract surgery and does not provide any additional clinical benefit for patients. The femtosecond laser cataract surgery was also more expensive and less effective in our cost-effectiveness analysis. Although femtosecond laser procedure is not associated with severe adverse events in our trial, this technology does not improve the rate of most frequent sight-threatening complications and postoperative visual or refractive outcomes are not different between the two surgical procedures.

Implications of all the available evidence

The FEMCAT trial was designed *to analyse all clinically significant outcomes related to cataract surgery, which is one of the most commonly performed operations in healthcare, and to minimize the risk of bias observed in previously published trials comparing the two surgical techniques.* By demonstrating that femtosecond laser cataract surgery is not superior to phacoemulsification cataract surgery and is not a cost-effective strategy for the French healthcare system, the results of our trial provide robust and useful information for patients, health-care providers and decision-makers before considering the implementation of this new and costly technology in routine practice. Further development and research should be conducted on femtosecond laser technology to provide a meaningful visual and health benefit for patients and to improve the increasing burden of cataract surgery at a sustainable cost for healthcare system.

MANUSCRIPT

Cataract is the leading cause of blindness worldwide, and the second cause of moderate or severe visual impairment after uncorrected refractive error.¹ Today, phacoemulsification cataract surgery (PCS) is the standard of care and consists of the manual opening of the crystalline lens anterior capsule (capsulorhexis) with a forceps, the removal of the opacified lens using ultrasound, and the implantation of an intraocular lens (IOL) in the remaining capsular envelope to restore visual function. By achieving excellent visual and anatomical outcomes, PCS is one of the most routinely performed operations worldwide, with approximately 17.7 millions procedures in 2018 (<https://www.market-scope.com>), and is also associated with a reduction in morbidity and mortality.²

Although PCS has provided important health benefits, this procedure is still facing some challenges. As life expectancy increases, the number of patients to be treated is rising. The French national register reported a 21.9% increase in cataract procedures from 2010 to 2016 with approximately 800,000 procedures performed in 2016 (<https://www.atih.sante.fr>). The overall complication rate can affect 4.2% to 8.6% of the eyes and is associated with worse postoperative visual outcomes.^{3, 4} Additionally, still 7.0% to 16.2% of eyes achieved a postoperative absolute refraction error more than one dioptre greater than planned preoperatively.^{1, 5, 6}

By providing focal tissue photodisruption within 5µm and minimal collateral damage, the recent development of femtosecond laser has opened new opportunities in ophthalmic surgery.⁷ Associated with a real-time imaging technology, femtosecond Laser-Assisted cataract surgery (FLACS) has enabled more precise anterior capsulotomy, corneal incisions and crystalline lens fragmentation without collateral damage to surrounding ocular structures. This technology has been suggested to improve cataract surgery outcomes compared with the manual PCS and has been presented as a breakthrough in cataract surgery.^{8, 9} Indeed, more

precise and reproducible corneal or capsulotomy incisions may reduce postoperative corneal changes and improve IOL positioning within the eye.¹⁰⁻¹² Moreover, lens fragmentation may reduce total ultrasound energy required, facilitate lens removal and could reduce surgical complications.^{9,13}

Nevertheless, despite the overwhelming number of studies comparing FLACS and PCS, few randomised clinical trials (RCT) have been published and the clinical benefit of FLACS over PCS for patients still remains controversial with inconclusive results in meta-analyses.^{14, 15} While Popovic et al. included a substantial number of consecutive case series in their meta-analysis, a recent Cochrane meta-analysis, including only robust RCT evidence, could not determine the superiority or the equivalence of FLACS due to very low certainty of evidence.^{14, 15} Most available RCTs were deemed not adequately powered and methodologically biased.¹⁴ A recent single centre RCT including one eye per patient did not observe any significant difference of visual or refractive outcomes between the two surgical techniques, but the authors acknowledged the analysis of safety outcomes was not adequately powered.¹⁶ Additionally, while this innovative technology is associated with significant additional costs, none of published RCT performed a cost-effectiveness analysis.

We, therefore, conducted an independent, superiority, multicentre and participant-blinded RCT comparing safety and efficacy as well as cost-effectiveness between FLACS and PCS.

MATERIALS AND METHODS

Study design

The FEMCAT *study was a prospective, multicentre, participant-blinded, superiority, randomised clinical trial involving 5 University Hospitals (UH) in France.*

This study aimed to compare safety and efficacy, as well as costs and cost-effectiveness, between two parallel patients groups of similar size: the conventional PCS and the innovative FLACS.

This research was approved by the French National Agency for Medicines and Healthcare Products and followed the Declaration of Helsinki's tenets. The FEMCAT trial was approved by the Ethical Committee of Bordeaux (France) in December 2012. The study protocol, statistical analysis plan and informed consent form are available at <https://nextcloud.chu-bordeaux.fr/index.php/s/a5P57HenXEi7Spq>.

Participants

Participants of the FEMCAT trial were consecutively enrolled from the outpatient clinic. We included all consecutive patients eligible for a unilateral or bilateral cataract surgery aged 22 years or older with the ability to give informed consent. Surgery was proposed when cataract was responsible for a best-corrected visual acuity (BCVA) worse than 0.3LogMAR or cataract related severe visual disturbance, such as halos, glare or severe photophobia. Detailed exclusion criteria are provided in the supplementary appendix (Figure S1).

Cataract was clinically graded using the Lens Opacities Classification System version 3 (LOCS III) grading scale.

All examinations were performed by experienced technicians specifically trained for the trial. Each centre used the same machines according to manufacturers recommendations for all patients enrolled in the trial and from the screening visit to the month-3 visit. Keratometry values, astigmatism power and axis were assessed using placido-based corneal topography. Anterior chamber depth, central corneal and macular thicknesses were measured using optical coherence tomography (OCT). Endothelial cell count was measured using specular microscopy.

