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Diagnostic Procedures in Ophthalmology

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Abstract

Determining the cause of intraocular inflammation has important implications both for the treatment and prognosis of uveitic diseases. This chapter describes ocular diagnostic procedures and their indications while mainly focusing on diagnostic vitrectomy. The chapter discusses the history of elective diagnostic procedures; main indications for invasive procedures in the diagnosis of uveitic disease; surgical principles and techniques for each of the diagnostic procedures; descriptions of the various laboratory techniques being used; and selected examples of conditions that may require the use of such techniques.

Keywords: Uveitis, diagnostic vitrectomy, tap

1. Introduction

The term uveitis refers to a large and varied group of disease entities, each with its own set of manifestations. While some may fit textbook characteristics, others may present in a way that baffles us as clinicians and leaves us with a wide differential diagnosis. Determining the cause of intraocular inflammation has important implications both for treatment and for prognosis of the disease. That is where the field of invasive diagnostic procedures comes into place. Through the use of different laboratory techniques, this diagnostic modality adds to the battery of other methods available to the clinician in order to reach the final diagnosis and provide proper management.

This chapter covers ocular diagnostic procedures, while focusing mainly on diagnostic vitrectomy.

2. History

The earliest attempts at elective pars plana vitreous surgery were directed toward cutting opaque vitreous. While still not used for the diagnosis of the etiology, papers as early as the 19th century report on the procedure in the context of ocular inflammation. Bull reported in 1890 on 17 cases in which a pars plana approach (first introduced by Von Graefe in 1863) involving a discission needle introduced through the pars plana was used to cut vitreous membranes resulting from inflammation or hemorrhage. [1]

The 1970s were a period of major advancement in the field of pars plana vitrectomy (PPV), with the introduction of advanced instrumentation, such as the vitreous-infusion-suction-cutter (VISC). Indications for diagnostic procedures and the diagnostic methods themselves were limited at that time. In a review paper from 1974, Michels et al. [1] described vitreous biopsy as a procedure that is rarely needed, with the most frequent indications being mycotic endophthalmitis and reticulum cell sarcoma. The diagnostic methods that were mentioned included only cytology and culture. Vitrectomy for endophthalmitis was also mentioned in this paper, and showed that performing the procedure for this entity was a novelty.

One of the first papers to describe diagnostic vitrectomy was published by Engel et al in 1981. [2] Findings resulting from early procedures in that era included ocular tumors such as reticulum cell sarcoma and leukemic infiltration, as well as infectious entities such as fungal endophthalmitis and acute retinal necrosis (ARN). The methods described there included cytology, histopathology, and ultrastructural studies, with “new” methods such as using a millipore filter and celloidin-bag cell-block techniques.

Since these early days, the field of diagnostic procedures has advanced rapidly. Methods including polymerase chain reaction (PCR), flow cytometry, and other advanced methods introduced in the general field of medicine have been adopted by ophthalmologists for use in ocular diagnostic procedures. With the introduction of these methods, the list of etiologies that can be recognized by invasive diagnostic techniques has also expanded, as will be described here.

3. Indications

Accurate diagnosis of the etiology behind intraocular inflammation is essential in order to provide the proper treatment and management and for prognostic reasons. While the general approach to uveitis patients includes history taking, review of systems, examination, and ancillary tests, at times none of these result in a conclusive diagnosis. In these atypical cases a diagnostic vitrectomy may lead to the correct diagnosis. An example for such an indication is primary intraocular lymphoma (PIOL), which requires a definitive tissue diagnosis to diagnose and commence treatment.[3]

Another indication for this procedure is failure of conventional therapy. While this might result from an intractable disease, it may also be the result of a misdiagnosis, requiring an invasive approach to reach the correct diagnosis.

A third indication is a sight-threatening disease, where the disease rapidity necessitates an invasive approach for diagnosis, and at times also for treatment. Examples for infectious entities that correspond to this description include infectious endophthalmitis [4] and ARN. [5]

4. Surgical principles and techniques

Prior to the procedures described henceforth, an informed consent should be obtained from the patient after discussing the potential for complications. Since modern diagnostic techniques may require special preparation (e.g. special stains or cultures) the laboratory or pathologist should be notified of the procedure and upcoming samples, and any requirements regarding the handling of the sample prior to delivery should be noted.

