Perspectives of Infectious Endophthalmitis

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Endophthalmitis is a term referring to the severe intraocular inflammation involving the vitreous cavity and/or anterior chamber of the eye. Endophthalmitis may lead to severe vision loss, even with appropriate and prompt treatment. Forty years ago, an eye developing infectious endophthalmitis was essentially lost, but now the possibility to save the eye is up to 85–90%. This improvement in management of endophthalmitis is due to three major factors:

- Immediate microbiological evaluation of intraocular fluids,
- Early laboratory diagnostic procedures including molecular biological testing,
- Therapeutic vitrectomy,
- Intraocular application of anti-inflammatory, anti-bacterial and anti-fungal drugs.

The aetiology of the endophthalmitis is mainly infectious, although sterile inflammation may rarely occur due to retained native lens material after an intraocular surgery or dehaemoglobinized intraocular haemorrhage or direct physical or chemical tissue injury or from immunologic or neoplastic processes.

Non-infectious Endophthalmitis

Occasionally, an exaggerated postoperative sterile inflammatory response may cause symptoms mimicking infectious endophthalmitis. These are due to: (a) Toxic lens syndrome (TLS) by definition, implicates the intraocular lens (IOL) itself, as the cause for a sterile inflammation possibly due to the poor manufacturing quality. In some instances, there was contamination of the implant by polishing substances. Another aetiological factor included defective sterilization methods. Certain wet-pack, as well as ETO sterilization, methods have led to several occurrences of TLS. At present, with improved manufacturing and sterilization processes, as well as much better quality control standards, these IOL production-related problems have largely been solved by most manufacturers. (b) Immune reaction to proteins from crystalline lens. This can be associated with hypopyon, although these reactions are less often associated with severe pain and prominent swelling of the eyelids than in infectious endophthalmitis. Nevertheless, some lens-induced reactions can be very destructive and have led to enucleation. Other kinds of lens-induced inflammations, namely, phacotoxic or phacolytic reactions, are less often so severe to produce endophthalmitis. These inflammations are characterized by a predominance of macrophages (with engulfed lens material) and lymphocytes. These are thought to be mediated by cellular immunity (type IV hypersensitivity). However, retained lens fragments and infectious endophthalmitis may occur concurrently. Therefore, it is mandatory to suspect an infection in any ocular inflammation following cataract surgery with retained lens material.1

The causative agents of infectious endophthalmitis include bacteria and fungi, with viruses and parasites being extremely rare. There is now an increasing recognition that virtually any bacterium or fungus can cause endophthalmitis, if introduced in sufficient quantities.2,4

Infectious endophthalmitis may be exogenous, when the microorganisms are introduced into the eye from the environment, or endogenous, caused by the haematogenous spread of microorganisms into the eye as a metastatic infection from an infected site elsewhere in the body. Exogenous endophthalmitis usually occurs following surgery (postoperative endophthalmitis) including bleb procedures for the treatment of glaucoma or trauma (posttraumatic) or from the contiguous spread of the infective agents in conditions such as microbial keratitis. Endophthalmitis following intravitreal injection is a new but rare entity since the introduction of interventional procedures of intraocular injections of therapeutic agents being a standard for management of certain intraocular diseases. Of these above, the postoperative form is the most common accounting for approximately 70–75% of the infectious endophthalmitis. Infectious endophthalmitis may be categorized by the cause of the infection and the characteristic onset of clinical symptoms and signs.4,5

Based on the time of onset of symptoms after surgery, three forms of postoperative endophthalmitis are clinically distinguishable, i.e. acute, delayed and chronic. The acute form is usually fulminant, occurs 2–4 days postoperatively and more frequently is caused by Staphylococcus aureus, Streptococcus spp. and Gram-negative bacilli. The delayed form, clinically a moderately severe disease, occurs 5–7 days postoperatively and generally due to Staphylococcus epidermidis and more rarely, fungal species. Chronic infection or late-onset endophthalmitis can occur as early as 1 month postoperatively. Propionibacterium acnes, S. epidermidis and fungi are the most important organisms in this late-onset group. These forms, however, are subject to great
The terms delayed and chronic have been used synonymously in various studies, and the definitions differ with respect to the time of onset of symptoms.6

