Review Article

Ancillary investigations in uveitis

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Ancillary investigations are the backbone of uveitis work-up both for anterior and posterior segment diseases. They help in making the diagnosis, ruling out certain differential diagnosis and monitoring inflammation during the follow-up. This review aims to be an overview describing the role of commonly used investigations for uveitis.

Key words: Uveitis; imaging; fluorescein angiography; optical coherence tomography

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Ancillary investigations are very useful in diagnosing the pathologies and monitoring inflammation in the anterior as well as posterior segment. The most commonly used ancillary investigations in uveitis include laser flare meter for anterior segment inflammation, ultrasound biomicroscopy (UBM) for ciliary body region, fundus photography and fundus fluorescein angiography (FFA) for retinal diseases, indocyanine green angiography (ICGA) for choroidal pathologies, fundus autofluorescence (FAF) for retinal pigment epithelium (RPE) and optical coherence tomography (OCT) as well as ultrasonography for posterior segment. This review aims to be an overview describing the role of each of these investigations.

Laser Flare Photometery

The laser flare/cell photometer is an instrument that comprises of a constant power helium-neon or diode laser light beam, which is directed into the anterior chamber at an angle of 45° to the antero-posterior axis.[1] A photomultiplyer-photodetector unit then detects back-scattered light from the incoming beam.[2] Two types of measurements are made; (i) Laser flare photometers where the level of back scattered light is reflected from small molecules such as proteins in the anterior chamber. The measurement units of backscattered light from small molecules are a number of photons per miliseconds (ph/ms)[2,3] (ii) laser flare-cell photometer where the back-scattered light from small molecules (proteins) as well as from larger particles such as inflammatory cells is measured. The measurement units for particles/cells are a number of particles/cells counted in a volume of 0.075 mm.[4,5]

Laser Flare Photometery (LFP) is the only quantitative method to measure intraocular inflammation and has been reported to be superior to slit-lamp flare and slit lamp cells to assess and monitor anterior chamber inflammation.[6-9] Laser flare photometer is useful in diagnosing subclinical inflammation, monitoring response to an intervention as well as to detect the disease relapses. A flare increase >20% over the flare level at the previous visit during the regular follow-up period, has a positive predictive value for disease relapse.[10] Beside monitoring the disease response to therapy and helping titrating the dosage of therapy, early changes in flare photometry values following therapeutic intervention also help in establishing an early diagnosis and documenting a therapeutic response in cases when there is a significant decrease of flare before any other clinical parameter change can be detected.[11]

Ultrasound Biomicroscopy

UBM is non-contact, non-invasive, quick to perform ancillary investigation that provides high resolution, cross sectional biomicroscopic images of the anterior structures of the eye. It uses ultrasound of high frequency (35-100 MHz) than conventional ultrasound and is based on the hypothesis that by increasing the frequency, greater microscopic resolution can be obtained.[12,13] This results in visualization of the limited tissue depths within its penetration of approximately 4-5 mm including cornea and anterior segment structures. Rotating the eye as far as possible can scan the conjunctiva, underlying sclera and peripheral retina as well.[14] Tran *et al*. studied[15] proportion of cases for which UBM gave essential information that allowed to reach a diagnosis or to influence the management in patients with uveitis. The UBM was performed in 77 uveitis patients where it was thought necessary and essential information was obtained in 32 of 77 patients (42%).[16] The patients benefitting most of UBM examination where those with opaque media, patients with hypotony and toxocara uveitis where UBM showed pseudocystic vitreous degeneration.[17] UBM plays a very important role in assessing ciliary body pre-operatively in patients with complicated cataract and hypotony. UBM has been reported to show inflammatory cells in the anterior and posterior chamber, exudates as well as swelling of the

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ciliary body in patients with acute anterior uveitis and these signs resolved on treatment.[17] We assessed ciliary body pre-operatively and reported that in eyes with normal ciliary body processes and cyclitic membrane, pars plana lensectomy with removal of ciliary membrane alone was sufficient to restore intraocular pressure (IOP) in the post-operative period. However, if pre-operative ciliary body atrophy was present, additional silicon oil tamponade was required to restore IOP.[18]

Fundus Photography

Fundus color photography helps in making a clinical diagnosis and serves as a very useful tool for monitoring the progression or regression of the inflammations of the retina and choroid. A good agreement has been shown between the retina specialists by interpretation of retinal photographs distinguishing between presumed ocular histoplasmosis and multifocal choroiditis without the need for any ancillary tests.[19] Fundus photography is routinely useful in all cases of posterior uveitis to document the lesions at the baseline that can be used for comparison with document the progression or healing of the lesions. Few of conditions where it is useful include macular edema, epiretinal membranes, retinitis, choroiditis, parasitic infections such as toxocariasis, cysticercosis, onchocerciasis, etc., retinal vasculitis, masquerade syndromes and for assessing media clarity.[20] Stereo photographs may be taken in cases with exudative retinal detachment, optic disc edema, macular and choroidal neovascularization, etc.