4.1. Anterior chamber tap

Unlike diagnostic vitrectomy, an anterior chamber tap may be done in an office setting and is less invasive. It is important to note that this procedure yields a smaller amount of fluid for analysis in comparison with diagnostic vitrectomy, and as such may be considered in cases in which a small sample may suffice for diagnosis.

The procedure is done under an aseptic technique. The area around the eye is cleaned with povidone-iodine and a local anesthetic is instilled into the eye. It may be done at the slit lamp or with the patient in a supine position by using binocular loupes. The eye is opened and fixated with a speculum. The conjunctival surface is washed with povidone-iodine solution. A 27-30-gauge needle on a tuberculin syringe is inserted to the anterior chamber using a limbal approach, and 200 to 250 μL of fluid can be obtained. At the end of the procedure, an antibiotic drop and povidone-iodine solution is instilled into the eye and a broad spectrum antibiotic drop is prescribed for several days. [6, 7]

Anterior chamber tap is a relatively safe procedure. Possible complications include trauma to the cornea, lens, and iris; hyphema; corneal abscess; and endophthalmitis. However these complications are rare. [7]

4.2. Vitreous aspiration needle tap

A vitreous specimen for analysis can be obtained by straight needle vitreous aspiration, or vitreous tap. This procedure has the advantages of being easier to perform, being less traumatic to the eye than diagnostic vitrectomy, and offering the ability to perform it in an office setting. Disadvantages of vitreous aspiration include: (1) the risk for retinal detachment from vitreoretinal traction during aspiration [8]; (2) a smaller amount of specimen in comparison to diagnostic vitrectomy as the procedure only yields about 300 μL of ocular fluid [9], which allows for fewer diagnostic tests and possibly a lower yield; (3) it is also not therapeutic, as a

diagnostic vitrectomy could be, since it does not clear a large amount of vitreous (and thus does not allow for better diffusion of intraocular medications, [10] removal of pathogens or improved media clarity [11, 12]).

The procedure is done under an aseptic technique. The area around the eye is cleaned with povidone-iodine and a local anesthetic is instilled into the eye. The eye is opened and fixated with a speculum and the conjunctival surface is washed with povidone-iodine solution. A large-caliber needle is usually needed, such as a 21-gauge hollow needle, mounted on a 1 ml syringe as an aspirating device, which permits better control during the procedure. The needle is directed posteriorly in the direction of the optic nerve head and vitreous humor is obtained. At the end of the procedure an antibiotic drop and povidone-iodine solution is instilled into the eye, and a broad spectrum antibiotic drop is prescribed for several days. [9]

4.3. Diagnostic vitrectomy

The aim of vitrectomy is to try to obtain the maximum possible amount of tissue from which a diagnosis can be made. A small sample volume may reduce the diagnostic yield. A variety of techniques involving the use of 20, 23, and 25 G PPV have been described in the literature. [13-19] An undiluted vitreous sample is obtained using a 3 or 5 mL syringe attached to the vitreous cutter. When the vitrector is cutting the vitreous, the assistant manually aspirates it until the eye softens, and the infusion is turned on. This provides between 1-2 mL of undiluted vitreous. Some authors propose using continuous infusion of air or perfluorocarbon liquid to substitute the vitreous removed from the eyeball which allows obtaining a larger amount of vitreous. [20, 21]

Following collection of undiluted specimen, fluid infusion is initiated and a second syringe is placed on the vitreous cutter to collect 3-10 mL of a diluted vitreous sample. [11, 15, 18] The surgeon may then proceed with core vitrectomy, induction of a posterior vitreous detachment, and peripheral vitrectomy using a standard approach if necessary. [16, 19, 21] Meticulous peripheral vitrectomy in the presence of significant media opacity, as may occur in many uveitis patients, is accompanied by potential complications and should generally be avoided. [21]

Complications

Diagnostic vitrectomy carries the possibility of complications encountered in vitrectomy for other indications, with some added due to the nature of the underlying etiology.