In several studies reported in the literature, the distribution of microorganisms isolated from the major categories of endophthalmitis has revealed certain genera and species unique to the clinical presentation. Factors such as difference in patient population and habitat of the region are known to influence the type of organisms associated with different categories of endophthalmitis. Our knowledge of the incidence and aetiology of endophthalmitis is based mainly on the Western literature, with the exception of a few of isolated ones available from India.7

**Laboratory Diagnosis in Infectious Endophthalmitis**

Advances in pharmaceutical and surgical therapy have tremendously improved the prognosis of endophthalmitis. Nevertheless, a prompt and accurate aetiological diagnosis of suspected endophthalmitis is still essential for the appropriate and timely treatment, which is central to a successful visual outcome. Hence, timely action has to be taken by the clinician to obtain intraocular fluids for microbiological studies and the microbiologist to detect and identify the aetiological agent. Conventional microbiological investigations comprise direct microscopy and culture to detect the bacterial and fungal agents. Though vitreous fluid (VF) has been documented to have more sensitivity to grow the organism in laboratory, aqueous humour (AH) should also be cultured as its collection is a simple office procedure and since on occasions, there has been growth only from AH. Culture of the intraocular specimen is considered the gold standard in the aetiological diagnosis of endophthalmitis. However, even under the most appropriate care, these traditional methods prove to be negative in 21–63% of the clinically typical cases of endophthalmitis. The reasons cited have been prior antibiotic therapy, small number of organisms in the samples, possible localized nature of the infections in the lens capsule and the fastidious growth requirement of some offending organisms. This high rate of culture negativity is especially observed in late-onset and chronic endophthalmitis. In such cases, the most appropriate antibiotic coverage is not discernible due to non-availability of an aetiological diagnosis. Additionally, in some cases, there may be a delay of several days before cultures are interpreted. All these findings suggest a need for a more sensitive and specific detection strategy in the diagnosis of endophthalmitis.

With the advent of molecular techniques such as polymerase chain reaction (PCR) and PCR-based DNA sequencing, there is an increased rate of detection and identification of the pathogen in clinical specimens, where microscopy is too insensitive or cultures take too long a time or fail. PCR has had a great impact on the speed and accuracy of microbiological diagnosis. This technique is extremely well suited for intraocular specimens because of the small sample volume, small number of organisms and the fastidious nature of many of the organisms known to cause endophthalmitis. In cases of suspected endophthalmitis, it is important for the clinician to know whether it is infectious or not, if infectious whether bacterial or fungal. Previous studies that have evaluated PCR using broad-range primers for the detection of bacterial aetiology in intraocular specimens have shown an improvement over conventional techniques in the diagnosis of endophthalmitis. Hence, firstly, a PCR using broad-range bacterial primers that code for the 16S rRNA gene common to all bacteria would facilitate the detection of any bacterium present in the clinical sample. This is particularly important because a wide variety of bacteria can cause endophthalmitis. A nested PCR approach increased the sensitivity.11–13

In case of bacterial endophthalmitis, intravitreal antibiotic administration is the method of choice in its treatment. At present, no single antibiotic covers efficiently all bacteria that cause endophthalmitis. Hence, a combination of two drugs are used—one active against Gram-positive bacteria (vancomycin/cefazolin) and the other active against Gram-negative bacteria (gentamicin/amikacin/ceftazidime). Some of these, especially aminoglycosides, show some amount of retinal toxicity. Hence, determination of Gram stain status of the infecting bacterium is extremely important. In a clinical setting, however, the Gram stain is usually negative. Molecular techniques such as PCR combined with DNA probe hybridization and a nested PCR have been described to determine the Gram status of the bacterium, directly using the DNA from the organism. Therefore, PCR using a set of broad-range primers that code for the 16S rRNA gene common to all bacteria to detect the presence of bacteria and subsequently DNA–probe hybridization using a non-radioactive system to discriminate the causative bacteria as Gram positive or Gram negative was evaluated. Knowledge of the Gram reaction of the bacterium in culture-negative intraocular specimens would be of immense help to the clinician to institute a more rational antibiotic therapy.