Fundus Fluorescein Angiography

FFA has established its role in the clinical practice of uveitis diagnosis as well as management. It is quite useful in differentiating active from inactive uveitis, diagnosing co-existent pathologies such as cystoid macular edema (CME), choroidal neovascularization etc., monitor response to therapy and identifying areas of capillary non-perfusion as well as retinal neovascularization.

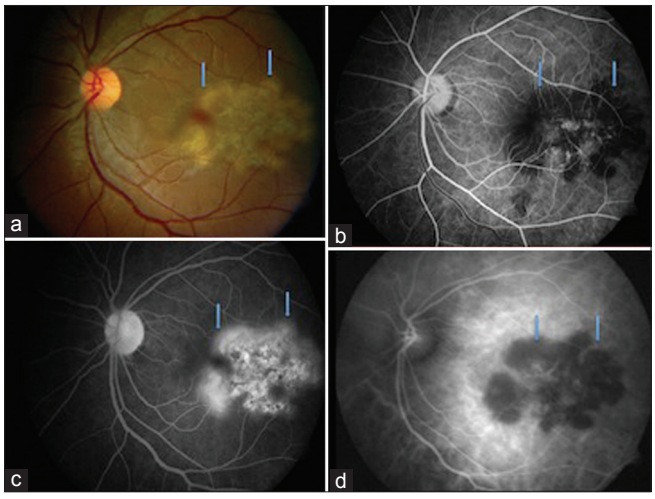

Figure 1: (a) Fundus photograph left eye showing macular serpiginous choroiditis. Arrows indicate the active edge; (b) Fluorescein angiogram shows initial hypofluorescence from the active edges (arrows) with (c) late phase showing diffusion of the dye from the active edge into adjacent retina. (d) Indocyanine green angiogram shows hypofluorescent lesion

FFA is quite useful in following situations

Retinitis/retinochoroiditis

FFA is, especially useful for studying the inflammatory lesions of the retina and retinal vessels. The free unbound fluorescein dye has very small molecules and thus leak out even from minimally inflamed retinal vessels including capillaries; thus making it an investigation of choice for retinal inflammations.[21,22] In retinitis/retinochoroiditis lesions, such as Toxoplasmosis and Behçet's disease, FFA demonstrates early hypofluorescence with dye leakage in the late phase including diffusion of dye into the healthy adjacent retina [Figs. 1 and 2]. Iijima *et al*.[22] reported following characteristics of acute ocular toxoplasmic retinochoroiditis that include a hyperfluorescent lesion with central hypofluorescence; the arterial occlusion passing through the necrotic lesion showing a dark silhouette; venous dilation and leakage and optic disc staining with dye leak.

Retinal vasculitis

Retinal vasculitis occurs in several inflammatory conditions including Behçet's disease, tuberculosis, sarcoidosis, toxoplasmosis, syphilis, systemic lupus erythematosis, Birdshot chorioretinopathy, acute retinal necrosis, idiopathic retinal vasculitis, aneurysms and neuroretinitis (IRVAN), frosted branch angitis and Eales'disease. FFA is particularly useful in the diagnosis and evaluation of the subclinical retinal capillary involvement [Fig. 3] and monitoring response to therapy during the follow-up in disease like Behçet's.[23]

Cystoid macular edema

CME is very commonly associated with most of the uveitis entities that on fluorescein angiogram is seen as telangiectasis of the parafoveal capillaries, with progressive leakage and accumulation of dye in the cystic spaces surrounding the fovea. This characteristic "petalloid" pattern of parafoveal hyperfluorescence is presumably related to the unique anatomy of the parafoveal retina.[24] This results in the typical chrysanthemum "flower petal" pattern seen on FFA. CME has been angiographically graded into the following grades by Miyake:[25]

Grade 0: No sign of fluorescein leakage; Grade I: slight fluorescein leakage into cystic spaces but not enough to