Cataract formation is a common complication after vitrectomy procedures reported to range from 12.5%-80% in 20-gauge PPV and 22.7%-79.3% in small gauge PPV. [22] The rate of cataract progression is higher in individuals older than 50 years. [23]

Retinal detachment is a possible complication of any PPV. In the setting of diagnostic vitrectomy, this complication may be related to the underlying etiology. For example, in cases of viral or fungal endophthalmitis it may already appear at the time of surgery, complicating the diagnostic procedure. [24]

Retinal detachment may also occur as a result of surgery. Iatrogenic retinal tears at the time of surgery may lead to retinal detachment. [25] This complication is especially true in ARN, where necrosis of the retina leads to its atrophy and subsequent retinal break formation. [26] It also may occur due to the development of new retinal breaks postoperatively.

Other, rarer complications of PPV include open-angle glaucoma, [27] retinal and vitreous incarceration, endophthalmitis, and vitreous hemorrhage. [28]

4.4. Chorioretinal biopsy

Chorioretinal biopsy should only be considered when the inflammatory process is localized primarily in the sensory retina, retinal pigment epithelium, or choroid and when in a previous workup neither aqueous nor vitreous samples provided the diagnostic answer. The main indication is diagnosis of a suspected intraocular lymphoma.

It is important to remember that this procedure involves a greater risk, including subretinal hemorrhage, vitreous hemorrhage, and retinal detachment, [29, 30] and should therefore only be used as a last resort.

The procedure may be done using 20 or 23 G 3-port PPV. Prior to the biopsy, undiluted and diluted vitreous samples are collected as described previously. The vitreous is separated over the biopsy site and intraocular diathermy is used to delineate the biopsy site and the border between the lesion and normal retina. A sample size of 1 x 1 mm or 2 x 2 mm is excised using vertical scissors or a diamond blade, while elevating the intraocular pressure temporarily to 70-90 mm Hg to prevent bleeding. The tissue is then grasped using intraocular forceps and removed through the sclerotomy site. Endolaser is applied around the biopsy site and the procedure is ended with long-acting gas or silicone tamponade. [21]

5. Diagnostic testing of vitreous specimens

With the advent of new laboratory techniques, a myriad of options are available for the clinician in the quest for obtaining a correct diagnosis of an unknown inflammatory, infectious, or neoplastic entity.

Of course not all tests should be performed in all cases, and tests should be chosen according to the suspected diagnosis.

5.1. Histopathologic evaluation

A sample is sent to a pathologist following the diagnostic procedure and is immediately processed. The specimen is generally divided into three portions: one third is fixed for routine histopathological evaluation, including light and electron microscopic examination. Another one third is frozen in optimal cutting temperature (OCT) embedding compound for immunopathology (phenotyping of cells by their surface markers) and molecular characterization. The last third portion is sent for culture of microorganisms. If the specimen is not adequate for

all three procedures, frozen sections are recommended, as they can undergo routine histopathology, immunohistochemistry, and molecular analysis. [20]

5.2. Cytology

Cytological evaluation reveals the phenotypes of infiltrating cells in the vitreous. The vitreous specimen is centrifuged and cells are smeared onto glass slides, and then immersed in 95% ethanol for Papanicolaou (Pap) staining or left to dry for Giemsa staining. [20]

The reported sensitivity of cytology in the detection of intraocular malignancy ranges from 31% to 66.7%. This relatively low yield may be due to the presence of immune cells, necrotic cells, fibrin, and debris in the specimen, which may confound the examination. [31] Other reasons include small sample volumes with a low number of malignant cells, inadequate preparation of the sample, and previous administration of corticosteroids. [20]

Cytologic evaluation may also be used to distinguish between a malignant process and an inflammatory disease. An example of an inflammatory etiology that may be diagnosed with the aid of cytology is sarcoidosis. Kinoshita et al. demonstrated multinucleated giant cells in the vitreous in 85.7% of cases and lymphocytes and epithelioid cells in all cases of intraocular sarcoidosis. [32]

An advanced technique for cytology is the use of cell blocks. They are superior to cell smears since cells are accumulated by centrifugation and stored as paraffin blocks. The large number of cells in a compacted area of one section on a slide glass as opposed to sparse cells on a smear leads to a more accurate diagnosis. Paraffin sections also have the advantage of being used for immunocytochemical diagnosis and clonal analysis, such as amplification by PCR of the immunoglobulin heavy chain gene. [33]

