Uveitis Society (India)

NEWSLETTER

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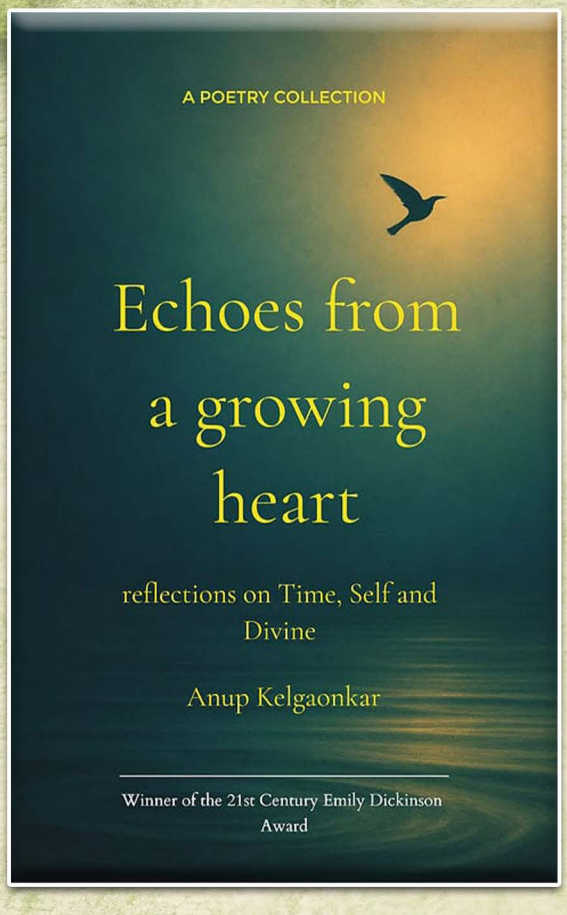
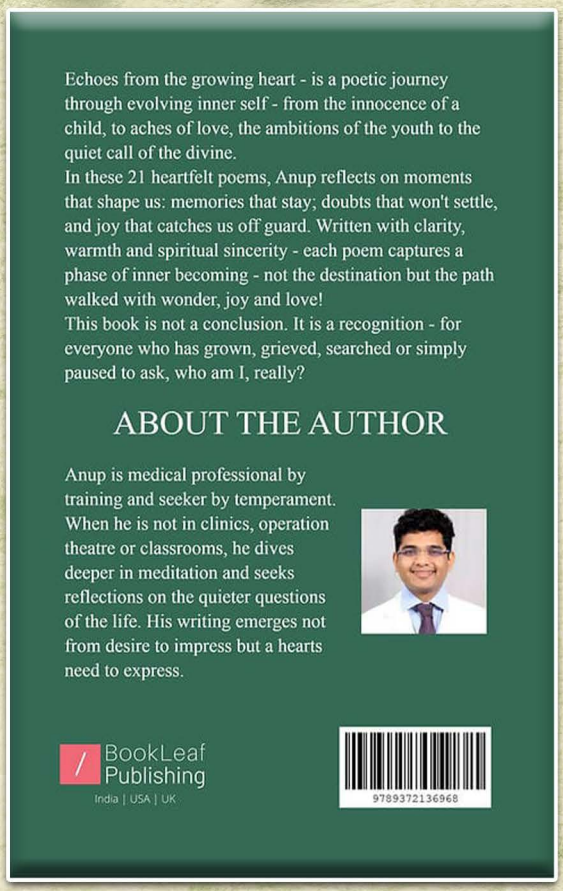
Laboratory testing in Uveitis





Judge me not

Judge me not for the colour of my skin,
Judge me not for the scars within.

Judge me not by the food I eat,
Judge me not by the tongue I speak.

Judge me not by the music I share,
Judge me not by the cloth I wear.

Judge me not for the words I say,
Judge me not for the notes I play.

Let the outer veil grow thin,
So we may touch the truth within.

The judgement ends, the wisdom grows,
As we search within, the divinity shows!

President
UVEITIS SOCIETY (INDIA)

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Dear Colleagues,

It gives me great pleasure to welcome you to this edition of our newsletter, which focuses on a crucial aspect of uveitis management- “Laboratory Testing in Uveitis”.

Uveitis is complex and challenges us to think beyond the eye. In many cases, clinical examination alone is insufficient to identify the underlying cause. Labs serve as a critical bridge, helping us move from the symptom to diagnosis, from uncertainty to clarity. Whether distinguishing infection from noninfection, uncovering hidden systemic diseases or monitoring disease activity and treatment responses, labs play an indispensable role. However the key lies not just in ordering tests, but in interpreting them judiciously, guided by the clinical context, epidemiology and evolving evidence. This issue offers practical insights into when and how to use investigations efficiently.

I congratulate and thank all the authors for having contributed to this issue and widening our understanding of many laboratory tests we routinely order in our practice. A big thanks to the editorial team and team Hallmark for their efforts in bringing out this edition of the USI newsletter for all of us.

Regards,
Dr. Kalpana Babu

Secretary
UVEITIS SOCIETY (INDIA)

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Dear friends
Here we are with the next edition of the newsletter of Uveitis society (India), on “Laboratory testing in Uveitis”

While clinical acumen remains central in uveitis, appropriate investigations form the backbone of a definitive diagnosis and effective management. Laboratory testing not only supports our clinical impressions but also helps us in course correction when we are not on the right track.

The challenge, however, is the “problem of plenty.” With innumerable investigations available, the questions that linger are: Which test, and when? And perhaps more importantly how to interpret them?

Who better than Dr Abhilasha, our editor, to curate this edition and identify the right authors for the right topics? With her characteristic meticulous planning, she has brought together a stellar panel of experts—truly the “who’s who” of uveitis and allied specialities—to provide clear, stepwise guidance on what, when, where, and how to order investigations.

On behalf of the Society, I extend my sincere appreciation to Dr Abhilasha for her tireless efforts in curating this issue, and to all the contributing authors for sharing their expertise.

I promise you, this edition will leave us more confident in our approach to laboratory testing, equipping us with practical algorithms to guide the next steps in managing our patients with uveitis.

As Secretary, I remain deeply grateful for her contribution and commitment to raising the standards of our collective learning. Enjoy reading—and happy investigating!

Warm regards,
Dr. Sudharshan S

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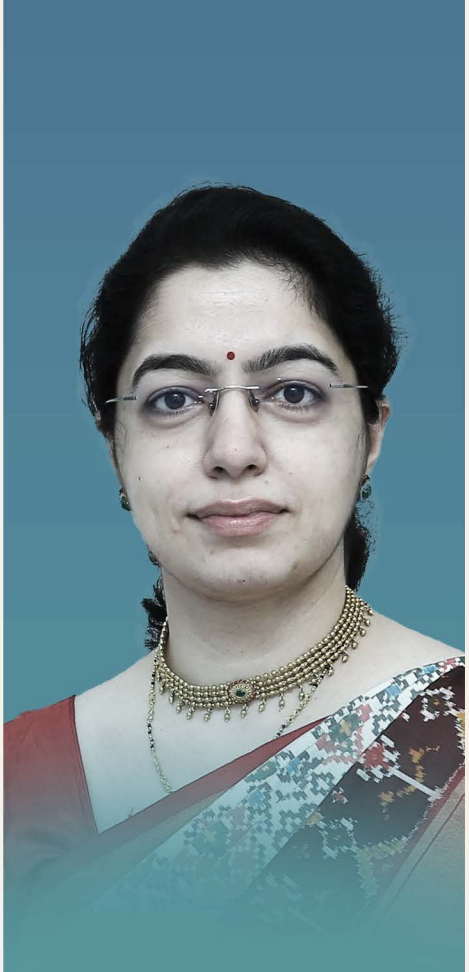

"Accurate diagnosis is half the cure"

– Sir William Osler

It gives me great pleasure to present this issue of the US(I) Newsletter, dedicated entirely to a critically important aspect of uveitis management: laboratory testing. In the nuanced field of ocular inflammation, reaching an accurate diagnosis often hinges not just on clinical acumen but on the judicious use of laboratory investigations. Accuracy is the key, not only in ordering tests but also in interpreting the results. This explains the cover page design of this important issue.

The inner coverpage poem by Dr. Anup Kelgaonkar from his award-winning compilation, "Echoes from a growing heart" celebrates the seeker in all of us, adding a lot of soul to the space.

The edition brings together a stellar lineup of experts who demystify the complexities of lab testing in various clinical scenarios we commonly face.

Prof. Dr. Amod Gupta sets the tone with a foundational overview on the Basics of Laboratory Testing in Uveitis, reminding us of the value of a targeted approach over indiscriminate ordering. He has shared his genius in tabular form. The 3 tables comprehensively detail the investigations in uveitis, based on anatomical classification, baseline testing and post treatment monitoring and can be used as a ready reckoner in the clinic.

Dr. Ramesh Jois and Dr. Keerthi Talari, both eminent rheumatologists, provide clarity on the role of RF, ANA and ANCA, helping bridge systemic autoimmune diseases with ocular manifestations. "Rheumatoid Factor and Antinuclear

Antibody in Ocular Inflammatory Disease" by Dr. Ramesh Jois is lucid and precise with several eye-opening facts for ophthalmologists. "Role of ANCA in GPA and Other Small Vessel Vasculitis" by Dr. Keerthi Talari, sheds light on the basics of testing and provides a practical guide for interpretation of the test. I'm sure this section will provide a whole new perspective to uveitis specialists.

Dr. Kalpana Babu thoughtfully examines the utility and limitations of Serum ACE and Lysozyme; frequently ordered yet often misunderstood tests. The well-researched article comprehensively sums up the basics of the tests and when and how to use them in the clinics. It is a treasure house for the management of sarcoidosis.

The role, technique and interpretation of PCR and TORCH screening in infectious uveitis, is beautifully addressed by Prof. Dr. Jyotirmay Biswas. Viral inflammatory disease, with its confounding presentations, is often a challenge to diagnose. Prof. Biwas has enriched his article with practical insights, illustrated with a case example. A good understanding of PCR is a useful addition to your diagnostic armamentarium.

Tuberculosis remains a major diagnostic consideration in our region. Dr. Alok Sen critically examines the nuances of Mantoux testing (1TU, 5TU, 10TU). More than a century after its introduction, the test continues to intrigue clinicians. The article sincerely explores the diagnostic value of the immunological test and the pitfalls in its interpretation in an endemic region. In sequel, Mantoux vs. Quantiferon debate, is addressed by Dr. Salil Mehta, offering guidance on test selection, interpretation and utility in the context of ocular tuberculosis. Together, both expert authors unriddle the diagnostic dilemma of ocular TB.

Prof. Dr. Rathinam Sivakumar explores the workup of post-fever retinitis, a growing clinical entity. This group of diseases with a common or similar phenotype often puzzle the uveitis specialist, some even worsen with empirical corticosteroids. Prof. Rathinam's wisdom and experience shared in her article will serve as a useful guide.

Dr. Anjani Gummadi, an eminent paediatric rheumatologist, offers a much-needed roadmap for laboratory testing in pediatric uveitis, where both the disease spectrum and approach differ significantly from adults. She has beautifully highlighted the importance of pattern recognition, history taking and a targeted approach towards lab investigations.

Together, these articles form a comprehensive, evidence-based guide to laboratory testing in uveitis. I hope that this issue serves as a practical resource for our members, one that encourages a more focused, thoughtful, and efficient diagnostic approach in clinical practice. My sincere thanks to all the authors for sharing their knowledge and for their time and efforts for writing such precious articles for the Newsletter.

We are also delighted to share the recent accolades and awards received by our esteemed members, a testament to the outstanding work and dedication of our community. These recognitions not only

honor individual achievements but also reflect the growing impact of uveitis specialists in the broader ophthalmology and immunology landscape. We extend our heartfelt congratulations to all awardees; you make us proud!

I sincerely thank the President, Dr. Kalpana Babu Murthy, Secretary, Dr. Sudharshan Sridharan, Vice President Dr. Padmamalini, Joint Secretary Dr. Soumyava Basu and Treasurer Dr. Parthopratim Dutta Majumder for their support and guidance.

Thanks to the Hallmark Team, Mrs. Veidhehi, Mr. Vinay, Mr. Sampath and Mrs. Pramila for their efforts in compiling and designing the Newsletter.

On a personal note, this marks my final issue as editor of the US(I) Newsletter. It has been a privilege to serve in this role and to contribute to the exchange of knowledge within our vibrant uveitis community. I am deeply grateful for the support, collaboration, and encouragement I have received from colleagues across the country and around the world. As I hand over the baton, I look forward to seeing the Newsletter continue to grow and evolve in new and exciting ways.

Sincerely,
Abhilasha.