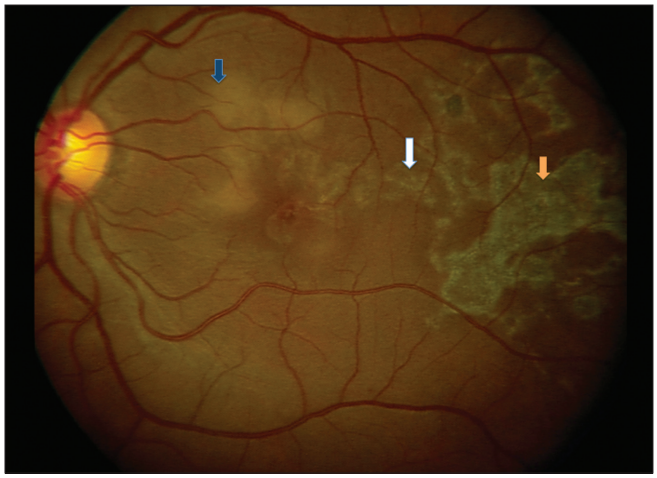

Figure 2: Fundus photograph left eye of a patient with tubercular serpiginous-like choroiditis showing active lesions (blue arrow), partly active (white arrow) and inactive (yellow arrow)

enclose the entire fovea centralis; Grade II: complete circular accumulation of the fluorescein in the cystic space but its diameter is smaller than 2 mm; Grade III: the circular accumulation of fluorescein is larger than 2.0 mm in diameter.

In 1984, Yannuzzi[26] proposed a slightly different classification as follows:

Grade 0: No perifoveal hyperfluorescence; Grade 1: incomplete perifoveal hyperfluorescence; Grade 2: mild 360° hyperfluorescence; Grade 3: moderate hyperfluorescent area being approximately 1 disc diameter across; Grade 4: severe 360° hyperfluorescence with the hyperfluorescent area being approximately 1.5 disc diameter across.

Although FFA is required in the evaluation of uveitic CME, OCT gives more accurate information as it helps to quantify retinal thickening and detect associated serous retinal detachment or vitreomacular traction.

Retinal vascular occlusion and neovascularization**:** FFA is useful in the evaluation of central retinal vein or artery occlusion, complication of retinal vasculitis.[27] FFA is also useful in demarcating the areas of retinal ischemia that is commonly associated with occlusive retinal periphlebitis seen in tuberculosis, sarcoidosis, Behçet's disease, Eales disease and idiopathic vasculitis.[28-30]

Retinal macroaneurysms

FFA is quite useful in detecting macroaneurysms in sarcoidosis and IRVAN.

Choroid

Although FFA is not an ideal investigation for the choroid, some information can be gained on choriocapillaris perfusion within the 1st min of angiography. There may be early choroidal hypofluorescence manifesting as choriocapillaris perfusion delay or non-perfusion in several choroiditis entities, including Vogt-Koyanagi-Harada (VKH) disease and inflammatory choriocapillaropathies, such as serpiginous choroiditis, acute posterior multifocal placoid pigment epitheliopathy (APMPPE) and multiple evanescent white dot syndrome (MEWDS). The fluorescein angiographic pattern is characteristic and diagnostic in VKH disease and sympathetic ophthalmia where it shows initial pinpoint hyperfluorescent dots or areas of delayed choroidal filling with late pooling of dye in subretinal space that maybe associated with optic disc hyperfluorescence.[31-34] In serpiginous choroiditis, the active borders show early hypofluorescence with progressive diffuse staining in late frames. The differential diagnosis of such lesions is choroidal tuberculosis and syphilis that too can produce secondary choriocapillaris perfusion disturbance. In addition, FFA may be useful in diagnosing choroidal neovascular membrane that complicates many of the posterior uveitides.

Indocyanine Green Angiography

ICGA is the method of choice to analyze choroidal inflammatory lesions including the choriocapillaris and the choroidal stroma.[34] ICGA shows occult choroidal lesions that are subclinical and not seen on fundoscopy or even FFA.

The hypofluorescent lesions on indocyanine green (ICG) can result from: (i) Choriocapillaris non-perfusion that shows patchy/geographic disposition in the early and intermediate phase and persists in the late frames; or (ii) Stromal inflammatory infiltration that show as more regular dots with an even distribution in the early phase. If these stromal lesions are full thickness, they shall remain hypofluorescent up to late frames, whereas partial thickness lesions become isofluorescent in late frames. Many a times, these lesions show fuzzy appearance of large choroidal vessels in the intermediate phase and may show diffuse choroidal fluorescence in the late phase.[35,36]

Hyperfluorescence on ICG may be (i) diffuse that results from increased leakage from large inflamed stromal choroidal vessels (ii) when seen at the level of disc indicates severe inflammation (iii) numerous late hyperfluorescent pinpoints are indicative of granulomatous disease.

