Endophthalmitis

Epidemiology and management of patients at
Oslo University Hospital from 2015 to 2021

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“We do not inherit the earth from our ancestors; we borrow it from our children.” - Unknown
2 Abbreviations

Anti-VEGF  Anti-vascular endothelial growth factor
BCVA      Best-corrected visual acuity
EO        Endophthalmitis
HSE       Norway’s South-Eastern health region
LogMAR    Logarithm of the minimum angle of resolution
nAMD      Neovascular age-related macular degeneration
OUH       Oslo University Hospital
PAIEV     Primary antibiotic injection followed by early vitrectomy
PCE       Post-cataract endophthalmitis
PCR       Polymerase chain reaction
PIE        Post-injection endophthalmitis
PV        Primary vitrectomy
PVE        Post-vitrectomy endophthalmitis
RCT       Randomized controlled trial
TASS      Toxic anterior segment syndrome
3 List of papers

Paper I.

Paper II.

Paper III.
Kathrine Blom, Ragnheiður Bragadóttir, Magne Sand Sivertsen, Morten Carstens Moe, Øystein Kalsnes Jørstad. Mask use by patients in the context of COVID-19 can increase the risk of postinjection endophthalmitis.
Kathrine Blom, Øystein Kalsnes Jørstad, Rowan Faber, Ingar Stene-Johansen, Mona Holberg-Petersen, Nils Olav Hermansen, Ragnheiður Bragadóttir. Primary vitrectomy or intravitreal antibiotics followed by early vitrectomy for acute endophthalmitis: A prospective observational study.

4 Thesis summary

4.1 Norsk sammendrag


Prosjektet førte til formalisering av et permanent endoftalmitt-register som gir oss en verdifull oversikt over våre endoftalmitt-tilfeller. Vi dokumenterte endoftalmitt etter kataraktkirurgi som den vanligste årsaken (43 %) i 2015 og 2016, etterfulgt av traumer, intravitreale injeksjoner og endogen spredning. To-års oddsradio for endoftalmitt etter kataraktkirurgi sammenlignet med etter intravitreale injeksjoner var høyere (p = <0.001). Mens kataraktkirurgi ofte utføres utenfor vår øyeavdeling (avtalespesialist, private klinikker), utføres intravitreale injeksjoner primært på Oslo Universitetssykehus. Da vi sammenlignet to-års oddsratio for endoftalmitt etter kataraktkirurgi med
Endoftalmitt etter intravitreale injeksjoner utført på Oslo Universitetssykehus med eksterne utøvere fant vi ingen forskjell ($p = 0.14$ for kataraktkirurgi og $p = 0.21$ for intravitreale injeksjoner). For første gang har vi dokumentert at endoftalmitt etter intravitreale injeksjoner har forbigått endoftalmitt etter kataraktoperasjon, og utgjør den vanligste årsaken til endoftalmitt ved Oslo Universitetssykehus (46%) fra 2019. Vi fant to tilfeller av streptokokker blant 35 positive mikrobiologiske prøver i 2015 og 2016 (begge var endogene, dvs. ikke relatert til inngrep), da endoftalmitt etter katarakt kirurgi fortsatt var vanligste årsak. Derimot fant vi ti tilfeller av streptokokker blant 30 positive mikrobiologiske prøver i perioden 2019 til mai 2021, da intravitreale injeksjoner hadde forbigått katarakt kirurgi som vanligste årsak til endoftalmitt. Empirisk er det dårligere prognose ved streptokokker enn de typiske stafylokokkbakteriene ved endoftalmitt etter katarakt kirurgi. Dette understreker viktigheten av overvåkning i et register.

Den jevne økningen i intravitreale injeksjoner utfordrer kapasiteten ved øyeavdelingene. Medisiner til intravitrealt bruk er meget kostbare og leveres i hetteglass. Selv om hetteglassene er påtenkt en enkelt injeksjon, inneholder de et overskudd av medikament. Deling av medikament fra hetteglass på flere injeksjonssprøyter er vanlig praksis, men kontaminering i relasjon til delingen er en reell fare og kan føre til alvorlige klynger av endoftalmitt. For å forbedre og sikre delingsprosedyren overførte Oslo Universitetssykehus denne oppgaven til sykehusapoteket. Intensjonen var at forhåndsfulde sprøyter for intravitrealt bruk ville både spare klinikertid, holde medikamentkostnader nede og gi bedre hygienisk standard. Vi evaluerte den første femårsperioden (2015-2019), da totalt 112 926 intravitreale injeksjoner (summen av klinikertilberedte sprøyter og ferdigfylte sprøyter fra sykehusapoteket) ble gitt under ellers identiske forhold. Vi fant ingen endring i risiko for endoftalmitt ($p = 0.32$). Dette støtter vår hypotese om at deling av medikament fra hetteglass til ferdigfylte sprøyter ved sykehusapoteket er trygt, så lenge korrekt farmasøytisk tilberedningsprosedyre
følges. Vår praksis gir effektiv injeksjonsbehandling og store kostnadsfordeler uten å øke risikoen for endoftalmitt, og har etter hvert blitt standard i Norge.


I 2019 ble et nytt regionalt behandlingstilbud for endoftalmitt innført i regi av Vitreoretinal Seksjon på Oslo Universitetssykehus. Ønsket var å sikre tidlig vitrektomi (antatt beste moderne behandling ifølge guidelines for forebygging og behandling av endoftalmitt publisert av European Society of Cataract and Refractive Surgeons) til alle pasienter med akutt endoftalmitt i Helse Sør-Øst. Der vitreoretinal kirurgi ikke var umiddelbart tilgjengelig (gjelder særlig lokale øyeavdelinger), ble aktuell øyeavdeling oppfordret til å umiddelbart injisere empirisk intravitreal antibiotika og deretter henvise pasienten til Oslo Universitetssykehus for tidlig vitrektomi. Vår prospektive observasjonsstudie av den første 18-månedersperioden fant ingen forskjell i synsutfall eller komplikasjoner mellom øyne behandlet med primær vitrektomi og primær intravitreal antibiotika etterfulgt av tidlig vitrektomi. Videre viste studien at implementering av PCR som rutineanalyse ved endoftalmitt (i tillegg til konvensjonell dyrkning) økte det diagnostiske utbyttet med 39 % (p = 0.02), uten signifikante forskjeller mellom prøver tatt før og etter intravitreal antibiotika.
andre ord viste vi at intravitreal antibiotika kan injiseres umiddelbart (uten tidskrevende og kirurgisk krevende prøvetakning fra øyet), uten å forringe mikrobiologisk diagnose. Dette forbedrer den kritiske behandlingstiden (tid til injeksjon av antibiotika) for endoftalmitt, i tillegg til å gi den nødvendige fleksibiliteten for å kunne tilby vitrektomi til alle endoftalmitt-tilfeller i Helse Sør-Øst.


4.2 English summary

Endophthalmitis is an inflammatory condition of the inside of the eye usually caused by an infection. Infectious endophthalmitis is a public health concern due to its potential to cause blindness, or even loss of the eye itself, and accompanied long-term morbidity. Most commonly, endophthalmitis occurs secondary to intraocular procedures, which supply a potential entry point for pathogens. Since endophthalmitis is closely related to surgery, it serves as an important quality indicator for ophthalmic surgery, including the two most frequently performed procedures: cataract surgery and intravitreal injection of drugs.
Although the Department of Ophthalmology at Oslo University Hospital performs a large amount of intraocular procedures each year, endophthalmitis cases have priorly not been systematically registred or analyzed, and it was crucial to establish a quality registry. Through this registry, we aimed to scrutinize all endophthalmitis cases admitted to the Department of Ophthalmology at Oslo University Hospital. We specifically looked at the epidemiology, treatment and outcome of each endophthalmitis case. Further, we explored whether there were associations between the endophthalmitis cases and alterations in our clinical practice over time.

Our project lead to the formalization of a permanent endophthalmitis registry and provided a valuable overview of our endophthalmitis cases. We documented post-cataract endophthalmitis to be the most common cause (43%) in 2015 and 2016, followed by trauma, post-injection, and endogenous endophthamitis. The two-year odds ratio for post-cataract endophthalmitis compared to post-injection endophthalmitis was found to be higher (p = <0.001). While cataract surgery is commonly performed outside of our department, intravitreal injections are primarily performed at Oslo University Hospital. When we compared the two-year odds ratio of post-cataract and post-injection endophthalmitis performed at Oslo University Hospital with other ophthalmological centers in the city of Oslo and Akershus County, we did not find any difference (p = 0.14 for post-cataract endophthalmitis and p = 0.21 for post-injection endophthalmitis).

In line with the increasing number of intravitreal injections, we documented post-injection endophthalmitis to supersede post-cataract endophthalmitis as the most common type of endophthalmitis at Oslo University Hospital (46%) from 2019. Moreover, we had two cases of streptococcal pathogens among 35 positive microbiological samples in 2015 and 2016 (both were endogenous, i.e., not related to surgery), when post-cataract endophthalmitis still was the most common type. On the other hand, we verify ten cases of streptococcal species among 30 positive
microbiological samples in 2019 through May 2021 when post-injection endophthalmitis had become the most common type of endophthalmitis. Empirically, streptococcal pathogens have a worse prognosis than the typical staphylococcal pathogens commonly seen with post-cataract endophthalmitis. This underscores the importance of careful monitoring.

The steady increase in intravitreal injections challenges the capacity of ocular health care services. The costly medical vials are intended for a single injection, but do contain an excess volume. Splitting of vials into several syringes is common practice. However, contamination in connection with splitting of vials can lead to devastating clusters of endophthalmitis. To improve and safeguard this practice, Oslo University Hospital transferred withdrawal and splitting of vials from the injection room to the hospital pharmacy, i.e., established pharmaceutical compounding of prefilled syringes for intravitreal use. Our intentions were that these prefilled syringes would save both clinician time and drug expenses and adhere to higher hygienic standards. We evaluated the five-year transition period (2015-2019) when a total of 112,926 injections (the sum of prefilled syringes and clinician-withdrawn syringes) were given under otherwise identical circumstances. Our study did not find an altered risk of post-injection endophthalmitis between these alternatives (p = 0.32). This supports our assumption that splitting of vials into prefilled syringes for intravitreal injections is safe, provided that an appropriate pharmaceutical compounding procedure is strictly followed. Importantly, pharmaceutical compounding also improves the cost-benefit of intravitreal injections and has now become the norm in Norway.

There are no broad updated consensus on endophthalmitis treatment. The cornerstone in management of endophthalmitis still relies on a randomized controlled trial published three decades ago: The Endophthalmitis Vitrectomy Study (1995). It concluded that vitrectomy only improved visual outcomes compared to intravitreal injection of antibiotics for eyes presenting with visual
function of light perception or worse. However, vitreoretinal surgery has since improved, but remains a centralized health care service in Norway and is not always achievable in an emergency setting. As a reflection of this, we documented intravitreal injection of antibiotics to be the most common treatment (54%) of cases admitted to Oslo University Hospital in 2015 and 2016.

In 2019, the Vitreoretinal Section at Oslo University Hospital took the initiative to offer a new treatment option for regional endophthalmitis cases. Our vision was to ensure early vitrectomy (“gold standard” treatment) to all patients with acute endophthalmitis in Norway’s South-Eastern health region. Local endophthalmitis cases that could not undergo immediate vitrectomy, due to lack of vitreoretinal availability, were encouraged to immediately inject empirical antibiotics intravitreally and then admit their endophthalmitis patients to Oslo University Hospital for early vitrectomy. Our prospective, comparative observational study in the following 18-month period found no differences in visual outcomes or complications between eyes treated by primary vitrectomy or primary antibiotic injection followed by early vitrectomy. Further, implementing PCR as a routine microbiological method in addition to conventional culture increased the diagnostic yield by 39% (p = 0.02), without significant differences between samples collected before or after intravitreal antibiotics. In other words, we showed that intravitreal antibiotics can be injected immediately (leaving out the traditional time-consuming and surgically demanding microbiological sampling) without sacrificing identification of the causative pathogen. This improves the critical treatment time and allows for flexibility in the timing of vitrectomy, making early vitrectomy accessible for all endophthalmitis cases in our region.

In conclusion, the studies present the epidemiology of endophthalmitis at the Department of Ophthalmology, Oslo University Hospital over time and support that our intraocular procedures have high standard in terms of
endophthalmitis risk. A notable exception was mask use by patients during the Covid-19 pandemic, which apparently increased the risk of post-injection endophthalmitis and was consequently abandoned. Our studies also support that endophthalmitis can be managed with either primary vitrectomy or primary intravitreal antibiotics followed by early vitrectomy, and that the combination of culture and PCR in connection with the vitrectomy increases the diagnostic yield, regardless of whether intravitreal antibiotics are injected before sampling.
5 Introduction

Endophthalmitis (EO) is an intraocular inflammatory condition usually caused by an infection.\textsuperscript{1} It represents one of the most feared ocular emergencies and is notorious for its rapid destructive progression, which can even lead to loss of the eye itself.\textsuperscript{2}

In general, the eye is divided into the anterior segment and the posterior segment. The gel-like structure that fills the main posterior part of the eye, is called the vitreous and serve as the main site of an EO. The vitreous is avascular and mainly consists of water (98-99%), in addition to collagen fibers and hyaluronic acid.\textsuperscript{3} A vital role of the vitreous is to maintain an oxygen gradient between the lens and the retina through ascorbic acid, which consumes oxygen, and the vitreous’s gel-like consistency, which limits convectional oxygen transport. A high oxygen level is maintained near the retina (e.g., high metabolic activity), while a low concentration is maintained near the lens (e.g., preventing oxidative damage).\textsuperscript{4} It is generally accepted that the vitreous is not regenerated throughout life, in contrast to the aqueous that fills the anterior segment, which is more liquid and continuously regenerated.\textsuperscript{3, 5} As a consequence, a smaller inoculum size (i.e., amount of pathogen) is needed to establish an EO via the more susceptible vitreous.\textsuperscript{6}

Moreover, the eye has immune privilege (i.e., it is isolated from the immune system), because of the blood-retina barrier (i.e., a physical barrier) and lack of direct lymphatic drainage (i.e., part of the immune system). Therefore, a pathogen will have favorable conditions for growth inside the eye.\textsuperscript{7-10} In EO there is initial exponential growth of bacteria with release of toxins and secondary inflammation (i.e., the inflammation is caused by the infection). These rapid processes lead to destruction of retinal cells and loss of vision within hours. If left untreated EO usually leads to blindness.\textsuperscript{11}
Sight is a critical factor for maintaining a good public health.\textsuperscript{12}\textsuperscript{13} The Ophthalmological Department at Oslo University Hospital (OUH) performs a large amount of surgical procedures each year. EO is the most feared complication to ocular surgery as it supplies a potential entry point for a pathogen.\textsuperscript{14}\textsuperscript{15} EO’s close relationship to ocular intervention makes it an important indicator for quality of treatment.\textsuperscript{16} Therefore, it is of paramount importance to have detailed knowledge on the risk factors of EO, as well as how to prevent, diagnose and manage this devastating disease: vigilance is key.\textsuperscript{17}

5.1 Etiology and risk factors for endophthalmitis

EO can be divided into exogenous (i.e., from an external source, such as a penetrating ocular injury or following ocular surgery) or endogenous (i.e., from an internal source, typically blood-borne spread from an infection elsewhere in the body). Further description can include causative pathogen (i.e., bacterial or fungal EO, while viral or parasitic infections are classified as uveitis).\textsuperscript{18}\textsuperscript{19}

Overall, exogenous EO secondary to ocular surgery is the most common form and can again be subdivided into acute or chronic where the separation goes by six weeks. The reported incidences of postoperative EO are relatively low, ranging from 0.03\% to 0.2\% for post cataract endophthalmitis (PCE) and 0.01\% to 0.08\% for post injection endophthalmitis (PIE).\textsuperscript{20}\textsuperscript{21} Still, EO is not an infrequent encounter, bearing in mind the high number of ocular surgeries performed yearly. In Norway, ophthalmologists performed 48 291 cataract surgeries in 2019 and 63 601 intravitreal injections in 2015.\textsuperscript{22}\textsuperscript{23} EO can also occur following corneal surgery, glaucoma filtering surgery, vitrectomy and, in principle, any other surgical procedure that penetrates the eye wall. The risk factors for contracting EO depends on the etiology of EO. In general, patient risk factors include local factors, such as blepharitis and nasolacrimal duct infections or obstructions, and systemic conditions, such as immunosuppression, diabetes
mellitus, and advanced age. In addition, risk factors for PCE include communication to the vitreous through a tear in the posterior capsule or vitreous loss, surgeon’s expertise, post-operative wound leak and properties of the intraocular lens implant (e.g., IOL with polypropylene haptics). Further, potential risk factors for PIE include spread of aerosolized droplets containing oral contaminants and inappropriate handling of the vials and syringes.