5.3. Microbiological analysis

Microbiological cultures are considered the “gold standard” for diagnosis of infectious uveitis. There are different types of media for isolation of the causative agent, including blood agar (for gram-positive or fastidious gram-negative bacteria [34]), MacConkey agar (for most gram-negative rods [34]), and Brucella agar for bacterial infections; Sabouraud dextrose agar for pathogenic fungi and yeast; and shell vial culture for viral infections. Along with the culture, the sample is sent for Gram staining and antibiotic sensitivity tests. [20]

Some fastidious organisms, such as *Propionibacterium acnes* and fungi require holding the culture for at least 1 month to avoid missing their diagnosis. [21]

The sensitivity of culture after diagnostic vitrectomy for diagnosis of chronic infectious uveitis has been reported between 16.7% and 96%. [21] In cases of acute endophthalmitis, the sensitivity of microbiological cultures and stains was shown to be 40-70%. [35] Higher yields are reported with vitreous rather than aqueous samples. [36] Processing both diluted and undiluted vitreous samples increases the sensitivity of vitreous cultures to 57.4%. [37]

The yield of positive cultures from vitreous samples is usually low in cases of fungal endophthalmitis. In a retrospective study by Tanaka et al., positive cultures were only found in 38% of vitreous specimens in patients with endogenous fungal endophthalmitis. [38]

While the utility of Gram stains is limited in comparison with culture (data from the Endophthalmitis Vitrectomy Study showed a yield of 66% for culture and 41% for Gram stain for patients undergoing vitrectomy [35]), they are useful for rapid initial diagnosis of intraocular infection and can help the clinician choose the appropriate antibiotic for the organism prior to culture results.

5.4. Molecular analysis

Molecular analysis of a vitreous specimen is used for two main indications: 1) to diagnose PIOL 2) to detect the DNA of microorganisms in cases of infectious uveitis.

The techniques currently in use for molecular analysis include PCR, an in vitro technique used to amplify small quantities of nucleic acid into analytic amounts [39] and microdissection, which allows the selection and molecular analysis of malignant or atypical lymphoid cells from vitreous samples with a small amount of preserved cells. [31]

In cases of infectious uveitis, several PCR techniques may be used. Over the years new modifications to the basic method, such as real-time PCR and multiplex PCR have been developed. Real-time PCR allows for the characterization of an active infection versus low-grade pathogenicity by quantifying the number of pathogen genomes in a sample. Multiplex PCR allows for the amplification and detection of a number of different sequences at the same time (such as two infectious agents from a single sample). [40]

The addition of PCR to microbiological analysis has been shown to increase the diagnostic sensitivity from 48% to more than 80%. [41] Prior short-term use of intravitreal antibiotics does not affect its ability to amplify DNA. In one series of patients with postoperative endophthalmitis treated with intravitreal antibiotics, PCR of vitreal specimens identified the causative organism in 10 of 16 patients (62%) versus only 3 (18%) with culture only. [42]

As the causative organism is not always known or suspected, a PCR technique that targets a specific microorganism is not always feasible. In such cases eubacterial PCR may be used. It targets the 16S ribosomal DNA (rRNA) common to all bacteria, thereby identifying a wider range of pathogens. [42-44] A similar approach detects the fungal genome in ocular fluids using probes that target the 18S rRNA present in the *Candida* and *Aspergillus* species, and probes that target the 28S rRNA also found in other species, including *Cryptococcus*, *Trichophyton*, *Mucor*, *Penicillium*, and *Pichia*. [45]

For PIOL diagnosis, PCR is used to detect monoclonality within the variable region of the third complementary determining region (CDR3) in the immunoglobulin heavy chain gene of malignant B cells. Single-band detection of immunoglobulin heavy chain rearrangement can be useful in PIOL. [20] In a study by Baehring et al., PCR was 64% sensitive for PIOL and identified immunoglobulin heavy chain gene rearrangements in four samples that were classified as negative for lymphoma based on cytopathology and flow cytometry. Cytology

had 24% sensitivity and flow cytometry had a sensitivity of 36%. [46] In addition, PCR may be used to detect bcl-2 gene translocations in PIOL that were shown to occur in younger patients, suggesting a more aggressive treatment approach. [47]