All participants provided informed written consent for enrolment in the study.

Surgical procedures

All operations were performed under topical anaesthesia and in ambulatory surgery setting as recommended in standard care. Twenty-one surgeons (Bordeaux UH: n=4; Brest UH: n=4; Lyon UH: n=4; AP-HP Paris-Cochin UH: n=5; Tours UH: n=4) experienced in cataract surgery enrolled patients and performed all surgical procedures.

Detailed surgical procedures are provided in Figure S2.

A 6 mm optic zone size foldable monofocal acrylic hydrophobic IOL was implanted in the capsular bag. IOL power was calculated using interferometry technology and the SRKT formula for all patients enrolled in the trial.

As each centre could have different brands of IOL available and to limit the risk of analysis biases related to potential different biomaterial characteristics or optical properties between IOL brands, each centre used the same IOL brand for all their included participants. Additionally, each centre used the same phacoemulsification machine for all their included participants.

FLACS arm procedure.

All femtosecond laser procedures were performed using Catalys precision system (Johnson & Johnson Inc., New Jersey, USA) according to company recommendations and after a certification process organised for all surgeons involved in the trial.

Participants were installed on the dedicated stretcher and 5mm diameter size capsulotomy, phacofragmentation and three-planes corneal incisions settings were systematically activated for all procedures. The non-applanating liquid optic interface was placed on the sclera centred

on the cornea and filled with balanced salt solution after suction activation. After docking to the disposable lens of the laser, a 3-dimensional imaging of the anterior structures of the eye was obtained using spectral-domain OCT imaging provided by the system and laser was activated. After laser treatment, the patient was positioned as is usual for a phacoemulsification procedure, under the microscope to open laser-created corneal incisions and surgical removal of dissected pieces of crystalline lens. Then, the IOL was implanted in the capsular bag and centred underneath the capsulotomy.

PCS arm procedure

Patients were positioned under the surgical microscope and PCS procedures were performed as usually described. Corneal incisions were manually created using calibrated blades and manual continuous curvilinear capsulorhexis was performed using a specific forceps. The cataract was then removed using the ultrasound probe of the phacoemulsification machine. After the complete removal of lens pieces with the aspiration probe of the phacoemulsification machine, the IOL was implanted in the capsular bag and centred underneath the capsulorhexis.

Randomisation and masking

A permuted-block randomisation stratified on centres was performed within 5 days before the surgery using a centralised web-based system. The allocation ratio was 1:1 with the patient as unit of randomisation and both eyes allocated to the same surgical procedure (PCS or FLACS) when both eyes were eligible for the study.

Detailed masking procedures are provided in Figure S2.

Participants were masked to the surgical treatment allocation until the last follow-up visit and a sham laser procedure was set up in the operating room for participants randomised in the PCS arm.

All medical and non-medical staff involved in the study were specifically trained in participant randomisation and blinding processes from the screening visit up to the last follow-up visit, including the day of surgery in the operating room and in the ambulatory surgery setting.

Outcomes

The primary clinical outcome measure was the difference between the two treatment arms in the proportion of eyes classified as a treatment success at the 3-month visit (V3) in modified intention-to-treat and as-treated analyses. Treatment success was a composite of any of the four following events at V3: 1) absence of severe intraoperative or postoperative complications up to V3, 2) BCVA of 0.0 LogMAR or better, 3) an absolute manifest refractive error ≤ 0.75 Dioptres, 4) postoperative change in corneal astigmatism power ≤ 0.5 Dioptres and astigmatism axis $\leq 20^\circ$. Complications considered for analysis were: intraoperative posterior capsule rupture (PCR) with or without vitreous loss, intraoperative zonular dialysis, posterior luxation or subluxation of the lens or the IOL, clinically significant and persistent corneal oedema at V3, and retinal detachment, clinically significant cystoid macular oedema (CME) or endophthalmitis up to V3.

The primary economic endpoint was the incremental *cost per additional patient presenting a treatment success from the French healthcare system perspective*. Patients were defined as presenting a treatment success when all their eyes treated within the FEMCAT trial were classified as treatment success *as defined in the primary clinical outcome*. *Costs of cataract surgery were estimated in each group by a bottom-up microcosting approach (Table S2). As*

PCS is already reimbursed by the *French National Healthcare System*, the differential cost between FLACS and PCS estimated through microcosting was taken into account to value the cost of FLACS in our analysis. In order to limit the learning curve effect on the estimation of FLACS cost, the microcosting study was conducted only for investigators who have had already included at least 20 patients in the study. All other inpatient and outpatient costs arising until the treatment success measurement were collected from the *French National Health Data System (SNDS, Système National des Données de Santé)*, a claim database encompassing 98.8% of the French population.¹⁷

As secondary outcomes, each component of the composite primary outcome was analysed separately as well as intraoperative complication during laser procedure or phacoemulsification phase and postoperative absolute manifest refractive errors.

An independent adjudication committee composed of three independent expert ophthalmologists masked to patient randomisation, analysed all adverse events related to the primary outcome measures.

Statistical analysis

The sample size calculation was based on the incremental cost-effectiveness ratio using a threshold of 16750€/ per additional patient presenting a treatment success. This threshold was estimated to be the maximum cost for the treatment of a severe perioperative ophthalmological complication using the reimbursement tariffs list provided by the French National Healthcare System. We anticipated a mean incremental cost of 312€ and a success rate of 75% with PCS and 82 % with FLACS. Based on the method proposed by Briggs et al., with a common standard deviation of costs of 100€, a 80% power and a 5% risk alpha, 1053 patients had to be included in the trial¹⁸. We anticipated that 90% of the patients would present a bilateral cataract, yielding 2000 eyes included in the trial.