5.2 Diagnosis of endophthalmitis

Acute postoperative EO usually present within the first week of surgery, but rarely the first day. Patients commonly complain of decreased visual acuity and eye ache or pain. The diagnosis of EO is made clinically based on typical manifestations of red eye, hypopyon, intraocular inflammation including the posterior segment and retinal hemorrhages. Notably, with the increasing volume of intravitreal injections with inoculation of pathogen to the posterior segment, fewer patients presents with hypopyon compared to PCE with inoculation of pathogen is to the anterior segment. In our own experience, vitritis (i.e., inflammation of the vitreous) in combination with retinal hemorrhages should raise a high suspicion of EO in predisposed individuals. An ultrasound of the eye normally reveal increased echogenicity in the posterior segment. Blood samples are usually negative, and not routinely taken, except in the case of endogenous EO (i.e., where bacteremia will show corresponding positive blood cultures, increased leucocytes and so on).

Routinely, samples from the vitreous and/or aqueous are gathered and sent for microbiological analyses to aid in the diagnosis and treatment of EO. The term “proven EO” can be used when a positive microbiological result has been secured. While samples often identify a causative pathogen, approximately 20-30% of culture samples come back negative, leaving the diagnosis completely clinical.
The main differential diagnoses of EO are sterile inflammation and viral or parasitic uveitis. It is worth to notice the distinction from toxic anterior segment syndrome (TASS), which is a sterile toxic reaction to solutions, material, or equipment used during surgery. A large series found TASS to occur in 0.22% of cases.\textsuperscript{29} In contrast to EO, TASS normally presents within the first day of surgery, mainly affects the anterior segment and lacks vitritis.\textsuperscript{30} The incidence of endophthalmitis-like reactions after anti-VEGF ranges from 0.005%-4.4%. Such reactions are harder to distinguish from EO than TASS because of overlapping presentation. Suggesting factors of infectious EO are retinal hemorrhages, hyperemia, severe pain and hypopyon.\textsuperscript{31} In addition, sterile subclinical inflammatory reactions after intravitreal anti-VEGF can occur in up to every fifth patient.\textsuperscript{31}

In contemporary ophthalmology polymerase chain reaction (PCR) has become an important addition to conventional microbiological methods.\textsuperscript{32,33} PCR is a method for amplifying and detecting DNA using a thermocycle (i.e., repeated increases and decreases in temperature) of two to three steps. In the first step of a thermocycle an increase in temperature (to \(\approx95^\circ C\)) allows the double stranded DNA in the sample to separate. In the second step, a decrease in temperature (\(\approx60^\circ C\)) allows the specific primers to anneal with the single stranded DNA strands, if complementary. In the third step, a slight increase in temperature (\(\approx72^\circ C\)) allows a heat-stable DNA-polymerase (e.g., Taq-polymerase) to copy the original DNA. For each thermocycle the DNA is doubled and for a PCR the thermocycle is normally run 20-45 times. That leaves us with \(2^{20-45}\) copies of the original DNA (!). In this way very small amounts of DNA are transformed into large quantities and this unique feature makes PCR extremely sensitive. Unlike culture, PCR does not rely on the pathogen in the sample to be alive and several studies have shown its superior results compared to conventional culture in vitreous (and/or aqueous) samples.\textsuperscript{34,35,36} However, 16S PCR (i.e., the type of PCR
we use for bacterial detection in EO) is laborious and do not supply us with the pathogenic antibiotic sensitivity pattern.

5.3 Background

For decades, cataract surgery has outnumbered other ocular procedures as the main cause of EO. Notably, up to 10 % of eyes were lost to PCE in the pre-antiseptic era (19th century). Later, from 1940s the increased use of antimicrobials started to improve treatment outcomes and the rate of EO dropped from 2% to below 1%. First in 1967, an American ophthalmologist, Charles Kelman, introduced phacoemulsification, through small clear corneal incision, which revolutionized cataract surgery by decreasing incision size from 10 mm to 1 - 3 mm, secondary decreasing the risk of post-operative infections. Although improvement in surgical techniques has lowered the frequency of PCE, the introduction of small gauge vitrectomy has not shown to do the same. Currently, phacoemulsification is the main method used for cataract surgery in the developed world. The incidence of EO after phacoemulsification surgery before year 2000 was in a large meta-analysis reported to be 0.097 per 1000. Administration of intracameral cefuroxime was introduced by a group of Swedish surgeons and first described in an observational study. The paradigm shift came in 2007, when a randomized controlled trial in Europe confirmed that administration of intracameral ceftazidime at the end of cataract surgery reduced the frequency of post-cataract endophthalmitis five-fold, from 0.3 – 1.2 % to 0.014 – 0.08 %. Along with several changes, such as decrease in operative complications and performance of surgery in an outpatient setting, the indication for modern ocular surgery has expanded.

Importantly, novel treatment as potential causes of EO have been introduced. In 2004, the U.S. Food and Drug administration approved the first anti-vascular endothelial growth factor (anti-VEGF) drug (pegaptanib) for
intravitreal injection treatment of neovascular age-related macular degeneration (nAMD). Off-label use of bevacizumab in 2005 and approval of ranibizumab for nAMD in 2006 then gave rise to a quick increase in intravitreal injections. The indication soon extended to other retinal diseases, such as retinal vein occlusion and diabetic macular edema. Together with an aging population (i.e., predisposed to age-related macular degeneration) and the obesity pandemic (i.e., more people predisposed to diabetes and thrombosis), the number of patients in need for intravitreal injections will continue to increase. Furthermore, intravitreal injections are administrated repeatedly at monthly to three-month intervals, often for many years, exposing these patients to a cumulative EO risk. In 2010, intravitreal injection for the first time outnumbered cataract surgery as the most prevalent ocular surgical procedure in Norway. The demand for intravitreal injections is still increasing, representing one of the biggest capacity challenges for contemporary eye care services.

### 5.4 Prevention

“Intellectuals solve problems, geniuses prevent them.”

- Albert Einstein

**Antisepsis:** A normal ocular microbiota helps prevent pathogenic species from colonizing the ocular surface. For acute postoperative bacterial EO, the most common pathogens belong to normal microbiota of the skin, conjunctiva and upper respiratory tract. Transiently sterilizing the periorcular skin, eyelid margins and ocular surface with Povidone-iodine before ocular surgery is widely documented to reduce the risk of EO. Chlorhexidine is generally regarded
inferior to Povidone-iodine for ocular antisepsis, and mainly used in the rare cases of true Povidone-iodine allergy.\textsuperscript{57}

*Antibiotic prophylaxis:* Administration of intracameral ceftazidime at the end of cataract surgery was a paradigm shift in the prevention of PCE. Additional evidence for the use of intracameral ceftazidime has since been presented.\textsuperscript{58-60} On the contrary, antibiotic prophylaxis in relation to the intravitreal injection procedure has not been shown to reduce the risk of EO.\textsuperscript{61} Oppositely, antibiotics can potentially give rise to more virulent pathogens colonizing the ocular surface and may even slightly increase the risk of PIE.\textsuperscript{25}

*Surgical environment and dressing:* While cataract, vitreoretinal, corneal and glaucoma surgery routinely take place in an operating theatre, intravitreal injections can also be performed in less sterile environments. In 2013, an expert panel reviewed the literature and published guidelines on how to perform intravitreal injections.\textsuperscript{61} While sterile gloves and drapes are used for most ocular surgeries, there is no hard evidence to support its use during intravitreal injections. Moreover, while clinician mask use is the rule during ocular surgery, the guidelines on intravitreal injections recommend *either* mask use *or* a strict no-talking policy to prevent aerosolized contamination of the injection site. Notably, there was no specific recommendation in the guidelines with regard to patient masking during intravitreal injection. As I will explain later in the thesis, this caused a particular dilemma when the COVID-19 epidemic broke out.

Most cases of EO occur sporadically. However, lack of proper preventive measures can lead to EO clusters through the use of contaminated equipment or material.\textsuperscript{62,63} Clinicians strive to limit contamination during ocular surgery and intervention, but there are also prognostic factors we cannot control, like the pathogen’s virulence and the patient’s immune system. While antibiotics can alter the microbiota and immunosuppressive medication can alter the patient’s immune
system, these factors are usually not intervened and remain static. On the contrary, one important prognostic factor we can influence is the timing of treatment. 18–64

5.5 Treatment of endophthalmitis:

“In an ideal world, a vitreoretinal surgeon and staffed operating room would be instantly available, but this ideal world rarely exists. Reality is, therefore, a balance of time over completeness.”

-ESCRS Guidelines 30

The management of EO has changed dramatically through history. 65 From 1918 to 1941, treatment of EO included everything from administration of antiserum, application of mercury oxycyanide, aqueous mercurochrome drops, local heat and intravenous typhoid vaccine to intramuscular injection of boiled milk. 65 A case reported in the Proceedings of the Royal Society of Medicine in March 1920 describes a 60-year-old female with PCE treated with hot baths and atropine. On discharge from the hospital, the eye was blind. 66

A major breakthrough in treatment of EO came in the 1970s when the intravitreal route of administrating treatment was established through experimental studies. 65 Intravitreal administration of antibiotic is still the mainstay of EO treatment today. Another breakthrough during the 1970s was that Robert Machemer (1933–2009), known as the father of modern pars plana vitrectomy, introduced this method. 67 Vitrectomy is the surgical name for removing the vitreous from the posterior segment of the eye using a vitrector (Figure 1). A vitrector is a device that both cuts and aspirates allowing easier handling of the gel-like vitreous.
Three ports are usually used. One port for infusion to maintain eye volume and pressure, one port for the vitreous cutter or other instruments (e.g., cutter, forceps, laser), and one port for the fiber optic light source. The surgeon visualizes the posterior segment through an external microscope equipped with a lens system. (© Geir Aksel Qvale, Department of Ophthalmology, Oslo University Hospital)

Treatment of EO remains a matter of debate. A dilemma is the limited evidence base for contemporary treatments of EO, which still mainly relies on the randomized controlled trial called the Endophthalmitis Vitrectomy Study (EVS) published in 1995. It included 420 PCE randomly assigned to treatment by either early vitrectomy (within 6 hours) or intravitreal antibiotics alone. The EVS showed that EO cases presenting with visual function of light perception or worse had improved visual outcomes (at nine-month follow-up) if treated with early vitrectomy compared to only intravitreal antibiotic injections. For eyes presenting with visual function better than light perceptions, however, vitrectomy did not show superior results compared to only intravitreal
antibiotics. Accordingly, the present-day, gold-standard treatment for severe EO (i.e., presenting with visual function of light perception or worse) is vitrectomy. On the other hand, no broad consensus exists for first-choice treatment of EO presenting with visual function better than light perception.

The only other RCT on EO treatment published in 2017 did not show beneficial visual outcome (at one-year follow-up) of adjuvant dexamethasone, injected together with empirical intravitreal antibiotics. This study included 324 PCE randomly assigned to receive either dexamethasone or placebo together with their intravitreal antibiotic treatment.

During vitrectomy a sample from the vitreous (and/or anterior chamber) is obtained in connection with the procedure, and empirical intravitreal antibiotic are administrated at the end of surgery. To carry out a vitrectomy, a vitreoretinal surgeon, specialized ophthalmic surgery nurse, operating room and special equipment are necessities, which in practice are not always immediately available.

The European Society of Cataract and Refractive Surgeons (ESCRS) guidelines from 2013 is another key reference. According to the ESCRS guidelines, intravitreal injection of empirical antibiotics is a reasonable silver-standard treatment alternative. However, treatment should first be administered after a vitreous (and/or aqueous) biopsy has been obtained. This recommendation may give rise to treatment delay. Previously, portable handheld vitrectors (Insight Instruments) were available in Norway, but since 2019 they have no longer been supplied, further complicating microbiological sampling before injection of antibiotics. A vitreous biopsy must now be obtained with machines used for either cataract or vitreoretinal surgery, which is larger, more advanced and requires special training to handle. Ultimately, recent debate on EO treatment questions both the role and timing of the vitrectomy and the intravitreal antibiotic
A summary of the introduction in the form of key points on contemporary challenges related to EO is given below (Table 2).

<table>
<thead>
<tr>
<th>Key points on endophthalmitis:</th>
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<tr>
<td>• <em>The most severe complication of ocular surgery</em></td>
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<td>• <em>Closely related to the quality of treatment</em></td>
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<tr>
<td>• <em>Increasing potential with increasing surgical volume</em></td>
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<tr>
<td>• <em>Vigilance is imperative for successful prevention</em></td>
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<tr>
<td>• <em>Early diagnosis and treatment are crucial prognostic factors</em></td>
</tr>
<tr>
<td>• <em>No general consensus on treatment exists</em></td>
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</tbody>
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**Table 2.** Key points on contemporary challenges of endophthalmitis.
5.6 Conditions specific for the Norwegian health sector

The mission of the public health care system in Norway is to deliver equal health services to all citizens. Pragmatically, with a relatively small and outspread population, large rural areas and geographical obstacles, specialized treatment is centralized to regional services. This includes vitreoretinal surgery, which only takes place at OUH in Norway’s South-Eastern region (HSE) (Figure 2).

