Evolving diagnostic approaches in infectious uveitides

The current special issue of the *Indian Journal of Ophthalmology* focuses on uveitis and related intraocular inflammations to reveal the importance of infections presenting with clinical features of uveitis, challenges in clinical diagnosis, diagnosis supporting laboratory investigations, and treatment with antimicrobial agents. Most infectious uveitides are a key cause of blindness due to their chronic and recurrent nature and associated intraocular morbidity resulting from ocular sequelae. In part, this is from failure to detect infectious agents in about 50% of the cases.

In the diagnosis of infectious uveitides; it is important to recognize the regional/geographic incidence, as well as the prevalence of infectious diseases and their endemic nature. Updated clinical knowledge on the periodic resurgence of infections such as mosquito-borne diseases, dengue, West Nile virus, Chikungunya virus, and others epidemiologic details are important, and such details play major roles in the proper diagnosis of infectious uveitis entities in endemic and in nonendemic countries, as well as in the later diagnosis of uveitis etiology in migrated individuals from endemic regions. Reactivation of latent tuberculosis in nonendemic countries and tubercular uveitis prevalent in immigrants living in USA are emphasized by the Center for Disease Control in the United States.

Uveitis Society of India members provided several review articles on various infectious uveitides. These timely articles emphasize several bacterial and viral infections prevalent in India and recent advances in diagnosis and treatment. Due to the high prevalence of infectious uveitides with known and unrecognized infectious causes in the Indian subcontinent and other parts of Asia and Asia-Pacific region, ophthalmologists correctly make every attempt to either confirm or exclude infectious etiology of intraocular inflammation. Although clinical history and findings supplemented with current imaging techniques have helped to some extent in the diagnosis of infectious uveitis (more so in differentiating viral, bacterial, fungal, and parasitic infections), in most cases, the causative infectious agent may not be identified and remain elusive. Such patients with presumed etiologic diagnosis of infectious cause are managed with antimicrobials to avoid delay in therapeutic intervention, salvage vision, and minimize ocular morbidity.

Interestingly, several articles reveal attempts by the authors to establish an etiologic diagnosis mostly based on hypothesis driven laboratory investigations either to support clinical diagnosis or eliminate an infectious cause. Approaches used are mostly examinations of ocular fluid or biopsy material by microscopic examination using special histologic stains, Gram stain, Acid-fast, Gomori's methenamine silver, auramine-rhodamine, Calcofluor-white, and others. Although microscopic examination is rapid and inexpensive, it requires expertise of laboratory personnel in staining methods and challenges inherent in low sensitivity. Thus, negative histology results are not helpful in supporting clinical diagnosis, requiring culture and PCR of ocular fluid examination.

Classic laboratory diagnosis by cultures of infectious agents is well studied. However, its sensitivity is limited for fastidious organisms and prolonged time required for the results in culture of acid-fast bacteria and fungi. Moreover, its use is limited in the detection of viruses, which are an important cause of anterior, intermediate, and posterior uveitides. Prior use of antimicrobials is known to interfere with the growth of organisms.

Molecular techniques no doubt help in clinical armamentarium in the diagnosis and management of infectious uveitis of known pathogens. These techniques include direct PCR, multiplex PCR, and targeted universal multiplex PCR. The latter employs universal primers for conserved 16S ribosomal RNA for bacteria and internal transcribed spacer sequence for detection of fungi. Although these molecular techniques can detect microbes rapidly and are relatively inexpensive, they depend on the clinical hypothesis of an infectious agent driving the uveitis. Other drawbacks of these techniques include low specificity, false positive, and false negative results. Moreover, these techniques require primers that may not be available for all infectious agents and cannot detect unknown infectious agents. Negative results raise questions about whether the uveitis of presumed infectious etiology is caused by a microbe for which primers were not used.

Other techniques of sequencing of pathogen nucleic acids have been employed and, in particular, targeted toward next generation sequencing for pathogens. It is a sensitive approach in detection of selected organisms when combined with targeted sequencing of 16S rRNA for bacterial detection. However, universal or broad range primers of conserved 16S ribosomal RNA gene amplification by sequencing may not be sufficiently broad to detect all bacteria. The techniques are more complex, limited to a small portion of the genome and prone to contamination with environmental microbes. Moreover, this and the above molecular approaches are hypothesis driven, where an infectious agent is considered causing uveitis and related intraocular inflammation. Moreover, antimicrobial sensitivity testing cannot be performed for pathogens detected by this method.

Unlike the above laboratory approaches, metagenomic next generation sequencing (mNGS) is gaining popularity not only in the diagnosis of known agents of infectious uveitis but for unexpected and undiscovered organisms.[1] This robust sequencing combined with metagenomics and bioinformatics is a promising, sensitive, and rapid technique in the diagnosis of an infectious agent. By virtue of comparing amplified genetic material extracted from intraocular fluid or tissue to a database of thousands of bacteria, viruses, fungi, and other pathogens, mNGS provides detection of known and unknown infectious agents. This evolving molecular technique is unbiased; it can detect any portion of an infectious agent genome and potentially provide quantification. mNGS is high-throughput sequencing technology by virtue of its parallel sequencing of thousands of DNA fragments generated by shotgun DNA fragmentation. This allows for an unbiased detection of pathogens. Moreover, the technique could improve the ability to diagnose infectious uveitis of currently known pathogenic agents and previously unsuspected bacteria or fungi or viruses.

In infectious uveitis, mNGS can overcome a common limitation of insufficient sample volume to run pathogen-specific PCR testing and requires tiny amount of 20 microliters. Moreover, the sequencing can provide phenotypic behavior of the identified pathogen and drug resistance modeling.