With 2000 eyes included in the primary clinical endpoint analysis, we had power to distinguish a 6-point difference in the success rate between both groups with an anticipated 75% rate of success in the PCS group, a 90% power and a 5% risk alpha (Chi-square test). We did not have to deal with multiple tests as cost-effectiveness analyses do not require statistical tests¹⁹.

Efficacy and safety analyses were conducted at the level of the eyes. As the randomisation was implemented at the individual level, statistical comparisons used mixed logistic regression models, or mixed linear regression models. All analyses were adjusted on centres and on bilateral/unilateral cataract surgery. The analyses were conducted in modified intention-to-treat (mITT). Patients for whom at least one major eligibility criterion was not met were excluded from the analysis. Exclusion decisions were taken by the scientific committee, blinded from surgical treatment allocation and from patient clinical examination during the follow-up. Missing data were replaced by failure. Analyses were also conducted on as-treated principle, and on available data. Maximum bias analyses were also conducted by replacing missing data by failure in a group and by success in the other, and vice-versa.

The cost-effectiveness analysis was conducted in mITT at the patient level as explained above. Missing data regarding treatment success were replaced by failure. Missing cost data were imputed using Fully Conditional Specification (FCSp, PROC-MI on SAS®) conditionally on gender, age, investigative centre, number of operated eyes and treatment success, and stratified on the randomisation group. Variability was assessed using bootstrap, stratified on the randomisation group and on randomisation stratas, with the same FCSp approach for each bootstrap iteration.²⁰ This bootstrap distribution of the incremental cost-effectiveness ratio (ICER) was used to estimate the cost-effectiveness probability of FLACS compared with PCS. A cost-effectiveness acceptability curve was elaborated by estimating

the cost-effectiveness probability for ceiling ICERs varying from 0 to 100 000€ per additional patient presenting a treatment success.

Statistical analysis was performed using SAS software version 9.3 (SAS Institute) and a p-value less than 0.05 was considered statistically significant.

An independent data monitoring committee oversaw the proper conduct of the study. The FEMCAT trial is registered at the US national institutes of health under the clinical trial identifier NCT01982006.

Role of the funding source

The study funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Between Oct 9, 2013 and Oct 30, 2015, 920 patients were enrolled for eligibility (Figure 1). Of the 909 randomised patients (n=1497 eyes), 455 (n=753 eyes) were allocated in the FLACS arm and 454 (n=752 eyes) in the PCS arm. 73 patients (73 eyes) allocated to the FLACS arm did not receive a femtosecond laser cataract surgery and 63 of them received a phacoemulsification procedure, 55.6% (n=35/63) of conversion were related to laser technical failures the day of surgery or before, 7.9% (n=5/63) had a poor pupil dilation the day of surgery and 12.7% (n=8/63) were related to inability for the patient to settle on the stretcher and under the laser optic lens or inability to place the suction ring.

Thirty-two eyes were excluded from the FLACS group, and 55 from the PCS group, yielding 704 FLACS eyes and 685 PCS eyes in the mITT analysis (Figure 1).

Demographic and ophthalmological baseline characteristics of the two treatment groups were similar in terms of age, sex distribution, preoperative visual acuity, absolute manifest refractive errors, cataract severity, ocular biometry measurements or IOL power implanted (Table 1). The mean age at surgery was 72.3±8.6 years and 62.3% (n=551) of participants underwent a bilateral cataract surgery. A wide range of cataract severity grade was included in the two groups as mentioned by nuclear opalescence and colour grades.

In the m-ITT analysis, the overall success rate was 42.3% (n=588) and was not statistically different between the two treatment groups with rates of 41.1% (n=289) and 43.6% (n=299) for FLACS and PCS groups respectively (Adjusted Odds-Ratio (OR): 0.85, 95% Confidence Interval (CI): 0.64-1.12, p=0.25) (Table 2). The overall success rate remained non significantly different between groups in the analysis performed on available data, in as-treated analysis, or in maximum bias analyses (Table S1).

Venn diagrams, elaborated on available data, illustrate the relationship between the four components of the composite primary outcome and their contribution in the overall success rate for FLACS (figure 2A) and PCS (figure 2B) groups. We observed a similar contribution of the four components outcome measures in the overall success rate of the two surgical techniques.

We included 108 patients in the microcosting analysis, 51 in the FLACS group and 57 in the PCS group. The mean costs of cataract surgery estimated through microcosting were 1119.7€ (162.2) and 565.5€ (61.4) for FLACS and PCS respectively.

The mean inpatient and outpatient costs obtained from the SNDS were 3418.6€ (1868.4) in patients treated with FLACS and 3667.5 (3775.1) in patients treated with PCS.

After multiple imputation of missing data and adding the microcosting between-group difference as a constant to the costs in the FLACS group, the total mean cost of care was

3975·5€ (95%CI: 3825·9;4151·2) in patients treated with FLACS and 3670·2€ (95%CI: 3360·3;4101·1) in patients treated with PCS. Details of costs are provided in table S3.

At the patient level, treatment success rate was 30·9% in the FLACS group and 33·7% in the PCS group. Hence, FLACS was more expensive (+305·3€ in average) and less effective (-2·8 percentage point), yielding an ICER of 10 703·2€ saved per additional patient presenting a treatment success with PCS compared with FLACS.

The bootstrap distribution of the ICER is shown in figure 3. 77·0% of the bootstrapped ICER are located in the upper-left quadrant of the cost-effectiveness graph. At a ceiling ICER of 16 750€ per additional patient with a treatment success, the cost-effectiveness probability of FLACS compared with PCS was 6·7%. For ceiling cost-effectiveness ratios ranging from 0 to 100 000€ per additional patient with a treatment success, the probability that FLACS is cost-effective compared with PCS never exceeds 15·7% (figure S3). Sensitivity analyses are presented in table S4. In all these analyses FLACS was more expensive and less effective than PCS.