A debate in Norway has been the issue of who should perform cataract surgery and intravitreal injections. The services in our public health care system are in practice divided between hospitals and semi-private practices. Treatment in the semi-private sector is partly covered by the government through operating grants from the regional health authority and activity based reimbursement related to diagnosis or procedure codes, in addition to patient co-payment. For ophthalmologists working in the semi-private sector, surgical procedures are potentially profitable. While cataract surgeries are frequently performed in the semi-private sector, intravitreal injections is not. Arguments for keeping cataract surgeries at the hospital are to provide a large enough volume to maintain specialized competence for the most complicated cases, and educate doctors in training. For intravitreal injections the main arguments are the potential for complications and missed or delayed treatments that can occur during closed-hours and vacations at ophthalmologists working in the semi-private sector. Even though the numbers of cataract surgeries still are increasing in Norway, they are more limited due to their one-time procedure, while intravitreal injections are both increasing and administrated repeatedly, and are therefore in abundance.
Figure 2. The map shows Norway’s four health regions. Vitreoretinal surgery only takes place at the university hospitals in Oslo, Stavanger, Bergen, Trondheim and Tromsø. (© Geir Aksel Qvale, Department of Ophthalmology, Oslo University Hospital)
6 Aims

The overall aim of this thesis was to ensure the quality of the EO management at the Department of Ophthalmology, OUH. To reach this aim, we scrutinized all EO cases admitted to our department between 2015 and 2021, examined their epidemiology and explored whether the incorporation of new clinical procedures influenced EO outcomes.

**6.1 Paper I: The current state**

To deliver high-standard eye services to the public, we need knowledge about the current state, which again can provide guidance for further improvements. Medicine is continuously evolving and demands clinicians to be dynamic and incorporate necessary changes in their clinical practice guidelines. Resources in the public health are limited and need to be used with care.77

Paper I retrospectively investigates all EO cases admitted to our department over a 2-year period, thereby supplying knowledge about the current state. By comparing these results to other publications, Paper I also provides a basis for evaluating our own risk factors, causes, treatment and outcomes.

**6.2 Paper II: Pharmaceutical compounding – yea or nay?**

Intravitreal injections put a high strain on ocular health care services. To simultaneously improve the cost-benefit and limit the potential for EO through contamination, OUH has incorporated splitting of anti-VEGF vials into prefilled syringes in the hospital pharmacy, so-called pharmaceutical compounding. Paper II looks at a five-year transitional period, in which patients were treated with either clinician-withdrawn or pharmaceutically compounded syringes. The aim of Paper II was to evaluate whether our new practice affected the frequency of EO (i.e., if it was safe and could be permanently incorporated).
6.3 Paper III: Mask up, folks?

In connection with the COVID-19 pandemic, patients were advised to wear masks during the intravitreal injection procedure. However, preclinical experiments suggested that this could paradoxically increase their risk of PIE by contaminating the injection site with aerosolized droplets. This suggestion caused controversy in the ophthalmological community, and in paper III we sought to answer this important question.

6.4 Paper IV: Moving forward

In November 2019, we implemented a new EO management guideline in HSE; based on the best available evidence, local ophthalmological departments were advised to immediately inject empiric intravitreal antibiotics and then send the patient to OUH for vitrectomy. As PCR was routinely performed in connection with the vitrectomy, a vitreous biopsy locally should only be acquired if achievable within the hour. The aim of Paper IV was to evaluate our new management of EO.
7 Results

7.1 Paper I: Endophthalmitis is Oslo, Norway

In Paper I we sought to provide an overview of EO in our clinic. Infectious EO is a public health concern, harboring a high potential for blindness and long-term morbidity. We retrospectively reviewed the medical record of all EO cases admitted to OUH over a two-year period (2015-2016). The study included 46 eyes of 44 patients. We registered whether preceding cataract surgery or intravitreal injection took place at OUH or other ophthalmological centers. We found post cataract endophthalmitis (PCE) to be the most common EO cause (43%) (incidence 0.21 per 1000; 95% CI 0.04-1.19 per 1000), followed by trauma, in line with other reports. Post injection endophthalmitis (PIE) (incidence 0.08 per 1000; 95% CI 0.03-0.23 per 1000) and endogenous EO shared third place. The two-year odds ratio for PCE versus PIE was 8.0 (95% CI 2.7-24.0; p < 0.001) probably reflecting a difference in invasiveness between the two procedures. There was no difference in odds ratio of PCE and PIE between OUH and other ophthalmological centers in Oslo and Akershus County. The paper included cases represented and treated from all of HSE, and silver standard treatment (i.e., intravitreal injection of empirical antibiotics) was most common (54% of cases). Microbiological samples were positive in 76% of the cases, with S. epidermidis being the most frequent pathogen, also in line with other reports.78 Twenty-two eyes (48%) achieved a clinical significant improvement of BCVA, whereas six eyes had a clinical significant deterioration of BCVA after treatment.
7.2 Paper II: Does Pharmaceutical Compounding of Vascular Endothelial Growth Factor Inhibitors for Intravitreal Use Alter the Risk of Post-injection Endophthalmitis?

In paper II we evaluated a five-year transitional period (2015-2019), in which 68,150 pharmaceutically compounded, prefilled syringes (60%) and 42,286 clinician-withdrawn syringes (40%) at OUH were administrated intravitreally under otherwise identical circumstances. There were a total of 11 post-injection endophthalmitis (PIE) cases in this period. We found a lower point estimate of PIE associated with prefilled syringes (incidence 0.07 per 1000; 95% CI 0.03-0.017) compared to clinician-withdrawn syringes (incidence 0.13 per 1000; 95% CI 0.06-0.29), but there was no statistically significant difference in relative risk (p = 0.32). With regard to different anti-VEGF drugs, we found one PIE case among 4991 injections with ranibizumab (incidence 0.20 per 1000; 95% CI 0.04–1.13), five PIE cases among 56,044 injections with bevacizumab (incidence 0.09 per 1000; 95% CI 0.04–0.21), and five cases among 51,891 injections with aflibercept (incidence 0.10 per 1000; 95% CI 0.04–0.23). We conclude that the use of pharmaceutical compounding for anti-VEGF in our clinic did not negatively affect the PIE risk and should be continued.

7.3 Paper III: Mask use by patients in the context of COVID-19 can increase the risk of postinjection endophthalmitis

Paper III takes a closer look at post injection endophthalmitis (PIE) and patient mask use during the Covid-19 pandemic. We defined the pandemic period as March 2020 through January 2021. In consequence of local and national rules and recommendations, mask use by patients occurred sporadically until August 2020 and then gradually became common. In the pandemic period, a total of 25,904 intravitreal injections were given and 14,649 of these given after August
2020. We identified a total of seven PIE cases and six of these occurred after August 2020. In the pre-pandemic control group, there were five PIE cases among 68,150 injections with prefilled syringes. The relative risk of PIE in the pandemic period compared to the pre-pandemic period was 3.68 (95% CI 1.17–11.60; p = 0.026). From August 2020 through January 2021, when mask use by patients gradually became common, the relative risk of PIE was 5.58 (95% CI 1.70–18.29; p = 0.005). These findings support the hypothesis that mask use by patients could increase the risk of PIE by contamination of the injection site. Consequently, OUH abandoned patient masks during intravitreal injections.

7.4 Paper IV: Primary vitrectomy or intravitreal antibiotics followed by early vitrectomy for acute endophthalmitis: a prospective observational study

In paper IV we aimed to evaluate our new treatment for EO by comparing cases treated by primary vitrectomy (PV) and primary intravitreal antibiotics followed by early vitrectomy (PIAEV). The study included 41 EO cases from 24 different municipalities. Causes of EO included 19 cases of PIE, 18 cases of PCE, three cases of PVE, and one case of blebitis-related EO. The female to male ratio, PCE to PIE ratio, and the mean age between the two treatment groups (PV or PIAEV) did not significantly differ (p = 0.39; p = 0.25; and p = 0.48). All cases in the PIAEV group presented with severe visual impairment (i.e., logMAR ≥ 1.0) compared to 71% in the PV group (p = 0.02). In the PV group vitrectomy was performed within four hours of admission in 17 of 20 cases, while for PIAEV cases the median time from antibiotic injection to vitrectomy was 23 (5-170) hours. At three-month follow-up, 15 of 19 cases in the PV group (one case was lost to follow-up) and 15 of 21 cases in the PIAEV group achieved a clinical significant improvement (i.e., a change of ≥ 0.3 logMAR or 15 letters) in BCVA (p = 0.58). Complications in the form of rhegmatogenous retinal detachment and phthisis distributed evenly between the two groups.
Microbiological analysis were positive for pathogen in 73% of the samples (95% CI 57%-86%). There were mainly gram-positive bacteria, one case of *P. aeruginosa* (gram-negative), and no fungal infections. No samples were culture-positive but 16S PCR negative. On the other hand, seven samples were culture-negative but 16S PCR-positive (*p* = 0.02). Within the PIAEV group a microbiological sample was taken *before* intravitreal antibiotic injection in eight cases, and for 13 cases the sample was taken *after* intravitreal antibiotic injection. Altogether, PCR increased the diagnostic yield by 31% (95% CI 9%-61%) for samples collected *before* intravitreal antibiotics and 60% (95% CI 15%-95%) for samples collected *after* intravitreal antibiotics.

We conclude that for acute EO, cases treated by PV or PIAEV have comparable three-month outcomes. By combining culture with PCR in connection with vitrectomy, injection of antibiotics can be prioritized over vitreous biopsy, thereby improving the critical treatment time without sacrificing diagnostic microbiology.
8 Methodological considerations

8.1 Research design and study population

The studies generally adhered to the SQUIRE 2.0 guidelines, which provide a framework for reporting knowledge about how to improve the quality, safety, and value of healthcare. All studies had an observational design, reflecting real-life clinical events. Equally, the majority of other studies on EO are observational.

The studies were registry-based and mainly collected data from medical records. In this way, they were retrospective studies, relying on data registered by others. This introduces a possibility for misclassification bias (i.e., bias through wrongly assigned diagnosis codes – both intended and unintended), which can decrease both the sensitivity and specificity of registry studies. Nevertheless, this can be regarded as a bias that appears randomly. Also, because of the severity of EO, it is unlikely that some cases did not seek medical attention, almost completely rejecting the possibility of underestimating the number of EO through registry-based inclusion (i.e., false negatives). On the other hand, retrospective studies are time-efficient, as data gathering has already taken place. It is also a good method for studying rare diseases.

Since EO is a rare disease it introduce the research challenge of statistical power. The low prevalence of EO limits our samples sizes and statistical power. This can make it difficult to detect true differences within subgroups (i.e., a type I error of finding a difference where there is not, or a type II error of rejecting a true difference), and restrict neutralization of random bias (i.e., bias that is not systematic and will be reduced by increasing the sample size).

In paper I we included all EO cases admitted to OUH with ICD-10 diagnosis code H44.0 (purulent EO) or H44.1 (other EO). We refined our inclusion criteria in the later papers by excluding certain types of EO. In paper IV
we also cross-checked our registry with data from The department of microbiology, OUH. In paper II and IV, we excluded traumatic and endogenous EO, and also cases that did not receive antibiotic treatment within a week. Consequently, there is a higher possibility of false positive EO cases in the first paper, particularity because some cases may have been uveitis.\textsuperscript{82} Still, the amount of negative microbiological results in the study, 24%, was in line with other reports (20-30%), which indicates that we can rule out a high amount of false positive cases in paper I.\textsuperscript{11} Taken together, we regard all cases included in the studies to have high validity.

\textbf{8.2 Control group}

A main limitation of the studies is the lack of a true control group. This is particularity the case for paper V, in which all cases were ultimately treated with vitrectomy. The observational study design makes room for variation, as the cases are not managed according to a strict study protocol. However, since EO is a potentially blinding eye infection, ethical considerations forbids potentially inferior treatments. In addition, to conduct a RCT on EO is challenging given the low prevalence and heterogeneity of EO.

For paper II the choice of using either prefilled or clinician-withdrawn syringes for intravitreal injection was strictly practical. Initially, the hospital pharmacy could not fully meet the demand for prefilled syringes. In a transition period prefilled syringes were used if available, with clinician-withdrawn syringes as the alternative. No patient-related characteristics or doctors’ personal preferences influenced on this choice. Otherwise, the circumstances surrounding the intravitreal injection were identical. For this reason, we were able to adequately compare the risk of EO associated with prefilled and clinician-withdrawn syringes.
Similarly, for paper IV the choice to treat EO with PV or PIAEV was practical and determined by whether vitreoretinal surgery could be carried out immediately. The choice of initial treatment was not related to disease severity or patient characteristics. However, as about half the EO cases were referred from other ophthalmological departments in the region, variation between the cases can be expected but not always accounted for.

### 8.3 Data collection and storage

Data were obtained by searching the Norwegian Patient Register (NPR) for International Classification of Diseases (ICD-10) codes and Nordic Medico-Statistical Committee (NOMESCO) classification of surgical procedures (NCSP) codes. ICD-10 codes included H44.0 (purulent EO) and H44.1 (other EO). NCSP codes included CKD05 (intravitreal injection). The number of intravitreal injections in paper I and II were collected from the NPR, and the number of cataract surgeries in paper I were reported to us by the regional health authority.

Data were anonymized by cross-lists applied by and stored in MedInsight (i.e., OUHs official digital solution for cross-lists). The data were stored in password-protected Excel databases and could only be accessed by the researchers. One person registered all data. Verification by a second person would have been preferable.

We registered numerous parameters in the database, including age, gender, municipality, affected eye, cause of EO, type of anti-VEGF (if any), concurrent ocular disease, previous eye surgery, time and place of ocular surgery and complications (if any), lens status, vitreous status, BCVA, and time of admission. Further variables included type, time and place of EO treatment, microbiological analyses of diluted and undiluted aqueous and vitreous samples, microscopy findings, culture and PCR results, and visual outcome. The data were registered
as categorical or numerical variables, in addition to free text for individualized customization.

8.4 Visual outcomes

Visual acuity as the main outcome in EO research has some limitations. First, concurrent ocular pathology may influence the visual acuity (e.g., age-related macular degeneration, which is common in the aging population and even a prerequisite for PIE). Secondly, cataract development is a well-known complication of vitrectomy that can influence the visual outcome. In paper I we registered the visual outcomes from the last visit recorded. This lead to a large variation in follow-up time. A standardization in time for the end-of-study visit would be preferable, and we set this to three months in paper IV. For paper I and IV some patients were unavoidably lost to follow-up because of the observational nature of the studies. Last, visual acuity is a subjective outcome, as it depends on the patient, the examiner, and their cooperation.

Properly measuring and reporting the visual function in a study setting is a complex procedure. Best-corrected visual acuity is by far the most commonly reported primary outcome, but it does not take into account contrast lightening, and pupillary size among other factors influencing our visual function. There are also other ways to measure the visual function, such as reading vision, contrast sensitivity, and visual field. The visual function can also be assessed from the patient’s perspective through patient-reported outcome measures.

Snellen decimal visual acuity is the most frequently used description of visual acuity in Norway. It is, however, a non-geometric value and must be converted to logarithm of the minimum angle of resolution (logMAR) for statistical analyses. While numerical Snellen values is easily transformed to logMAR (i.e., the logMAR value is the negative logarithm of the decimal value), low-vision measurements such as hand motion, counting fingers, light perception
and no light perception remain challenging to accurately convert into numerical values. Notably, low vision commonly occurs for patients with EO. We used relatively conservative values of 2.0, 2.3, 4.0, and 9.0 in our study. Other studies on EO may use different values, complicating comparison of data.\textsuperscript{87,88}

### 8.5 Microbiological methods

In paper I the microbiological results were not differentiated by analyzes but were regarded as positive regardless of whether the diagnosis was made by culture, PCR or both analyzes. In paper IV we refined our method and differentiated culture from PCR results, which also allowed us to compare these methods. Additionally, a second microbiologist verified the microbiological results in paper IV.