Based on ICG, choroidal inflammation can be reclassified as inflammation of the choriocapillaris (choriocapillaritis) or inflammation of the choroidal stroma (stromal choroiditis).[37] The entities causing them are as follows:[37]

Choriocapillaris inflammation (primary inflammatory choriocapillaropathies)

- MEWDS/Acute idiopathic blind spot enlargement
- APMPPE
- Multifocal choroiditis/Punctate inner choroidopathy
- Serpiginous choroiditis
- Rare entities: Acute macular neuroretinopathy, acute zonal occult outer retinopathy

Stromal inflammation (stromal choroiditis) further subdivided into two categories

- Primary obligatory stromal choroiditis
  - VKH disease
  - Sympathetic ophthalmia
  - Birdshot chorioretinopathy
- Stromal choroiditis as a random location of a systemic disease
  - Sarcoidosis
  - Tuberculosis
  - Syphilis
  - Other infectious choroiditides

Fundus Autofluorescence

FAF using confocal scanning laser ophthalmoscope (cSLO) is becoming a popular technique of imaging as it allows detecting low intensity autofluorescence (AF) produced by fluorophores such as lipofuscin present in the retinal pigment epithelial cells.[38,39] The lipofuscin originates from the photoreceptor outer segments and its accumulation in the RPE cell lysosomes are an indicator of the quality of the RPE cell metabolism. Since RPE is involved in most of the posterior segment inflammations, FAF imaging provides useful information of the metabolic state of RPE that may be indicative of disease activity. FAF imaging has been used to monitor disease activity in vertiginous-like choroiditis. Recently, we reported four stages where Stage 1 with active edge shows an area of hyper AF at the borders of active edge. As the disease starts healing, the hyper-AF is replaced with hypo-AF. Stage 2 disease shows healing lesions with mixed AF that are predominantly hyperfluorescent. In Stage 3, the lesions that are now progressively healing show mixed AF that are predominantly hypofluorescent. As the

lesions become totally healed with scar, they show Stage 4 pattern, which is totally hypofluorescent.[40] Application of wide-field FAF imaging has shown several peripheral abnormalities including multifocal hypofluorescent spots, hyperfluorescent spots and unique lattice-like pattern in patients with chronic VKH.[41]

Wide-field FAF has recently been reported to correspond to visual field defect-related to alterations of the RPE in uveitis cases.[42]

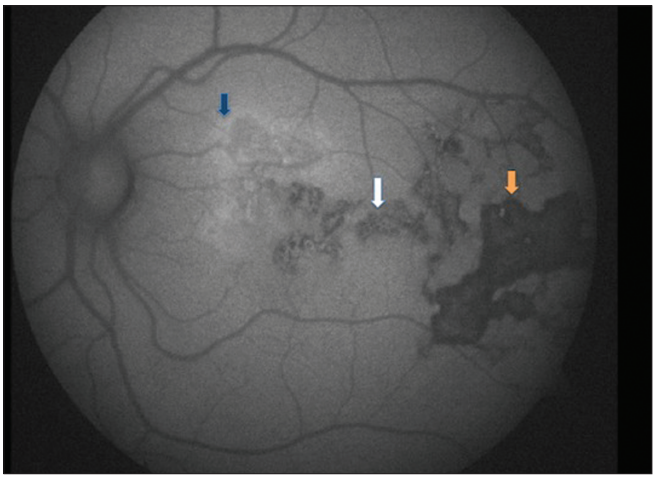

Figure 3: Fundus autofluorescence image of the same eye as in Fig. 2 shows Stage 1 hyper autofluorescence around the active lesion (blue arrow); the partially healed lesions show Stage 2 mixed autofluorescence (predominant hyper-auto fluorescent) while yellow arrow indicates a Stage 3 lesion that is predominately hypo-auto fluorescent