In the secondary outcomes analysis, the overall rate of surgery without complication up to month-3 visit was 94·2% and was not significantly different between the two groups (OR: 1·17, 95%CI: 0·26-5·36, p=0·84) (Table 2). 80·2% of the eyes had a BCVA of 0·0 LogMAR or better and 81·4% exhibited an absolute manifest refractive error \leq 0·75 dioptres with OR of 0·74 (95%CI: 0·28-1·96, p=0·541) and 0·78 (95%CI: 0·32-1·9, p=0·583) respectively. Finally, the lowest success rates were observed for postoperative changes in corneal astigmatism with values of 56·7% in the FLACS group and 58·4% in the PCS group (OR: 0·89, 95%CI: 0·66-1·19, p=0·429).

In the FLACS group, suction loss during the installation phase occurred in 12·9% (n=79) of procedures and laser procedure could not be performed in 9 eyes (1·5%), which underwent a conventional phacoemulsification procedure (Table 3). Laser anterior capsulotomy

abnormalities and incomplete laser corneal incision were reported in 6 eyes (1.0%) and 42 eyes (6.8%) respectively. We did not observe any cases of collateral damage such as iris trauma or PCR on surrounding ocular structures during laser procedure. None of them resulted in an intraoperative complication during the phacoemulsification phase or postoperatively up to month-3 visit.

During the phacoemulsification phase we did not observe any significant difference in the frequency of PCR, crystalline lens or IOL luxation, vitreous loss or zonular dialysis between the two groups. Postoperatively, CME was the most frequent complication and we did not observe any endophthalmitis cases.

BCVA and UCVA significantly improved between preoperative measurements and month-1 or month-3 visits without significant difference between groups. When analysing postoperative absolute manifest refraction errors at different thresholds, 55.6% of the eyes were within 0.25 dioptres and 7.6% were outside 1 dioptre of the intended refractive outcome. There was no significant difference for any of the absolute manifest refraction error thresholds between the 2 groups.

Other surgical and clinical outcomes, and learning curve effect are provided in tables S5 and S6. We did not observe any difference of central corneal thickness or corneal endothelial cell count between the two groups. We did not observe any difference of success rate between FLACS and PCS either for the subgroup of the first 20 eyes or in the subgroup of the following eyes operated on by each surgeon.

DISCUSSION

Our study did not show any significant differences of visual, refractive, corneal astigmatism changes or anatomical outcomes between femtosecond laser and phacoemulsification techniques for cataract surgery. We also showed that nearly 10% of FLACS procedures could

not be performed because of technical problems. The weight of each of the four outcome measures in the overall success rate was also similar between the two surgical techniques demonstrating the absence of clinical benefits of femtosecond laser technology in cataract surgery for patients in our trial. Additionally, FLACS was more expensive and less effective in our cost-effectiveness analysis.

The overall complication rate and visual or refractive results were consistent with data from published cataract surgery nationwide register and large cohort studies.^{3, 4, 6} We did not observe any severe adverse events during femtosecond laser procedure and most of complications of the FLACS group occurred during the phacoemulsification phase or postoperatively. While we did not observe any conversion from PCS to FLACS, we observed a high rate of conversion from FLACS to PCS mainly related to either technical failure or inability for the patient to settle under the laser. Anterior capsule tear and PCR are usually the most frequent intraoperative complications and remain an important concern in cataract surgery.^{21, 22} Indeed, these complications can lead to worse postoperative visual and anatomical outcomes including vitreous loss and subluxated or luxated lens, and subsequent higher risk of retinal detachment or postoperative CME. In our study, the incidence of anterior capsule tear was not significantly different between the two groups and none of these cases resulted in additional complication. Despite the technical performances of FLACS in achieving precise and complete capsulotomy, the laser beam can be impaired by corneal folds or cavitation bubbles and can result in an incomplete capsulotomy.²³ In the as-treated analysis, the PCR rate was also not significantly different between the two groups and our results confirm meta-analyses findings.^{14, 15} FLACS may result in comparable or increased surgical manoeuvres in the eye.²⁴ In our study, while FLACS decreased ultrasound time and energy, aspiration time increased and surgical time was longer than in PCS group. These findings might explain the similar risk of surgically induced PCR in the two groups.

Postoperative CME is another common sight-threatening complication with rates ranging from 1.17% to 2.35% in the largest studies.^{25, 26} We observed a higher incidence of clinically significant CME than that observed in the literature. This could be related to the independent adjudication committee who validated all adverse events of our trial. The three experts identified CME using perioperative data, BCVA and macular OCT parameters. The OCT interpretation may have led to an overestimation of CME cases as compared with the methodology commonly described in the literature. Schultz et al. also showed femtosecond laser releases more prostaglandin in the anterior chamber than phacoemulsification.²⁷ This might increase the risk of CME since this complication is in part mediated by prostaglandins.²⁸ We did not observe such an increase compared to PCS in our trial.