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Basics of laboratory testing in uveitis

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Table 1: Laboratory investigations indicated by clinical signs of uveitis according to anatomical classification

Symptoms	Classification of uveitis	Characteristic Clinical Signs	Ancillary Investigations	Lab Investigations
Anterior Uveitis				
Painful, red eyes, acute onset, unilateral	Viral anterior uveitis	Unilateral, raised IOP Dull corneal sensations, pigmented keratic precipitates; Transillumination iris atrophy and posterior synechiae. Recurrent	None	PCR for CMV, HSV, VZV, CHIKV, PCR+ in ~85%; more likely in acute and more severe uveitis

Painful, red eyes, most acute onset, unilateral	AAU	Young M > F, recurrent, alternating, sudden onset, acute course, intense flare, cells, and fibrin. Synechiae break easily with cycloplegics. Leave behind a ring of pigment on the crystalline lens	UW-FFA to detect subclinical retinal vessel leak	HLA B27 MRI SI joints Seek the opinion of a physician if IBD, Psoriasis, or Reiter's disease is suspected
Painful, acute onset, bilateral	TINU	F>M, mostly under 20years, sudden onset, bilateral redness, pain, photophobia, vitreous cells, fine KPs. Acute TINU: pain in the flanks, hematuria/oliguria Ch TINU: fatigue, weight loss, loss of appetite, nausea	FFA may show peripheral vascular leak. Optic disc or macular edema	ESR S Creatinine Urine B-2 macroglobulin Urine for low-grade proteins, eosinophils, RBCs, Sugars (Normoglycemic) Renal Biopsy for lymphocytes attacking renal tubules, plasma cells, and eosinophils HLA-DRB1*0102 (the highest association of HLA type for any disease) HLA-DQA1*01/ DQB1*05

Unilateral Painless progressive loss of vision due to cataract, Floaters are typical after cataract surgery	Fuchs' Uveitis	Young patients with Unilateral PSC, diffuse iris atrophy, loss of pupillary ruff, iris transillumination defects, no synechiae, stellate keratic precipitates seen all over the corneal endothelium Vitreous floaters, High IOP +/- NO CME	None	None required. Mainly as a research tool or in an atypical presentation GW coefficient in West for Rubella Abs PCR for CMV in Asians, HSV, RV, VZV, CHIKV mNGS for rare viral infections.
Painless progressive bilateral loss of vision Due to BSK, cataract, glaucoma or hypotony	JIA Oligoarti cular (< 4 joints) for both persistent and extended) Persistent oligoarticular Extended oligoarticular	Girls 3-5 years; Bilateral insidious onset; Within 4 years of onset of asymmetric arthritis; Uveitis and joint inflammation are independent events; In 10% uveitis may precede the onset of arthritis F: M::3:1 F: M:: 3:1		ANA + in 60% HLA DR typing Susceptible alleles: DRB1*08, DRB1*11, DPB1*0201, DQA1*04, DQB1*04 Protective alleles: DRB1*04, DRB1*07 Susceptible: DRB1*13 Protective: DRB1*04 Susceptible: DRB1*01 Protective: DRB1*04 Susceptible Alleles for Uveitis: DRB1*11; DRB1*13 Protective allele: DRB1*01

	JIA Polyarticular ≥ 5 joints RF Negative	F: M:: 3:1; Symmetrical or asymmetric, small and large joints. It is a potential seronegative RA Uveitis in ~10%		ANA + in 40% RF Look for -RF HLA-DR typing for Susceptible alleles: DRB1*08 DPB1*03; DQA1*04 Protective alleles: DRB1*04 DRB1*07
	JIA Polyarticular ≥ 5 joints RF+	F: M::4:1; symmetrical metacarpoph alangeal joints. Erosive arthritis; Joint deformities Seropositive RA Uveitis in ~10%		RF+ ANA + Anti-CCP Ab + Susceptible alleles: DRB1*04 DQA1*03 Protective alleles: DQA1*02
Acute onset, painful, unilateral	JIA-ERA Ref: Srivastava et al. 2015	M: F::4:1; Lower limb joints more often affected; SI joints, hip or shoulder. Enthesitis or gut inflammation AAU		HLA B27 Susceptible allele: HLA-B* 27:05 Protective allele: HLA-B*27:06
Painless, or minimally painful, unilateral or bilateral insidious onset, blurring of vision	Granulo matous Anterior uveitis. Very often, panuveitis may have a very significant anterior uveitis component	Granulomatous Keratic precipitates may have broad posterior synechiae, Busacca or Koeppe nodules, Berlin nodules in the AC angle, and spillover vitreous cells	FFA if you suspect VKH disease, especially in the chronic recurrent stage. OCT	Tuberculin skin test, CECT chest, VDRL, TPHA,

Intermediate Uveitis				
Often, painless blurring of vision, unilateral or bilateral, floaters and diminution of vision	Intermediate uveitis	Inflammatory cells are predominantly in the vitreous cavity, with spillover cells. Most cases may be idiopathic In TB endemic regions, TB may present as intermediate uveitis; Sarcoidosis, Syphilis, Lyme disease, and MS may present as intermediate uveitis Lyme disease (Borrelia Burgdorferi) presents as Erythema migrans	FP for media haze FFA to look for CME; UW-FFA to detect peripheral retinal vasculitis seen in MS. OCT	HRCT chest CECT chest if planning an EBUS-guided biopsy of lymph nodes for diagnosing Sarcoidosis or TB, Biopsy of extrapulmonary nodes or skin lesions PET-CT may be required to find an active node for biopsy MRI brain to look for periventricular plaques (Dawson's fingers) of MS HLA-DRB1*1501 (strong association with MS), Serology for syphilis; serology for Lyme disease in endemic areas
Acute onset Unilateral or bilateral Painful or painless	Endogenous endoph thalmitis	Disproportional Media haze: retinal details not visualised History of hospitalisation, intravenous infusions, indwelling catheters, and hyperalimentation	FP USG	Blood and urine culture Vitreous smears and cultures PCR for VZV/HSV/ Toxoplasma
Gradual unilateral loss of vision	Toxocara endophth almitis	Seen in children	FP USG	Toxocara ELISA, Aqueous tap for PCR and eosinophils

Painless unilateral or bilateral progressive blurring of vision	PVRL	Mostly elderly >65, although in India, we see them at a younger age, too	FP FAF OCT MRI	Aqueous/ Vitreous fluid for IL-10/IL-6 ratio; Vitreous biopsy for cytopathology.
	Amyloid	Glass wool or cobweb-like vitreous opacities	USG for highly reflective mobile echoes FFA for any retinal vessel leak	Vitreous biopsy; Congo red stain for characteristic "Apple-green birefringence" Genetic testing for systemic transthyretin amyloidosis
Posterior Uveitis				
Painless blurring of vision	Toxoplas mosis	Often unilateral, a "Headlamp in the fog" appearance of necrotising retinochoroiditis. Intense vitreous inflammation overlying the retinitis lesions. Kyrieleis plaques along retinal arterioles. Active lesion next to a healed retinochoroidal scar. Bilateral, multifocal in immunocom promised patients	FP FFA OCT shows increased choroidal thickness under the retinal lesion; Vitreous cells in front of the retinal lesion	HIV Toxo serology IgM and IgG PCR in HIV patients for Toxo and other infectious agents, as more than one infection may exist GW coefficient in immunocompetent individuals
Painless blurring of vision	Necrotising retinitis (ARN, PORN)	Rapidly progressive, Peripheral circumferential or confluent wedge-shaped necrotising retinitis, retinal vascular sheathing and/or occlusion; vitritis+	UW-FP	PCR for VZV and HSV HIV, especially if no or minimal vitritis

Painless blurring of vision	CMV retinitis	Immunocom promised; HIV; IMT; Transplants; Intravitreal/ periocular corticosteroids No or minimal vitreous inflammation; Wedge-shaped retinitis lesions with indistinct borders; hemorrhagic and/or granular; relatively slow progression Non-HIV patients may show significant vitritis and arterial occlusion	UW-FP	PCR for CMV, HSV, VZV and Toxoplasmosis Syphilis serology
Painless blurring of vision	Tuberculosis	Broad posterior synechiae. Vitritis; Retinal periphlebitis with or without discrete chorioretinal lesions/scars. SLC; Choroidal granuloma usually in the posterior pole,	FP FAF FFA OCT shows choroidal granuloma infiltrating the outer retina, SRF+	Immunological evidence of TB (TST; QuantiFERON-Gold TB test; Radiological evidence of TB-lymphadenitis (HRCT/CECT/ PET-CT); Biopsy of available lymph node. RT-PCR (Smear and Cultures for MTB are rare + from ocular TB) Physician exam to find any site of extrapulmonary TB

Painless blurring of vision	Sarcoidosis	Multifocal choroidal granulomas, Perivascular candle wax drippings, Macroaneurysm	FP ICG/FFA OCT	X-ray chest; CT chest, USG abdomen; PET scan optional; CBC; Liver enzymes; creatinine; BUN; Uric acid; creatine kinase; calcium; albumin; CRP. protein-electrophoresis; ACE; sIL-2R Urine analysis for proteinuria, hematuria and hypercalciuria; RF; ACPA; ANA or ENA; IgG4 Biopsy from mediastinal or extrapulmonary lymph nodes or skin lesions to show a non-caseating granuloma Positive tests for TB rule out Sarcoidosis Syphilis must be excluded

Sudden loss of vision	SSPE	Rapidly progressive multifocal full-thickness necrotic lesions sparing the retinal vessels, Scarring with extreme thinning of the affected retina Declining cognitive function; myoclonus jerks	FP OCT shows ballooning of ILM; necrosis of retina with sparing of Bruch's membrane.	Measles antibodies from CSF and serum Oligoclonal bands in CSF Increased (>20%) CSF γ-Globulin levels. EEG for periodic complexes MRI shows asymmetrical hyperintense periventricular and subcortical lesions
Sudden loss of vision	Unilateral acute idiopathic maculopathy (UAIM)	History of fever with hand, foot, and mouth rash Central scotoma due to a monofocal placoid lesion May have retinal vasculitis	FP FAF stippled OCT shows loss of interdigi tation and the ellipsoid zone	Coxsackie virus serology, IgM and IgG, especially for serotype A16 PCR
Panuveitis				
Sudden painless loss of vision is often bilateral; can be unilateral	Behçet's disease	Anterior uveitis or Anterior and intermediate uveitis or Posterior uveitis with retinal vasculitis and/ or focal retinal infiltrates or Panuveitis with retinal vasculitis and/or focal retinal infiltrates. Recurrent oral ulcers observed at least 3 times in a 12-month period, PLUS any of the two criteria- Recurrent Genital ulceration/scarring, EN, uveitis or positive pathergy Note: Presence of choroiditis significantly reduces the possibility of Behçet's disease	FP FFA classic Fern pattern leakage due to leakage from all vessels Multimodal imaging should be done to rule out subclinical inflammation in patients with Behçet's disease	HLA-B*51 increases risk by 6-10-fold. Not all + for HLA-B*51 develop Behçet's disease On its own, it is not diagnostic of Behçet's disease. See Table 3 for labs for monitoring therapy

Sudden painless loss of vision is often bilateral	VKH disease	Predominantly Young women; No history of penetrating injury; bilateral onset of inflammation preceded by a prodromal stage. Bilateral multifocal exudative RD in the acute exudative stage or panuveitis with any of the two- headache, tinnitus, or meningismus (neck stiffness, with malaise), dysacusis or CSF lymphocytic pleocytosis. In chronic recurrent stage granulomatous response, nummular choroidal scars in the inferior periphery of the fundus; Sunset glow fundus in late stages; peripapillary atrophy. Melanin-laden macrophages in CSF are highly characteristic of VKH disease and distinguish it from syphilis	FP FFA, USG, and OCT to detect increased choroidal thickness; OCT to monitor the response to treatment; ICG to monitor the resolution of subclinical choroidal inflammation	Exclude Syphilis and Sarcoidosis HLA-DRB1*0410 is strongly associated with VKH HLA-DRB*0404; HLA-DR53 and DQ4 HLA-DRB1*0401 is a protective allele See Table 3 for labs for monitoring therapy
Penetrating trauma or surgery in one eye preceding ocular inflammation	Sympathetic Ophthalmia;	May have the same set of signs as in VKH disease. Bilateral ocular inflammation involving the anterior chamber and vitreous or panuveitis with choroidal involvement.	FP FFA, OCT	

Progressive Visual symptoms in patients with DM, or Immunocom promised patients with indwelling catheters or infusions, GI or Urogenital surgery, may be unilateral or bilateral	Fungal panuveitis	AC reaction ± hypopyon; puff balls in vitreous in candida; moderately severe vitreous inflammation with membranes, choroidal abscess; chorioretinitis with cloud burst signs characteristic of candida;	FP for media haze and monitoring response to therapy	Diagnostic challenge. Needs a Microincisional vitreous biopsy for cytopathology, KOH and Calcofluor smears, PCR and fungal cultures. Cultures to be maintained for 2-3 weeks to rule out Nocardia, which may mimic fungal abscess.
Note: TB (in endemic regions), Syphilis, Sarcoidosis, may present as anterior, intermediate, posterior or panuveitis and must be ruled out in all cases. Toxoplasmosis uveitis may present as panuveitis in addition to its common presentation as posterior uveitis				

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Table 2: Baseline laboratory investigations prior to starting therapy for uveitis

Baseline lab tests	Rationale for the lab tests
HIV	
CBC with differential	Detect cytopenia
Blood sugar levels	If planning corticosteroids
Triglycerides	If planning long-term oral corticosteroids to check for hypertriglyceridemia
Urea and electrolytes	If planning long-term oral corticosteroids to monitor for adrenal insufficiency
Thiopurine methyltransferase/ Nudix Hydrolase 15 (TPMT/NUDT15)	If planning to use Azathioprine to assess the genetic risk of severe myelosuppression
DEXA scan for Bone density	To look for osteoporosis
25-hydroxyvitamin D test	Long-term corticosteroids interfere with calcium absorption and interfere with bone metabolism. Vitamin D deficiency may exacerbate bone loss
Liver Function Tests (LFTs)	Many IMTs are hepatotoxic
Renal Function Tests (RFTs)	Before starting Cyclosporine, Azathioprine, and methotrexate can also affect the kidneys.
Urine analysis	Baseline renal status
QuantiFERON-TB Gold; TST	If planning biologics, to rule out latent TB before biologics
X-ray chest	If planning biologics, to rule out latent TB
Pregnancy test	If planning Methotrexate/ mycophenolate, as these are teratogenic
Hepatitis B and C serology	If planning Biologics, can reactivate hepatitis
G6PD	If planning to use Sulpha drugs
ANA/Anti ds-DNA	Risk of autoimmune disorders if planning biological therapy