The preliminary observations in our centre on the aetiology of endophthalmitis in our setting indicate a higher incidence of fungal endophthalmitis compared to the published literature. Fungal endophthalmitis has a latent period of weeks to months and being clinically similar to *P. acnes* and *S. epidermidis* endophthalmitis, is often
Pathogenesis\textsuperscript{14}

Since the source of the pathogen in exogenous endophthalmitis is mainly the conjunctival surface or from the external environment, the first step in the pathogenesis is the adherence to the ocular surface. The epithelium of the conjunctiva and the cornea is susceptible to attachment by microbial proteins. However, for exogenous intraocular infection to occur, a breach in the corneoscleral surface is necessary. This is the reason behind intraocular surgery and penetrating trauma, being the main pre-disposing factors in the development of endophthalmitis. Inside the vitreous cavity, the microbes are ingested by wandering phagocytic cells that can ingest and destroy them. The vitreous does not contain soluble anti-infective factors. After tissue injury and microbial invasion, the ocular blood vessels respond by increasing capillary dilatation and permeability with intraocular migration of inflammatory cells and antimicrobial proteins. Even if factors such as complement system do not completely eradicate intravitreal microbes, they can provide some protective effects against small numbers of bacteria. The reasons for loss of visual acuity are destruction of intraocular tissue due to direct action of the infectious agents, release of toxins and enzymes from bacteria and destruction caused by the local inflammatory response to the infectious agent. In addition, WBCs from the host defences also produce proteolytic enzymes in an effort to digest the invading bacteria, which are toxic to the eye. When these two processes run unchecked, the net effect is destruction of the visual and structural potential of the eye. On many occasions, the eyes with endophthalmitis are successfully sterilized but are completely disabled by the damage that had been inflicted by the secreted toxins. Sequestration of the organism within the closed compartment of the capsular bag or between IOL optic and intact posterior lens capsule may play an important role in the pathogenesis of delayed endophthalmitis. The anaerobic Gram-positive bacillus \textit{P. acnes} enjoys a nearly perfect anaerobic growth condition in this environment. Other potential physical refuges from marauding leucocytes may be provided by early postoperative fibrin reactions. In case of infection with \textit{P. acnes}, lipolytic enzymes produced by the organism may support an inflammatory response—this is the reason behind it in \textit{acnes vulgaris}. In the case of \textit{S. epidermidis}, such refuge may be rendered by the potential of this bacterium to produce a protective glycocalyx or extracellular slime matrix following initial polymer surface adhesion and reproduction. This protection works two-fold—first, against the host’s immune response protecting the bacterium from opsono-phagocytosis, and second, against anti-bacterial drugs physically preventing them to penetrate the matrix.

Aetiological Agents\textsuperscript{15,16}

It is recognized that specific organisms are more likely to cause endophthalmitis in different clinical categories of presentation. The most common...
organisms responsible for postoperative endophthalmitis include Gram-positive bacteria accounting for 57–90% of the cases followed by Gram-negative bacteria (3–22%). Among the Gram-positive bacteria, *S. epidermidis* is the most common causative agent in a majority of the cases. The other common Gram-positive bacteria include *S. aureus*, *Streptococcus pneumoniae*, *Streptococcus viridans* and *Streptococcus pyogenes*. Among the Gram-negative bacteria isolated, *Pseudomonas aeruginosa* is the most common, although others such as *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Escherichia coli* and *Enterobacter aerogenes* have been implicated. *P. acnes* endophthalmitis is now recognized as the causative agent in the majority of the cases of chronic indolent intraocular inflammation after ECCE with implantation of posterior chamber IOL. Postoperative fungal endophthalmitis is uncommon, but many different fungi often considered as saprophyles or opportunistic pathogens (e.g. *Aspergillus*, *Penicilium*, *Paecilomyces*) have been associated with postoperative endophthalmitis.