Our refractive results are also in accordance with large studies and the European registry report showing that 72.7% and 93.0% of the eyes are within 0.5 dioptres and 1 dioptre respectively.^{3, 6} In our study, despite comparable complication rates and BCVA results between the two groups, FLACS did not achieve a lower mean absolute manifest refractive error than PCS whatever the absolute manifest refractive error thresholds we tested. As all eyes included in the study were scheduled to receive the same standard IOL, biomaterial properties and optical performance of the IOL could not have biased the comparison between the two groups. Although, laser capsulotomy could improve IOL centration or tilt within the capsular bag by providing a complete overlap of the IOL edge by the anterior capsule, it is unlikely this technique improves reproducibility of postoperative anterior chamber depth and IOL axial positioning.^{10, 12} Indeed, Norrby et al. demonstrated that the main cause of postoperative refractive error is related to the preoperative estimation of the postoperative IOL axial position.²⁹

To our knowledge, no such cost-effectiveness analysis of FLACS compared to PCS within a clinical trial has been published so far. We demonstrated in a large RCT using a direct

estimation of the costs of cataract surgery and a large claim database that FLACS was more expensive and less effective than PCS even through all our sensitivity analyses. A FLACS cost lower than the one we used in our analyses would reduce the ICER but still would not make FLACS a cost-effective procedure in our trial. Thus, our results confirm Abell et al. findings that FLACS was not cost-effective using a decision tree model performed on a hypothetical cohort of patients undergoing a cataract surgery.³⁰

We believe that the FEMCAT trial provides a high level of evidence about the comparison of FLACS and PCS and that our results are generalisable to cataract surgery practice. Our population sample had a comparable mean age and sex distribution to the population of cataract surgery in the French national register (<https://www.atih.sante.fr>) and we also included a wide range of cataract severity grade. The FEMCAT trial was designed to minimize the risk of bias commonly observed in studies comparing the two surgical techniques. Indeed, quality of current evidence is impaired by risks of performance, detection or selection bias. Furthermore, most RCTs carried-out within-person comparisons or included only one eye per patient.¹⁴ This raises concerns about generalisability of these findings in clinical practice and the real clinical benefit of the new surgical technique for patients. In the FEMCAT trial, the patient was the unit of randomisation and we performed a sham laser procedure to ensure a single blinding. As surgeons were not blinded, we also designated an independent adjudication committee to increase objectivity in safety evaluation. Moreover, we maintained the between-group comparability by standardising all the associated procedures, such as IOL characteristics or phacoemulsification machines. Finally, as visual outcomes are as important as refractive or anatomical outcomes for the success of cataract surgery, we also chose to give an equivalent weight to these outcomes by using a composite criterion as a primary outcome and then compare separately these four outcomes between the two techniques. As PCS is known to be very effective and reproducible, this methodology

should be sensitive in comparing the two techniques and detect the superiority of FLACS over PCS if it really exists. A potential limitation of our trial is that we did not reach the expected sample size. Although this had no impact on the validity of our estimates, it reduces the power of our comparison tests. However, the observed difference on the primary outcome between FLACS and PCS was far lower than the hypothesis on which our sample size calculation was based, and the observed differences on all outcomes were in favour of PCS. Another potential limitation could be the learning curve effect associated to the innovative technology for surgeons experienced in cataract surgery. Although this may have influenced our results, we organised a standardised certification process for all surgeons whatever their experience in ophthalmic surgery, and the complication rate during laser procedure was low and did not result in specific perioperative complications. Additionally, we did not find any difference of success rates between the first twenty eyes and the following eyes operated on by each surgeon. Hence, we assumed the learning curve effect associated with FLACS was limited and did not significantly influence the main results of our trial. Finally, although we did not observe any differences of clinical outcomes between the two surgical procedures at any follow-up visits, we could not exclude a difference in patient-reported outcomes particularly up to month-1 visit.

In conclusion, the FEMCAT trial is the largest RCT comparing the two technologies and provides useful information for patients, health-care providers and decision-makers before considering the implementation of this new and costly femtosecond laser technology in routine practice for cataract surgery. Our results show that FLACS fails to provide an additional clinical benefit for patients as compared to PCS and was also not a cost-effective strategy for the French Healthcare system. Despite the high level of technical performances associated with FLACS, this technology does not address specific issues and unmet needs to

improve anatomical, visual and refractive outcomes in cataract surgery or its increasing economical burden in population with longer life expectancy.

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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 ($VA \leq 0.3$ 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 μ L) and/or VF samples from tap (200–300 μ L) or vitrectomy (500 μ L) 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 IVI whereas culture before IVI 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 IVI 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 administered 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*

Enrollment

Assessed for eligibility: n = 920 patients

- Patients non included: n = 11
- Not meeting inclusion criteria (n = 3)
 - Declined to participate (n = 7)
 - Withdrawal of informed consent (n = 1)

Allocation

Randomised: n = 909 patients
N = 1480 eyes

- Withdrawal of informed consent before surgery: n = 2 patients
- Allocated to femtosecond laser (N=2 eyes)
 - Allocated to phacoemulsification (N=2 eyes)

Randomised excluding 2 withdrawal of informed consent: n = 907 patients
N = 1476 eyes

Allocated to **femtosecond laser cataract surgery (FCS)**
n = 454 patients
(N = 736 eyes)

- Received allocated surgery: N = 663 eyes
- Did not receive allocated surgery: N = 73 eyes
- Received phacoemulsification: N = 63 eyes
- N = 35 eyes laser not working the day of surgery or the day before
- N = 5 eyes with poor pupil dilation the day of surgery
- N = 8 eyes physical impossibility of the patient to settle under the laser or inability to place the suction ring
- N = 15 eyes other reason
- Did not receive any surgery: n = 10 eyes
- n = 1 patient lost to follow-up (N = 1 eye)
- n = 2 patient dropout (N = 4 eyes)
- n = 1 patient not meeting inclusion criteria (N = 1 eye)
- n = 1 patient file lost (N = 2 eyes)
- n = 1 patient dead (N = 2 eyes)

Allocated to **phacoemulsification cataract surgery (PCS)**
n = 453 patients
(N = 740 eyes)

- Received allocated surgery: N = 732 eyes
- Did not receive allocated surgery: N = 8 eyes
- Received femtosecond laser: N = 0
- Did not receive any surgery: N = 8 eyes
- n = 4 patients *dropout* (N = 7 eyes)
- n = 1 patient not meeting inclusion criteria (N = 1 eye)