Table 3: Lab tests after initiating systemic therapy for uveitis

Lab test after initiating therapy	Frequency of tests	Rationale of test
CBC	2-4 weeks initially and then every 1-3 months	Monitor for leucocytosis, myelosuppression and anaemia if receiving IMT
Blood glucose/HbA1c	Every 3-6 months	Detect corticosteroid-induced hyperglycaemia
Liver function tests	2-4 weeks initially and then every 1-3 months	Hepatic function if receiving IMT
Creatinine and BUN	Every 1-3 months	Renal function, especially if receiving calcineurin inhibitors and Methotrexate
Lipid profile	Annual	Detect Hyperlipidemia
Dexa Scan	Every 6 months	To detect osteoporosis

Note:

1. Patients receiving corticosteroids should be periodically monitored for BP and DM
2. Patients should be periodically screened for secondary infections if receiving IMT and biologics by a team of rheumatologists, internal medicine, infectious disease specialists, and ophthalmologists.
3. Screening for lymphomas and skin cancer may be considered for patients on long-term IMT and biologic use
4. Urine microscopy for hematuria, if cyclophosphamide is used

Awards and Accolades

Dr. Aneena Anna Thomas
Narayana Nethralaya, Bengaluru
Image-nation contest: First runner up
4-6th July 2025, Retina Imaging Congress, Trivandrum

Rheumatoid Factor and Antinuclear Antibody in Ocular Inflammatory Disease

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Rheumatoid Factor

Rheumatoid factors (RF) are autoantibodies that target the Fc region of IgG. They were the first autoantibodies described for autoimmune diseases. Traditionally although RFs are associated with Rheumatoid arthritis, they have been described in a wide variety of other conditions.

What should an Ophthalmologist know about RF?

1. RF may be positive in healthy control subjects (1% in younger persons and prevalence increases with age, exceeding 25% in individuals older than 85 years).
2. RF may be positive in other Rheumatological conditions such as Sjogren's Syndrome, Mixed Cryoglobulinemia, Systemic Lupus, Mixed Connective Tissue Disease amongst others.
3. RF may be positive in non-rheumatological diseases such as Sarcoidosis, Primary Sclerosing Cholangitis and certain Malignancies.
4. RF may be positive in certain Chronic infections such as Hepatitis C (causative agent for Cryoglobulinemic Vasculitis), Subacute Endocarditis, Epstein Barr Virus infections, Tuberculosis, etc.
5. Hence RF is NOT a diagnostic test for Rheumatoid arthritis. RFs are positive in ~70% of patients with Rheumatoid arthritis but have been shown to have lower specificity for Rheumatoid arthritis compared with anti-CCP antibodies (85 vs. 95%, respectively). The sensitivity of IgM-RF for RA is estimated to range from 41% to 66% in early RA and increases to 62% to 87% in established RA
6. In a patient presenting with polyarthritis (higher pre-test probability) a positive RF increases the sensitivity and specificity to classify the patient as having Rheumatoid arthritis.

7. RF has a pathogenetic role in Rheumatoid arthritis. They may appear much earlier (even ten years) prior to the onset of symptoms. Treatment is NEVER initiated till symptom-onset and mere positivity for RF are not treated with Disease modifying anti-rheumatic drugs.

8. Patients with Rheumatoid arthritis can be negative for RF and positive for anti-CCP antibody or rarely both antibodies maybe negative.

9. Rather than mere positivity for RF, moderate to high titres (>50 IU/L to > 100 IU/L) have a better predictive value for the classification as Rheumatoid arthritis.

10. RF should be measured by quantitative methods such as nephelometry. Qualitative tests such as latex agglutination are better avoided.

11. Seropositivity with RF has been associated with more aggressive and erosive arthritis and hence suggesting that strict disease control is essential in these sub-set of patients.

12. Seropositivity in RA has been associated with greater chances of developing extra-articular manifestations in patients with poorly-controlled arthritis particularly Rheumatoid nodules and Vasculitis.

13. Presence of high titres of RF defines better response to certain treatment like Rituximab and poorer response to anti-TNF therapy (eg. adalimumab)

14. Rheumatoid arthritis can present to an ophthalmologist with episcleritis, scleritis or ocular sicca. Uncontrolled or poorly controlled arthritis patients are at high risk of developing necrotizing scleritis, scleromalacia and perforation. Treatments used in rheumatoid arthritis such as hydroxychloroquine on prolonged use (> 5 yrs) at higher doses (> 5 mg/kg/day) have an increased risk of developing retinopathy. Hence regular ophthalmological screening with OCT, Visual field test, fundus examination (autofluorescence) and if required ERG is essential in patients who take long-term Hydroxychloroquine.

15. Ocular or oral sicca, recurrent dental caries, hypostatic purpurae on the legs (purpurae developing on prolonged standing or sitting or travel), raised ESR with normal CRP, positive RF, raised globulins, positive SSA / SSB antibodies and a suggestive minor salivary gland biopsy are some of the features suggestive of a diagnosis of Sjogren's Syndrome.

Further Reading:

Togashi T , Ishiara R, Watanabe R et al. Rheumatoid Factor: Diagnostic and Prognostic Performance and Therapeutic Implications in Rheumatoid Arthritis. J Clin Med. 2025, 14, 1529.

Antinuclear Antibody

Table 1: Incidence of ANA in various conditions

Conditions	Incidence (%)
Systemic Lupus Erythematosus	99
Systemic Sclerosis	95
Sjogren's Syndrome	50-95
Inflammatory Myositis	40-80
Mixed Connective Tissue Disease	100
Juvenile Idiopathic Arthritis	20-50
Anti-phospholipid Antibody Syndrome	40-50
Autoimmune Hepatitis	50-75
Primary Biliary Cirrhosis	30-50
Idiopathic Thrombocytopenic Purpura	10-30
Thyroid Disease	30-50
Infections and Malignancy	Varies
Normal / Healthy Individuals	30

Antinuclear antibodies (ANA) are one of the most common laboratory tests performed in order to make diagnosis of connective tissue diseases. It has a pathogenetic role in the causation of disease. The standard method used to test for ANAs is Hep-2 substrate-based Indirect Immunofluorescence (IIF). A titre of 1:80 and higher is considered positive but tires of 1:160 or greater is considered clinically relevant.

IIF is observer - dependent and hence has to be interpreted with caution. Based on the ANA appearance on IIF, distinct patterns are identified such as Diffuse, Cytoplasmic, Nuclear, Nucleolar, Speckled, Centromere etc. Although centromere pattern is more commonly associated with Systemic Sclerosis, none of these patterns are disease - specific or pathognomonic. Hence the clinical utility of these patterns is limited.

The prevalence of ANA in various diseases is summarized in Table 1.

ANAs do not correlate with disease activity. They are more useful to make a diagnosis rather than for longitudinal follow-up. Hence ANA test should not be repeated to follow up these patients.

A positive ANA (titre of 1:640 or higher) may predate the onset of a rheumatological disease by many years. Hence the diagnosis of the rheumatological disease should not be made solely on the basis of a positive ANA test in the absence of relevant clinical features.

A positive ANA test in the absence of clinically significant rheumatological disease might reflect pre-clinical rheumatic disease or

may also be due to a non-rheumatologic autoimmune or inflammatory disease. It could also be the normal autoantibody repertoire in an otherwise healthy individual. Enzyme-linked immunosorbent assays (ELISA) provide a highly sensitive method for quantitative estimation of individual autoantibodies such as anti-dsDNA, SS-A (Ro) or SS-B (La). Moderate to high titres of these antibodies are clinically relevant to monitor disease state (anti-dsDNA titres in Lupus) or risk profiling (risk of neonatal heart block in Ro positive mothers).

There are many individually named ANA's and these have specific disease associations. These are listed in Table 2.

Table 2: Disease association of various ANA antibodies

Anti-nuclear Antibody	**Disease Association**
Anti-ds DNA	SLE (Especially nephritis)
SS-A / Ro-60	Sjogren's Syndrome, SLE, Neonatal lupus, Neonatal Congenital Heart Block
SS-B / La	Sjogren's Syndrome, SLE
Ro-52	Interstitial Lung Disease
Jo-1	Dermatomyositis / Polymyositis
Ku	Polymyositis / Systemic Sclerosis Overlap
Scl-70	Systemic Sclerosis (Diffuse variety)
Centromere	Systemic Sclerosis (Limited variety)
Sm	SLE
RNP (U1-Sn RNP)	Mixed Connective Tissue Disease, SLE
Ribosomal-P protein	SLE
PM-Scl	Systemic Sclerosis / Myositis Overlap
PCNA	SLE
Histones	Drug-induced Lupus
DFS-70	No clinical association

ANA for the Ophthalmologist:

Although ANA is a very common test performed for ocular inflammatory diseases, NOT all patients with inflammatory ocular disease need an ANA test.

1. The commonest ocular involvement in patients with rheumatological diseases is probably Ocular Sicca.
2. Ocular Sicca is seen more frequently in patients with Rheumatoid arthritis, Sjogren's Syndrome, Mixed connective tissue disease, Systemic Sclerosis, Sarcoidosis amongst others.
3. Investigations relevant for Ocular Sicca include Rheumatoid factor, anti-CCP antibody and ANA profile.
4. Ocular Sicca maybe the only clinical feature of many patients with Sjogren's Syndrome. Some patients might also complain of oral sicca, skin

/ vaginal dryness, purpurae on the legs on prolonged standing or sitting. Positive SS-A and / or SS-B antibody along with a raised ESR, normal CRP, positive Rheumatoid factor and hypergammaglobulinemia are the main laboratory abnormalities in patients with Sjogren's Syndrome.

5. Presence of SS-B antibody / La alone is of lesser predictive value for Sjogren's Syndrome.

6. However, it is important to be aware that SS-A / Ro is positive only in 50-70% of patients with Sjogren's Syndrome and SS-B / La in about 25-40%. A suggestive ultrasound scan of salivary glands and labial biopsy (minor salivary gland) are useful to make a diagnosis of iSjogren's syndrome n patients with negative ANA antibodies.

7. ANA is not useful in patients presenting with anterior / intermediate uveitis.

8. Routine ANA determination as a part of the diagnostic work-up of uveitis patients is not recommended.

9. ANA test (not the profile) will be particularly useful in patients presenting with Retinitis which is sometimes a feature of Lupus. A negative ANA profile does not rule out Lupus since many of the individual autoantibodies may be negative in Lupus.

10. Systemic evaluation for involvement of other organ systems is very important in Lupus. In majority of lupus patients, clinical manifestations involving haematological and renal systems are asymptomatic. Hence referral to a Rheumatologist is very important to ascertain the diagnosis and severity of systemic involvement.

11. Many diseases related to various other ANA autoantibodies (eg. Systemic sclerosis, Inflammatory myositis) do not have major ophthalmologic manifestations.

12. ANA maybe positive in children with pauci-articular juvenile idiopathic arthritis. These children have the highest risk of developing chronic asymptomatic iridocyclitis.

13. Hence all children with oligo articular Juvenile arthritis should have baseline eye screening with slit-lamp examination to diagnose asymptomatic chronic iridocyclitis and should undergo periodic follow up with an ophthalmologist.

Further Reading:

Firestein and Kelley's Text Book of Rheumatology. Elsevier's Publication. 12th Edition Vol.2; Chapter 55: 868-83

Awards and Accolades

Dr. Anup Kelgaonkar,
LV Prasad Eye Institute, Bhubaneshwar.
Prof. Amod Gupta Young Researcher Award in recognition of his work on Toxoplasma retinitis and its various atypical presentations at USICON, 4th-6th October 2024, Chandigarh.