In regard to PCR, for the target DNA to amplify we need the correct complementary primer (i.e., a polynucleotide that serves as the start point for DNA-synthesis). At OUH, we order our “own” primers for 16S PCR used in EO that included 830 of the 1542 base pairs on the 16S gene.

While target specific PCR (e.g., Staphyloccocus aureus) can be limited by false negative results (i.e., you only find what you look for e.g. Staphylococcus aureus), quite the opposite is true for broad range PCR were false positive results is the main problem (e.g., 16S PCR targets all bacteria). PCRs high sensitivity together with the universal targeting of bacteria, and the possibility for contamination during every diagnostic step (e.g., sampling, transport, preparation and analyzing) means that strict practice are crucial for securing valid results. Our measures to limit contamination include sterile elbow-long gloves and designated lab coats, DNA and master mix (i.e., a solution containing primers and other essential ingredients for a PCR) are each handled at laminar flow benches at separate appointed rooms, all surfaces are disinfected before use, and an one-hour
ultraviolet decontamination program is performed on the SelectNA instrument before use.

Further, that sampling take place in sterile environment, samples are directly delivered to the PCR laboratory (before culture), and every plate run in the PCR machine includes positive, negative controls, and samples of the master mix. We receive both primers and master mix from the German Company Molzym known for producing DNA free reagents.

8.6 Statistical analyses

We converted decimal visual acuity scores to logMAR for all analyzes. A clinically meaningful improvement in visual acuity was defined as ≥ 0.3 logMAR (equal to a 15-letter gain). Overall, the results are presented as mean (standard deviation) or median (range). We used binominal 95% confidence intervals for proportions, McNemar’s test for within-group comparison and Chi-Square test for between-group comparison. Relative risk was used to compare risk between groups, and the odds ratio to compare associations of exposure to outcome (i.e., EO). Statistical significance was generally defined as a p-value of < 0.05.

8.7 Ethical aspects, data gathering and storage

The research and papers were all approved by the institutional data protection officer at OUH, in adherence with the Norwegian legislation for quality assurance in the health service. Observational, registry-based research for this purpose is a key method for surveillance, increasing our knowledge to ensure good quality of clinical practice, and without asking for extra time or risk from the individual patient. As long as the personal integrity of all patients is preserved, the advantages outweigh the disadvantages.89 90
9 Discussion

“Sight is the noblest sense of man.”

-Albrecht Dürer

Because EO functions as an important quality indicator for all intraocular surgical procedures, it is of great importance to continuously monitor the status of EO with regard to incidence and etiology. Admittedly, OUH did not have its own EO registry before the present project. Moreover, although it is a notoriously sight-threatening condition, there is lack of broad consensus regarding contemporary management of EO. Addressing this lack of knowledge and general agreement about management can ultimately contribute to an improved health care service for a vulnerable patient group. An overall improvement in the public health.

9.1 Paper I: An overview of endophthalmitis at Oslo University Hospital

The purpose of the first study was to paint the picture by providing insight on the current state of EO in our department. We documented the incidences of EO, which were comparable to other reports. Additionally, we provided “proof of concept” for our registry-based research method, which we even refined in the later studies. Our observational approach provided valuable real-life data on EO. Further, we found no difference in 2-year odds ratio of post cataract endophthalmitis (PCE) and post injection endophthalmitis (PIE) between other ophthalmological centers and OUH (p = 0.14, P = 0.21). This supports conducting cataract surgery in both hospitals and semi-private sector. Although the Norwegian Ophthalmologic Association provide guidelines on management of
In the beginning of the project, PCE was the most prevalent cause of EO (43% of cases in paper I). A few years later, we then found PIE to be the most common cause (46% of cases in paper IV), along with the steady increase in intravitreal injections. Intravitreal injections are associated with a higher rate of streptococcal species.\textsuperscript{18,91} In paper I we documented three streptococcus cases, and none of them were associated with an intravitreal injection. In paper IV, on the other hand, we registered ten cases from streptococcal species. Streptococcal species generally cause more serious EO than staphylococcal species, which are commonly associated with PCE. In addition, PIE cases can present with different clinical symptoms because the infection is inoculated into the posterior segment of the eye. Such a fact underscores the need for surveillance of EO through registries, as it suggests PIE may require even more aggressive treatment than PCE.

In paper I a slight majority (54%) of EO cases were treated with the “silver line” approach, for which a biopsy is taken before intravitreal injection of empirical antibiotics. These patients did not routinely undergo secondary vitrectomy. Approximately half of the cases (48%) achieved a clinical significant improvement in visual acuity, whereas six cases (13%) experienced a clinical significant deterioration. At the same time, the study included a mixed etiology of EO, with choice of treatment at the discretion of the clinician (indeed, a vitreoretinal surgeon was not always available). We can therefore not draw a firm conclusion with regard to best management or outcome of EO based on this study.
9.2 Paper II: The influence of compounding pharmacy on the risk of endophthalmitis

The purpose of paper II was to evaluate the safety of pharmaceutical compounding of prefilled syringes for intravitreal use, and whether this practice altered the risk of EO. Anti-VEGF is the most common drug class for intravitreal use. The approved anti-VEGF drugs are all expensive (i.e., the market price for a vial of aflibercept is 9 277 NOK and for ranibizumab is 7 726 NOK at the time of writing). Although each vial is intended for one injection, it actually contains an excess volume for a single dose. Withdrawal and splitting of vials into multiple syringes therefore take place. Notably, this practice is not according to the manufacturers’ label, and contamination during the procedure can potentially lead to a cluster of PIE cases on the responsibility of the eye care service.

Pharmaceutical compounding of anti-VEGF drugs is a controversial topic (Figure 3). Some argue that the practice increases the risk of EO, whereas others claim the opposite. The high costs involved introduce the possibility for economic motives. Besides saving costs, pharmaceutical compounding can also save clinician-time, introduce superior hygienic standards and improve precision and number of patient-doses withdrawn from each vial. In Norway, the practice of intravitreal injections remained unregulated for many years. In 2016, ophthalmologists and researchers at OUH then took the initiative to transfer the task of splitting and withdrawing anti-VEGF vials from the clinicians to the hospital pharmacy. In the same period two clusters of PIE in Norway achieved broad media attention, and the Norwegian health authority finally decided to regulate the practice, dictating that splitting of anti-VEGF vials from now on should only take place in a hospital pharmacy setting. Paper II provides important evidence for the safety of such prefilled syringes for intravitreal use. OUH has the largest volume of intravitreal injections in Norway, allowing us to gather solid data that are of interest also for other smaller ophthalmological
departments, ensuring the safety of the current practice. Importantly, there have not been new clusters of PIE in Norway since we implemented pharmaceutical compounding of prefilled anti-VEGF syringes.

**Figure 3.** This picture shows a prefilled, pharmaceutically compounded syringe delivered by the hospital pharmacy at Oslo University Hospital. On one side of the package, you find information like type of anti-VEGF, production number and expiration date (A) and on the other side you can see the prefilled syringe (B).


Managing retinal diseases is particularly challenging in a time of pandemic. On the one hand, patients are often elderly and at high risk for severe illness from COVID-19. On the other hand, postponed anti-VEGF treatment or vitreoretinal surgery can adversely affect their prognoses. Consequently, retina specialists must uphold their core clinical services while applying precautions and balancing risks and benefits for each patient. Safety measures typically include both clinicians and patients wearing masks, for which there is widespread consensus that it protects against COVID-19 transmission. Retina specialists are already
accustomed to wearing a mask during surgery. To reduce the risk of PIE, this is recommended also when performing intravitreal injections. In the context of COVID-19, it may seem intuitive to extend this recommendation to include the patient wearing a mask during the intravitreal injection procedure. However, the question was raised as to whether mask use by patients could paradoxically increase the risk of PIE through contamination of the injection site with aerosolized droplets. Consequently, mask use by patients during the COVID-19 pandemic was surrounded by controversy with regard to EO.

In this study we documented a statistically significant increased risk of EO coinciding with the COVID-19 pandemic and the introduction of mask use by patients. Notably, the patients’ adherence to wearing a mask use was primarily determined by changing rules and regulations from both local and national authorities. Although a large majority of patients ultimately wore masks, we cannot quantify small no mask proportion with certainty. Accordingly, the risk estimates for PIE inevitably include some intravitreal injections performed without the patient wearing a mask, which introduces a potential source of error. Also, because patients wore their own masks during the intravitreal injection procedure, the study incorporates various masks (cloth and surgical). It should be noted that other studies did not show a similar increase in EO risk related to mask use. However, these studies may have methodological biases, which we have pointed out in the appendix. First, in the study by the Post-injection Endophthamitis Study Group, neither patients nor clinicians wore masks, introducing a potential selection bias (i.e., is the outcome related to the patient or the clinician not wearing a mask?). On the other hand, in our single-center study the physician always wore masks. Second, the relative risk of EO in their no mask group was significantly higher than in ours (p = 0.003), potentially concealing an increased risk of EO through patient masks. While the PIE rate in our no-face-mask control group was one in 13 630 injections, it was substantially higher in the
report by the Post-injection Endophthalmitis Study Group, with one in 3 464
injections. Third, the study was retrospective and included multiple centers but
without possible inter-center variations in the injection protocol taken into
account. On the other hand, a Japanese study found higher incidence of
postvitrectomy endophthalmitis during the COVID-mask period. At the end of
the day, the debate on mask use by patients and PIE remains unsettled.

9.4 Paper IV: Improving the diagnosis and treatment of endophthalmitis in
South-Eastern Norway

“We must use time as a tool, not as a couch.”

- John F. Kennedy

The literature does not offer any updated consensus on EO treatment and
treatment differs to the level of the given doctors discretion. The EVS remains
the only level I evidence, and its methods have been criticized. First, its narrow
inclusion criteria excluded the most severe EO cases, which may otherwise have
benefitted from vitrectomy and might shift the visual outcomes in favor of the
vitrectomy group. Also, the procedure in the two treatment arms have been
criticized for resembling each other (e.g., the majority of biopsies were essentially
a “mini vitrectomy”, and vitrectomies only required 50% removal of the vitreous).
Importantly, the EVS only included PCE, and its results cannot be extrapolated to
other forms of EO. Finally, major advances have been made to the vitrectomy
procedure since the study was conducted in the 1990s.

In contemporary ophthalmology, there is a trend towards early vitrectomy
for EO, regardless of the presenting visual function. However, like for all
surgery, vitrectomy for EO does not come without a risk. First, vitrectomy
accelerates cataract formation, with the majority of patients experiencing visually significant cataract within one year of vitrectomy. Besides cataract formation, vitrectomy can be complicated by the development of retinal breaks and detachments. Still, refraining from surgical removal of toxins, membranes and pus from an infected eye also poses a risk for the same complications. A second observation in favor of vitrectomy is the fact that many cultures remain positive after injection of intravitreal antibiotics. This is to a lesser extent the case for vitrectomized eyes. A vitrectomy effectively removes destructive inflammatory debris and allows for better distribution of the antibiotics in the posterior segment. Importantly, a potential biofilm (i.e., a secreted exopolysaccharide that make the bacteria able to withstand antibiotic action) is also effectively cleared away during vitrectomy.

Despite arguments in favor of vitrectomy for EO, practical obstacles to surgery remain. In many instances it may be impossible to perform a vitrectomy within six hours (the vitrectomy timeframe in EVS). This is particularly the case in Norway, where vitreoretinal services are entirely centralized.

Antibiotics are the mainstay of treatment for bacterial infections, but in EO particular problems arise with regard to retinotoxicity and limited penetration from systemic administration, which ultimately limit medical treatment options for EO. There are relatively few studies on the pharmacokinetics of intravitreally administrated antibiotics, and those that exist are mostly animal studies. Although the source and pathological spectrum of EO varies between different world regions, there is solid evidence for the choice of empirical antibiotics, even supported by our findings.

Several studies propose that immediately injecting empirical antibiotics is the single most important factor in managing EO, but they are hesitant about how to ensure a microbiological diagnosis. Crucial time can be lost while striving to obtain a vitreous biopsy for culture. Several pathogens (including the common
S. epidermidis and S. aureus) show initial accelerative growth the first hours and days, and then reach a plateau before declining, even without antibiotic treatment.\textsuperscript{120} \textsuperscript{121} As the inflammatory response peaks shortly after reaching the plateau, it would be desirable to initiate treatment before this stage. Also, when culture results are ready, it may be too late to adjust treatment nevertheless. One can also speculate as to whether causative bacteria that are resistant would grow on culture in spite of antibiotics before sampling.

PCR is becoming more available and common as a diagnostic method. A recent study on multi-mono PCR (i.e., including many target specific primers for the most common pathogens causing EO in addition to universal 16S) shows potential for quick and easy bacterial identification from undiluted antibiotic naive vitreous samples in vitro.\textsuperscript{70} Accordingly, PCR can increase the diagnostic yield and present the results earlier than conventional culture. Yet obtaining a vitreous biopsy remains an obstacle and produces a time lag to initiation of treatment.

A major advantage of PCR is that it detects pathogenic DNA, regardless of whether the pathogen itself is dead or alive (Figure 4). By combining PCR with culture, we were generally able to determine both the microbiological diagnosis and the resistance pattern, even for patients who first received antibiotics. In this way, we can alter the steps in traditional EO treatment (i.e., culture first, antibiotics second) by prioritizing injection of antibiotics without delay. Ideally, antibiotics should be injected before the plateau phase of bacterial growth to inhibit the inflammation and prevent destruction of retinal cells.\textsuperscript{69} \textsuperscript{72}

The purpose of our final study (paper IV) was to evaluate our new management of EO and ensure its quality. Importantly, we found comparable three-month outcomes in the primary vitrectomy (PV) and primary intravitreal antibiotic injection followed by early vitrectomy (PIAEV) groups. Moreover, 13 cases received intravitreal antibiotic injection \textit{before} microbiological sampling.
Even so, a causative pathogen was identified in 84% (i.e., a higher proportion than general in paper I and V, 76% and 73%), indicating that prior intravitreal antibiotics does not negatively affect the microbiological diagnosis when combining PCR with culture. In fact PCR increased the diagnostic yield of samples by 31%-60%.

Our new approach to EO allows for more flexibility in the timing of vitrectomy, a prerequisite when patients must travel large distances to their nearest vitreoretinal service. In paper IV vitrectomy was performed within four hours of admission in the PV group. In the PIAEV group the median time from antibiotics to vitrectomy was 23 hours. Intuitively, one might draw the conclusion that vitrectomy should be performed the sooner the better. However, an interesting recent study showed better visual outcomes for patients undergoing vitrectomy after one day compared to within the day. The authors hypothesized that a slight delay in surgery could induce a posterior vitreous detachment (by ongoing inflammation), and improve media clarity making the surgery less exposed to complications.