Follow-Up

- Complete follow-up : n = 405 patients
- Dropout : n = 22 patients
- Death : n = 3 patients
- Lost to follow-up : n = 23 patients
- Other reason : n = 1 patient

- Complete follow-up : n = 408 patients
- Dropout : n = 21 patients
- Death : n = 3 patients
- Lost to follow-up : n = 19 patients
- Other reason : n = 2

Analysis

n = 870 patients,
N = 1389 eyes

- Analysed: n = 440 patients (N = 704 eyes)
- Excluded from analysis: n = 14 patients (N = 32 eyes)
 - Primary outcome available : N = 582 eyes

- Analysed: n = 430 patients (N = 685 eyes)
- Excluded from analysis: n = 23 patients (N = 55 eyes)
 - Primary outcome available : N = 581 eyes

A

BCVA = 0.0 LogMAR

0.2%

0.7%

Absolute refractive error \leq 0.75D

0.7%

1.1%

1.1%

2%

0.7%

17.7%

1.5%

53.7%

3.9%

6.3%

8.2%

1.9%

Change in corneal astigmatism at 3 months \leq D AND change in axis of corneal astigmatism \leq 20°

OutsideUnion - 0.2%

No intraoperative or postoperative complications within 3 months of follow-up

B

BCVA = 0.0 LogMAR

0.6%

0.9%

Absolute refractive error \leq 0.75D

0.6%

0.6%

0.7%

1.9%

1.7%

18.8%

0.9%

55.6%

2.8%

6.1%

7.6%

0.7%

No intraoperative or postoperative complications within 3 months of follow-up

Change in corneal astigmatism at 3 months \leq 0.5 D AND change in axis of corneal astigmatism \leq 20°

OutsideUnion - 0%

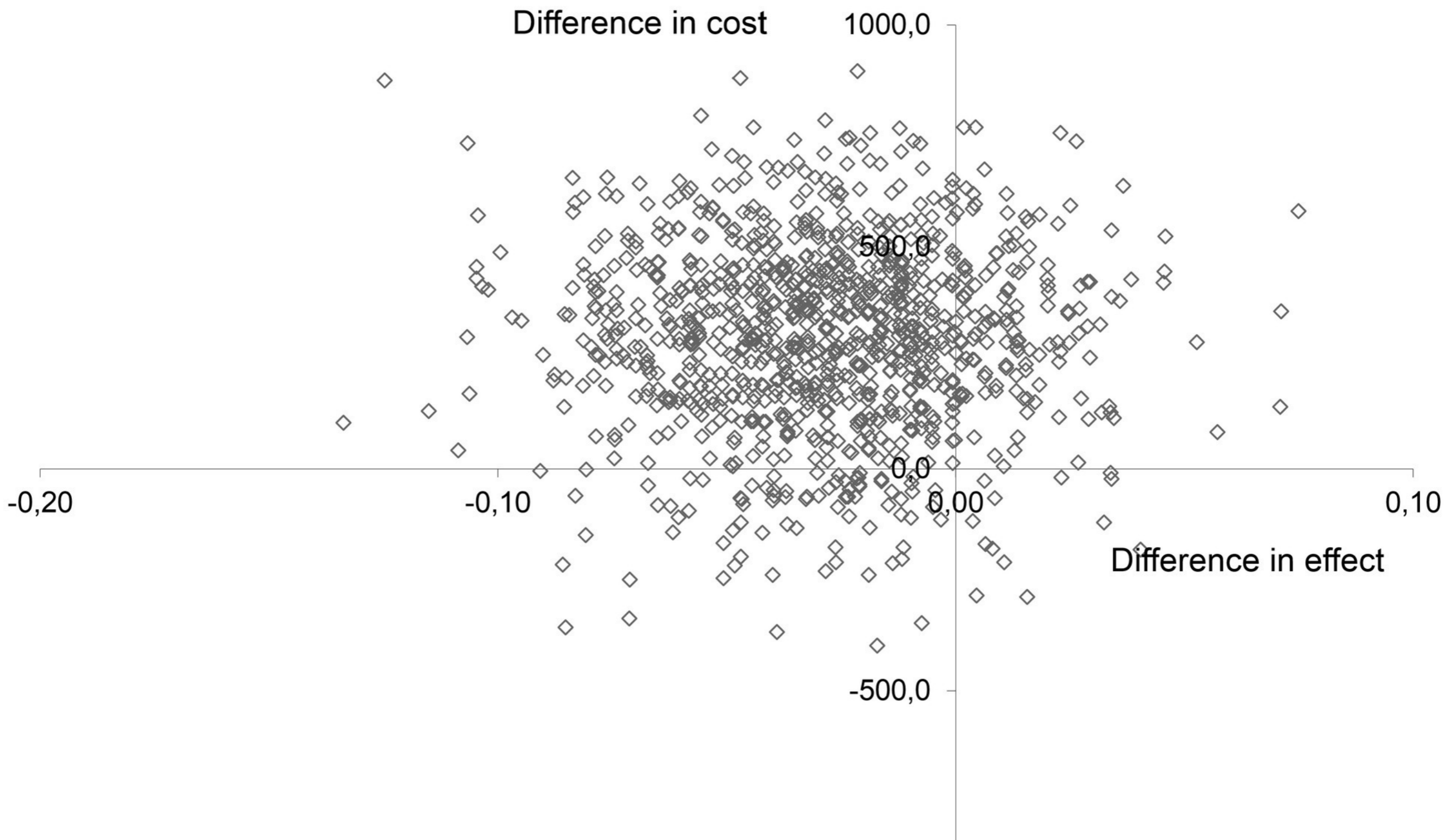


Table 1. Baseline characteristics of the patients (SD: Standard Deviation, IOL: IntraOcular Lens, UCDVA: UnCorrected Distance Visual Acuity, BCDVA: Best Corrected Distance Visual Acuity, LogMAR: Logarithm of the Minimum Angle of Resolution, LOCS: Lens Opacities Classification System, NO: Nucleus Opacities, NC: Nucleus Colour, C: Cortical opacities, P: Polar opacities, Q1: First quartile, Q3: Third quartile, CCT: Central Corneal Thickness)