Role of ANCA in GPA and Other Small Vessel Vasculitis

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Introduction

Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies targeting neutrophil granule proteins, primarily proteinase 3 (PR3) and myeloperoxidase (MPO). They play a central role in the diagnosis, classification, and management of ANCA-associated vasculitis (AAV), including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA). From an ophthalmology standpoint, ANCA-associated vasculitis is an important differential in ocular inflammation such as scleritis, peripheral ulcerative keratitis, retinal vasculitis, and orbital inflammation.[2,3,7]

Basics of ANCA Testing

ANCA are detected by indirect immunofluorescence (IIF), which demonstrates staining patterns (cytoplasmic c-ANCA and perinuclear p-ANCA), and antigen-specific immunoassays such as ELISA, which detect PR3 and MPO antibodies. The 2017 international consensus recommends antigen-specific immunoassays as the primary diagnostic method, with IIF as complementary in certain situations.[1,8]

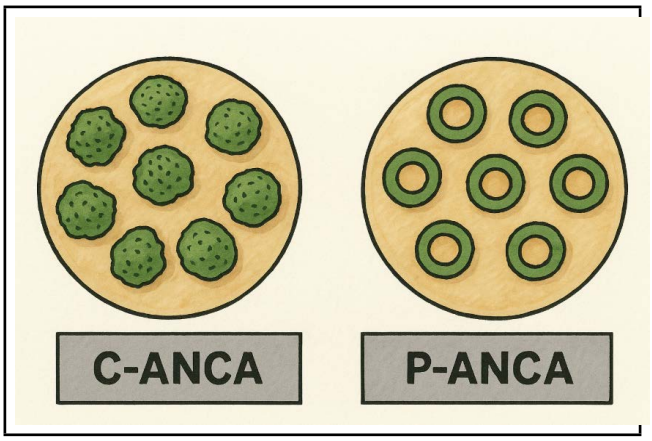

Figure 1. *Indirect immunofluorescence patterns of ANCA. Comparison of c-ANCA (PR3) showing diffuse cytoplasmic staining and p-ANCA (MPO) showing perinuclear staining. (DALL-E used for image generation)*

ANCA in GPA and Other Vasculitides

GPA is strongly associated with PR3-ANCA, with positivity seen in 70–90% of cases. Ocular involvement in GPA occurs in up to 50% of patients and can be the presenting feature. MPA, associated more with MPO-ANCA, can present with retinal vasculitis and scleritis. EGPA is MPO-ANCA positive in about one-third of cases, with ocular disease being less common.[4,5,7] (Table 1)

Ocular Manifestations (Table 2)

ANCA-associated vasculitis may involve multiple ocular structures:

- Orbit: orbital pseudotumour, proptosis (especially GPA)
- Sclera/Cornea: necrotising scleritis, peripheral ulcerative keratitis
- Uvea: anterior/posterior uveitis
- Retina/Choroid: retinal vasculitis, choroiditis
- Optic nerve: ischemic optic neuropathy, optic neuritis[3,7]

Practical Approach for Ophthalmologists

When evaluating patients with severe or atypical ocular inflammation (necrotising scleritis, peripheral ulcerative keratitis, unexplained retinal vasculitis), ophthalmologists should consider systemic ANCA-associated vasculitis. Work-up should include systemic evaluation, ANCA testing (PR3/MPO assays), renal function tests, urinalysis, and rheumatology referral.[3,4,6](Table 3)

Laboratory Guidance and Harmonization

The 2017 international consensus and subsequent updates recommend PR3- and MPO-ANCA immunoassays as first-line tests, with IIF reserved for special cases. Harmonization of assays across laboratories is essential for reproducibility. The 2022 EULAR update and 2024 KDIGO guidelines emphasize timely testing, early diagnosis, and the importance of integrating ANCA results with clinical context.[1,4,6,8]

Conclusion

ANCA testing has transformed the diagnosis of GPA and other small vessel vasculitides. For ophthalmologists, awareness of ANCA's role is vital when faced with severe ocular inflammatory disease. Early suspicion, appropriate laboratory testing, and multidisciplinary care are crucial to preserving vision and reducing systemic morbidity.[2,3,4]

Table 1: Key Differences Between PR3-ANCA and MPO-ANCA Vasculitis

Feature	PR3-ANCA (c-ANCA)	MPO-ANCA (p-ANCA)
Main Disease Association	Granulomatosis with Polyangiitis (GPA)	Microscopic Polyangiitis (MPA), EGPA
Frequency in AAV	60–80% of GPA	60–80% of MPA, 30–40% of EGPA
Ocular Involvement	Common: scleritis, orbital masses, keratitis	Less frequent: retinal vasculitis, scleritis
Relapse Risk	Higher relapse rates	Lower relapse rates
Pathogenesis	Strong neutrophil activation, granulomatous inflammation	More necrotizing vasculitis, less granulomatous
Prognosis	Higher risk of relapse but better long-term survival	More renal-limited disease, slightly worse renal outcomes

Table 2: Ocular Manifestations of ANCA-Associated Vasculitis

Ocular Structure	Typical Manifestation	Clinical Relevance
Orbit	Orbital pseudotumour, proptosis	Mimics thyroid eye disease, sarcoidosis
Cornea	Peripheral ulcerative keratitis (PUK)	Vision-threatening, linked to systemic flare
Sclera	Necrotising scleritis	Marker of active systemic disease
Uvea	Granulomatous/non-granulomatous uveitis	May be isolated or part of systemic disease, rare
Retina	Retinal vasculitis, vein occlusions	Can resemble Behçet's or lupus retinopathy
Optic Nerve	Optic neuritis, ischemic optic neuropathy	Rare but devastating for vision

Table 3: Practical Laboratory Interpretation of ANCA Testing for Ophthalmologists

Scenario	Likely ANCA Result€	Pitfalls / Pearls
Necrotising scleritis with systemic symptoms	PR3-ANCA positive	May be first sign of GPA; biopsy helpful
Retinal vasculitis with pulmonary-renal symptoms	MPO or PR3 positive	Distinguish from lupus or Behçet's
Orbital pseudotumour	PR3-ANCA positive	Consider biopsy to exclude lymphoma/sarcoid
False positives	IBD, autoimmune liver disease, infections, drugs	Use ELISA confirmation and clinical context

€ The sensitivity in each condition varies and should be kept in mind

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Awards and Accolades

Dr. Dipankar Das

Sri Sankaradeva Netralaya, Guwahati

AIOS Achievement Award 2024 at APAO- AIOC

at Yashobhoomi, New Delhi, 3-6 April, 2025

Serum Angiotensin Converting Enzyme and Lysozyme: Usefulness and limitations

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Introduction

Sarcoidosis is a chronic granulomatou inflammation of unknown etiology, whic mainly affects the lungs and lymp nodes, as well as extrapulmonary orgar including the ocular tissues. The prevalenc course largely varies with regior and populations globally. The clinic manifestations of sarcoidosis depend o the affected organs and the degree severity. The diagnosis is made based o characteristic clinical presentations, seru biomarkers, radiography and biopsy. Nor caseating granulomas composing T cell macrophages, epithelioid cells, and giant cells on biopsy, negative for acid fast bacilli and fungi are the characteristic findings in sarcoidosis. The serum biomarkers include lymphopenia, serum angiotensin converting enzyme, serum lysozyme and serum IL2 assays. Ocular involvement can be the presenting or the only manifestation of sarcoidosis. The latter makes it challenging for diagnosis and treatment. The revised criteria of the international workshop on ocular sarcoidosis (IWOS) for diagnosis of ocular sarcoidosis includes

serum biomarkers like serum angiotensin converting enzyme and serum lysozyme.[1] The diagnostic utility of these markers is often debated due to the varying sensitivity and specificity.

Serum Angiotensin Converting Enzyme (ACE)

ACE is a dipeptidyl carboxy peptidase, which catalyzes the conversion of angiotensin I to angiotensin II and helps metabolize bradykinin. ACE is found in the normal lung on the luminal surface of capillary endothelial cells. It also originates from active epithelioid cells, giant cells, alveolar macrophages and fibroblasts.

ACE is encoded by a single gene located on chromosome 17q23.[2] ACE has three main genotypes: DD, ID, and II. Hence, the gene polymorphism of ACE affects the serum ACE level, in which DD genotype has the highest and the II genotype has the lowest value.[3] Serum ACE levels of different genotypes of ACE in normal subjects and in patients with sarcoidosis has been summarized in table 1.Serum levels of ACE are most commonly increased in sarcoidosis. Increased levels are also found in tuberculosis, leprosy, fungal infections like coccidiomycosis, histoplasmosis, liver disorders such as alcoholic cirrhosis and primary biliary cirrhosis, kidney disorders such as nephrotic syndrome hyperthyroidism, Hodgkin disease, lymphoma, diabetes, gaucher's disease, multiple sclerosis, amyloidosis, HIV infection and pregnancy.

In sarcoidosis, the epitheloid and giant cells that compose granuloma can express ACE, increasing the level of ACE. Serum ACE can also reflect the severity of sarcoidosis granulomas and can be used to monitor disease activity and treatment response, with high specificity especially when it is twice as high as the normal limit

How is it measured?

The concentration of the angiotensin converting enzyme in the blood is measured by colorimetric or fluorescent assay or an ELISA test.

Factors which affect ACE levels

Despite its relatively high specificity, the sensitivity of ACE is inconsistent, limiting its standalone diagnostic value. Table 2 provides a summary of the key factors influencing measured ACE levels.

Sensitivity and specificity

The diagnostic performance of serum angiotensin-converting enzyme (ACE) in ocular sarcoidosis varies considerably across studies. Reported sensitivity ranges from approximately 38% to 84%, while specificity is generally high, between 83% and 98%.[4,5] Recent meta-analysis evaluating the performance of serum angiotensin-converting enzyme (ACE) in diagnosing sarcoidosis reported a sensitivity of 60% and a specificity of 93%. For predicting active sarcoidosis, the summary estimates showed a sensitivity of 76% and a specificity of 80%.[6]

Serum ACE is considered a valuable marker for diagnosing sarcoidosis due to its relatively high specificity, particularly when levels are markedly elevated—such as twice the upper limit of normal.[7]

The effectiveness of serum ACE in conjunction with other investigations has been summarised in Table 3.

Serum ACE in Ocular Sarcoidosis: Diagnostic Performance

In a 2021 study by Papasavvas et al., serum ACE levels were found to be significantly higher in patients with ocular sarcoidosis (OS) compared to controls. The mean ACE level in the OS group was 49.17 ± 29 IU/L, versus 27.4 ± 15.34 IU/L in patients with non-granulomatous uveitis ($p \leq 0.00018$). Despite this statistically significant difference, only 27% of OS patients had ACE levels above the diagnostic cutoff, yielding a sensitivity of 27%. However, the test maintained a high specificity of 96.6%, reinforcing its value for confirming (but not excluding) OS.[8]

Birnbaum AD et al. in their retrospective study reviewed 63 patients with biopsy-proven uveitic sarcoidosis seen between 1989 and 2009. African American patients (62%) presented at a younger age and more frequently had granulomatous anterior uveitis. The levels of serum markers angiotensin-converting enzyme and lysozyme were elevated in 40% and 42% of patients tested, respectively. Elevated serum ACE or lysozyme was found in 58% of tested patients. Chest CT was more sensitive (100%) than chest X-ray (69%) in detecting sarcoid-related changes. The study highlights that demographics, serum markers, and imaging together improve diagnostic accuracy for ocular sarcoidosis.[9]

Utility of serum ACE in monitoring response to treatment

Serum ACE may serve as a useful biomarker for monitoring treatment response in sarcoidosis, as levels typically decline with effective therapy and often rise again during disease relapse. Corticosteroid therapy was shown to reduce elevated serum ACE levels back to normal, correlating with clinical improvement.[10] More recent longitudinal observations confirm that serum ACE levels decrease after initiation of immunosuppressive therapy—such as corticosteroids or methotrexate—and increase again upon recurrence of disease .[11]

Serum Lysozyme

Lysozyme is an enzyme that hydrolyses glycosidic bonds, and it is revealed to degrade the peptidoglycans in the bacterial cell wall.[12]

Sensitivity and specificity

The serum lysozyme assay has an estimated sensitivity of 60–78% and a specificity of 76–95%.[13]

Serum lysozyme in Ocular Sarcoidosis: Diagnostic Performance

Papasavvas I et al. assessed the serum levels of ACE and lysozyme in patients with proven ocular sarcoidosis and suspected cases, comparing them with a control group of non-granulomatous uveitis patients. The findings revealed that while both ACE and lysozyme levels were elevated in ocular sarcoidosis patients, lysozyme demonstrated higher sensitivity (83.7%) compared to ACE (27%) for diagnosing ocular sarcoidosis. Additionally, lysozyme exhibited a specificity of 90%, whereas ACE had a specificity of 96.6%. These results suggest that serum lysozyme may be a more reliable biomarker than ACE in supporting the diagnosis of ocular sarcoidosis, particularly in the absence of systemic involvement.

Combining ACE and lysozyme increases diagnostic yield. Papasavvas et al. reported that 21.6% of OS patients had both markers elevated, while 10.8% had neither,

highlighting the complementary role of these tests.[8]

Factors which affect lysozyme levels

Lysozyme may be increased in patients with latent tuberculosis and latent syphilis, and its interpretation alone should be treated with caution.[14]

Conclusion

Table 4 summarises the usefulness and limitations of serum ACE and lysozyme.

- Lysozyme is generally more sensitive and often superior to ACE for detecting ocular sarcoidosis.
- ACE, despite lower sensitivity, offers good specificity, making it helpful in confirming cases albeit with limitations.
- Combined testing significantly enhances diagnostic accuracy.
- Always interpret results in clinical context—i.e., signs of granulomatous uveitis, imaging findings, and potentially histology

Table 1: Serum ACE levels of different genotypes of ACE in normal subjects and in patients with sarcoidosis.[1]

Serum ACE level	**Genotypes**			
	II	**ID**	**DD**	
Normal	21.4 ± 7.9	23.9 ± 7.2	27.3 ± 7.5	IU/L
Sarcoidosis	10.8 ± 3.1	13.8 ± 4.3	17.2 ± 4.0	IU/L

Table 2: Factors influencing variability in measured ACE levels

Factor	**Effect on Serum ACE Level or Measurement**
ACE I/D Genetic Polymorphism(Insertion/ Deletion in intron 16; rs1799752)[16,17]	The DD genotype is associated with highest serum ACE, DI with intermediate, and II with the lowest levels. Genotype-specific reference ranges improve clinical interpretation.
Concurrent Use of ACE Inhibitors(e.g., captopril, enalapril, lisinopril)[18]	ACE inhibitors reduce ACE enzyme activity by competitively blocking conversion of angiotensin I to II. This leads to lower measurable serum ACE activity, potentially masking elevated levels from pathology.
Age-Related Physiological Differences[19,20]	Children have higher serum ACE activity than adults; adolescents show intermediate levels and sex differences begin to emerge. In adults, older men (>55 years) have lower ACE activity compared to younger men; no consistent change seen in women.
Smoking [21-23]	In chronic smokers- Lower serum ACE activity was noted in healthy smokers compared to nonsmokers. This suggests that habitual smoking can suppress baseline ACE levels.