Essentially, our new management of EO addresses the practical obstacle related to centralization of vitreoretinal services. It also promotes an advanced interdisciplinary approach to EO, routinely incorporating diagnostic microbiology with PCR. The new clinical practice for EO is well established in our health region, and it has generally been welcomed by the local eye services we collaborate with. Nonetheless, we agree with other recent studies that there is demand for a new RCT on the treatment of EO, and the time is now!
Figure 4. Detection of bacterial DNA by 16S PCR. The figure shows a real-life example of a 16S PCR run at Oslo University Hospital including amplification plots of several samples (A), melting curves of several samples (B) and part of one single sample result consisting of a 830 long base pair sequence (C) that is matched with the GenBank (Basic local alignment search tool) to reveal the pathogen.
A universal truth is that things change. Although the focus on EO is not new, several of our findings have had a direct effect on our clinical practice.

First, detailed knowledge about EO is fundamental for delivering the best possible ocular health care to our patients. Second, performing research creates a top of mind awareness of EO, sharpening our clinical practice. Third, a permanent EO quality-registry at OUH has been established, and will be continued even after this thesis is finished. We believe such a registry is an important quality measure and should be implemented in all eye clinics performing surgical procedures; vigilance is key for successful prevention of EO. Fourth, OUH was the first hospital in Norway to implement pharmaceutical compounding of prefilled syringes for intravitreal use. This contributed to national guidelines, and other ophthalmological departments in Norway soon followed. Similar methods for pharmaceutical compounding have also been applied in other Nordic countries, such as Finland and Denmark. Fifth, we abandoned further mask use by patients in the setting of intravitreal injections after documenting that this was associated with an increased risk of PIE. Sixth, our studies contributed to establishing PCR as a routine in addition to culture in the diagnostic work-up of EO. Seventh, the studies sparked a rewarding partnership between our microbiological and ophthalmological departments, to the benefit of both patients and researchers. Eight, we improved the critical treatment time for EO by prioritizing immediate injection of antibiotics over culture locally. Last, but not least, OUH can now offer early vitrectomy to all EO cases, despite serving a large region.
11 Future perspectives

In the Norwegian health care system, public eye departments are the main deliverer of retinal care and perform most intravitreal injections. On the contrary, cataract surgery is also performed by ophthalmological centers in the semi-private and private sector. Similar to cataract surgery, there could be room for performing intravitreal injections in other ophthalmological practices under the prerequisite that national regulations and clinical practice guidelines are established and followed. Pharmaceutical compounding of prefilled syringes for intravitreal injections is the current norm in Norway, but there is still a potential for improving this practice. For instance, syringes containing silicon oil, which is shown to deposit in the eye, can now be replaced by silicone-free syringes.123

Along with rapid technological improvements, we expect PCR diagnostics to further improve, allowing for even quicker EO diagnosis at less expense. Also, PCR and other technologies might soon allow for rapid assessment of antibiotic susceptibility, making pathogen-specific antibiotic treatment within the same day a possibility.124 Further, we expect causative pathogen spectrum and pathogen characteristics to change along with the change in source of EO (i.e., more streptococcal species is seen with PIE). Surveillance is therefore of utmost importance. We advocate all suppliers of eye care services to keep track of their EO cases until permanent regional, and eventually national, EO registries can secure us with this key information.

We hope for a new RCT on EO management that will establish an updated consensus on EO management. The current state of globalization and digitalization can facilitate a multinational study. The study should evaluate modern complete pars plana vitrectomy at different times (within six hours and between 24-48 hours) against antibiotics only. Preferably, it can manifest the ideal timing of the vitrectomy. Nonetheless, until a consensus for EO management is
established, the duplication of our EO treatment strategy to other regions in Norway and possibly abroad seems reasonable. At least we expect more clinicians to initiate EO treatment earlier with immediate antibiotic injections, without obtaining prior microbiological samples.
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13 Papers I – IV
ABSTRACT.
Purpose: To study the incidence, aetiology, pathogenic causes, treatment and visual outcomes of endophthalmitis (EO) at the Department of Ophthalmology, Oslo University Hospital (OUS), Norway.
Methods: Retrospective registry study. Medical records of all EO patients treated at OUS over a 2-year period were reviewed.
Results: The study identified 46 EO eyes of 44 patients; 19 eyes had postcataract surgery EO (PCE), and 6 eyes had postinjection EO (PIE). Of 4778 primary cataract surgeries performed at OUS, there was one PCE (incidence 0.21 per 1000; 95% CI 0.04–1.19 per 1000). Of 38 134 intravitreal injections performed at OUS, there were 3 PIE (incidence 0.08 per 1000; 95% CI 0.03–0.23 per 1000). Among 15 751 cataract surgeries performed at other ophthalmic centres in Oslo and Akershus County (OOC), there were 15 PCE (incidence 0.95 per 1000; 95% CI 0.58–1.57 per 1000). Of 3000 intravitreal injections performed at OOC, there was one PIE (incidence 0.33 per 1000; 95% CI 0.059–1.89 per 1000). For neither PCE nor PIE, there were significant differences in odds ratios between OUS and OOC. The odds ratio for PCE versus PIE was, however, 8.0 (95% CI 2.7–24.0; p < 0.001). Cultures were positive in 35 of 46 eyes (76%). The most common pathogen was Staphylococcus epidermidis. Twenty-two eyes (48%) achieved a clinically significant improvement in visual function (≥0.3 logMAR) following treatment.
Conclusion: The overall risk of PCE and PIE was low. It was, however, higher for PCE than PIE, probably reflecting the relative difference in invasiveness between the procedures.

Key words: cataract surgery – endophthalmitis – iatrogenic endophthalmitis – intravitreal injection

Introduction
Endophthalmitis (EO), the most severe intraocular eye infection, is an ophthalmological emergency (Durand 2013, 2017). The clinical diagnosis of EO is based on typical symptoms and signs, including pain, decreased visual acuity, red eye and severe intraocular inflammation. The risk of irreversible visual loss and debilitation is significant if not treated adequately. Disease severity is influenced by the virulence of the infectious agent, duration before diagnosis and intervention and the patient’s immune status. Aetologically, EO is divided into exogenous and endogenous. Exogenous EO is caused by an external source, such as a penetrating eye trauma or surgery, whereas endogenous EO emerges from systemic dissemination of pathogens, such as infectious endocarditis or dental infections.

Among exogenous EO, iatrogenic causes are the most common. Accordingly, the incidence of iatrogenic EO can be interpreted as a quality measure of practices and precautions for the prevention of this severe complication of various ophthalmological procedures. In recent years, the incidence of postcataract surgery EO (PCE) has decreased dramatically due to improved surgical techniques, povidone-iodine antisepsis and prophylactic intracameral antibiotics (Barry 2014) (Ulf & Mats 2015) (Line et al. 2015). The reported incidence of PCE ranges from 0.03% to 0.2% (Sheu 2017). In the last decennium, intravitreal injections of antivascular endothelial growth factor (anti-VEGF) drugs have become the mainstay of treatment of neovascular age-related macular degeneration (AMD). In addition, macular oedema secondary to retinal vein occlusions, diabetes and non-AMD chorioidial neovascularization are commonly treated with anti-VEGF injections. Patients are usually treated repeatedly over many years (Yu & Ta 2014; Ulf & Mats 2015; Relhan et al. 2017). Accordingly, they are exposed to a cumulative risk of postinjection EO (PIE), and the reported incidence of PIE ranges from 0.01% to 0.08% (Rayess et al. 2016).

The purpose of this study was to investigate the incidence, aetiology, pathogenic causes, treatment and visual outcomes of EO referred to the Department of Ophthalmology, Oslo University Hospital (OUS), Norway's largest tertiary eye care centre.

Materials and Methods
The study was designed as a retrospective registry study approved by
the institutional data protection officer.

For data collection, the medical records of all patients diagnosed with EO (ICD-10 code H44.0: purulent EO or H44.1: other EO) at OUS from 01.01.2015 through 31.12.2016 were reviewed. For each EO case, the aetiology, pathogenic cause (if identified), treatment and visual outcome were recorded.

Cataract surgery at OUS routinely takes place in an operating theatre. The personnel use surgical masks, caps, sterile coats and sterile gloves during the procedure. An ophthalmic drape with incise film and an eye speculum are administered. Povidone-iodine 5% is used as antiseptic; the ocular exposure time is 3 min. At the end of the surgery, 0.1 ml of cefuroxime 10 mg/ml is used as intracameral antibiotic prophylaxis.

Similarly, intravitreal injections take place in an operating theatre. The personnel use surgical masks and caps; in addition, the doctor performing the injection uses sterile gloves during the procedure. An ophthalmic drape with adhesive aperture and an eye speculum are administered. Povidone-iodine 5% is used as antiseptic; the ocular exposure time is 90 seconds. No antibiotic prophylaxis is used. During the study period, preparation for ranibizumab administration was in accordance with its labelling information, whereas pharmacetical compounding of aflibercept in prefixed syringes was implemented (Sivertsen et al. 2018). Similar to ranibizumab, bevacizumab was prepared in the operating theatre; bevacizumab vials were, however, routinely split into multiple syringes. In patients where both eyes were injected, injections were from separate vials for each eye.

Treatment of EO at OUS is in accordance with the ‘ESCRS guidelines for prevention and treatment of EO following cataract surgery’, utilizing either ‘gold’ or ‘silver’ standard therapy independently of aetiology. ‘Gold’ standard is defined as performing a diagnostic and therapeutic formal three-port core or complete vitrectomy followed by injection of intravitreal antibiotics. ‘Silver’ standard is defined as performing only a diagnostic vitreous biopsy followed by injection of intravitreal antibiotics (Barry 2013).

Empirically, vancomycin 1 mg (0.1 ml solution of 10 mg/ml) and ceftazidime 2 mg (0.1 ml solution of 20 mg/ml) are routinely injected intravitreally, whereas gentamicin 20 mg is deposited subconjunctivally. On clinical suspicion of fungal EO, 0.1 ml amphotericin B liposomal solution of 0.05 mg/ml is injected intravitreally.

Oslo University Hospital (OUS) provides local ophthalmic care for 1 070 000 people living in the city of Oslo and the surrounding Akershus County. To estimate the local risk of PCE and PIE, the number of surgical procedures performed at OUS and other ophthalmic centres in Oslo and Akershus (OOC), as reported to the regional health authority’s medical reimbursement system, was used as a denominator. Oslo University Hospital (OUS) also receives regional and, sporadically, extraregional EO cases, but these were not included in the local risk assessment.

Decimal best-corrected visual acuity (BCVA) was converted to the logarithm of the minimum angle of resolution (logMAR) for statistical analysis. In the setting of profound low vision, counting fingers and hand motion (HM) were assigned logMAR values 2.0 and 2.3, whereas light perception and no light perception were reported as such. A clinically significant change in visual function was defined as ≥0.3 logMAR; furthermore, a change from light perception to at least HM or from no light perception to at least light perception was defined as a clinically significant change. For cumulative 2-year PCE and PIE, the incidence, Wilson score 95% CI and odds ratios were calculated. Statistical significance was defined as p < 0.05.

Table 1. Causes of endophthalmitis at Oslo University Hospital (OUS) in 2015 and 2016.

<table>
<thead>
<tr>
<th>Cause</th>
<th>Number of patients (N = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract surgery (primary*)</td>
<td>19 (43%)</td>
</tr>
<tr>
<td>Traumatic</td>
<td>8 (18%)</td>
</tr>
<tr>
<td>Postinjection</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>Endogenous</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>Persistent fistula after</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>previous surgery</td>
<td></td>
</tr>
<tr>
<td>Trabeculectomy</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Corneal transplant</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Secondary lens implantation in</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>aphakia</td>
<td></td>
</tr>
<tr>
<td>Vitrectomy</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

* Exchanging natural lens to artificial lens.
† Endophthalmitis (EO) believed to occur through a persistent limbal port following previous peritomy, removal of intraocular lens, anterior vitrectomy and implantation of Verisyse lens.

Results

The study identified 46 eyes of 44 patients (2 patients with bilateral EO); twenty-two patients in 2015 and 22 patients in 2016. The most common cause of EO was cataract surgery, constituting 19 eyes (43%). Other causes of EO are listed in Table 1.

Among 4778 primary cataract surgeries performed at OUS in 2015 and 2016, one eye with PCE was identified (incidence 0.21 per 1000; 95% CI 0.03–0.34 per 1000). Likewise, among 38 134 intravitreal injections performed at OUS, 3 eyes with PIE were identified (incidence 0.08 per 1000; 95% CI 0.03–0.23 per 1000). Among 15 751 cataract surgeries performed in OOC, 15 eyes with PCE were identified (incidence 0.33 per 1000; 95% CI 0.059–1.89 per 1000).

The 2-year odds ratio for PCE at OOC versus OUS was 4.6 (95% CI 0.6–34.5; p = 0.14). The 2-year odds ratio for PIE at OOC versus OUS was 8.0 (95% CI 2.7–24.0; p < 0.001).

Among the 25 eyes with EO complicating primary cataract surgery or intravitreal injections, one eye with PCE and 3 eyes with PIE originated in OUS, whereas 15 eyes with PCE and one eye with PIE originated in OOC. Three eyes with PCE and 2 eyes with PIE originated from centres outside Oslo and Akershus. In the 3 eyes with PIE originating in OUS, one eye received bevacizumab, one eye received aflibercept, and one eye received ranibizumab; the total number of injected eyes was 18 210 for bevacizumab, 16 238 for aflibercept and 2622 for ranibizumab.