5.5. Flow cytometry

Flow cytometry is a diagnostic technique that allows for simultaneous analysis of several different cell surface markers. It involves centrifuging diluted vitreous and re-suspension in cell culture medium. The cells are then counted and stained with antibodies to detect cellular surface markers that identify leukocytes. [20]

It has been shown to be useful in the diagnosis of PIOL. [16] It relies on the fact that most PIOLs are composed of monoclonal populations of B-lymphocytes that stain positively for B cell markers (CD19, CD20, CD22) and have restricted expression of κ or λ chains [48]

Davis et al [49] correlated different flow cytometric markers with lymphoma, infection, and idiopathic uveitis. They found that the most sensitive marker for lymphoma was a κ : λ ratio ≥ 3 or ≤ 0.6 , while CD22 and CD20 were specific but not sensitive for lymphoma. For infection they found that the CD8, CD14, and CD11c markers that indicate monocytes and cytotoxic CD8⁺ T lymphocytes were specific, but not sensitive. A CD4:CD8 ratio of ≥ 4 was highly sensitive and specific for inflammatory uveitis.

5.6. Cytokine measurement

B-cell malignancies can secrete high levels of interleukin-10 (IL-10), an immunosuppressive cytokine. Inflammatory conditions are associated with high levels of interleukin-6 (IL-6), a proinflammatory cytokine. [50, 51] IL-10 in PIOL tends to be high, with IL-10:IL-6 ratios greater than 1.0 being suggestive of the disease. This ratio may serve as a useful adjunctive test in the diagnosis of suspected PIOL, while also showing whether there is a significant response to treatment. [31]

Cassoux et al [52] found that mean IL-10 values were 2205.5 pg/mL in the vitreous and 543.4 pg/mL in the aqueous humor in patients with PIOL, while in uveitis patients mean values were 26.6 pg/mL in the vitreous and 21.9 pg/mL in the aqueous. This difference was highly significant.

Since the measurement of cytokine levels is fairly easy, measurement of IL-10 and IL-6 levels is recommended for patients with suspected PIOL. [20]

5.7. Antibody measurement

This indirect method of diagnosing infection is often negative early in the course of the disease as well as in immunocompromised patients. [31] Intraocular-specific antibody secretion has been shown to confirm the etiology in 23-32% of cases. [53, 54]

A helpful concept in antibody measurement is the Goldmann-Witmer coefficient (GWC). It can be calculated to compare intraocular antibody production with serum antibody levels. A

ratio of greater than 1.0 is abnormal and ratios of 2-3 are considered significant. [55] Its accuracy has been shown in the case of toxoplasmosis. [56] Errera et al have shown that GWC testing had better sensitivity than PCR in ocular toxoplasmosis, especially when the test was carried out in younger patients with quiet eyes, with smaller sized chorioretinal lesions. In contrast, they have shown that this test was not helpful in viral retinitis in comparison to PCR, as the sensitivity and positive predictive value (PPV) were lower for GWC. [57]

6. Selected examples

6.1. Infectious etiologies

6.1.1. *Bacterial and fungal endophthalmitis*

In cases of suspected bacterial or fungal endophthalmitis, Gram stain and culture (aerobic, anerobic, and fungal) of the vitreous sample are performed in order to identify the causative organisms and their susceptibilities.

As mentioned above, PCR analysis of aqueous and vitreous fluid have also been applied in case series of patients with acute and delayed postoperative endophthalmitis, sometimes with better detection of the causative agent than cultures.

6.1.2. *Mycobacterium tuberculosis*

Diagnosis of ocular tuberculosis (TB) is possible with the use of various tests for the detection of systemic TB, including chest radiography, Purified Protein Derivative (PPD) tuberculin skin test, Interferon Gamma Release assays (IGRA), and analyses of extraocular sites. [58, 59] Intraocular fluid analysis may help.