	The suppressive effect persists even after quitting, particularly within the first decade post-cessation. Only after more than 10 years did ACE levels rebound to match those of lifelong non-smokers.
	In contrast, acute smoking triggers transient spikes in serum ACE, likely due to direct endothelial release in the lungs, highlighting a dynamic short-term effect.
ABO blood group[24]	Blood group B and AB are associated with higher plasma ACE activity, while group O and A have lower levels; influences ACE independently of ACE I/D genotype.
Thyroid disease[25]	Hyperthyroid patients show elevated ACE, which normalizes with successful treatment.
	Hypothyroid individuals exhibit lower ACE, which increases with levothyroxine replacement.
	These effects appear quantitative, potentially due to hormone-driven synthesis or release of ACE, not necessarily enzyme activity per se.

Table 3: Performance of Serum ACE when combined with other investigations

Combination	Indication	Findings (Sensitivity, Specificity, etc.)
ACE + Gallium-67 scan[26]	Ocular sarcoidosis (clinical suspicion, normal/equivocal CXR)	- Sensitivity: 73% - Specificity: 100% when both positive
ACE × Chitotriosidase (new parameter derived by multiplication of ACE by Chitotriosidase activities -double product)[27]	Systemic sarcoidosis (suspected)	Sensitivity: 90.5% - Specificity: 79.3%
ACE and/or sIL-2R[28]	Sarcoid uveitis (Japanese cohort)	- ACE alone: Sensitivity 44.2%, Specificity 100% - sIL-2R alone: Sensitivity 69.2%, Specificity 93.0%
		- Combined (either elevated): Sensitivity 75.0%, Specificity 93.0%
ACE + lymphopaenia[29]	Sarcoid uveitis (uveitis cohort)	- ACE alone: Sensitivity 45.8%, Specificity 88.8%

		- Lymphopaenia alone: Sensitivity 15.3%, Specificity 96.7%
		- Combined (both): Sensitivity 18.9%, Specificity 99.0%, PPV 73.9%, NPV 89.5% - In age ≤50 subgroup: Sensitivity 31.3%, Specificity 99.7%, PPV 90.9%, NPV 94.3%

Table 4: Summary of diagnostic Value, usefulness and limitations of Serum ACE and Lysozyme in Sarcoidosis

Marker	**Usefulness**	**Limitations**
Serum ACE	- High specificity	- Low sensitivity; many false negatives.
	- Reflects granuloma burden	- Affected by ACE-inhibitor use and by ACE gene I/D polymorphisms; genotype correction may be needed.
	- Helpful as a diagnostic adjunct and for monitoring activity.	- poor positive predictive value and poor correlation with disease severity.
	- High negative predictive value	
Serum Lysozyme[30,31]	- Sensitivity higher than ACE: Detects sarcoidosis even when ACE is normal	- Much less specific than ACE; elevated in other diseases
	- Correlates with number of organs involved and radiographic stage; useful for monitoring disease progression and activity.	- Low specificity and modest sensitivity limit its standalone use in diagnosis.
	- Reflects biologically active granuloma mass and normalizes with treatment; can guide corticosteroid dosing.	- Not easily available

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Awards and Accolades

Dr. Jyotirmay Biswas

Sankara Nethralaya, Chennai

1. *Achievement Award, American Academy of Ophthalmology.*
2. *Asia Pacific Eye 100: Top 100 Ophthalmologists award, APAO-AIOC, New Delhi, 2025*
3. *Dr. David BenEzra Award, IOIS Congress, 26th June, Rio de Janeiro*
4. *Dr. P B Menon Oration award, Kozhikode Ophthalmic Society, 1st September 2024*
5. *Gold medal award for Dr. B N Datta Oration by Association of cytologists and histopathologists, 1st February, 2025, Surat.*
6. *Sri K C Somany memorial lecture, EYEconic annual eyecare fest, 20th July 2025, Kolkata.*

Use of Polymerase Chain Reaction and TORCH Screening in Infectious Uveitis

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A tailored diagnostic approach is a vital part of uveitis management. The polymerase chain reaction (PCR) has revolutionized the field of molecular diagnostics since its introduction of modern form in 1988.[1] In uveitis, PCR is utilized in the diagnosis of infectious uveitis especially in cases of posterior uveitis with opaque media, PCR has a diagnostic role.[2] In cases of non-infectious uveitis, PCR is utilized in human leukocyte antigen (HLA) typing.[3]

PCR allows use of a very small amount of starting material to produce analytic quantities of DNA. Hence, this is a powerful tool in molecular biology and all practicing ophthalmologists should have a clear knowledge about utilising this technique.

PCR has a benefit of being performed in nearly any ocular specimen. The commonly used specimens are aqueous or vitreous aspirate. For diagnostic purposes, the required quantity is 50 microlitres of aqueous or 100-500 microlitres of undiluted vitreous aspirate. One of the important steps in PCR testing is proper transport of samples in a frozen aseptic manner to the laboratory for processing as repeated freeze-thaw cycles degrade the RNA and DNA.

PCR serves as an adjunct test in cases with granulomatous anterior uveitis to establish tubercular etiology wherein other

supportive investigations like Mantoux, interferon gamma release assay or high-resolution computed tomography (HRCT) of chest is indecisive. A PCR positivity when performed early in the disease process also helps in deciding initiation of anti-tubercular treatment.[4] In cases with suspicious tubercular origin like choroidal granuloma, choroidal abscess, serpiginous like choroiditis and multifocal choroiditis, it is advised to do PCR for improved diagnostic outcome.[5]

The real-time PCR correlates well with the clinical involvement extent and helps in confirmation of diagnosis in over 50% of cases with suspected viral retinitis.[6] The utility of PCR in viral anterior uveitis with secondary glaucoma was found to be significant in timely and accurate identification of virus inciting the pathology thereby improving the visual outcome.[7]

PCR based restriction fragment length polymorphism (RFLP) technique was used to demonstrate Aspergillus fumigatus fungus in an enucleated paraffin section of an eyeball in an 8-month-old child with suspected endogenous endophthalmitis.[8] The gradient PCR testing can also be used as a novel technique in detection of dirofilaria species.[9]

Even challenging situations like Nocardia scleritis, PCR-based gene sequencing plays a major role establishing at a confirmatory diagnosis enabling timely management.[10]

The real-time PCR comes handy in HIV patients with inconclusive cause of intraocular inflammation. Earlier reports in literature suggest that a high intraocular fluid: plasma HIV RNA load ratio is indicative of HIV induced uveitis and decrease in the HIV load in aqueous tap is found to have a prognostic value.[11]

Evolution is constant in medical field and PCR is no exception to this rule. PCR has advanced from single gene PCR to multiplex PCR and now has reached the stage of chip-based real-time PCR.[12]

Role of PCR:

PCR is an effective investigative tool in the field of ophthalmology. Its varied applications include

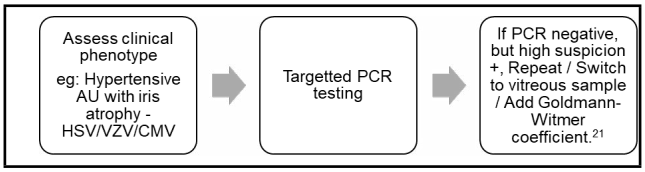
- Tracing the disease to its etiological agent in infectious uveitis. This dates back to 1990 wherein toxoplasma gondii was isolated in ocular tissue through PCR testing.[13]
- The viruses that cannot be cultured were demonstrated through PCR analysis.[14]
- PCR helped in successful detection of several cases of fungal endophthalmitis.[15]
- Role of establishing infectious association of diseases – demonstrating rubella virus in Fuchs uveitis and Mycobacterium tuberculosis in Eales disease.[17]
- HLA typing in non-infectious uveitis.
- IgH gene rearrangement identification through PCR in ocular masquerades.

PCR has an advantage of being a simple, rapid, sensitive and specific tool in diagnosis of ocular infectious, autoimmune and masquerade conditions.[18] However, PCR testing has few pitfalls to be noted. It has a high false positive rates due to likely laboratory contamination during the amplification process and it has little value in scenarios wherein the differentials have not been well established owing to capacity to detect organisms for which the primer set is included.

TORCH complex is a group of congenital infections acquired perinatally and includes Toxoplasmosis, Others (syphilis, hepatitis), Rubella, Cytomegalovirus and Herpes simplex.[19] The commonly followed screening test for this group of infections is assessing the IgM and IgG titres in serum. This was mainly designed for testing congenital infections and not for uveitis.

Role of TORCH Screening:

The assessment of serum IgM levels suggest active infection and IgG is considered a marker of past exposure. However, presence of IgM in serum alone is inadequate as a diagnostic tool.[20] Hence a blanket TORCH panel is very misleading investigation in the path to diagnosis of uveitis.



A Simple Algorithm on Decision for Testing PCR / TORCH Panel:

While testing, points to remember are:

1. **Tailor** the testing based on **suspected phenotype** and **timing** of the test.
2. Don't consider **blanket TORCH panel** for uveitis.
3. Time the sample **early in the disease** process before or as soon as the empirical treatment is initiated for better results.

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Practical insights: Prof. Dr. Jyotirmay Biswas

I do PCR in infectious uveitis, in particular viral retinitis. As the virus is often impractical to get for cytology or culture, the PCR is the right and rapid test to **precisely identify** viral infection in necrotising retinitis, particularly viral retinitis. There are two types of PCR nested and real-time PCR. I feel real-time PCR is more valuable because it gives copy number indicating the replicating DNA. In case of viral anterior uveitis, I started doing the PCR more often, particularly when there is a coin-shaped keratic precipitate or anterior uveitis without synechiae and high spike of intraocular pressure in the range of 40s or 50s. The possibility of the viral aetiology is quite high. I do both nested and real-time PCR for all the three viruses - HSV, HZV and CMV.

In case of granulomatous uveitis, I tried to make the diagnosis by ancillary tests like Mantoux, QuantiFERON-TB Gold test, HRCT chest and serum angiotensin-converting enzyme. If there is a strong suspicion like of tuberculosis like granulomas of the iris or ciliary body, I do AC tap for PCR when there is anterior chamber inflammation. If there are no cells in the anterior chamber, I do not do anterior chamber tap because the DNA is not available in the anterior chamber in such cases to get it amplified.

Here we give an example of a case of granulomatous anterior uveitis with necrotizing retinitis in a patient with history of Non-Hodgkin Lymphoma and received 6 cycles of chemotherapy Fig 1-4.