Table 2 shows the pathogenic causes of EO identified by culture. Cultures were positive for pathogen(s) in 35 of 46 eyes (76%). Eighteen different pathogens were identified, and 3 eyes had more than one culture positive for pathogen. Staphylococcus epidermidis, present in cultures of...
Table 2. Type of surgery, pathogenic cause in positive cultures, and originating centre

<table>
<thead>
<tr>
<th>Type of surgery</th>
<th>Pathogen</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cataract</td>
<td>Staphylococcus epidermidis</td>
<td>OUS</td>
</tr>
<tr>
<td>Cataract</td>
<td>UK</td>
<td>OUS</td>
</tr>
<tr>
<td>Cataract</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>Staphylococcus epidermidis</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>Staphylococcus epidermidis</td>
<td>OUS</td>
</tr>
<tr>
<td>Cataract</td>
<td>Staphylococcus epidermidis</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>Staphylococcus epidermidis</td>
<td>OUS</td>
</tr>
<tr>
<td>Cataract</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>Staphylococcus epidermidis + Staphylococcus capitis</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>Staphylococcus epidermidis</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>Staphylococcus warner</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>Enterococcus faecalis</td>
<td>OOC</td>
</tr>
<tr>
<td>Cataract</td>
<td>Corynebacterium</td>
<td>OUS</td>
</tr>
<tr>
<td>Traumatic</td>
<td>Propionibacterium acnes</td>
<td>OOC</td>
</tr>
<tr>
<td>Traumatic</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Traumatic</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Traumatic</td>
<td>Staphylococcus epidermidis</td>
<td>OUS</td>
</tr>
<tr>
<td>Traumatic</td>
<td>Pseudomonas aeruginosa</td>
<td>OUS</td>
</tr>
<tr>
<td>Traumatic</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Traumatic</td>
<td>B-haemolytic streptococci group C + Dermabacter hominis</td>
<td>OUS</td>
</tr>
<tr>
<td>Traumatic</td>
<td>Acinetobacter Iwofii</td>
<td>OOC</td>
</tr>
<tr>
<td>Intravitreal injection (affiberecept)</td>
<td>Staphylococcus aureus</td>
<td>OUS</td>
</tr>
<tr>
<td>Intravitreal injection (ranibizumab)</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Intravitreal injection (dexamethasone)</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Intravitreal injection (affiberecept)</td>
<td>Staphylococcus epidermidis</td>
<td>OUS</td>
</tr>
<tr>
<td>Intravitreal injection (bevacizumab)</td>
<td>Staphylococcus epidermidis</td>
<td>OUS</td>
</tr>
<tr>
<td>Intravitreal injection (bevacizumab)</td>
<td>Staphylococcus warner</td>
<td>OUS</td>
</tr>
<tr>
<td>Endogenous</td>
<td>UK</td>
<td>OUS</td>
</tr>
<tr>
<td>Endogenous</td>
<td>Staphylococcus pneumonia</td>
<td>OUS</td>
</tr>
<tr>
<td>Endogenous</td>
<td>UK</td>
<td>OOC</td>
</tr>
<tr>
<td>Endogenous</td>
<td>Beta-haemolytic Streptococcus group G</td>
<td>OOC</td>
</tr>
<tr>
<td>Endogenous</td>
<td>Candida albicans</td>
<td>OOC</td>
</tr>
<tr>
<td>Endogenous</td>
<td>Beta-haemolytic Streptococcus group B</td>
<td>OUS</td>
</tr>
<tr>
<td>Persistent fistula after previous surgery</td>
<td>Staphylococcus aureus, Moraxella nonliquefaciens, staphylococcus capitis</td>
<td>OUS</td>
</tr>
<tr>
<td>Trabeculectomy</td>
<td>UK</td>
<td>OUS</td>
</tr>
<tr>
<td>Corneal transplant</td>
<td>Staphylococcus aureus</td>
<td>OUS</td>
</tr>
<tr>
<td>Secondary lens implantation in aphakia</td>
<td>Enterococcus faecalis</td>
<td>OOC</td>
</tr>
<tr>
<td>Vitrectomy</td>
<td>Enterococcus (unspecified)</td>
<td>OUS</td>
</tr>
</tbody>
</table>

OOC = Other Ophthalmological Centres, OUS = Oslo University Hospital, UK = unknown: sample taken external to OUS or not found.

Among the 6 eyes of 6 patients with clinically significant deterioration in visual function, 3 had BCVA of HM at admission. The first of these three patients had culture-negative PCE and was initially treated with ‘silver’ standard followed by vitrectomy. The second patient had traumatic EO and positive culture media for two pathogens: beta-haemolytic Streptococcus group C and Dermabacter hominis. This patient was initially treated with ‘gold’ standard followed by evisceration. The third patient had endogenous EO and positive culture media for beta-haemolytic Streptococcus group G and was initially treated with hourly dexamethasone eye drops followed by ‘silver’ standard. Finally, 3 patients had light perception at admission. Two of these had endogenous EO and positive culture media, each for one pathogen: beta-haemolytic Streptococcus group B and Streptococcus pneumonia. The third patient had postvitrectomy EO and positive culture media for enterococci (unspecified species). Final 3 patients were all treated with ‘silver’ standard.

Discussion

In the present paper, we identify all EO patients referred to Norway’s largest tertiary eye care centre over a 2-year period. Moreover, we determine the risk of EO complicating cataract surgery and intravitreal injections, two frequent ophthalmological procedures. Endophthalmitis (EO) is a heterogeneous clinical entity. In accordance with other studies, cataract surgery was found to be the most frequent cause (Durand 2017; Rahmani & Elliott 2018). The overall incidences of PCE and PIE were low. There was, however, a higher risk of PCE than PIE. A reasonable explanation for this observation is the difference in invasiveness between the two procedures. Nevertheless, whereas cataract surgery at OUS includes the use of intracameral cefuroxime as EO prophylaxis, intravitreal injections are performed without the use of pre- or postinjection antibiotics.

At OUS, experience may vary among surgeons in training. Moreover, complicated cataract cases associated with a higher risk of PCE are typically referred to OUS for surgery. Accordingly, the incidence of PCE could be expected to be higher at OUS. We found, however, no significant
difference in the risk of PCE between OUS and OOC.

Similar to other reports, the most common pathogen was found to be Staphylococcus epidermidis (Teweldemedhin et al. 2017). Staphylococcus species are frequently identified as part of the normal bacterial flora of human skin, ocular surface and respiratory tract. Gram-positive bacteria are reported to constitute >95% of culture-positive cases of PIE (Durand 2013). Likewise, among the 3 eyes with PIE originating in OUS, there were 2 cases of Staphylococcus, whereas one case was culture-negative. Generally, this could imply that PIE is often caused by patients’ normal ocular flora or by contamination from the clinical staff. Moreover, in all PIE cases, the anti-VEGF injection of choice was pre-compounded in prefilled syringes. Among 3 eyes with PIE originating in OUS, all received different medication: one aflibercept, one bevacizumab and one ranibizumab. Bevacizumab was injected almost 7 times as frequently as ranibizumab (18 210 versus 2622) in this period.

‘Silver’ standard was found to be the most frequent EO treatment. Endophthalmitis (EO) is an ophthalmological emergency; undelayed antibiotic treatment is of paramount importance, whereas the significance of vitrectomy is debatable. The results of the Endophthalmitis Vitrectomy Study in 1995 indicated that it was not necessary to perform immediate vitrectomy in PCE eyes with vision better than light perception (‘Results of the Endophthalmitis Vitrectomy Study. A randomized trial of immediate vitrectomy and of intravenous antibiotics for the treatment of postoperative bacterial EO. Endophthalmitis Vitrectomy Study Group’, 1995). However, the study design has been criticized by vitreoretinal surgeons, arguing that an abscess should be drained as soon as possible (Kuhn, 2005). At OUS, emergency vitrectomy is performed if the patient is admitted during workdays, when retinal surgeons, arguing that an abscess should be drained as soon as possible. In conclusion, EO is a rare occurrence. Cataract surgery was the most common cause. Moreover, the EO risk was higher following cataract surgery than intravitreal injections, probably reflecting the relative difference in invasiveness between the procedures. The most frequent pathogen identified was Staphylococcus epidermidis. About half of the treated eyes gained a clinically significant improvement in visual function following treatment.

Nevertheless, about half of the EO eyes achieved a clinically significant improvement in visual function following treatment, whereas eyes with deterioration in visual function all presented with profound low vision at admission.

A limitation of the study is its retrospective nature and real-life lack of a systematic EO protocol. Prophylactic measures may differ between the ophthalmic centres. The wide range in follow-up time hinders standardized evaluation of visual endpoints. Finally, the EO sample size is small; larger numbers are needed to identify any small differences between groups.

In conclusion, EO is a rare occurrence. Cataract surgery was the most common cause. Moreover, the EO risk was higher following cataract surgery than intravitreal injections, probably reflecting the relative difference in invasiveness between the procedures. The most frequent pathogen identified was Staphylococcus epidermidis. About half of the treated eyes gained a clinically significant improvement in visual function following treatment.

Figure 1. Change in visual function from admission to last recorded visit. Twenty-two eyes showed clinically significant improvement of best-corrected visual acuity (BCVA), and 6 eyes showed clinically significant decreased BCVA.

The most frequent pathogen identified was Staphylococcus epidermidis. About half of the treated eyes gained a clinically significant improvement in visual function following treatment.

References

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Does Pharmaceutical Compounding of Vascular Endothelial Growth Factor Inhibitors for Intravitreal Use Alter the Risk of Post-injection Endophthalmitis?

Kathrine Blom, Ragnheiður Bragadóttir, Magne Sand Sivertsen, Morten Carstens Moe & Øystein Kalsnes Jørstad

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Letters to the Editor

Mask use by patients in the context of COVID-19 can increase the risk of postinjection endophthalmitis

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The close proximity puts ophthalmologists at particular risk for contracting COVID-19 from patients and vice versa. To reduce the risk of postinjection endophthalmitis (PIE), clinicians commonly wear masks when performing intravitreal injections (IVI). In the context of COVID-19, it may seem intuitive to extend this practice to include the patient wearing a mask. However, observations raise the question as to whether patient masks could paradoxically increase the risk of PIE; masks can leak exhaled air towards the eyes and contaminate the injection site with aerosolized droplets (Hadayer et al. 2020; Raervis et al. 2021).

The suggestion that mask use by patients puts them in peril of PIE is alarming. Consequently, we have compared the risk of PIE at Oslo University Hospital (OUH) since the beginning of the COVID-19 pandemic with the pre-pandemic risk. We defined the pandemic period as March 1, 2020 through January 31, 2021. Mask use by patients was influenced by rules and recommendations from the Norwegian Institute of Public Health and the City of Oslo in particular and occurred sporadically until August. It then gradually became common, and ultimately a majority of our patients wore masks during the IVI procedure. In both the pre-pandemic and pandemic period, the IVI were generally performed ambulatory and took place in positive air pressure cleanrooms. Povidone iodine 5% was used as antiseptic (no antibiotic prophylaxis was used). The standard procedure was to drape the patient after the initial application of povidone iodine, but in the pandemic period clinicians temporarily chose to first drape patients not wearing a mask.

There were five cases of PIE of 68.150 IVI with compounded syringes at OUH in the pre-pandemic period (Blom et al. 2020). A total of 25 904 IVI were given in the pandemic period from August 2020 through January 2021. Seven cases of PIE were identified, six of these after August 2020. Microbiological analyses were positive for pathogens in most of the cases; the bacteria all belonged to the microbiota of the skin and upper respiratory tract.

Table 1 displays a summary of the PIE cases. The relative risk (RR) of PIE in the pandemic period compared to the pre-pandemic period was 3.68 (95% CI 1.17–11.60; p = 0.026). From August 2020 through January 2021, when mask use by patients gradually became common, the RR of PIE was 5.58 (95% CI 1.70–18.29; p = 0.005).

Our findings support the hypothesis that patient masks could redirect exhaled air towards the eyes and contaminate the injection site. Additional aspects may also contribute to an increased risk of PIE. First, the widespread use of masks by the general public has led to a new form of dry eye disease: mask-associated dry eye (MADE). A healthy tear film possesses antimicrobial properties, and air leakage from poorly fitting masks that dries the ocular surface may disrupt the eye’s innate immunity and increase its susceptibility to infections (McDermott 2013). Second, a normal ocular microbiota helps prevent pathogenic species from colonizing the ocular surface (Petrillo et al. 2020). Regular mask use may alter the microbiota by contaminating the surface with microbes of higher pathogenic potential.

An increased RR notwithstanding, PIE remains a rare occurrence at OUH. The risk of contracting COVID-19, on the other hand, is still imminent. Accordingly, we do not encourage

Table 1. A summary of the postinjection endophthalmitis cases at Oslo University Hospital in the pre-pandemic and pandemic period.

<table>
<thead>
<tr>
<th>Pre-pandemic period n = 68 150</th>
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M, male; F, female; nAMD, neovascular age-related macular degeneration; RVO, retinal vein occlusion; S. epidermidis, Staphylococcus epidermidis; S. aureus, Staphylococcus aureus; S. gordonii, Streptococcus gordonii.
patients to stop using masks but instead advise clinicians to be vigilant about sealing the injection site and counsel patient about properly adjusting their masks and treatment for dry eyes. Ultimately, COVID-19 vaccination would mitigate the risk of transmission in the setting of IVI and allow for safe reimplementation of a procedure for which patients do not wear masks.

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Neovascular age-related macular degeneration inactivated during systemic administration of an immune checkpoint inhibitor pembrolizumab for lung cancer

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Editor,

I t has long been known that the immune system is involved in the pathophysiology of neovascular age-related macular degeneration (nAMD). Here, we report a case of nAMD resistance to regular intravitreal injection of vascular endothelial growth factor (VEGF) inhibitor, in which disease activity subsided following systemic administration of an immune checkpoint inhibitor for lung cancer.

A 77-year-old man presented with decreased vision in his left eye. The best-corrected visual acuity (BCVA) of the left eye was 20/32 and fundus examination revealed submacular haemorrhage. Optical coherence tomography (OCT) examination showed retinal pigment epithelium detachment (PED) involving the fovea (Fig. 1A, B). The patient was diagnosed with nAMD, and intravitreal injection of the VEGF inhibitor aflibercept was started. Although no obvious reduction in PED size was obtained during the induction phase, and we judged the patient to be a non-responder to VEGF inhibitor treatment, intravitreal injection of aflibercept was continued at regular intervals of 4 to 8 weeks. A total of 15 doses were administered for a period of 2 years, but the obvious reduction of PED size was not obtained, and BCVA decreased to 20/63 (Fig. 1C). Two years after starting treatment for nAMD, he was diagnosed with squamous cell carcinoma in the lung (Fig. 1D), and systemic therapy with the immune checkpoint inhibitor, pembrolizumab, was initiated. Six weeks after starting therapy for lung cancer, he visited our hospital for scheduled intravitreal injection. Fundus examination of the affected eye showed that the PED, which had been resistant to treatment with VEGF inhibitor had decreased from 1.5 disc diameter (DD) to 0.5 DD. The exudative lesions in the fovea disappeared and BCVA improved to 20/40 (Fig. 1E). CT scan of the lung cancer at the same time also showed a marked reduction in tumour size (Fig. 1F). Treatment with pembrolizumab continued for one year during that his lung cancer did not progress and nAMD remained stable, despite no additional intravitreal injections of VEGF inhibitor.

Cancer escape from immune destruction has been associated with immunosuppressive mechanisms that inhibit T-cell activation by binding to programmed cell death protein 1 (PD-1), an immune checkpoint molecule on T cells. Pembrolizumab, an anti-PD-1 monoclonal antibody, is one of the immune checkpoint inhibitors that block PD-1 to promote T-cell activation, enabling the activated T cells to attack and diminish the cancer. This immune checkpoint inhibitor is used as a new therapeutic agent for advanced cancers.

Several types of immune cells have been reported to be involved in the pathophysiology of nAMD. CD8+ T cells have been found to be abundant in human eyes with drusen, and in eyes with advanced AMD and fibrovascular scarring (Ezzat et al. 2008). A study has shown that CD8+ T cells in the blood of nAMD patients express markers indicating accelerated ageing and T-cell differentiation (Subhi et al. 2017). In addition, CD8+ T cells have been reported to be involved in the reduction of experimental choroidal neovascularization in mice (Mochimaru et al. 2007). Our patient was a non-responder to VEGF inhibitor treatment. However, nAMD appeared to be inactivated shortly after receiving systemic immune checkpoint inhibitor for lung cancer. Activation and local invasion of CD8+ T cells associated with immune checkpoint blockade has been reported to be important for cancer regression (Tuneh et al. 2014). We speculate that treatment with an immune

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Editor,
INTRODUCTION

Time is of the essence in acute endophthalmitis (EO); there is exponential growth of bacteria initially, and prompt diagnosis and treatment are keys to successful management (Barry et al., 2013; Clarke et al., 2018). The randomised controlled Endophthalmitis Vitrectomy Study (EVS) concluded that immediate vitrectomy was only beneficial in eyes with...
light perception or worse, but it had narrow inclusion criteria, was conducted in the 1990s, and may not reflect modern surgical outcomes (1995; Barry et al., 2013; Clarke et al., 2018; Flynn Jr. & Scott, 2008; Grzybowski et al., 2018; Maguire, 2008). Undeniably, surgical removal of the infected vitreous adheres to the fundamental treatment principle of evacuating pus (hence the Latin aphorism “Ubi pus, ibi evacua”). The European Society of Cataract & Refractive Surgeons (ESCRS) deems vitrectomy to be the present-day gold standard treatment of EO (Barry et al., 2013), and retina specialists now frequently perform early vitrectomy for EO, regardless of the presenting visual function (Soliman et al., 2019). However, a dilemma surrounds this approach to EO treatment: vitreoretinal surgery can be practically impossible to carry out within a narrow time frame (6h in the EVS). Consequently, the ESCRs considers swiftly acquiring samples of the aqueous and vitreous for microscopy and culture before injecting empirical antimicrobial therapy intravitreally as a reasonable silver standard alternative.