Characteristics	Total	FLACS group	PCS group
Total	870 (100%)	440 (100%)	430 (100%)
Bordeaux University Hospital	256 (29.4%)	129 (29.3%)	127 (29.5%)
Brest University Hospital	95 (10.9%)	47 (10.7%)	48 (11.2%)
Lyon Croix-Rousse University Hospital	130 (14.9%)	67 (15.2%)	63 (14.7%)
Paris Cochin AP-HP University Hospital	167 (19.2%)	83 (18.9%)	84 (19.5%)
Tours University Hospital	222 (25.5%)	114 (25.9%)	108 (25.1%)
Patients by Centres - n (%)			
Mean age at surgery - years (SD) (min-Q1-med-Q3-max)	72.3 (8.6) (25-67-73-79-93)	72.4 (8.6) (37-67-73-79-92)	72.1 (8.7) (25-67-73-78-93)
Sex-female subjects - n (%)	543 (62.4%)	272 (61.8%)	271 (63%)
Rate of bilateral cataract surgery - n (%)	551 (63.3%)	276 (62.7%)	275 (64.0%)
Rate of right eyes - n (%)	695 (50%)	354 (50.3%)	341 (49.8%)
Mean preoperative UCDVA - LogMAR (SD)	0.66 (0.45)	0.64 (0.44)	0.69 (0.46)
Mean preoperative BCDVA - LogMAR (SD)	0.24 (0.25)	0.24 (0.24)	0.25 (0.26)
Mean preoperative absolute manifest refractive error -	2.2 (2.1)	2.1 (2.1)	2.2 (2.0)

Dioptres (SD) (min-max)		(0-16)	(0-16)	(0-16)
LOCS classification mean values (SD) (min-Q1-med-Q3-max)	NO	3.3 (1.2) (0.0-2.6-3.1-4.0-6.9)	3.4 (1.3) (0.0-2.6-3.1-4.1-6.9)	3.3 (1.2) (0.5-2.5-3.2-4.0-6.9)
	NC	3.4 (1.2) (0.5-2.8-3.2-4.1-6.9)	3.5 (1.2) (0.5-2.7-3.2-4.2-6.9)	3.4 (1.2) (0.5-2.8-3.2-4.0-6.9)
	C	2.3 (1.5) (0.0-1.0-2.1-3.4-5.9)	2.4 (1.5) (0.0-1.0-2.2-3.6-5.9)	2.3 (1.4) (0.0-1.0-2.0-3.2-5.9)
	P	1.4 (1.3) (0.0-0.5-1.0-2.0-6.0)	1.4 (1.3) (0.0-0.4-1.0-2.0-6.0)	1.5 (1.4) (0.0-0.5-1.0-2.0-5.9)
Mean Axial length - mm (SD) (min-max)		23.60 (1.14) (20.92-27.82)	23.62 (1.17) (20.98-27.82)	23.58 (1.12) (20.92-27.55)
Mean Anterior Chamber depth – mm (SD) (min-max)		3.10 (0.40) (1.78-4.93)	3.09 (0.41) (1.78-4.93)	3.11 (0.40) (1.95-4.14)
Mean Preoperative Keratometry - Dioptres (SD)	Mean keratometry	44.4 (2.7)	44.2 (2.5)	44.5 (2.8)
	Flat keratometry	43.8 (2.5)	43.8 (2.1)	43.9 (2.9)
	Steep keratometry	44.4 (2.6)	44.2 (2.5)	44.5 (2.7)
	Cylinder absolute power	0.7 (0.4)	0.7 (0.4)	0.7 (0.4)
Mean preoperative CCT – microns (SD)		539.3 (34.0)	539.6 (33.4)	539.0 (34.7)
Mean preoperative endothelial cell count (SD)		2547.8 (407.5)	2569.6 (405.2)	2526.2 (409.0)
Mean IOL Power implanted - Dioptres (SD)		21.1 (3.2)	21.0 (3.1)	21.1 (3.3)

Table 2. Primary and secondary efficacy and safety analyses between the 2 groups in modified Intention-To-Treat and As-Treated populations. (BCDVA: Best Corrected Distance Visual Acuity, D: Dioptre, LogMAR: Logarithm of the Minimum Angle of Resolution, CI: Confidence Interval)

Success rates at month 3 visit (V3)	Modified Intention-To-Treat Analysis*					As-Treated Analysis				
	Total	FLACS group	PCS group	Odds-Ratios** (95% CI)	P value	Total	FLACS group	PCS group	Odds-Ratios** (95% CI)	P value
Overall success rate - n (%)	588 (42.3%)	289 (41.1%)	299 (43.6%)	0.85 (0.64-1.12)	0.250	588 (50.6%)	268 (50.0%)	320 (51.0%)	0.88 (0.66-1.17)	0.369
Overall rate of uncomplicated surgery - n (%)	1308 (94.2%)	664 (94.3%)	644 (94.0%)	1.17 (0.26-5.36)	0.840	1292 (94.1%)	597 (94.0%)	695 (94.2%)	1.02 (0.24-4.24)	0.980
Overall rate of BCDVA at 0.0 LogMAR - n (%)	1114 (80.2%)	554 (78.7%)	560 (81.8%)	0.74 (0.28-1.96)	0.541	1114 (84.5%)	504 (83.0%)	610 (85.7%)	0.71 (0.25-1.99)	0.514
Overall rate of absolute manifest refractive error $\leq 0.75D$ - n (%)	1131 (81.4%)	564 (80.1%)	567 (82.8%)	0.78 (0.32-1.9)	0.583	1131 (86.2%)	519 (86.1%)	612 (86.3%)	0.99 (0.36-2.71)	0.986
Overall rate of postoperative change in corneal astigmatism power $\leq 0.50D$ and change in corneal astigmatism axis $\leq 20^\circ$ - n (%)	799 (57.5%)	399 (56.7%)	400 (58.4%)	0.89 (0.66-1.19)	0.429	799 (73.7%)	366 (72.9%)	433 (74.4%)	0.86 (0.62-1.17)	0.334