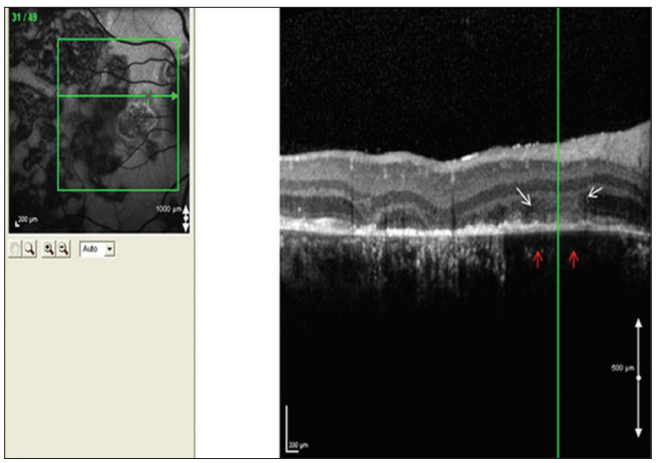

Figure 5: Simultaneous autofluorescence and optical coherence tomography scan through the active lesion of vertiginous choroiditis show an ill-defined area of increased auto fluorescence (left panel) with fuzzy area of hyperreflectivity in outer retinal layers (white arrows) involving the retinal pigment epithelium, photoreceptor outer segment tips, photoreceptor inner segment-outer segment junction, external limiting membrane and outer nuclear layer (right panel). The inner retinal layers showed mild distortion. There was absence of any backscattering from the inner choroid (red arrows). (Reproduced from Gupta V, Gupta A, Dogra MR, editors. Atlas Optical Coherence Tomography of Macular Diseases and Glaucoma. 4th ed., Ch. 25. Jaypee-Highlights Medical publishers; 2012. p. 591-655.)

OCT

OCT has been found to be useful in the imaging of intraocular inflammations in establishing the diagnosis and monitoring response to therapy. It helps in the localization of the pathology by defining the extent, depth and thickness of the inflammatory lesion. It is a very useful tool in quantifying macular edema including CME.[43] When compared with FFA, OCT was found have 89% sensitivity for diagnosing CME.[44] Markomichelakis *et al*.[45] described three patterns of uveitic macular edema: (1) Diffuse macular edema seen as sponge-like thickening of the retina with low-reflectivity seen in 54.8% eyes; (2) CME

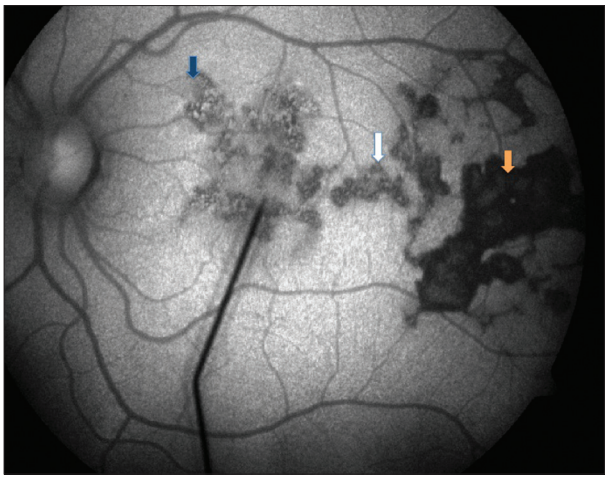

Figure 4: Fundus autofluorescence image of the same eye as in Figures 2 and 3, done after 2 months of treatment with antituberculosis therapy and systemic corticosteroids. The lesions show progressive healing with previously active lesions now showing Stage 2 autofluorescence (Blue arrow). White arrow shows Stage 3 and yellow arrow shows Stage 4 healing patterns

