Diagnosis of Fungal Endophthalmitis

Subjects: Ophthalmology

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In large case series of fungal endophthalmitis (FE), the most frequent etiologic agents for all types of FE are molds (usually Aspergillus species, while Fusarium is the prevalent etiology in keratitis-related FE). Candida was the organism found in most cases of endogenous FE. Lately, polymerase chain reaction (PCR) was increasingly used for the diagnosis of FE, allowing for very high diagnostic sensitivity, while the costs become more affordable with time. The most important shortcoming of PCR—the limited number of pathogens that can be simultaneously searched for—may be overcome by newer techniques, such as next-generation sequencing. There are even hopes of searching for genetic sequences that codify resistance to antifungals.

Keywords: fungal endophthalmitis ; polymerase chain reaction ; Diagnosis

1. Introduction

Endophthalmitis is a serious ophthalmic condition, carrying the risk of permanent visual loss. Knowledge of its diagnosis and treatment is of essence for every ophthalmologist. In endophthalmitis the internal structures of the eye are invaded by replicating microorganisms, resulting in an inflammatory response [1]. The term endophthalmitis is usually reserved for bacterial or fungal infections, while inflammation of viral or parasitical cause is considered a form of uveitis. The causative organism may be directly inoculated into the eye (exogenous endophthalmitis, usually posttraumatic or post intraocular surgery) or may enter through hematogenous spread from distant foci (endogenous endophthalmitis). In fungal endophthalmitis (FE), the causative organism is either a mold or yeast.

The diagnosis and treatment of FE are challenging due to a series of particularities, especially the difficult, time consuming etiological diagnosis and the problems of antifungal therapy (availability, efficacy, potential toxicity). The relative rarity of the FE has led to the fact that there is no level 1 evidence to guide its management [2].

2. Etiology and Pathogeny

In the largest retrospective study of patients with FE, which took place in India, 39.0% were caused by *Aspergillus* species, 15.1% by *Candida* species, 15.9% by *Fusarium* species, and 30.0% by other fungi [3]. Another large retrospective review of endophthalmitis cases from North India also found that *Aspergillus* was the predominant cause of FE (36.1%), followed by *Fusarium* (26.4%) [4]. A classic scenario is an ocular trauma that took place in a rural setting, perhaps involving contamination with soil or vegetal matter.

In a recent review of 59 articles, the most common etiology of post-cataract FE was *Aspergillus*, followed by *Fusarium*. [5].

In a retrospective study of patients from South India diagnosed with *Aspergillus* endophthalmitis, 46% of cases were associated with penetrating trauma, 33% were acute -onset postoperative cases, 6.5% delayed-onset postoperative cases and 11% endogenous [6]. *Aspergillus flavus* was the commonest infecting species. Also, in retrospective series of posttraumatic fungal endophthalmitis (patients from eastern China), 74.3% of cultures have grown *Aspergillus* species [7].

In a large series of patients with fungal keratitis, 9.4% developed an endophthalmitis. The risk factors for endophthalmitis were: topical steroid use, previous corneal laceration suturing, large corneal ulcer size (≥10 mm diameter), hypopyon and aphakia, while the advent of corneal perforation was not a significant risk factor. The most frequent etiologies were *Fusarium* (40.5%) and *Aspergillus* (16.2%) [8]. In another series of patients from northern China, 73.3% of FE associated with fungal keratitis were caused by *Fusarium* species [9].

Endophthalmitis after intravitreal injections is a very rare occurrence (0.02 to 0.5%) [2], and fungal etiology appears to be extremely rare [10]. There were reports of outbursts of FE following intravitreal injections contaminated with *Bipolaris*

hawaiiensis [11][12], but recently there are fewer and fewer cases like that, probably due to the fact that ophthalmologists have performed less compounding pharmacy-prepared intravitreal injections.