Results in bold are statistically significant ($p < 0.05$)

*Analysis performed with missing data considered as failure

** Logistic Regression Model with patient random effect adjusted on centres

Table 3. Comparison of intraoperative and postoperative complications up to month-3 visit (V3) included and comparison of visual and refractive outcomes between Femtosecond Laser-Assisted Cataract Surgery (FLACS) group and Phacoemulsification Cataract Surgery (PCS) group (IOL: IntraOcular lens, UCDVA: Uncorrected Distance Visual Acuity, BCDVA: Best Corrected Visual Acuity, LogMAR: Logarithm of the Minimum Angle of Resolution, SD: Standard Deviation)

Intraoperative and postoperative complications up to month-3 visit (V3)		Total	FLACS group	PCS group
Intraoperative complications during laser procedure - n (%)	Suction loss during installation phase ^a	79 (12.9%)	79 (12.9%)	-
	Suction loss during laser procedure	17 (2.8%)	17 (2.8%)	-
	Incomplete Laser procedure	61 (9.9%)	61 (9.9%)	-
	Laser capsulotomy abnormalities (tears, tags, incomplete opening requiring a manual forceps)	6 (1.0%)	6 (1.0%)	-
	Non perforated laser corneal incisions requiring manual corneal incisions	42 (6.8%)	42 (6.8%)	-
	Posterior capsule rupture+/- subluxated lens	0	0	-
	Laser induced iris trauma	0	0	-
Intraoperative complications during phacoemulsification phase - n (%)	Manual anterior capsulotomy (tears, tags, incomplete)	2 (0.3%)	-	2 (0.3%)
	Posterior capsule rupture	21 (1.5%)	10 (1.4%)	11 (1.6%)
	Vitreous loss/ anterior vitrectomy	17 (1.2%)	10 (1.4%)	7 (1.0%)
	Subluxated or luxated crystalline lens or IOL	3 (0.2%)	1 (0.1%)	2 (0.3%)
	Corneal incision leakage requiring a suture	37 (2.9%)	23 (3.7%)	14 (2.1%)
	Zonular dialysis	8 (0.6%)	4 (0.6%)	4 (0.6%)

	IOL implantation outside the capsular bag or no implantation	15 (1.1%)	8 (1.1%)	7 (1.0%)
Postoperative complications up to month-3 visit (V3) included - n (%)	Cystoid macular oedema ^b	48 (3.5%)	21 (3.0%)	27 (3.9%)
	Persistent corneal oedema ^c	7 (0.5%)	6 (0.9%)	1 (0.1%)
	Endophthalmitis	0	0	0
	Retinal tears or retinal detachment	1 (0.1%)	1 (0.1%)	0
	Subluxated or luxated IOL	0	0	0
Visual outcomes at screening and month-3 (V3) visits				
Mean UCDVA - LogMAR (SD)	Preoperative	0.66 (0.45)	0.64 (0.44)	0.69 (0.46)
	Postoperative (V3)	0.13 (0.19)	0.14 (0.19)	0.13 (0.18)
	Improvement (V3-preoperative)	-0.53 (0.44)	-0.50 (0.42)	-0.57 (0.45)
Mean BCDVA - LogMAR (SD)	Preoperative	0.24 (0.25)	0.24 (0.24)	0.25 (0.26)
	Postoperative (V3)	0.02 (0.07)	0.02 (0.08)	0.02 (0.05)
	Improvement (V3-preoperative)	-0.22 (0.25)	-0.21 (0.24)	-0.23 (0.26)
Refractive outcomes at screening and month-3 (V3) visits				
Mean Absolute error of manifest refraction spherical equivalent – Dioptre (SD)	Preoperative	2.2 (2.1)	2.1 (2.1)	2.2 (2.0)
	Postoperative (V3)	0.4 (0.9)	0.5 (1.1)	0.4 (0.5)
	Improvement (V3-preoperative)	-1.8 (2.1)	-1.7 (2.3)	-1.8 (2.0)
Absolute error of manifest refraction spherical equivalent - n (%)	≤0.25D	730 (55.6%)	357 (54.2%)	373 (57.1%)
]0.25-0.5D]	274 (20.9%)	138 (20.9%)	136 (20.8%)
]0.5-0.75D]	127 (9.7%)	69 (10.5%)	58 (8.9%)
]0.75-1D]	81 (6.2%)	39 (5.9%)	42 (6.4%)
	> 1D	100 (7.6%)	56 (8.5%)	44 (6.7%)

^aBefore the activation of the femtosecond laser beam

^bDefined as the presence of a postoperative cystoid macular oedema using optical coherence tomography with a thickening of central macular thickness higher than 10 microns and cystic areas of low reflectivity within the outer plexiform layer of the retina (not documented preoperatively)

and without any preoperative macular abnormalities) and a decrease in visual acuity without other ocular abnormalities within 90 days of surgery. Adverse event classified as Cystoid macular oedema by the independent adjudication committee.

^cDefined as a persistent postoperative thickening of central cornea higher than 20 microns between the screening visit and month-3 visit using optical coherence tomography without other corneal abnormalities within 90 days of surgery. Adverse event classified as persistent corneal oedema by the independent adjudication committee.