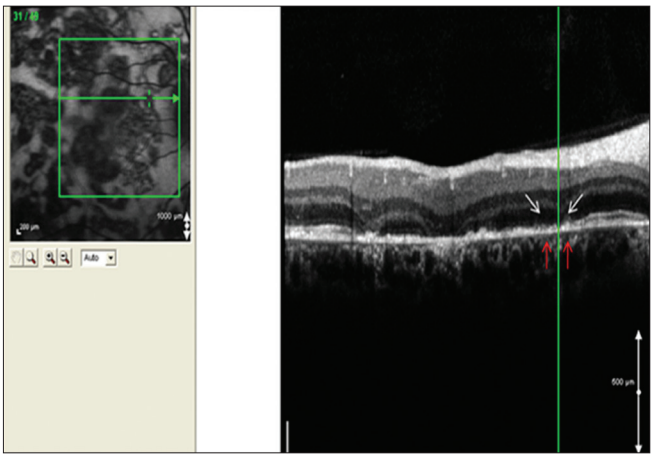

Figure 6: Same eye 3 months later. As the lesions healed further, they appeared stippled with predominantly hypo auto fluorescence (left panel). The spectral domain-optical coherence tomography scan showed loss of retinal pigment epithelium, photoreceptor outer segment tips, inner segment-outer segment junction and external limiting membrane (white arrows) (right panel). The increased backscattering of the choroid persisted (red arrows). (Reproduced from Gupta V, Gupta A, Dogra MR, editors. Atlas Optical Coherence Tomography of Macular Diseases and Glaucoma. 4th ed., Ch. 25. Jaypee-Highlights Medical publishers; 2012. p. 591-655.)

with clearly defined intra-retinal cystic spaces seen in 25%; and (3) Serous retinal detachment with fluid accumulation between RPE and neurosensory retina seen in 5.9% of cases. In addition, 14.3% of the eyes in their series had diffuse macular edema and retinal detachment. It is also very useful in studying the vitreoretinal interface and identifying vitreo-foveal traction in uveitic eyes. In patients with VKH and sympathetic ophthalmia, the OCT is very useful in monitoring serous retinal detachments. During the early stage of VKH disease, the RPE may be elevated because of underlying granulomas; thus, producing choroidal striations.[46] In our experience, the retina inner to external limiting membrane did not show any remarkable structural alteration in VKH and sympathetic ophthalmia patients and the changes seen in the outer retina segment in sympathetic ophthalmia were reversible.[47]

We prospectively compared high definition spectral domain-optical coherence tomography (SD-OCT) (CIRRUS™ HD OCT Carl Zeiss) with time domain OCT (STRATUS™ version 4, Carl Zeiss) for imaging macula in patients of uveitis and found that SD CIRRUS™ HD OCT had an advantage over the time domain stratus OCT in uveitic eyes by providing better identification of both normal and pathological structure in patients with poor media clarity.[47]

Multimodal Imaging

The multimodal imaging is performed with spectralis Heidelberg Retinal angiography (HRA) + OCT that is the combination of a cSLO and a SD-OCT and has a dual-beam scanning system. One laser captures the reference image while other simultaneously captures the SD-OCT scan and the cSLO part of the device allows acquiring reflectance images, angiography images (both fluorescein and ICG) and AF images. The SD-OCT part allows acquiring cross-sectional and volume images. The Spectralis HRA-OCT allows capturing of following individual images: (1) Infrared reflectance imaging (2) Red-free imaging (3) Fluorescein angiography (4) ICGA (5) AF and (6) OCT imaging [Figs. 3-6]. The different imaging modes can be used either alone or simultaneously in different combinations. Spectralis offers a unique technique of enhanced depth imaging (EDI) that produces high-resolution cross-sectional images of the whole thickness of the choroid and is very useful for studying diseases involving the choroid. The contour, architecture and thickness of the choroid can be assessed using EDI. Fong *et al*.[48] reported a loss of focal hyper reflectivity in the inner choroid in patients with VKH, a feature that is consistently observed by independent masked observers. The presence of this feature was seen in both acute as well as convalescent phases and authors hypothesized that it could represent permanent structural change to small choroidal vessels.

Ultrasonography

Ultrasonography may be useful in the evaluation of intraocular inflammatory conditions, especially when visualization of the fundus is poor due to media haze. Ultrasonography is useful in assessing the location, extent and density of vitritis. The 20-MHz frequency probes can detect the typical snow bank in intermediate uveitis.[49] Ultrasonography is also useful in the detection of posterior vitreous detachment, a common finding in eyes with vitreous inflammation.[50] Ultrasound can be used to monitor serous retinal detachments in VKH disease and sympathetic ophthalmia. However, currently, OCT is a preferred modality for monitoring serous detachment. The diagnostic ultrasound still has a role in acute VKH disease where it typically shows diffuse, low-to-medium reflective choroidal thickening most evident in the posterior pole. It is also an important diagnostic modality in diffuse posterior scleritis where it shows high-reflective sclero-choroidal thickening. Scleral edema associated with fluid within Tenon's space results in an echoluscent region just posterior to the sclera results in the classic "T" sign.[50,51]

Summary

Ancillary investigations are important for establishing the clinical diagnosis and in most cases, these investigations supplement the information obtained on clinical examination and laboratory investigations. The choice of investigation would depend upon the site of inflammation and many a times, a number of ancillary tests may need to be performed in combination to get the useful information.

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