While the contemporary role of vitrectomy for acute EO remains to be resolved, the Ophthalmological Society has been encouraged to pursue also other improvements in the management of EO (van Meurs & van Dissel, 2018). Intravitreal injection of antibiotics only requires basic ophthalmological skills and can be quickly administered in an outpatient setting, but the silver standard alternative still necessitates surgical training and access to a portable or full-size vitrectomy to obtain a vitreous biopsy. As time is of the essence, this raises a second dilemma in the approach to EO: must the crucial intravitreal injection of antibiotics be withheld until a vitreous biopsy has been acquired? The diagnostic yield of culture is in fact frequently maintained after the first dose of intravitreal antibiotics (Kosacki et al., 2020), and, additionally, culture results are often not the key determinant of treatment choice in EO (Fliney et al., 2018). Moreover, modern polymerase chain reaction (PCR) allows for rapid and accurate identification of a wide range of organisms, dead or alive (Cornut et al., 2014). Accordingly, it has been proposed to update the EO guidelines by emphasising immediate injection of antibiotics (Clarke et al., 2018; van Halsema et al., 2022; van Meurs & van Dissel, 2018).

Norway’s south-eastern health region serves about 3.1 million people and covers an area of nearly 111,000 km². As an emergency vitreoretinal surgery in this large territory only takes place at the Department of Ophthalmology at Oslo University Hospital (OUH), primary vitrectomy is unachievable in many EO cases. Taking into account the rationale for vitrectomy, we have taken the initiative to improve the management of acute EO in our region. Since 2019, local eye care services have been encouraged to immediately inject empirical intravitreal antibiotics before admitting their acute EO cases to OUH. A vitreous biopsy should only be obtained locally if it is achievable within the hour at the time of antibiotic injection. On arrival at OUH the patients then undergo vitrectomy, preferably within the day, and broad-range 16S PCR as well as bacterial culture is routinely performed as an aid to diagnosis. Ultimately, this approach to EO has the potential advantages of improving both critical treatment time and access to vitrectomy without sacrificing identification of the causative organism.

The purpose of this 18-month observational study was to compare outcomes of acute EO managed with either primary vitrectomy (PV) or primary intravitreal antibiotics followed by early vitrectomy (PIAEV) combined with PCR-based diagnostics.

2 | MATERIALS AND METHODS

This was a prospective, comparative observational study of acute EO patients admitted to the Department of Ophthalmology at OUH. The study was approved by the institutional data protection officer as quality assurance of our EO treatment (ref. 19/23628). The inclusion criterion was a diagnosis of acute EO (ICD-10 code H44.0: purulent EO or H44.1: other EO) from 1 November 2019 to 31 May 2021. Because endogenous or traumatic EO generally received varying treatment, such cases were excluded. EO cases that did not receive intravitreal antibiotics and vitrectomy within 1 week (typically because they were regarded sterile) were also excluded.

2.1 | Treatment of acute endophthalmitis

OUH serves as the local ophthalmology department for citizens of Oslo and nearby municipalities. For our local EO cases, PV (within 6h) was often achievable, and if not, empirical antibiotics were injected intravitreally pending vitrectomy. By contrast, the other ophthalmology departments in south-eastern Norway do not perform vitreoretinal surgery. Instead, they were encouraged to admit patients with acute EO to OUH after injecting empirical antibiotics intravitreally. For these EO cases we performed early vitrectomy, preferably within the day of admission. Consequently, the EO cases in the study were treated with either (1) PV or (2) PIAEV.

2.2 | Vitrectomy

Pars plana (23 or 25G) vitrectomy was performed with a Stellaris PC or Stellaris Elite platform (Bausch + Lomb; Laval, Canada) using retrobulbar anaesthesia. Preoperative ultrasound was performed in cases where visualisation of the fundus was limited. In cases without a posterior vitreous detachment, an attempt was made to induce one with the vitrector. Care was taken to evacuate pus and remove as much of the vitreous as possible whilst simultaneously being careful not to create iatrogenic retinal breaks. Shaving of the vitreous base was performed within the limits of the surgeon's ability to visualise the vitreoretinal interface. Pus in the anterior chamber was removed at the surgeon's discretion. Follow-up procedures were performed later as needed once the inflammation was controlled.

2.3 | Antimicrobial therapy

The initial antimicrobial therapy for EO was chosen empirically and routinely consisted of intravitreal injections of vancomycin 1.0 mg (0.1 ml of 10 mg/ml
solution) and ceftazidime 2.0 mg (0.1 ml of 20 mg/ml solution). In EO cases treated with primary intravitreal antibiotics, injection of antibiotics was repeated at the end of the vitrectomy procedure. Additionally, gentamicin was injected subconjunctivally at the end of the procedure by some surgeons. Amphotericin B 5 μg (0.1 ml of 0.05 mg/ml solution) was administered intravitreally on clinical suspicion of fungal EO. Intravitreal antimicrobial therapy was repeated at the doctor's discretion. Systemic antimicrobial therapy for EO was not administered at OUH.

2.4 | Microbiological sampling

For EO cases that received immediate intravitreal antibiotics, microbiological samples were only obtained before the injection if achievable within the hour. During vitrectomy, undiluted and diluted vitreous samples for microbiology were always collected at the beginning of the procedure. In addition, an anterior chamber sample was sometimes collected. Consequently, the microbiology samples in the study were collected either (A) before or (B) after administration of intravitreal antibiotics.

2.5 | Conventional microbiological investigations

The vitreous specimens were cultured aerobically on chocolate agar and anaerobically on blood agar. Chocolate agar plates were incubated in 5% CO₂ at 35°C for 7 days. Anaerobic plates were also incubated at 35°C for 7 days. Subcultures from Brain Heart Infusion broth were performed on chocolate agar on days two and five. Yeast and mould cultures were incubated for 14 days. Bacteria were identified by typical colony morphology and Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS Bruker), using MALDI Biotyper MSP Identification Standard Method 1.1. MALDI-TOF MS gave a spectral score of 2.0 or higher, consistent with accurate species identification. Antimicrobial susceptibility testing was performed by disk diffusion (MAST), using EUCAST breakpoints.

2.6 | DNA isolation

Vitreous specimens were obtained in sterile tubes. Bacterial and fungal DNA were extracted from 0.2 ml fresh fluid samples using a MolYsis-SelectNA™plus kit in combination with an automated SelectNA™plus DNA extraction instrument (Molzym).

2.7 | Broad-range PCR

Universal primers (Molzym) targeting the bacterial 16S rDNA, D1/D2 region of 28S fungal rDNA, and fungal Internal Transcribed Spacer 2 (ITS2) region were used for broad-range PCR. The 16S rRNA gene was amplified by forward primer (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primer (5'-GGCCTTACTTCAGGAGGT-3'). The ITS2 region was amplified by forward primer (5'-GCATATCAATGCGCGAGGAAAG-3') and reverse primer (5'-TCCTCCGCTTATTGATGTC-3'). SYBR Green-based qualitative real-time PCR was carried out with the Mastermix 16S/18S Dye (Molzym). The PCR assays were performed with an AriaDx real-time PCR instrument (Agilent Technologies) with the following cycling conditions settings: initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 15 s, and extension at 72°C for 35 s, followed by a melting curve analysis (70°C–95°C). Samples with a melting temperature value between 87°C and 91°C were considered positive.

2.8 | Nucleotide sequencing and sequence homology analysis

Cycle sequencing was performed by forward and reverse priming using PCR primers and a BigDye Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific). The amplification was performed with a Perkin Elmer 2720 and the following cycling conditions settings: 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 150 s. Samples were kept at 4°C until purification with the BigDye Terminator. Nucleotide sequences were automatically determined using an Applied Biosystems 3130xl Genetic Analyser (Thermo Fisher Scientific). The obtained sequences were compared with the Basic Local Alignment Search Tool (BLAST) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The identity of the organisms was determined using similarity cut-off values of >97% for genus and >99% for species.

2.9 | Data collection

The data were prospectively registered in an anonymised database in Excel 2019 (Microsoft Corporation). We recorded patient characteristics, aetiology, pathogen, treatment, door-to-treatment time, complications (if any), and three-month visual outcome for each case. We registered whether the patient received (1) primary vitrectomy or (2) primary intravitreal antibiotics followed by early vitrectomy. We also recorded microbiology results for microscopy, culture, PCR, and whether the samples had been collected (A) before or (B) after administration of antibiotics.

2.10 | Statistical analyses

Best-corrected visual acuity (BCVA) was converted to logarithm of the minimum angle of resolution (logMAR) for statistical analyses. In the setting of profound low vision, counting fingers and hand motion were assigned logMAR values 2.0 and 2.3, whilst light perception and no light perception were reported as such. We defined a
clinically meaningful improvement in BCVA as ≥0.3 logMAR, equivalent to 15 letters. We used the chi-square test or Fisher's exact test (for small samples) to analyse frequencies between groups, the McNemar's test for within-subject analyses, and the independent samples t-test to analyse means. We determined 95% binominal confidence intervals (CI) for proportions. We defined statistical significance as \( p < 0.05 \). Results are presented as either mean (standard deviation) or median (range).

3 | RESULTS

Sixty EO patients were admitted to OUH in the inclusion period. Forty-one EO cases were included in the study. Nineteen cases did not fulfil the inclusion criteria (10 were endogenous, two were traumatic, and seven did not receive intravitreal antibiotics and vitrectomy within 1 week). All the included EO cases were unilateral, i.e., there were no correlated observations. Twenty EO cases received PV, whilst 21 EO cases were treated with PIAEV.

3.1 | Baseline characteristic

There were 14 women and 6 male patients in the PV group, and 12 female and 9 male patients in the PIAEV group (\( p = 0.39 \)). Mean age was 77.8 (8.9) years in the PV group and 71.2 (13.2) years in the PIAEV group (\( p = 0.48 \)). The patients were referred from 24 different municipalities. The patient living the farthest away had a 395 km distance to OUH, corresponding to an approximately 5-h drive. The two major causes of EO were cataract surgery (PCE; 18 cases) and intravitreal injection (PIE; 19 cases). Additionally, there were three EO cases following vitrectomy (PVE) and one EO case following blebitis. There was no significant difference in frequencies of PCE and PIE between the two groups (11 PCE and eight PIE in the PV group and seven PCE and 11 PIE in the PIAEV group; \( p = 0.25 \)). None of the 41 EO patients had known predisposing risk factors for EO.

Four of 20 cases in the PV group and 10 of 21 cases in the PIAEV group presented with a visual function of light perception (\( p = 0.06 \)). For the remaining cases mean BCVA was 1.54 (0.83) logMAR in the PV group and 2.19 (0.36) logMAR in the PIAEV group (\( p = 0.01 \)). Fifteen of 20 cases in the PV group and all 21 cases in the PIAEV group presented with logMAR 1.0 (i.e., severe visual impairment) or worse (\( p = 0.02 \)). Table 1 summarises the baseline characteristics.

3.2 | Door-to-treatment time

For the PV group the procedure was performed within 4 h of admission in 17 of 20 cases. For the three remaining
causes, the vitrectomy procedure was delayed because of poor posterior visualisation (case 22), initial suspicion of sterile inflammation (case 11), and relatively low-grade PIE (case 16). For the PIAEV group, median time from primary intravitreal antibiotics to vitrectomy was 23 (5–170) hours.

3.3 | Antimicrobial therapy

Routine empirical intravitreal injection of vancomycin 1.0 mg and ceftazidime 2.0 mg was administered in 38 of 41 cases. One of these cases received a second intravitreal antibiotic injection after 42 h (case 17). In one PCE case, amphotericin B 5 μg was injected in addition to vancomycin and ceftazidime (case 9). In one PVE case, only vancomycin was given because the eye was filled with 22% SF6 gas (case 40). In one PIE case, gentamicin 20 mg was initially combined with ceftazidime by mistake (case 19); approximately 1.5 h later, the vitreous cavity was irrigated, and the eye then received standard treatment with vancomycin 1.0 mg and ceftazidime 2.0 mg.

3.4 | Three-month visual outcomes

One patient was lost to follow-up before the 3-month visit (case 18). At the 3-month visit, four cases had no light perception. For the remaining 36 cases, mean BCVA was 0.66 (0.75) logMAR. Fifteen of 19 cases in the PV group and 15 of 21 cases in the PIAEV group achieved a clinically meaningful improvement in BCVA ($p = 0.58$). Fourteen of 19 cases in the PV group and 10 of 21 cases in the PIAEV group achieved a BCVA of logMAR 0.5 or less (i.e., better than moderate visual impairment; $p = 0.09$). Fifteen of 18 PCE cases and 14 of the 18 PIE cases achieved a clinically meaningful improvement in BCVA ($p = 0.67$). Figure 1 illustrates the visual function at admission and at 3 months. Table 2 summarises the main results.

3.5 | Complications

Within 3 months, three eyes developed rhegmatogenous retinal detachment (cases 2, 19, and 22), and three eyes developed phthisis (cases 20, 25, and 41).

3.6 | Microbiology

Diagnostic microbiology was performed according to the described methods in all 41 cases. In 21 of 41 cases, an anterior chamber sample was available along with the vitreous samples.

A causative microorganism was identified in 30 of 41 (73%) cases (95% CI: 57%–86%); 23 cases were culture- and PCR-positive, and seven additional cases were culture-negative, but PCR-positive ($p = 0.02$). Accordingly, PCR increased the diagnostic yield through a positive result in 39% of the culture-negative samples (95% CI: 17%–64%). Notably, no cases were culture-positive, but PCR-negative. An anterior chamber sample was available for 16 of the 30 positive cases; nine of these samples (56%) were positive (95% CI: 30%–80%), i.e., the diagnostic yield of anterior chamber samples was inferior to vitreous samples.