**Bleb-Associated Endophthalmitis**

Patients with surgically produced filtering blebs for glaucoma or blebs resulting inadvertently after intraocular surgery are susceptible to the development of endophthalmitis months or years after surgery. The most common isolates are *Streptococcus* spp. and *H. influenzae*.

**Posttraumatic Endophthalmitis**

Endophthalmitis following penetrating eye injuries has a relatively poor prognosis due to the underlying eye trauma and frequency of association with more virulent bacteria such as *Bacillus* spp. At the time of penetrating trauma, organisms from the environment or from the perocular surface of the patient gain access to the globe causing infection. Factors associated with an increased risk of developing endophthalmitis following penetrating trauma include a retained intraocular foreign body (IOFB), a delay in wound closure of less than 24 hours, a rural setting for the injury and a disruption of the crystalline lens. The spectrum of organisms isolated in post-traumatic compared to other categories of endophthalmitis is different with a high incidence of *Bacillus* spp., especially, *B. cereus*. In addition, Gram-positive cocci—both staphylococci and streptococci—are more common than Gram-negative bacilli and fungal isolates. *Aspergillus* (especially, *Aspergillus fumigatus*), *Fusarium solani* and *Sporothrix schenckii* are among the most important fungi implicated to endophthalmitis due to non-surgical trauma.

**Endogenous Endophthalmitis**

Endogenous endophthalmitis or metastatic endophthalmitis is defined as an inflammatory process of the internal ocular spaces secondary to an infective focus elsewhere or generalized sepsis. Endogenous endophthalmitis is characterized by haematogenous spread of microorganisms from a focus to the ocular blood vessels, followed by their crossing of the “blood ocular” barriers, their deposition and subsequent multiplication in the ocular tissue and the host’s inflammatory response. Structural defect in the globe is not necessary for the infection. Predisposing factors can invariably be identified. Most factors predisposing to sepsisemia can also predispose to metastatic endophthalmitis as well. Prior antibiotic therapy, diabetes mellitus, intravenous (IV) drug abuse, recent surgery, IV catheters, cardiac anomalies and corticosteroid therapy are found in a majority of these patients. Fungi are the most important cause of endogenous endophthalmitis. *C. albicans* is the most frequently reported agent of metastatic endophthalmitis. Metastatic endophthalmitis due to *Aspergillus* may remain localized to the eye, as in drug abusers or it may occur in the course of disseminated aspergillosis.

**Laboratory Diagnosis of Endophthalmitis**

The specimens that aid in the diagnosis of endophthalmitis include anterior chamber fluid, vitreous cavity contents, iris, IOL, remnants of lens and lens capsule, and at times the eviscerated contents of the globe or an enucleated globe. Intraocular fluid specimens for culture are obtained using the operating microscope. A diagnostic AC tap is performed by passing a 25-G needle attached to a tuberculin syringe through the limbus into the AC. Approximately 0.1–0.2 ml of aqueous is aspirated in the syringe. The aqueous and vitreous specimens are usually sent to the laboratory in the same syringes used for collection with a sterile rubber cork stuck onto the needle. Specimens are ideally processed within half an hour of collection. If the laboratory is not located nearby, the smears can be made and cultures inoculated in the operation theatre itself and then transported. Transport media of the conventional kind have no place in the transport of aqueous and vitreous fluids.