In patients with clinical presumed diagnosis of infectious uveitis, samples of intraocular fluid, tissue biopsy (either fresh or formalin fixed paraffin embedded tissue material) can serve as a sample for initial nucleic acid extraction. DNA or RNA and the later extraction require transcription of RNA to generate complementary DNA to proceed with the shotgun fragmentation of DNA. The samples contain abundant quantitates of human background DNA that is known to interfere with detection of infectious agents(s) DNA present in very low quantity, thus removal of human DNA by various methods is required prior to proceeding with sequencing. The shotgun DNA fragmentation of the sample allows sequencing of thousands of DNA fragments effectively to generate the sequencing library. There are several commercially available platforms for such sequencing, each one with inherent advantages and disadvantages. Among them Illumina platform (San Diego, CA, USA) has been used in a majority of publications.

The DNA extraction, shotgun fragmentation of DNA, and sequencing library require attention with care to avoid microbial contaminants present in the reagents and laboratory environment. The sequenced DNA fragments require analysis using bioinformatics and alignment to reference the database for taxonomic classification, followed by identification of the infectious agent. The microbial detection sensitivity depends on the extraction of genomic material from the samples, sequencing library platform, and other steps of mNGS.

In the future, mNGS could revolutionize the unbiased diagnosis of infectious agent(s) in uveitis and related intraocular inflammations. This technology could provide infectious etiology, currently undetected by current methods, in culture negative endophthalmitis and in those infectious uveitides with prior antimicrobial treatment.[2] Clinical adaptation of this novel technique may take time and require proper validation and confirmation of diagnosis by various current methods. It will require an understanding and confirming of its beneficial role in targeted antimicrobial treatment and prognosis from therapeutic interventions.

There are several case reports of mNGS revealing detection of the virus, bacteria, fungi, and parasite genomes in central nervous system infections, pulmonary infections in culture negative blood samples.[1,3,4] In ocular fluid, the technique identified Cryptococcus neoformans, Toxoplasma gondii, Herpes simplex 1 virus. The results were confirmed with classic current available methods. Interestingly, Doan T and her colleagues from the University of California, San Francisco were able to diagnose a chronic intraocular rubella virus infection in a patient with a diagnosis of long-standing idiopathic uveitis.[3] Moreover, the technique provided details that the virus was related to the German rubella virus strain isolated in 1992. Clinical history revealed that a year earlier the patient developed fever and rash while living in Germany. Moreover, the number of mutations detected in the virus supports long-term viral replication in the eye, and the eye as a long-term reservoir of the virus. To add, in 1967 Murphy *et al.*

reported the rubella virus residing in the congenital rubella cataract lens epithelial cells.[5] Histologically, the cataract reveals pyknotic nuclear debris within the epithelial cells.

It is known that incidence and prevalence of infectious uveitis varies in different parts of the world, and it is recognized that tuberculous uveitis is a major cause of intraocular infection with significant morbidity and loss of vision in tuberculosis endemic countries. This is also apparent from the current issue of this journal. However, there are other infectious causes of uveitis. No doubt there are several cases in which an infectious agent could not be detected as stated above from a hypothesis driven approach in diagnosis of intraocular infections. To make progress in the diagnosis of an etiologic agent in an infectious uveitis prevalent in a country or region, an approach combining mNGS to understand pathogen genomic factors with patient epidemiologic data gathered from a large national medical claims database, or similar such big data, could elucidate novel pathogen–host factor interactions and to target diagnosis and effective treatment.

In a recent study conducted at the USC Roski Eye Institute, Dr. Brian Toy employed a large nationwide medical claims database in the United States to demonstrate an infectious uveitis incidence of 18.9 and prevalence of 60.6 per 100,000 people.[6,7] Interestingly, the data revealed that the overall risk of the infection increased with age for each decade over the age of 18 years old. Moreover, the data showed that the ocular infection incidence and prevalence were higher than previously estimated and that there were significant geographic and racial disparities. A similar epidemiologic approach combined with an unbiased diagnosis of the infectious agent with mNGS may advance the field of uveitis. Such an approach could provide prevalence of an etiologic specific diagnosis in identifying host susceptibility factors, as well as preventive and treatment strategies. Moreover, understanding effective treatment options may lead to cost effective therapeutic intervention and minimize recurrent inflammation. The approach can mitigate the sequelae of inflammation and visual morbidity from the infection process. Such mitigations at the national level are achievable when combining big data-based epidemiologic studies with the powerful technology of mNGS.

In conclusion, infectious uveitides represent a collection of disparate intraocular inflammations initiated by diverse infectious agents that are currently known from culture methods, and undetected or unidentified pathogens by commonly employed direct and multiplex PCR techniques. Introduction of mNGS, which employs unbiased and hypothesis-free detections of all pathogens in a clinical sample, could prove to be a powerful technique in the precise detection of infectious agent(s). Moreover, the novel mNGS technique by virtue of providing data within 48 h and with very small volume of sample is a major advantage in the management of infectious uveitis in clinical practice. In uveitis, such mNGS technology combined with big data epidemiologic study has great potential in modifying risk factors and is most cost effective in clinical practice to treat infections expeditiously. Moreover, mNGS-based diagnosis and intervention can minimize recurrent inflammation and mitigate its sequelae of visual morbidity. Although big data and mNGS may offer exciting diagnostic and epidemiologic clinical opportunities, it is doubtful that currently such technologies, which are not

readily available in clinical settings, can replace an astute ophthalmologist in making a diagnosis and treating infectious uveitides.

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	DOI: 10.4103/ijo.IJO_2487_20