Recently, there have been a few reports of cases of FE in patients hospitalized for COVID-19 pneumonia [13][14][15]. In a retrospective report of 24 patients from India, diagnosed with COVID-19 and endogenous endophthalmitis, 78.5% were of fungal etiology [13].

In a review of several series of patients with endogenous FE, the predominant microorganisms were yeasts (71.4% to 76.1%), most frequently *Candida* species (50% to 65%), while *Aspergillus* was the most frequent mold (in 11.7% to 16.4% of cases) [16]. However, *Aspergillus* was the most prevalent (29.7%) and *Candida* species followed closely (26.6%) in the largest retrospective study from India [3]. In cases of infantile endogenous endophthalmitis, *Candida* species have been characterized as the primary responsible organisms in multiple case series and reports in the United States [17]. The most common systemic risk factors were: prematurity, respiratory disorders, intraventricular hemorrhage, birth trauma, necrotizing enterocolitis, intrauterine hypoxia and birth asphyxia.

The pathogeny of endogenous endophthalmitis is hematogenous dissemination from distant foci; it affects primarily the choroidal space, due to the comparatively large blood flow, and then it spreads into the retina and vitreous [3].

In histopathology studies, *Candida* species seem to sequester preferentially in inflammatory nodules (another explanation for the fact that negative cultures should be interpreted with caution) [18]. There is, however, a clinicopathologic study of enucleated eyes with endogenous FE where the primary focus of infection with *Candida* was the vitreous; whereas subretinal or sub-retinal pigment epithelium infection (with invasion of retinal and choroidal vessel walls) was noted in eyes with aspergillosis [19]. In a murine model of fungal endophthalmitis, the infected retina exhibited induction of inflammatory mediators (TNFα, IL-1β and IL 6) with increased polymorphonuclear neutrophil infiltration. Histological analysis revealed heavy cellular infiltrates in the vitreous cavity, disruption of normal retinal architecture and retinal cell death [20].

3. Diagnosis

3.1. Clinical Diagnosis

In most cases FE does not have an acute clinical presentation. In one study, the mean latent periods were 7 days for post-traumatic FE, 20 days for postoperative and 30 days for endogenous FE [21]. In a retrospective series of posttraumatic FE (patients from eastern China), the time from trauma to the diagnosis of endophthalmitis was 2–4 weeks in 37.1% of patients and over 4 weeks in 42.9% [7]. Delayed diagnosis or initial misdiagnosis was reported in 16% to 63% of cases [22], some cases being initially treated as non-infectious uveitis. Eyelid edema, conjunctival injection, anterior chamber cells, flare or hypopyon, vitreous inflammation and chorioretinitis are frequent but non-specific signs. Focal vitreous opacities (“string of pearls”) are more suggestive for fungal etiology. Endogenous FE may start as flat choroidal lesions that progress to the vitreous cavity and lead to “puff ball” abscesses [3]. Vision loss can be mild in cases with peripheral vitreous lesions (“snowballs”, “snowbanks”) and severe in extensive vitreous and/or anterior chamber inflammation [5].

3.2. Imaging

B-scan ultrasonography is mandatory in eyes where there is no visualization of the posterior segment. While vitreous strands and membranes with reduced mobility are usual findings in endophthalmitis, the presence of a choroidal mass projecting into the vitreous (in the clinical context of an endophthalmitis) is suggestive for FE [3].

3.3. Laboratory Diagnosis

The ophthalmologist confronted with a possible diagnosis of endophthalmitis should perform an anterior chamber and/or vitreous tap before initiating treatment. It is known that anterior chamber tap has a lower diagnostic yield [16]. As often as possible, researchers prefer to sample undiluted vitreous during the beginning of a vitrectomy, using the technique described in the surgical management chapter. While the clinician may not initially suspect fungi as the etiology in a case of endophthalmitis, it is good practice to ask the laboratory to search for bacteria and fungi in the provided sample.