**Microscopy of the Smears**

The specimens can be concentrated using a cytopin machine to obtain a compact smear. It results in a uniform layer of flattened, well-preserved cells that are particularly well suited for cytological examination. Though Gram stain provides rapid diagnostic information, it may be inappropriately negative or inconsistent with culture results in approximately two-thirds of the cases.
The other commonly used staining method is potassium hydroxide–calcofluor–white. This is an extremely sensitive technique in the detection of fungal elements in the intraocular specimen. It is rapid and easy to perform, but it is not a permanent preparation. This fluorochrome also combines with polysaccharides such as chitin that are present in fungal cell walls and in the exocyst of Acanthamoeba.

**Cultures**

Culture of the intraocular specimen is considered the gold standard in the diagnosis of endophthalmitis. Inoculation of media for culture of bacteria and fungi is done first, because the number of organisms is likely to be low and every chance is given for them to multiply. A variety of media are generally included for the favourable growth of both aerobic and anaerobic bacteria. However, the number of media used depends on the volume of sample available. Sometimes, emergency surgeries for an open globe are performed at odd hours and weekends, and these standard culture media may not be available. In such cases, it is recommended that inoculation of standard blood culture bottles or liquid media provided by the microbiology department to the operation theatres should be done. Such inoculation can be performed by surgeons immediately in the theatre and minimizes handling of the specimens, thereby reducing the risk of contamination. The criterion laid down for a positive culture is the growth from the AH/VF on two or more media, confluent growth on one or more solid media at the inoculation site or growth in a single medium correlating with direct smear findings or repeated isolation of the same organism from two or more intraocular specimens of the patient.

**Antimicrobial Susceptibility Testing**

The standard procedure of Kirby–Bauer disc diffusion is the procedure generally utilized in the antibiotic susceptibility testing of intraocular aerobic bacteria. The results of this test relate to the level of the drug achievable in the serum and not to the concentration of the drug in the intraocular tissues which is likely to be very high as fortified antimicrobials are introduced into the eye for treatment of infectious endophthalmitis. Therefore, results of the antimicrobial susceptibility testing should be carefully interpreted taking into consideration the clinical response to the treatment.

**Molecular Techniques**

The emergence of new molecular methods adapted to the field of medical microbiology has led to improved diagnostic procedures, providing promising tools for the rapid and sensitive detection of bacterial and fungal pathogens. These DNA detection techniques depend on the identification of unique and specific nucleotide sequences of an organism by use of nucleic acid hybridization or DNA sequencing methods. PCR is the simplest and most widely laboratory tested of the in vitro amplification methods. This powerful technique has attracted widespread attention and become essential to clinical diagnostic laboratories, as well as to basic research in molecular biology and evolution. The acceptance of PCR is greatly facilitated by three developments: (a) commercial availability of a heat-stable DNA polymerase, (b) availability of highly efficient thermal cyclers and (c) availability of oligonucleotide primers made possible by the widespread availability of highly efficient chemistries and automated DNA synthesizers.

The acquisition of pathogen-specific DNA sequence information is now much more accessible to laboratories leading to a virtual explosion in specific sequence data useful for designing diagnostic probes, and much of this diagnostic information can now be accessed through sequence databases.

PCR product authenticity can be determined by a number of simple methods:

- Agreement between observed and expected sizes of the PCR product,
- Confirmation of the position of a single restriction site within the amplified DNA,
- Dot-blot hybridization or application of the Southern procedure or DNA sequencing.

**Prevention of Infectious Endophthalmitis**

Given the poor visual outcome of many cases of postoperative endophthalmitis, the importance of prevention of this dreaded surgical complication should seriously be considered. As the majority of causative microorganisms in acute postoperative endophthalmitis come from the patient’s own periocular flora, efforts to reduce their numbers should decrease the prevalence of endophthalmitis. Preoperative topical antibiotics are proved to reduce the periocular bacterial flora, but without conclusive evidence of reduction in the prevalence of endophthalmitis. Preparation of the eye at the time of surgery is most critical. Irrigation of the surface of the eye with 5% povidone–iodine prior to surgery shows good evidence of reduction in the infection rate. Isolation of the eyelid margin and eyelashes from the surgical field with adhesive draping minimizes contamination of the surgical field and reducing the risk of infection.