Traditional fluid analysis with Ziehl-Neelsen staining and culture on Lowenstein-Jensen medium is not ideal, as the former has low yields and the latter takes up to 6-8 weeks, limiting its clinical utility. [58, 59] The yield of PCR analysis of aqueous and vitreous fluid has been shown to range from 37.7% to 72% in a series of Indian patients. [60-62] It was shown that 77-80% of PCR-positive patients in these series were PPD positive, and 90-100% of PCR-positive patients who were treated with antitubercular treatment had resolution of inflammation. Similar rates were shown by a Mexican group, [63] where PCR testing in 22 patients with a known diagnosis of TB uveitis showed a yield of 77.2%. All patients improved with antitubercular treatment.

6.1.3. *Toxoplasma gondii*

While diagnosis of ocular toxoplasmosis is typically made by a characteristic clinical presentation and supported by positive serology, there are cases that pose a diagnostic dilemma in which an invasive ocular diagnostic procedure may be needed. For example, in immunocompromised and elderly patients the disease may mimic viral necrotizing retinitis. [64, 65]

Culture of *T. gondii* from the vitreous may be lengthy and ranges from 2 to 23 days for positive cultures. [66] The rapid detection of toxoplasmosis DNA using PCR techniques on aqueous fluid has a yield of 13% to 55% according to literature, with positive results occurring more often with larger chorioretinal lesions, immunosuppressed patients, and active anterior segment inflammation. [21] Antibody levels in the aqueous may supplement PCR results by calculating the GWC, as described above. In one series, calculation of the aqueous GWC for toxoplasmosis antibody at the onset of clinical manifestation had a yield of 57%, rising to 70% after 3 weeks. [67]

The utility of GWC is decreased in immunocompromised patients. In one series of 34 immunocompetent patients with negative PCR tests for toxoplasmosis, 25 had a positive GWC, whereas none of the immunocompromised patients exhibited a positive test. [56] In a similar fashion, another series showed 93% positivity with use of this test in immunocompetent patients, in comparison with a yield of only 57% in immunocompromised patients. [68]

While these results deal with aqueous analysis, less data appears in the literature regarding diagnostic vitrectomy for this purpose. Available data shows a trend towards improved yields for PCR from vitreous specimens. [21]

6.1.4. Viral retinitis

The diagnosis of infectious viral retinitis caused by herpes simplex virus (HSV), cytomegalovirus (CMV), or varicella zoster virus (VZV) is not always straightforward. As with the other infectious entities just mentioned, growth on culture may take a long time. PCR analysis of aqueous and vitreous fluid plays an important role thanks to its high sensitivity, low false-positive rates, and the rapidity of the assay. [69, 70]

The sensitivities of PCR for VZV, HSV, and CMV were reported to exceed 90%, with specificities in excess of 95%. [39] Knox et al. performed PCR on specimens from 38 eyes of 37 patients with an inflammation of unknown etiology suggestive of an infectious posterior segment disease. In 24 of these cases CMV, HSV, or VZV were detected. [71] Sugita et al. collected 68 aqueous humor samples and 43 vitreous fluid samples from 100 patients with uveitis. The samples were assayed for human herpes viruses using multiplex PCR and real-time PCR. Out of 16 patients with ARN, either HSV1, HSV2, or VZV genomes were detected. In another 10 patients with anterior uveitis with iris atrophy, the VZV genome was detected. Epstein-Barr virus was detected in 17% of samples, and (CMV) was detected in three patients with anterior uveitis of immunocompetent patients and in one immunocompromised CMV retinitis patient. [72]

As was shown above for toxoplasmosis, calculation of the GWC may also be of use for HSV, VZV, and CMV, although variable results have been reported in the literature. In one series of immunocompromised patients with posterior uveitis and panuveitis, analysis of an aqueous sample demonstrated a detection rate of 94% for PCR aimed for the detection of CMV and VZV versus only 18% with GWC. [73] Another series demonstrated an identification of 92% of HSV-associated and 87.5% of VZV-associated infectious uveitis using GWC, in comparison with 54% of HSV and 75% VZV cases that were identified using PCR. [74]

6.2. Non-infectious inflammatory conditions

6.2.1. Sarcoidosis

The frequency of sarcoidosis involving the posterior segment varies in different series. One group reported that as many as 89% of patients with ocular sarcoidosis demonstrated posterior segment involvement, with vitritis as the most common manifestation, present in 69% of these patients. [75] As the manifestations of sarcoidosis are varied, a diagnosis of this inflammatory entity is not always straightforward, and may require an invasive procedure such as diagnostic pars plana vitrectomy.