The usual workup includes direct microscopy (using stains as calcofluor white, Gram and Giemsa) and cultures on media such as blood agar, brain heart infusion, thioglicollate broth, potato dextrose agar and Sabouraud’s dextrose agar. It is necessary to incubate the media for 2 weeks before reporting a culture as negative [3].

Kehrmann et al. compared culture techniques in patients with suspected endophthalmitis and found that 100% of grown fungi were detected by blood culture bottles, while broth solution recovered 64% and solid media 46% of grown fungi [23]. It is also usual practice to use blood culture bottles for immediate seeding of undiluted vitreous samples.

In cases of FE correlated with fungal keratitis, corneal scraping is also routinely recommended [5]. In a case series of *Fusarium* endophthalmitis, isolates were initially identified microscopically and the species subsequently confirmed by sequencing the elongation factor alpha (EFα) and internal transcribed spacers [24].

In endogenous FE it is recommended to perform blood cultures, even if they have a low diagnostic yield (9.2% to 25.6%) [16]. In order to maximize the yield, 3 consecutive blood samples should be taken during fever spikes and before systemic treatment [16][25].

The main advantage of culture techniques is that they are available in any hospital and the laboratories have extensive experience using them. Thus, microbiological culture remains the gold standard for the diagnosis of most intraocular infections. However, fungi may have a fastidious nature that makes them difficult to grow in culture (or, in some cases, unculturable). The rate of positive cultures in presumed FE has varied largely, between 30% and 70% [16].

Galactomannan (GM) is a cell wall carbohydrate that is mostly specific for *Aspergillus* species. While the manufacturer has only validated galactomannan detection in serum and bronchoalveolar lavage fluid, Dupont et al., have reported detection in a vitreous sample, suggesting that it might have a diagnostic role in cases with negative cultures and when PCR is not available [26].

β-d-glucan (BDG) is a major constituent of most fungal cell walls, including Candida and *Aspergillus* species. It can be detected in blood of patients with invasive fungal infections such as invasive candidiasis [27]. Chen et al., reported the testing of BDG in samples of intraocular fluids as a meaning of raising a high suspicion of FE (although the BDG concentrations in intraocular fluids of healthy individuals have not been established) [28]. Ammar et al., chose to define serum BDG ≥ 80 pg/mL as test positive and found a sensitivity of 66.7% for fungal chorioretinitis and 100% for endophthalmitis, while specificity was 74.4% [29]. A combination of PCR and BDG testing in patients with culture proven candidaemia and control patients revealed a sensitivity of 90% and a specificity of 79.5% [30].

The use of polimerase chain reaction (PCR) techniques has increased the yield of detection (up to 100%) [31] and has reduced the time necessary for etiological diagnosis. However the number of pathogens that can be simultaneously searched for is limited, due to differences in amplification efficiencies of different primer sets and to the limited number of fluorescent labels [32]. In a retrospective study on eyes suspected of endophthalmitis or infectious uveitis, cultures of aqueous humor or vitreous had 17% sensitivity, while PCR had 85% (remaining relatively inexpensive) [33]. It seems that PCR performed from aqueous humor and vitreous samples have similar diagnostic yields, which may ease the task of the first ophthalmologist who takes the patient into charge: it may be technically and logistically easier to collect a sample of aqueous humor before the initiation of treatment.

To date, the T2Candida panel (from T2 Biosystems) is the only commercial PCR assay platform with extensive clinical validation for the detection of *Candida* [34]. It detects the five major pathogenic *Candida* species: *C. albicans, C. tropicalis, C glabrata, C. krusei* and *C. parapsilosis.* With the advent of the COVID-19 pandemic, technology and expertise to perform PCR assays has known an unprecedented boom, and researchers hope to access it more frequently in the future for the diagnosis of other infectious diseases.

While researchers were able to detect bacterial or fungal DNA in many cases of culture-negative endophthalmitis, the results have to be regarded with caution, and the clinician must balance the clinical and laboratory data while observing the response to therapy. Prior antimicrobial therapy is frequently incriminated for culture-negative endophthalmitis (while the microorganism may be dead, its DNA is still detectable). Other possible explanations are: the presence of fastidious microorganisms, scant (undetectable) bacterial pathogens or even true sterile endophthalmitis associated with antigenic response to a non-infectious pathogen [32].