Intracameral cefuroxime given at the completion of cataract surgery appears to have decreased the incidence of endophthalmitis by five-fold. Postoperative topical antibiotics and subconjunctival antibiotic injection achieve bactericidal levels in the aqueous for up to 12 hours after...
surgery but not in the vitreous. While these methods are commonly used, evidence that they reduce the rate of endophthalmitis is lacking.

Endophthalmitis, an inflammatory condition of the intraocular cavities (i.e. the aqueous and/or vitreous humour), is commonly caused by infection. Non-infectious (sterile) endophthalmitis may result from various causes such as retained native lens material after a surgery such as cataract extraction or from toxic agents. Panophthalmitis is inflammation of all coats of the eye including intraocular structures. Two types of infectious endophthalmitis clinically observed are exogenous and endogenous (i.e. metastatic). Exogenous endophthalmitis results from direct inoculation of an organism from the outside as a complication of ocular surgery, foreign bodies, and/or blunt or penetrating ocular trauma. Endogenous endophthalmitis results from the haematogenous spread of organisms from a distant source of infection. Endophthalmitis is an ophthalmologic emergency as the patient is likely to develop decreased or permanent loss of vision. Encleation to eradicate a blind and painful eye may be required. History should be focussed towards practices or procedures that may have been responsible for endogenous or exogenous endophthalmitis. An emergency referral to an ophthalmologist is indicated if endophthalmitis is diagnosed. The most important laboratory investigations for endophthalmitis are Gram stain and culture of the aqueous and vitreous obtained by the ophthalmologist. PCR including real-time polymerase chain reaction (RT-PCR) has improved diagnostic results over traditional smear and culture methods. Treatment depends on the underlying cause of endophthalmitis. Empiric antimicrobial therapy must be comprehensive and should cover all likely pathogens in the context of the clinical presentation. Final visual outcome is heavily dependent on timely recognition and treatment. Multiple different approaches to treatment have been made as per the requirement of individual patient. The rate of preservation of visual acuity is the most significant outcome. The prognosis is extremely variable because of the variety of microorganisms involved and also related to the patient’s underlying health conditions such as diabetes. The visual acuity at the time of the diagnosis and the causative agent are the most predictive of the outcomes. Given the poor visual outcome of many cases of post-operative endophthalmitis, the importance of prevention of this dreaded surgical complication should seriously be considered. As the majority of causative microorganisms in acute postoperative endophthalmitis come from the patient’s own periocular flora, efforts to reduce their numbers should decrease the prevalence of endophthalmitis. Preoperative topical antibiotics are proved to reduce the periocular bacterial flora, but without conclusive evidence of reduction in the prevalence of endophthalmitis. Irrigation of the surface of the eye with 5% povidone–iodine prior to surgery shows good evidence of reduction in the infection rate. Intracameral cefuroxime given at the completion of cataract surgery appears to have decreased the incidence of endophthalmitis by five-fold.

Summary
In recent years, our knowledge of the causes, pathogenesis, laboratory diagnosis and treatment of endophthalmitis has vastly improved. We have a better understanding of the fact that relatively avirulent or non-pathogen or commensal organisms can cause serious infections when introduced into the eye. On one hand, serious efforts are being made to design rapid diagnostic modalities such as PCR to detect the etiological agent and to facilitate prompt institution of specific therapy. On the other hand, several studies are on to investigate newer molecules with better antimicrobial activity and lesser ocular toxicity. Progress has been very evident in both these areas.

References


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