An increased CD4+ helper T-cell type 1 lymphocyte subset in bronchoalveolar lavage (BAL) fluid and a high CD4/CD8 ratio are helpful for the diagnosis of sarcoidosis. [76] Kojima et al. demonstrated that this ratio may also be applied for vitreous specimens, when a CD4/CD8 ratio of vitreous-infiltrating lymphocytes greater than 3.5 provided a diagnosis of ocular sarcoidosis with a sensitivity of 100% and a specificity of 96.3% (in comparison with a sensitivity of 53% and specificity of 94% in analysis of BAL fluid). [77]

6.3. Neoplastic processes

6.3.1. Primary intraocular lymphoma

PIOL is considered one of the masquerade syndromes, or diseases that mimic inflammatory conditions in presentation, leading to a diagnostic dilemma. [78] When the diagnosis of a neoplastic process such as PIOL is suspected, reaching a diagnosis is of utmost importance in terms of prognosis and the choice of treatment.

A definitive tissue diagnosis is required to make the diagnosis of PIOL. If lymphoma is identified from a lumbar puncture, an invasive diagnostic ocular procedure may not be required. If, on the other hand, lumbar puncture results are inconclusive and neuroimaging is not consistent with CNS lymphoma in a patient with a high index of suspicion for PIOL, invasive diagnostic procedures are appropriate. [3]

Histologic identification of malignant lymphoid cells is the gold standard for diagnosing PIOL. [3] As stated above, it is pertinent to communicate with the pathologist before the procedure, as any delay in delivery of the sample may result in death of acquired cells.

The characteristic features of PIOL using microscopic analyses include large atypical lymphoid cells with scarce cytoplasm, prominent nucleoli, frequently large segmented nuclei, and a high nuclear to cytoplasm ratio. [3] Cytology has a sensitivity ranging from 31% to 66.7% for detecting intraocular malignancy, and one report showed a sensitivity of 83.3% for detecting PIOL. [31, 49] In addition to cytology, immunohistochemistry, cytokine analysis, flow cytometry, and gene rearrangements by PCR are also performed on the specimens. [3]

6.3.2. Tumor metastasis

Tumor metastasis is the most common cause of intraocular malignancy in adults. [78] While their typical appearance and preexisting history of cancer typically lead to diagnosis, uveal metastases masquerading as intraocular inflammation have been reported. [21]

A few cases were reported on the use of aqueous sampling for cytology which led to the diagnosis of metastases masquerading as anterior uveitis. [79-84] Of patients undergoing diagnostic vitrectomy for uveitis of unknown cause, detection of metastasis from cytology results was rare in the literature, [11, 85, 86] with only one case reported in each of these series. In case reports of patients with the rare occurrence of tumors metastatic to the retina and vitreous, these conditions present as intermediate uveitis, vitreous hemorrhage, or retinal vasculitis with vitreous cytology and retinal biopsy assisting in diagnosis if no primary malignancy is identified. [21]

In a series of 159 cases by Shields et al, [87] transocular fine needle aspiration (FNA) biopsy led to an adequate sample collection in 88% of cases, with a sensitivity rate of 100% and specificity rate of 98%, leading to diagnosis of intraocular malignancies such as uveal melanoma, uveal metastasis, retinoblastoma, lymphoma, and leukemia. In another series of 39 patients with uveal metastasis undergoing ocular biopsy of the tumor, 25 G vitrectomy had a yield of 100% for cytologic diagnosis. It indicated the site of origin in 24 out of 27 patients without a known primary tumor. [88]

7. Summary

Diagnostic procedures in ophthalmology have gone a long way from the early days of pars plana vitrectomy, when instrumentation and diagnostic methods were limited and the amount of entities that could be diagnosed by invasive methods was restricted.

As this chapter has shown, the approach to a patient with a cryptic diagnosis, a rapidly deteriorating disease, or treatment failure has changed in the last decades and ophthalmologists now have in their arsenal a battery of tools to help in the diagnosis of cases that were once considered unsolvable or untreatable.

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