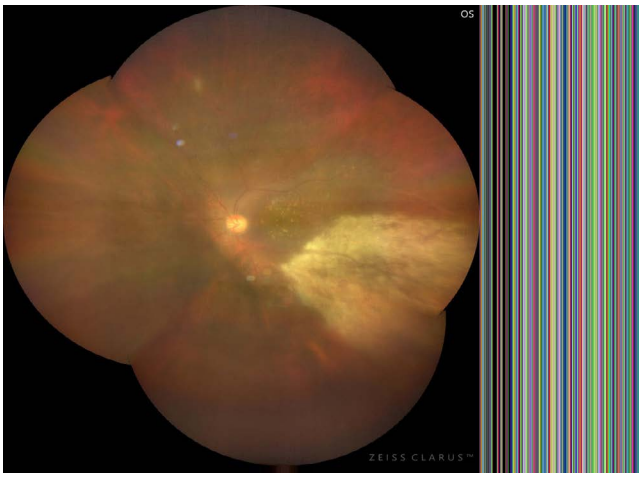

***Figure 1**. Slit lamp photograph (OS) shows large Keratic precipitates on the posterior surface of the cornea*

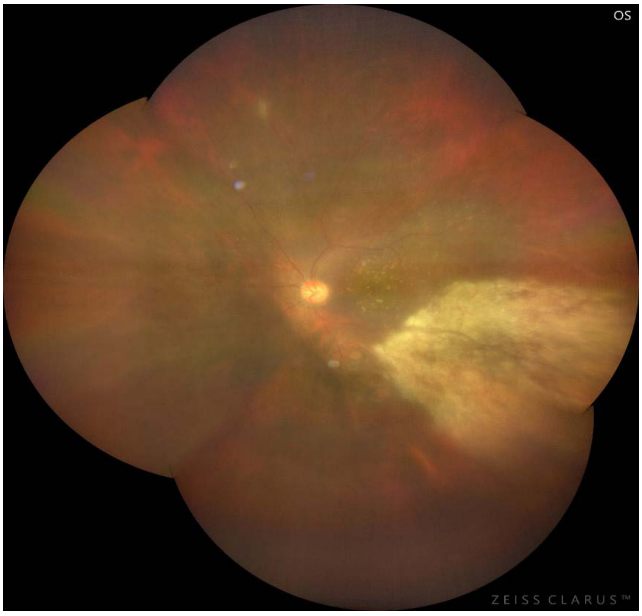

***Figure 2.** Color fundus photograph (OS) showing retinitis lesion infero-temporally*

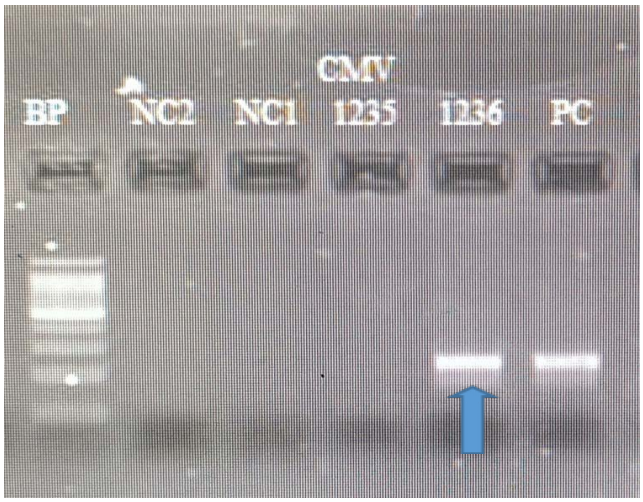

***Figure 3.** Nested PCR amplification of CMV, Lane 1236 shows a positive band.*

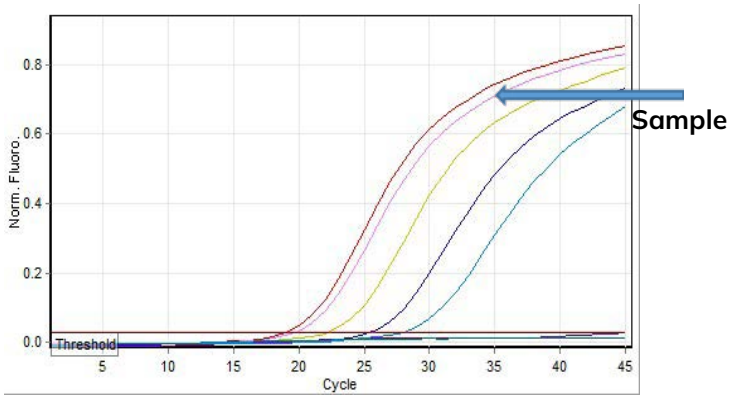

***Figure 4.** Real-time PCR amplification curves showing standards (S1–S3), sample (MB-NO-12345/25, Ct = 19.82; ~6200 copies/µL), and controls.*

Awards and Accolades

Dr. Manu Sharma

1. *Postgraduate Institute of Medical Education and Research, Chandigarh Carl Herbort Travel Award For Best Free Paper On Ultrawide Field Imaging In Takayasu Arteritis- What We Were Missing, USICON, 4-6 October, 2024, Chandigarh*
2. *First Prize In Photo Contest, For A Image Titled- Snowflake Appearance Of Fundus, USICON, 4-6 October, Chandigarh*

Mantoux Test Revisited: Comparative Insights into 1 TU, 5 TU, and 10 TU Tuberculin Strengths in the Diagnosis of Tuberculosis

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Introduction

Tuberculosis (TB) continues to pose a significant global health challenge, with India accounting for a substantial share of both latent and active disease.[1] Among its extrapulmonary manifestations, tubercular uveitis (TBU) is of particular concern due to its diagnostic complexity and potential for vision loss. There is a high variation in the prevalence of TBU, ranging from 0.2 to 10.5% in the reported series of uveitis patients from different regions of the world. In comparison, the reported incidence in India is around 22.5%.[2,3]

In endemic areas, the Mantoux test, also known as the Tuberculin Skin Test (TST), remains a long-established diagnostic tool for detecting both latent and active tuberculosis infection. First introduced by Charles Mantoux in 1907, the test is based on a **Type IV delayed-type hypersensitivity reaction** to purified protein derivative (PPD) injected intradermally. The degree of induration reflects prior sensitisation to *Mycobacterium tuberculosis* or related mycobacterial antigens.

The diagnostic utility of the Mantoux test depends significantly on the strength of PPD used, expressed in tuberculin units (TU). This review aims to evaluate the comparative diagnostic performance of 1 TU, 5 TU, and 10 TU strengths of the Mantoux test, with a specific focus on their interpretation, limitations, and relevance in the diagnostic algorithm of uveitis in TB-endemic settings.

Immunological Basis

The Mantoux test hinges on a classic Type IV delayed-type hypersensitivity reaction, a hallmark of cell-mediated immunity. It assesses the host sensitisation to *Mycobacterium tuberculosis* by detecting memory T-cell responses to intradermally injected tubercular antigens.[4]

Purified protein derivative is a heterogeneous, standardised mixture of over 200 low-molecular-weight proteins, lipids, and polysaccharide fragments derived from filtrates of tubercle bacilli culture (heat-sterilised, protein-precipitated). The first standardised PPD was PPD-S, prepared by Florence Seibert in the 1930s and later adopted as the international reference by WHO and the CDC.[5] In India and several other countries, PPD-RT23 with Tween 80 (derived from the Danish Statens Serum Institute) is commonly used due to its consistent potency and stability.

PPD of non-tuberculous (i.e. atypical) mycobacteria is identified by a letter other than S, serves both research and comparative diagnostic purposes, like PPD-A from *Mycobacterium avium*, PPD-B from *M. intracellulare* (Battey bacillus), and PPD-G from *M. scrofulaceum*.[4] These variants are not routinely available in India and lack standardised interpretation thresholds. Cross-reactivity with environmental mycobacteria may lead to false-positive Mantoux reactions, especially in tropical and endemic areas.

Upon intradermal administration of PPD, local antigen-presenting cells - primarily dermal dendritic cells (Langerhans cells) - capture and process the antigens. These cells present processed peptides via MHC class II molecules to primed $CD4^+$ Th1 lymphocytes in individuals previously exposed to *M. tuberculosis*. Activated T cells secrete pro-inflammatory cytokines - IFN-γ, TNF-α, and IL-2 - culminating in mononuclear cell infiltration, endothelial activation, and local tissue induration at the site of injection. The reaction peaks at **48–72 hours**, with the diameter of induration (not erythema) being the critical parameter.[4]

Tuberculin Units and Strength Variability

The strength of PPD determines the biological efficacy of the Mantoux test used, expressed in tuberculin units (TU). These units indicate the amount of antigen needed to produce a standardised delayed-type hypersensitivity response in sensitised guinea pigs and are used to calibrate diagnostic potency across formulations.

Globally, PPD-S, developed by Florence Seibert in the 1930s, remains the international reference standard and is typically administered at a dose of 5 TU/0.1 mL, as endorsed by the WHO and CDC. In contrast, the formulation more widely used in India is PPD-RT23 with Tween 80, manufactured by the Statens Serum Institute, Denmark.[5] Commercially available kits - produced by Span Diagnostics, Arkray, and others - offer RT 23 in 1, 5, and 10 TU

concentrations. The choice of TU strength significantly influences test interpretation, particularly in TB-endemic regions and in extrapulmonary TB presentations like uveitis. (Table 1) However, one must remember that the **cutoff for higher-strength TST is not established**, and the standard cutoff of 10 mm can lead to high false positives.[6] Therefore, it is essential to pay attention to both preparation and dosing when interpreting test results. In India, the National TB Elimination Programme (NTEP) officially recommends the use of 2 TU of PPD-RT23 for routine Mantoux testing, including in extrapulmonary TB settings. Notably, **2 TU of PPD-RT23 is biologically equivalent to 5 TU of PPD-S**. WHO explicitly cautions against dose escalation beyond 5 TU PPD-S in routine practice, citing increased false-positive rates and reduced diagnostic reliability.[6]

Administration and Interpretation

The Mantoux test is performed by intradermal injection of 0.1 mL PPD, typically on the volar aspect of the left forearm, using a 26 or 27-gauge tuberculin syringe. The bevel should face upward to produce a discrete 6–10 mm wheal. Subcutaneous placement must be avoided, as it can lead to invalid results due to antigen dispersion. No local dressing or massage should be applied. The test site is evaluated **48 - 72 hours** after intradermal injection. The transverse diameter of palpable induration (not erythema) is measured in millimetres using either the ballpoint pen method or gentle palpation by trained personnel. A record should also be made of local reactions, such as vesiculation, bullae formation, lymphangitic spread, ulceration, or necrosis

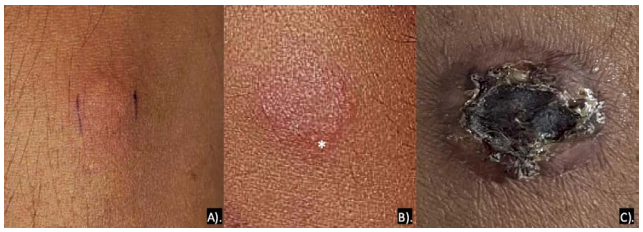

Figure 1. *- A) 16mm induration in a case of TBU at 48 hours. B). Blisters with >15mm induration at 48 hours C). Skin necrosis with ulceration at 2 weeks.*

Their presence signifies a marked hypersensitivity reaction and is strongly suggestive of significant prior sensitisation to *Mycobacterium tuberculosis*.

Interpretation depends on induration size, patient risk factors, and PPD strength used. Induration cut-offs may require adjustment - using higher TU leads to generally higher induration values. For standard 2 TU PPD-RT23 (recommended by NTEP) or 5 TU PPD-S (CDC/WHO), the following cut-offs are applied (Table 2).

Diagnostic Value in Tubercular Uveitis (TBU)

The diagnostic value of the Mantoux test in Tubercular Uveitis (TBU) lies in identifying prior sensitisation to *Mycobacterium tuberculosis* in patients with otherwise unexplained intraocular inflammation. In TB-endemic regions, it remains an accessible and widely used immunological marker to support the diagnosis of presumed ocular tuberculosis, particularly when microbiological confirmation is lacking. Clinical observations show that positive Mantoux results often correlate with treatment response, even in the absence of systemic TB or radiologic findings, suggesting an underlying immune-mediated ocular hypersensitivity to mycobacterial antigens.[7]

The diagnostic yield of the Mantoux test is significantly influenced by the strength of tuberculin derivative used. The 1 TU dose, previously employed in epidemiological studies, demonstrates limited sensitivity and is considered inadequate for clinical decision making in uveitis. The 5 TU dose, endorsed by international guidelines (CDC/WHO), offers a more balanced sensitivity-specificity profile. On the contrary, the 10 TU PPD-RT23 formulation, though not internationally standardized, is commonly used in some of the Indian setups, aiming to enhance sensitivity in detecting latent or extrapulmonary TB. However, this increased sensitivity comes at the cost of reduced specificity - especially in those exposed to NTM or BCG-vaccinated individuals - raising the risk of false-positive results.

Clinical Phenotypes that Increase the Likelihood of Ocular TB

Overreliance on the Mantoux test without corroborative clinical or imaging evidence may lead to misdiagnosis and inappropriate initiation of anti-tubercular therapy. Thus, interpretation should be guided by the clinical context.

The diagnostic value of the Mantoux test, is significantly enhanced when interpreted in the context of specific clinical phenotypes highly suggestive of tubercular uveitis. These phenotypes, often identified in the Collaborative Ocular Tuberculosis Study (COTS) and other research, act as a "clinical filter" that increases the pre-test probability of the disease, thereby strengthening the significance of a positive Mantoux result.[2,8]

Serpiginous-like choroiditis

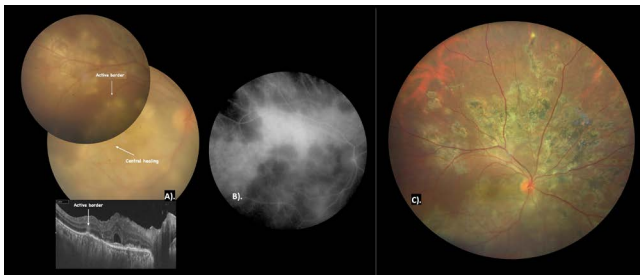

Figure 2. *Case 1 - 27 years old male with BCVA 5/60, having anterior segment cells and keratic precipitates with A). diffuse hypopigmented yellowish lesion with central healing and active borders. B). Fluorescein angiography showing geographic areas of hypofluorescence. His mantoux test had 21mm induration with fibrocalcific scars in left upper lobe on HRCT chest. C). Fundus photograph of right eye showing complete serpiginous like scarring on completing treatment with steroids and ATT.*

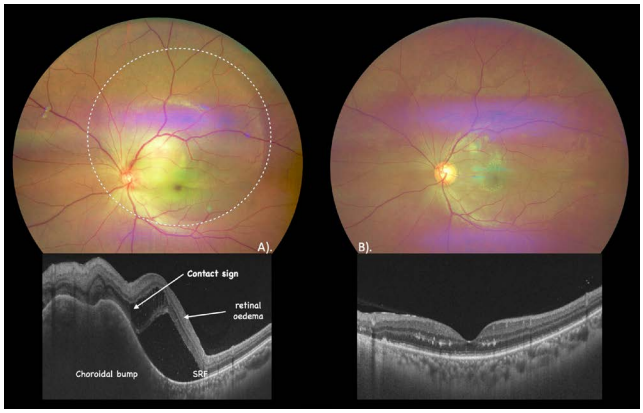

Figure 3. *Case2 - 37 Years old male presenting with DOV in LE A)- fundus photograph showing solitary choroidal granuloma (dotted circle) with overlying retinal edema and surrounding SRF. B). Fundus photograph of LE having BCVA- 6/9 at one month after initiating ATT along with oral steroids and intravitreal anti-VEGF.*

Choroidal Granuloma, Obliterative retinal vasculitis with or without vasocentric choroiditis and Granulomatous anterior uveitis with broad-based posterior synechia are clinical presentations that are most strongly associated with ocular TB, increasing the post-test probability of a positive Mantoux test.