Among the 30 positive cases, we identified nine different causative bacteria: *Staphylococcus epidermidis*...
### TABLE 2 Main results

<table>
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<tr>
<th>Case</th>
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<th>Pathogen</th>
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<td>−</td>
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<td>−</td>
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<td></td>
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<td>+</td>
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<td>4</td>
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<td>29*</td>
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<td>−</td>
<td>−</td>
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<td></td>
<td>30*</td>
<td><em>S. aureus</em></td>
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<td></td>
<td>33*</td>
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<td>−</td>
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<td></td>
<td>34*</td>
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<td>−</td>
<td>+</td>
<td>0.08</td>
<td></td>
<td>35*</td>
<td><em>S. gordonii</em></td>
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<td>+</td>
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<tr>
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<tr>
<td>18</td>
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<td>+</td>
<td>+</td>
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<td>38*</td>
<td><em>S. epidermidis</em></td>
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<td>+</td>
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<tr>
<td>19</td>
<td>20</td>
<td>−</td>
<td>−</td>
<td>HM</td>
<td>RD</td>
<td></td>
<td>39</td>
<td>−</td>
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<td>−</td>
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<tr>
<td>20</td>
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<td>+</td>
<td>NLP P</td>
<td></td>
<td>40*</td>
<td><em>S. pneumoniae</em></td>
<td>−</td>
<td>+</td>
<td>0.18</td>
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Abbreviations: CF, counting fingers; HM, hand motion; NLP, no light perception; P, phthisis; RD, rhegmatogenous retinal detachment.

* = microbiological samples taken after antibiotic injection.
in 10 cases, *Staphylococcus aureus* in seven cases, *Streptococcus gordonii* in four cases, *Streptococcus mitis* in three cases, *Enterococcus faecalis* in two cases, *Staphylococcus cohnii* in one case, *Pseudomonas aeruginosa* in one case, *Streptococcus pneumoniae* in one case, and group G β-hemolytic streptococcus in one case. These bacteria could be empirically divided into low-pathogenic (*S. epidermidis, S. gordonii, S. mitis, and S. cohnii*) or high-pathogenic (*S. aureus, E. faecalis, P. aeruginosa, S. pneumoniae, and group G β-hemolytic streptococcus*). There was no significant difference in frequencies of low- and high-pathogenic bacteria between the two groups (six low- and seven high-pathogenic bacteria in the PV group and 12 low- and five high-pathogenic bacteria in the PIAEV group; *p* = 0.18).

### 3.7 Microbiological sampling before versus after administration of intravitreal antibiotics

Microbiology samples were collected before administration of intravitreal antibiotics in 28 cases. Fifteen of these 28 cases were both culture- and PCR-positive, and four additional cases were culture-negative but PCR-positive, i.e., PCR increased the diagnostic yield through a positive result in 31% of the culture-negative samples (95% CI: 9%–61%). Microbiology samples were collected after administration of intravitreal antibiotics at the time of vitrectomy in 13 cases. Eight of these 13 cases were both culture- and PCR-positive, and three additional cases were culture-negative but PCR-positive, i.e., PCR increased the diagnostic yield through a positive result in 60% of the culture-negative samples (95% CI: 15%–95%). As previously mentioned, no cases were culture-positive, but PCR-negative. Figure 2 displays the proportions of positive and negative microbiological results for samples collected before and after administration of intravitreal antibiotics.

### 4 DISCUSSION

This was a prospective, comparative observational study of primary vitrectomy (PV) or intravitreal antibiotics followed by early vitrectomy (PIAEV) for acute endophthalmitis (EO) in a regional vitreoretinal service. Broad-range 16S PCR in addition to culture was routinely performed as an aid to diagnosis. When PV was unachievable, injection of empirical antibiotics locally was prioritised over vitreous biopsy. Eyes that received PIAEV had worse visual function at baseline. The two groups were balanced with respect to other baseline characteristics and frequencies of low- and high-pathogenic bacteria. Most eyes in both groups achieved a clinically meaningful improvement in BCVA of at least 15 letters. PCR increased the diagnostic yield for samples collected both before and after administration of intravitreal antibiotics. Accordingly, omitting a vitreous biopsy and instead immediately injecting antibiotics improved the door-to-treatment time without sacrificing microbial identification.

An overarching principle of the Norwegian public healthcare system is to offer all citizens equal medical treatment, irrespective of age, sex, income, or place of residence. As we discussed in the introduction, many retina specialists prefer vitrectomy for acute EO. Yet, primary vitrectomy can be practically impossible to carry out within a narrow time frame. This is particularly the case in Norway, which has large rural areas and centralised vitreoretinal services (the most remote patient in this study lived 395 km from our clinic). Given these circumstances, primary injection of intravitreal antibiotics locally followed by admission for vitrectomy centrally is a pragmatic approach to effective treatment for EO. Although intravitreal antibiotics may be inferior to present-day vitrectomy, they do have an evident effect on EO and allow for more flexibility in the timing of vitrectomy than within the traditional 6-h time frame. A finding in support of this approach to EO is that similar proportions of eyes achieved a clinically meaningful improvement in BCVA in both groups. At the same time, EO remained potentially devastating, with cases of rhegmatogenous retinal detachment, blindness and phthisis despite every effort.

Unlike, for instance, the Endophthalmitis Vitrectomy Study, which only included PCE, this study had a heterogeneous aetiology of EO. PIE was most prevalent, for the first time superseding PCE as the leading cause of EO in our clinic (Blom et al., 2019). This is in line with a steady increase in the number of intravitreal injections in our health region (Jorstad et al., 2020; Kristiansen et al., 2020). It should be noted that the typical indication for an intravitreal injection is macular disease, e.g., nAMD, RVO, or DME. Such underlying macular pathology limits the visual prognosis and consequently complicates the evaluation of PIE outcomes. Still, most of the PIE cases in this study experienced a clinical meaningful improvement in BCVA.

A causative microorganism was identified in about three-fourths of the EO cases in this study, and all but one case of *P. aeruginosa* were Gram-positive bacteria. Vancomycin-resistant Gram-positive infections are rare in Western countries, and ceftazidime exhibits effects against both Gram-positive and -negative bacteria (Clarke et al., 2018; NORM-atlas, 2021). This supports empirical therapy with vancomycin and ceftazidime. Retinotoxicity, nevertheless, limits alternative antibiotics for EO. Notably, almost one-third of the microbiology samples were collected after administration of intravitreal antibiotics, but despite that many cultures remained positive. Keeping in mind the time-dependency of antibiotics, the relatively short time span between injection of antibiotics to vitrectomy and sample collection may explain this observation. The positive cultures after administration of intravitreal antibiotics also highlight that they do not achieve an immediate bactericidal effect in EO. This further supports definite removal of the infected vitreous through vitrectomy.

Incorporating broad-range 16S PCR into routine microbiological diagnosis of EO increased the diagnostic yield in this study. PCR proved superior to culture for samples collected after administration of intravitreal
antibiotics, as could be expected. However, PCR also identified the causative microorganism more often than culture in samples collected before administration of intravitreal antibiotics. Altogether, no cases were culture-positive but PCR-negative. Based on this observation, one may draw the conclusion that PCR can replace culture in the diagnostic workup of EO. However, it is important to keep in mind that PCR and sequencing have several limitations. Firstly, the method is laborious, expensive, and relies heavily on advanced laboratory services. Our department of microbiology performs PCR for EO three times a week, which may delay results, especially over the weekend. Secondly, PCR is sensitive not only to causative microorganisms but also to contamination, introducing the possibility of false positive findings. Consequently, vitreous samples for PCR must be carefully collected and handled and results interpreted in a clinical context as an interdisciplinary effort, as was the case for all microbiological results in our study. Finally, PCR and other emerging technologies might assess antibiotic susceptibility, but we still rely on culture to analyse antibiotic resistance in clinical practice (Li et al., 2017). As mentioned in the introduction, culture results are often not the key determinants of treatment choice in EO, but they do play an important role in surveillance of pathogen spectrum and antibiotic resistance. Taking all these factors into consideration, culture should not be omitted.

This study is subject to limitations. First and foremost, it lacks a conservatively managed control group for comparison. Three decades after the Endophthalmitis Vitrectomy Study it remains to be proven with a randomised clinical trial whether present-day vitreoretinal surgery is superior to conservative antibiotic treatment for eyes presenting with better visual function than light perception. Secondly, the study was observational, and the patients were treated according to a clinical practice guideline, not a rigorous study protocol. Finally, as symptom onset was often not accurately documented, we were unable to determine the time from symptoms to treatment, a potential prognostic factor.

In conclusion, this prospective, comparative observational study found that primary vitrectomy or primary injection of intravitreal antibiotics locally followed by admission for vitrectomy centrally allowed for early vitrectomy for all cases of acute endophthalmitis in a large region. Most eyes in both groups achieved a clinically meaningful improvement in BCVA. By combining culture with broad-range 16S PCR in connection with the vitrectomy procedure, intravitreal antibiotics could be injected before microbiological sampling, thereby improving the door-to-treatment time without sacrificing microbial identification.

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REFERENCES


14 Appendix
estimate of effect size was done. To detect a $\geq 40\%$ difference in myopia progression using within-group standard deviation of 0.5 D and 1:1 randomization, a sample size of 84 participants (42 participants per arm) was required to achieve 90% power with a 2-sided test of 5% (0.05 significance level). Considering the attrition rate of 15%, a total sample size of 100 participants (50 participants per arm) was required.

Refraction was carried out by a single optometrist in every center. Automated refraction was done under homatropine 2% instilled thrice at a 10-minute interval followed by subjective acceptance. Three readings using an automated refractometer were taken and the median was recorded. The final power was prescribed based on subjective acceptance and was recorded for analysis.

Because changes in spherical equivalent are nearly constant over time in the atropine group, the effect of significant change in myopia progression is possibly related to the cumulative effect of 0.01% atropine (Fig S1 in the original article). We analyzed the spherical equivalent change in 3 different quadrimesters (4-month periods) in the 2 groups. Change in the spherical equivalent between different time periods (from baseline to 4 months, 4–8 months, and 8–12 months) in the atropine treatment group was evaluated and was not found to be significant ($P = 0.09$) (Table 1).

We agree with the author that atropine could increase the pupil size, but a small change is difficult to appreciate clinically. In our study, we found statistically insignificant changes in pupil size with 0.01% atropine. Such clinically insignificant change on follow-up in the pupil size from baseline did not result in the unmasking of therapy to the blinded observer in this study.

This study evaluated the effect of 0.01% atropine versus placebo at 1 year. After completion of the study period and as per the study protocol, all patients were shifted to open-label 0.01% atropine drops.

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**References**


**Re: Writing committee for the Post-Injection Endophthalmitis Study Group, et al.: The influence of universal face mask use on endophthalmitis risk after intravitreal anti-vascular endothelial growth factor injections**

(Ophthalmology. 2021;128:1620–1626)

TO THE EDITOR: On the basis of a large retrospective study, the Post-injection Endophthalmitis Study Group concluded that universal face mask use (physician, ancillary staff, and patient) during intravitreal injections (IVI) did not alter the risk of presumed post-injection endophthalmitis (PIE), although the rate of culture-positive PIE was decreased. A smaller retrospective study by Bisorca-Gassendorf et al concluded similarly. This finding contradicts the original suggestion by Hadayer et al that mask use by patients could paradoxically increase the risk of PIE by leaking exhaled air toward the eye, potentially contaminating the injection site. Notably, the Post-Injection Endophthalmitis Study group drew their conclusion by comparing PIE rates with a no-talking policy seems hard to verify in a retrospective study, which raises concern about hygiene standards and increased relative risk of PIE in a single-center study.

A key element for preventing PIE is to mitigate the spread of aerosolized droplets containing oral contaminants and, irrespective of the current COVID-19 pandemic, clinicians are commonly advised to wear a mask when performing IVI. A strict no-talking policy may be equivalent to physician mask use. Still, compliance with a no-talking policy seems hard to verify in a retrospective study, which raises concern about hygiene standards in the control group. The Post-Injection Endophthalmitis Study Group reported a presumed PIE rate of 1 in 3464 injections in the “no face mask” control group, which we consider relatively high, and Bisorca-Gassendorf et al reported an even higher PIE rate among controls of 1 in 2139 injections. Clearly, an increase in PIE rate through patient masks is difficult to detect if the risk of contamination is elevated in the control group as well.

In contrast with the Post-Injection Endophthalmitis Study Group, we found a statistically significant association between mask use by patients and increased relative risk of PIE in a single-center study.

Our conclusion, however, was drawn by comparing PIE rates with a control group in which physicians and ancillary staff indeed wore
masks. Also, patients in the control group were draped, povidone iodine 5% was used as an antiseptic, syringes were pre-filled, and the IVI took place in positive air pressure rooms, which is, high hygiene standards were preserved. The PIE rate in our control group was 1 in 13 630 injections, which corresponds with a lower risk of PIE than in the reports by the Post-Injection Endophthalmitis Study Group (relative risk of 0.25; \( P = 0.003 \)) and Bisorca-Gassendorf et al\(^2\) (relative risk of 0.16; \( P = 0.001 \)). When mask use by patients gradually became common under otherwise unchanged hygienic conditions in our study, the PIE rate significantly increased (relative risk of 5.58; \( P = 0.005 \)), lending support to the hypothesis that patient masks could increase the risk of PIE.

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**References**


**REPLY:** We appreciate the comments by Blom et al on our study\(^1\) and agree that a key element to minimizing the risk of postinjection endophthalmitis is to mitigate the spread of aerosolized droplets containing oral contaminants.

In our study, for the “no face mask” group, neither physicians nor patients wore a face mask when administering intravitreal injections. Previous evidence suggested that a strict no-talking policy decreases the risk of endophthalmitis compared to no such policy\(^2\) and may be equivalent to physician face mask use in terms of endophthalmitis risk.\(^3\) Indeed, a global survey of nearly 400 retina specialists in 2018 reported that 68% use a no-talking policy during intravitreal injections compared with 32% who prefer wearing a face mask during intravitreal injections.\(^4\) Based on these findings, we believe that either a strict no-talking policy or physician face mask use may be an appropriate control group for the standard of care in the prepandemic era.

The authors suggest that the rate of postinjection endophthalmitis in the control group may be unduly high. However, the rates of endophthalmitis in our control group are within the range of multiple previously reported studies. Indeed, the largest cumulative reviews (those with >100 000 intravitreal injections) report endophthalmitis rates ranging from 0.016% to 0.036%, and no regional differences were noted.\(^5\) Furthermore, endophthalmitis risk within the current study was similar across all study sites, each of which had multiple treating physicians. The authors also suggest the lack of physician and ancillary staff face mask use in the control group may account for the perceived differences. However, another study evaluating physician face mask use found that endophthalmitis risk to be about 0.0298% (1 in 3351 injections),\(^3\) which is similar to the control group in this study (0.028%; 1 in 3464 injections).\(^1\)

The authors also report data from their single-center series of 68 150 intravitreal injections in the prepandemic period and 14 649 intravitreal injections in the postpandemic period. The authors suggest that their low endophthalmitis rate of 0.007% (1 in 13 630 injections) in the control group may be due to physician and ancillary staff face mask use. However, there are several methodological differences in their study, including the exclusive use of prefilled syringes with compounded medications, the routine use of positive pressure ventilation systems, and sterile draping, which could account for differences. In addition, it is unclear how endophthalmitis was defined and if cases of possible “noninfectious” endophthalmitis that were empirically treated with intravitreal antibiotics were ultimately excluded from the study. We agree that there are challenges conducting studies in which (thankfully) the event rates are low, and large multicenter studies that enable granular collection of data can be helpful in these scenarios.

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**ON BEHALF OF THE POST-INJECTION ENDOPHTHALMITIS STUDY GROUP**

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