While PCR techniques impose a limit on the number of pathogens that can be simultaneously searched for, next-generation sequencing (NGS) does not target specific species; it can detect all the different bacteria or fungi that are present in a sample, in one single assay. In a proof of concept study of 34 eyes with presumed infectious endophthalmitis, 44.1% were positive by microbial culture, while 82.3% were positive by the NGS technique. Among the culture negative endophthalmitis cases that showed presence of DNA of bacterial pathogens, 11 of 14 cases had polybacterial infections (4 had a bacterial and fungal infection) [32].

NGS is an emerging technique and it currently has a turnaround time of around 4–5 days. However, once it would become a routine test, Desmukh et al. predicted that a 48 h turnaround time would be feasible. The cost of NGS for metagenomics testing is somewhat comparable to the cost of current microbiological cultures, while promising in terms of reducing diagnostic time and (ultimately) hospitalization time [32].

While NGS, also termed high-throughput sequencing (HTS), has the potential to detect simultaneously and sequence virtually all the DNA seque nces present in a sample, it results in a large number of reads of both host and pathogen DNA. The detecting and interpreting of millions of sequences in order to identify the pathogen is highly challenging [35].

Targeted NGS uses a selective amplification of specific regions of interest inside the genome, prior to massive parallel sequencing. It provides easier downstream analysis and lower cost by allowing more samples to be tested in one run. In a study on vitreous samples from clinically presumed infectious endophthalmitis, Gandhi et al., used extraction and amplification of 16S RNA for the detection of bacteria and ITS 2 region for the detection of fungi. The rate of detection of fungal pathogens in culture-negative samples was 71.9%, again highlighting the prevalence of these pathogens in infectious endophthalmitis patients from South India [35].

The nanopore sequencer is a third-generation sequencing platform that identifies DNA from the change in electrical current resulting from a DNA strand being forced through a nanometer sized pore embedded in a membrane. In another very recent proof-of-concept study, Huang et al., have used nanopore targeted sequencing (NTS) in aqueous humor and vitreous fluid samples from presumed cases of infectious endophthalmitis [36]. NTS identified microorganisms in 94.4% of cases (half of which were culture-negative) [37].

A major critic for the use of genetic sequencing in the purpose of identifying pathogens is the lack of information about susceptibility to antimicrobial treatments. However, knowing the causative species can be helpful for the clinician, narrowing his choice of antimicrobials. There are exciting perspectives for culture-independent, molecular-based identification not only of pathogen fungi, but also of their antifungal resistance mechanisms [38]. To the date, there are no commercial PCR tests to detect mutations associated with antifungal resistance, but the latest developments in next-generation sequencing may allow in the future the detection of selected genes or regions associated with resistance [39] [40].

In summary, the clinician that suspects a diagnosis of FE should perform a vitreous tap (or vitrectomy) and ask for microscopical examination and cultures for bacteria and fungi. For suspected endogenous endophthalmitis, blood cultures should be performed. If the endophthalmitis is keratitis-related, corneal scraping is also helpful. Searching for galactomannan and β-d-glucan in serum is fast and inexpensive.

3.4. Screening for Endogenous Endophthalmitis

As late as 2016, the Infectious Disease Society of America recommended a screening ophthalmological examination of all patients with candidemia [41]. However, recent studies have found that rates of ocular involvement in these patients were as low as 2.9% [42][43]. The American Academy of Ophthalmology has very recently stated that a routine ophthalmologic consultation after laboratory findings of systemic *Candida* septicemia appears to be a low-value practice. They have recommended that an ophthalmologic consultation should be performed in a patient with signs or symptoms suggestive of ocular infection, regardless of *Candida* septicemia [44].

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