This combination of a highly suggestive clinical picture and a positive immunological test provides a strong rationale for initiating a trial of anti-tubercular therapy (ATT), even in the absence of systemic or microbiological confirmation. The diagnosis is further supported if the patient shows a favourable clinical response to the ATT.

Limitations and Pitfalls

Despite its longstanding use in uveitis work-ups, the Mantoux test is associated with several important limitations that constrain its standalone diagnostic value:

- **Lack of specificity:**
  - Does not differentiate between latent TB, active TB, or ocular hypersensitivity to mycobacterial antigens.
  - Prior BCG vaccination and environmental exposure to NTM frequently cause false-positive results, especially in endemic regions.
- **False negatives:**
  - Common in patients with immunosuppression, including those on systemic corticosteroids, cytotoxic agents, or suffering from systemic immune dysfunction.
  - Can also occur during the early phase of infection, before adequate sensitisation has developed.
- **Overinterpretation risk in uveitis:**
  - A positive Mantoux result is often overvalued in the absence of systemic or radiological evidence, potentially leading to misclassification of non-tubercular uveitis as presumed ocular TB and overtreatment.

Conclusion

The Mantoux test, although an imperfect diagnostic tool, remains a valuable immunological marker in evaluating suspected tubercular uveitis in endemic regions. Its diagnostic usefulness heavily depends on the tuberculin dose used, each with a unique sensitivity and specificity profile. The internationally recognised 5 TU dose offers a balanced diagnostic performance, but its effectiveness may be limited in TB-endemic settings where a high level of sensitisation exists. Conversely, the 10 TU dose increases sensitivity, which is vital for identifying latent and extrapulmonary TB. However, this comes with decreased specificity, especially in populations with high BCG vaccination rates or environmental mycobacterial exposure. Therefore, interpreting the Mantoux test requires consideration of the tuberculin dose and, more importantly, the clinical context.

Table 1

Strength	Preparation Commonly Used	Uses	Sensitivity	Specificity	Clinical Implications
1 TU	PPD-S, PPD-RT23	Formerly used for epidemiological surveys (ICMR) , especially in children	Low	High	Risk of false negatives; not recommended for routine diagnosis in adults or uveitis work-up
5 TU	PPD-S (WHO/CDC standard)	Global diagnostic standard for latent TB; recommended by CDC/WHO	Moderate	Moderate–High	Balanced trade-off; good for baseline screening; commonly used in IGRA-complemented settings
10 TU	PPD-RT23 (most Indian kits)	Used in clinical practice in India, especially for extrapulmonary TB	High	Low-Moderate	May increase false positives in BCG-vaccinated/ NTM-exposed populations **[6]**; increases sensitivity in endemic settings

Table 2

Induration Size	Positive in
5 mm or more	HIV-positive person, recent contacts of people with infectious TB, people who have fibrotic changes on a chest X-ray, patients with organ transplants, severely malnourished children, other immunosuppressed patients (e.g., on prolonged corticosteroids).
10 mm or more	People born in TB-endemic countries (Mexico, Philippines, Vietnam, India, China, Haiti, and Guatemala), IV drug users, people who live or work in high-risk congregate settings, people with certain medical conditions with high risk for TB (e.g., silicosis, DM), children younger than 5 years, infants, children, and adolescents exposed to adults in high-risk categories.
15 mm or more	Persons with no known risk factors for TB. Reactions larger than 15 mm are unlikely to be due to previous BCG vaccination or exposure to environmental mycobacteria.

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Awards and Accolades

Dr. Mayur R. Moreker

Bombay Hospital Institute of Medical Sciences, Mumbai

Dr. V. K. Chitnis Oration Award from Maharashtra Ophthalmological Society, 20th October 2024; Annual Conference of Maharashtra Ophthalmological Society (MOSCON 2024); Solapur

Quantiferon Gold Or Mantoux Testing? Or Both?

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Ocular tuberculosis (TB) is an important cause of visual morbidity globally. The diagnosis remains difficult, and a variety of clinical and immunological techniques exist to assist in the diagnosis. The two most common immunological tests are the Mantoux test and IGRA (Interferon Gamma).

The Mantoux test (also known as the tuberculin skin test (TST)) which was developed by Koch and Charles Mantoux, uses a standardised purified protein derivative (PPD), This intracutaneous injection of PPD (in India, PPD-RT-23 is used) incites a delayed (cellular) hypersensitivity reaction. Sensitized T-cells at the injection site release lymphokines that induce erythema and induration. A positive Mantoux is proof of previous exposure to tuberculosis i.e., latent tuberculosis, but not necessarily active disease. Common tuberculin strengths of 1 TU and 2 TU are available. This test measures the degree of hypersensitivity to PPD.

The TST has a sensitivity of roughly 70% in cases of active pulmonary TB. Its specificity in latent TB is poor in low-endemic areas. TST sensitivity and specificity for assessing ocular tuberculosis vary from 92% to 95% and 72% to 90%, respectively.[1] Advantages of Mantoux testing include: low cost, easy to administer and read and no need for sophisticated laboratory support.

Current interpretation guidelines

Five mm or more is positive in

- HIV-infected individuals
- Recent contacts of active cases
- Solid organ transplant recipients and other immunosuppressed patients

· Patients on long-term systemic corticosteroid therapy (> than six weeks) and those on a dose of prednisone ≥ 15 mg/ day or equivalent.

Ten mm or more is positive in injectable drug users

- Mycobacteriology lab personnel
- Persons with high-risk clinical conditions (e.g., diabetes mellitus or long-term corticosteroid therapy)
- Fifteen mm or more is positive in
- Persons with no known risk factors for TB.

IGRA (Interferon Gamma Release Assay) Tests

Two versions of IGRA tests are available. The first, QuantiFERON-TB-Gold (QFN-TB), assesses IFN-γ concentration by the ELISA technique. The blood sample is incubated for 24 hours with ESAT6 and CFP10. In the presence of active or latent tuberculosis, there will be antigen recognition with secretion of IFN-γ by the T Cells. The quantum of IFN-γ is assessed by optical density.

Some newer versions use an additional antigen (TB7.7). The second assay is the T-

SPOT-TB. This counts the number of IFN-γ-secreting peripheral blood T cells.

Since the antigens used (ESAT6, CFP10, and TB7.7) are exclusive to Mycobacterium tuberculosis, IGRAs may be useful in populations where BCG vaccines are still used. IGRAs exhibit sensitivity equivalent to that of TST for the diagnosis of active pulmonary TB (76%) and latent TB[1], and are reliable for immunocompromised patients. Disadvantages include a higher cost and a need for sophisticated laboratory support. Ang et al analysed both tests for a cohort of presumed ocular tuberculosis patients in Singapore and were able to report that QuantiFERON was more accurate in identifying true-positive cases than was T-SPOT.TB, and suggested that it should be the initial test that should be performed.[2]

Immunological tests in ocular tuberculosis

Due to its earlier introduction, most initial large-scale studies of ocular tuberculosis utilised the Mantoux test.[2] An early Italian study by Cimino and co-workers described the use of Mantoux testing to diagnose and successfully treat a cohort of 35 patients.[3]

The introduction of IGRA testing led to a several large-scale epidemiological studies that utilised IGRA as well. Ang et al studied the role of the Quantiferon gold assay in the assessment of tuberculous uveitis. They reviewed the records of 157 patients with suspected TB uveitis and were able to report that QFT is not superior to the TST in sensitivity but had a higher specificity in the identification of Mycobacterium tuberculosis.[4]

Mackensen et al utilised QuantiFERON testing to assess for a possible tubercular etiology in patients with serpiginous-like choroiditis. Of 26 patients, 21 were tested, of whom 11 tested positive. Four were subsequently treated with antitubercular treatment. They pointed out that a high proportion of tested patients had a tubercular etiology.[5]

Albini et al have suggested that the benefits of IGRAs relative to the TST as a screening investigation in a uveitic work-up in a low-endemic area are minimal, largely due to a low pre-test probability. In endemic areas, an increased pre-test probability and consequently increased post-test probability could mandate the use of IGRA testing.[6]

In an Indian context of endemic tuberculosis, the largest study to date was conducted in Chennai. Sudharshan et al analysed 50 patients with suspected tubercular uveitis. The Quantiferon test was positive in 29 patients, with positive radiological findings in four patients. 11 patients had both tests positive. Eighteen Mantoux-negative patients were positive on Quantiferon testing, but no patient with a positive Mantoux test tested negative on a Quantiferon test. They pointed out the important role of Quantiferon testing in the identification of latent TB.[7]

In a study of clinical practice, Babu et al analyzed the survey responses of 37 physicians (19 uveitis specialists, 9 rheumatologists, and 9 pulmonologists), the majority of whom agreed on the role of Quantiferon TB Gold assay in the diagnosis of latent TB. However, its use was limited due to cost and its inability to differentiate latent from active disease. 51 % of physicians suggested a combination of the Mantoux test and Quantiferon TB Gold test.[8]

In summary, a dual test strategy in a high-endemic area (like India) might be ideal. This would increase the specificity for detecting tubercular uveitis, with a high accuracy, with a positive likelihood ratio of 6.1 (95%CI, 1.6–23) for the Quantiferon/ Mantoux combination.[1]

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Awards and Accolades

Dr. Padmamalini Mahendradas

Narayana Nethralaya, Bengaluru

1. *APAO Achievement Award at APAO- AIOC, April 2025, New Delhi.*
2. *The International Ocular Inflammation Society Picture award 2025 for the best image at the annual Congress of the IOIS, Rio de Janeiro, 25-28 June 2025.*

Laboratory testing in post fever retinitis: A guide.

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Post-fever retinitis is an infectious or para-infectious uveitis caused by bacteria or virus. It is commonly seen in tropical countries.[1] Ocular manifestations are seen within days to weeks after the onset of febrile illness. The etiopathogenesis of this uveitic entity is unclear. Irrespective of the etiology of the fever, clinical manifestations are similar with predominant signs at the posterior pole of the retina. A response to steroids indicates an immunological pathology. When the patient presents to the ophthalmologist, they are afebrile and systemic features are resolved. However, it is necessary to rule out the treatable causes which might have caused the post febrile retinitis. The ophthalmologist also needs to be aware of the recent outbreaks or endemic infections in the region, which may be the probable cause. There is no specific guideline for the management of post febrile retinitis. Diagnosis of fever is challenging due to non-specific systemic symptoms such as fever, myalgia and headache. This article outlines the laboratory investigations for the diagnosing the etiology.

Rickettsial retinitis

There are specific history and clinical signs such as maculopapular skin rash, presence

of eschar, history of recent forest visits, insect bite and exposure to animals which aid in the diagnosis of rickettsia.

The rickettsial organisms can be isolated from body fluids on different cell lines. But the serological tests are the popular. The serological tests for the diagnosis of rickettsial diseases include:

1. Microimmunofluorescence
2. Latex agglutination
3. Indirect hemagglutination
4. Immunoperoxidase assay
5. Indirect immunoperoxidase assay (IPA)
6. Immunofluorescence assay (IFA)
7. ELISA- Immunoglobulin M (IgM), Immunoglobulin G (IgG)
8. Polymerase Chain Reaction (PCR)

These serological tests are considered gold standards. However, they are not available in many laboratories and require high level expertise.[2] Hence, it is recommended for regional research and in areas where seroprevalence of rickettsial diseases has been established.

IgM antibodies, indicates recent infection with rickettsia. A high IgM antibody titre is observed at the end of first week, whereas IgG antibodies appear at the end of second week. Baseline titres need to be established due to the regional variations.

Polymerase chain rection is a rapid and specific test for diagnosis. It can detect rickettsial DNA in whole blood. The results are positive within the first week (first 7-10 days) for blood samples because of presence of rickettsemia.[2]

Supportive laboratory investigations: These investigations are required depending on the severity and development of complications. These investigations can help in deciding upon appropriate treatment of patients.

1. Complete blood counts: Total leucocytes count during early course of the disease may be normal but later in the course of the disease, leucocytosis and thrombocytopenia are seen.
2. Liver function test: Raised transaminase levels.
3. Chest X-ray: Bilateral infiltrates

It should be considered in a case of retinitis with a recent history of fever with skin rash. The visual prognosis is good and there are no recurrences

Typhoid retinitis

Typhoid retinitis is an immune mediated retinitis. This is a rare sequela to typhoid infection which occurs secondary to direct invasion of the Salmonella typhi.

The diagnosis for typhoid is by the method of culture and antibody detection by the Widal test. Isolation of Salmonella typhi is the gold standard, with culture from blood. However, facilities for culture are not readily available or are limited in many areas. Isolation of Salmonella typhi by culture though has specificity, it lacks sensitivity and speed.[3]

The Widal test, which uses the agglutination technique for the diagnosis of typhoid and paratyphoid fevers. In endemic areas where culture facilities are lacking or limited, the Widal test differentiate typhoid from other

causes of fever. A four -fold rise in antibody titers between acute and convalescent sera, is essential. Usually, interpretation based on a single serum specimen which may not reflect the diagnostic value of the test. When paired sera are obtained, a decrease in titer is observed when comparing the convalescent titer to the acute titer.[3] This is because most patients consult during the convalescent phase, after initial treatment by the general practioners fail.

Also while interpreting the Widal test it has to be performed with the background normal titer of the population in the particular area. It is not uncommon to find what is considered positive in a non-endemic area may be considered normal in an endemic area. The interpretation of the tests may also vary among the endemic areas.

Due to the poor accuracy of available diagnostic tests, attempts have been made to develop polymerase chain reaction(PCR) -based assays to detect bacterial DNA.[4] PCR-based laboratory methods to confirm clinical diagnosis of typhoid fever are not commonly reported. This is of importance in endemic areas, where antimicrobial pre-treatment or immune interference are common and thereby reduce culture sensitivity.

A study to compare polymerase chain reaction with blood culture and Widal test for the diagnosis of typhoid in patients taking antibiotics was done by Choudhry et al This study demonstrated that the positivity rate of polymerase chain reaction was significantly higher as compared to blood culture or Widal test for diagnosing typhoid in patients who were already taking antibiotics.[5] This is useful in cases of post-fever immune mediated ophthalmic sequelae.

Acharya et al. found that high Widal titers were associated with an increased risk of bilateral involvement, extensive ocular involvement and poor visual acuity.[6]

Dengue retinitis

Dengue fever related ocular involvement is rare, occurring in less than 10% of patients with symptomatic dengue.[7] Dengue foveolitis and retinal vasculitis have been reported.[8]

Dengue is diagnosed by NS1 antigen assays within the first week of illness, subsequently dengue serology IgM and IgG antibody testing are done.[9]

Real-Time (RT)-PCR assay is a nucleic acid amplification assay that detects DENV (dengue virus) serotypes 1, 2, 3 or 4 RNA from human serum or plasma.[10]

Complete blood counts are also essential in patients with dengue as there is correlation of thrombocytopenia and ocular complications. Also, visual recovery correlates with improvement of thrombocytopenia.[11] Though the exact mechanism is not clear, it is thought to involve antibody production, immune-complex deposition or production of autoantibodies.

Various dengue serotypes can cause infection in the same patient later in life and do not provide cross immunity. It is important for physicians to be aware and educate the

patients about possible ocular symptoms after the dengue has been treated.

West Nile retinitis

The most common ocular manifestation of West Nile Virus is bilateral multifocal retinitis and retinal vasculitis.

Diagnosis of West Nile Virus (WNV) infection is based on the ocular signs, and is confirmed by positive serologic testing or PCR. Serology for West Nile Virus is less specific due to the cross-reacting antigens among flavivirus infections. Therefore, molecular diagnostic assays are used for confirmation. Real-time (RT)-PCR and RT-LAMP (Reverse Transcription Loop-Mediated Isothermal Amplification) assays have a high sensitivity even with a low copy number of templates. There are no concerns about cross reactivity. Sequencing of RT-PCR amplified product and genotyping corroborated the RT-PCR results. Results from molecular techniques are discordant from serology findings because of the delay in the appearance of antibodies against WNV. Antigen capture ELISA and molecular techniques will be positive even in early cases, whereas antibody-based ELISA testing will be positive only after approximately 2 weeks, when the antibody response is high.[12]

Chikungunya retinitis

Chikungunya infection produces a sudden onset of fever, joint pains and erythematous skin rashes. The common ocular sign such as multifocal retinitis is a delayed presentation.[13]

Virus isolation using PCR and serological tests (IgM and IgG antibodies) are used to diagnose chikungunya infections.[14] Virus isolation and real-time polymerase chain reaction (RT-PCR) are useful during the initial viremic phase of the illness. Antibodies can be detected from the serum in the later phase of the disease.

Zika virus retinitis

Zika virus infection especially patients with neurological symptoms, may have changes in the retinal vasculature. These changes can affect vision and should be monitored closely.[15]

The laboratory diagnosis of Zika infection is limited to research laboratories. The Zika virus cross-reacts serologically with other flaviviruses. (Table 1). Active infection, resolved infection and vaccination for any flavivirus, will cause false positive results.

Table 1: Flavivirus with serology cross reactivity

	Flavivirus with serology cross reactivity
1	Dengue virus: serotypes1,2,3,4
2	West Nile virus
3	Zika virus
4	Yellow fever virus

Detection of viral RNA during acute infection using nucleic acid amplification tests provides accurate results. Testing of whole blood and urine is also recommended due to the high viral loads. However, nucleic acid amplification testing has limited use

because many patients are asymptomatic or present for evaluation after the infection has resolved. The challenge is development of accurate antibody tests for the diagnosis of recent Zika infection. Research is needed to identify Zika virus epitopes that do not cross react with other flavivirus antigens. With the ongoing research it is hoped that rapid progress will be made in developing diagnostic tools.[16]

Post fever retinitis resolves without any relapses. The visual prognosis depends on macular ischaemic damage and optic nerve involvement. Early referral by the treating physician would result in a better visual outcome.

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Awards and Accolades

Dr. Reema Bansal

Postgraduate Institute of Medical Education and Research, Chandigarh

Major General Amir Chand Silver Medal by PGIMER, Chandigarh, for PhD category for "Proteomics of vitreous fluid in intraocular tuberculosis" on 10th August 2024

Laboratory Testing in Paediatric Uveitis: A Roadmap

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Paediatric uveitis is a vision-threatening disorder that is often more challenging to manage than adult uveitis. Children may be asymptomatic at onset, leading to delayed diagnosis and a higher risk of chronic inflammation, complications, and long-term visual loss. In addition, interpretation of laboratory investigations in children has its own limitations and pitfalls.

Laboratory evaluation is an integral component of management, helping to identify systemic disease, guiding therapeutic decisions, and ensuring safety monitoring. However, testing should follow a structured, pattern-based approach. Indiscriminate or "wholesale" ordering increases the likelihood of false positives, confusion, and unnecessary interventions. The predictive value of each test depends on the clinical context and the pre-test probability of a particular condition.

Pathway to Diagnosis

There is no standard approach for laboratory investigations in children with uveitis. The diagnostic approach in paediatric uveitis relies first on **pattern recognition** through detailed history and ophthalmic examination. Systemic review is essential, with attention to features such as arthritis, joint swelling or back pain, rash, oral/genital ulcers, history of infections, weight loss, cough, fever, pet or cat exposure, tuberculosis contacts. These clues help direct the laboratory work-up, which may then be divided into **baseline investigations for all children** and **targeted tests guided by clinical pattern**.

Basic Work-Up for All Paediatric Uveitis

A minimal baseline panel is essential in every child to assess systemic health and establish safety before immunosuppressive therapy

1) **CBC with differential count**
   a) Anemia- Suggestive of chronic disease
   b) Leucocytosis- underlying systemic inflammation or infection
   c) Eosinophilia – Suggestive of parasitic infection, sarcoidosis
   d) Lymphocytosis- Tuberculosis, viral
   e) Cytopenias - SLE/MAS, drug induced, leukemia (masquerade syndromes)
2) **ESR and/or CRP-** these inflammatory markers are useful for baseline in rheumatology-associated and infectious conditions
3) **Liver and renal function tests** – to assess systemic disease and provide baseline before therapy
4) **Urinalysis**
5) Tuberculosis screening- Mantoux/ interferon gamma release assays(IGRA), chest X-ray are important in children with TB exposure, granulomatous uveitis, or before biologics

Targeted Investigations Based on Clinical Pattern

Beyond the baseline panel, further testing is dictated by the anatomic subtype of uveitis and systemic clues. For example, a child with chronic bilateral anterior uveitis would have antinuclear antibodies (ANA) testing because JIA associated uveitis is a likely diagnosis, and a teenager with severe acute anterior uveitis in one eye would be ordered testing for HLA-B27 blood test. **Avoid** sending panel for rheumatological tests such as rheumatoid factor, anti-CCP, ACE level for uveitis etiology.

Anterior uveitis

1) **Non-granulomatous, acute, unilateral, painful** – think of **HLA-B27-associated** enthesitis-related arthritis and order **HLA-B27 by PCR**
2) **Chronic, bilateral uveitis** with or without arthritis- check **ANA (**IFA)
3) Bilateral nongranulomatous anterior uveitis with fatigue, weight loss, renal involvement- consider TINU and **urinalysis** may show sterile pyuria/ proteinuria; **urine β2-microglobulin may be helpful.**
4) **Granulomatous** uveitis
   a) Mantoux/IGRA, Chest X-ray **CXR** (± HRCT) **for tuberculosis screening-** It is important to consider TB in developing countries where there is a high background prevalence of TB
   b) **Genetic analysis for Blau/ Early onset sarcoidosis** (EOS)
   c) Serum ACE, chest radiography for sarcoidosis Serum Calcium, Urine Calcium:creatinine ratio.
5) Atypical/refractory/ non responsive→ aqueous PCR for HSV/VZV/CMV

Intermediate uveitis

1) Renal function tests **and Urinalysis** may show sterile pyuria/proteinuria; **urine β2-microglobulin** is highly useful when TINU is considered
2) TB workup- Mantoux/IGRA, Chest X-ray
3) Serum ACE, chest radiography for sarcoidosis

Posterior uveitis/retinitis or panuveitis

1) Work up for TB/Blau/EOS in Granulomatous panuveitis
2) Vasculitis, oral/genital ulcers- HLA-B*51 is supportive but not diagnostic of Behçet disease
3) Infectious work up- toxoplasmosis (IgM, IgG), toxocariasis serology, Bartonella serology ± PCR (with cat exposure), syphilis with Treponemal (TPHA-Treponema Pallidum Haemagglutination Assay) and non-treponemal (VDRL-Venereal Disease Research Laboratory) tests, HSV/VZV/CMV PCR
4) Vasculitis: ANA, ANCA, APLA, ENA testing only if systemic features are present

Monitoring & Therapy-Related Labs

Children often require immunosuppressives in non-infectious uveitis with methotrexate, biologics

1) **Before immunosuppressives**: CBC, LFT, RFT, **Infectious safety screen** HBsAg, anti-HCV TB screening with Mantoux/ IGRA and Chest X-ray
2) Monitoring during therapy: Regular CBC/LFT, Quantiferon/Chest X-ray TB rescreening on prolonged anti-TNF agents.

Interpretation of rheumatological lab markers in paediatric uveitis

Certain commonly used laboratory markers have different implications in paediatrics

1) **ANA by IFA:** ANAs are traditionally used for uveitis risk stratification in children with JIA. It is a useful test in a young patient with chronic anterior uveitis. It can be positive in JIA-U and idiopathic uveitis as well. However, ANA negativity does not exclude the possibility of JIA-uveitis.
2) **ACE Level**: Serum ACE have variable sensitivity/specificity in children and should not be used alone to rule in/out disease as the diagnostic performance of ACE in paediatric sarcoid uveitis was found to be poor. ACE levels tend to be elevated in the paediatric population in general compared with the adult population, so elevated ACE alone may not be diagnostic of juvenile sarcoidosis. Also, level can be normal in localized sarcoidosis ex ocular involvement and a normal ACE level does not exclude the disease, hence do not use to screen all patients.
3) **HLA testing** is not routine- **HLA-B27** positivity is seen in ERA/AAU: HLA-B*51 is supportive in oral/genital ulcers, skin lesions, pathergy, vasculitis
4) **Immunodeficiency work up**- Immunoglobulin profile, lymphocyte subset analysis, HIV serology in children with uveitis and recurrent infections
5) **Genetic analysis** considered in certain paediatric-specific red flags conditions-

- Very young child with uveitis, granulomatous dermatitis, boggy arthritis, often <5 years- consider Blau syndrome and do genetic testing for NOD2
- Recurrent fever + uveitis- Consider autoinflammatory syndromes (ex-cryopyrin associated periodic syndromes (CAPS), deficiency of adenosine deaminase 2 (DADA2), haploinsufficiency of A20 (HA20))
- Recurrent infections + uveitis- consider primary immunodeficiency disorders.

Conclusion

- In children, special attention must be given to conditions that are less common in adults, such as JIA-uveitis, TINU, Blau syndrome, parasitic infections, and autoinflammatory disorders.
- Laboratory testing in paediatric uveitis should be guided by clinical pattern rather than exhaustive panels
- **Start with a minimal baseline** on nearly all children: CBC, ESR/CRP, urinalysis and infectious screening dictated by geography and exposure
- **Stepwise escalation** toward investigations like ANA, ACE, HLA typing, infectious serologies, or genetic panels based on specific findings (e.g., chronicity, bilateral disease, systemic features)
- **JIA-associated uveitis** with positive ANA remains the commonest non-infectious cause in many cohorts.
- Consider rare paediatric-specific causes (Blau, autoinflammatory diseases, immunodeficiencies) and do genetic analysis
- Do not order ACE, ANCA, RF, CCP in all children with uveitis
- Interpretation of markers like ANA, ACE, and HLA must be contextualized within paediatric limitations.
- Close follow-up with lab monitoring is vital in children on long-term immunosuppression.

Suggested reading:

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Awards and Accolades

Dr. Sara Rizvi

Narayana Nethralaya, Bengaluru

1. *2nd prize in Poster Presentation on 'Rosai-Dorfman Disease: Unexpected Twist in the Tale of Epibulbar Masses'at Annual Conference of USI, October 2024, Chandigarh.*
2. *1st prize in FFA/ICG category in APAO-APOIS Imaging Competition at AIOS-APAO conference, April 2025, New Delhi.*
3. *1st prize in 'Artistic Photography in Ophthalmology' and 2nd prize in 'Clinical Ophthalmology' categories in KOS Photography competition at KOSCON, November 2024, Udupi.*

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