Commentary: Understanding immunological tests for uveitis – *ten essentials*

Immunological tests have become *sine qua non* in day-to-day uveitis practice. These tests are meant to detect different attributes of the patient's systemic immune response that could be linked to the cause of inflammation in the eye. The systemic immune response could be antibody-mediated (humoral), cell-mediated, or it could merely be proteins produced by the activated immune cells. The immune response could be directed against external agents such as microbes or against self-antigens. In this issue of the IJO, Rathinam *et al*., have provided a comprehensive overview of the range of different immunological tests currently available in our armamentarium.[1] However, to use these tests meaningfully, it is important to remember certain fundamental principles about the application of these tests. These are listed below.

1. All immunological tests have inherent sensitivity (proportion of patients *with* the disease who have a positive test) and specificity (proportion of patients *without* the disease who

have a negative test) to diagnose a particular disease. Tests used for screening typically have high sensitivity and those required for confirmation of diagnosis high specificity

2. The diagnostic utility of a test is represented by its positive and negative predictive values which inform the likelihood of having or not having a disease, based on the test's sensitivity, specificity, and the probability of the disease *before* the test is performed (pre-test probability). The pre-test probability is determined by the *prevalence* of the disease in the given population, patient demographics, characteristic clinical signs, and the results of any previous diagnostic tests. A relevant example here is the Mantoux or tuberculin skin test (TST). The cut off value for Mantoux induration in a non-endemic country for tuberculosis (TB) is 15 mm (Centre for Disease Control guidelines), whereas in a TB-endemic country with higher pre-test probability, it is 10 mm. In patients with recent TB-contact or other risk factors for TB, the cut off can be even lower at 5 mm.[2] Conversely, in a patient with typical clinical signs of HLA-B27 anterior uveitis (low pre-test probability), even a 20 mm Mantoux induration would have a low positive predictive value
3. Majority of the immunological tests – for infectious as well as non-infectious uveitis – are antibody detection assays. These are mainly immunofluorescence assays (using fluorescent antibodies against target antibodies) or by enzyme-linked immuno-sorbent assays. The results are expressed either in titers of dilution, or in specific units (e.g., international units)
4. Some of these antibodies (e.g., rheumatoid factor [RF], antinuclear antibody [ANA]), when pathological, are always associated with characteristic disease entities, whereas others such as anti-neutrophil cytoplasmic antibody (ANCA), may have non-specific clinical presentations, even in pathological state. Examples of the latter situation are ANCA-associated vasculitis or ANCA-associated scleritis[3]
5. Some tests require a sequential approach. For example, a relatively low specificity test like ANA typically needs to be confirmed with a test of higher specificity – anti-double-stranded DNA (anti-dsDNA), for the diagnosis of systemic lupus erythematosus. The same principle applies to RF and anti-cyclic citrullinated peptide (anti-CCP) respectively, for the diagnosis of rheumatoid arthritis. Sometimes the more specific test is also more sensitive. In that case, a reverse algorithm is applied as in ocular syphilis, where the treponema pallidium hemagglutination assay [TPHA] takes precedence over venereal disease research laboratory (VDRL) test
6. The values of some antibody tests (e.g., ANCA or TPHA) either change slowly, or do not change at all with treatment, whereas others like ANA or VDRL test come down with resolution of disease. The latter can be used to monitor the effect of treatment
7. Cell-mediated immunity is typically tested for diagnosis of systemic TB infection. It could be tested either in the patient (*in vivo*, TST) or in the blood sample (*in vitro*, QuantiFERON TB Gold test). Although they may vary in specificity, neither of the tests can distinguish between latent or active TB
8. Some tests also measure enzymes produced by activated immune cells e.g., angiotensin-converting enzyme by epithelioid macrophages in sarcoid granulomas, or lysozyme by neutrophils, again in sarcoidosis. These tests typically have low sensitivity and should not be used as screening tools
9. Antigen detection is typically performed for identification of specific serotypes of class I human leucocyte antigens (HLAs), which are associated with specific uveitis entities. The HLA antigens are proteins found on surfaces of nucleated cells that help distinguish self from non-self. The genes for these serotypes are typically tested by polymerase chain reaction and reported as positive or negative. The most common HLA alleles tested are HLA-B27 (seronegative spondyloarthropathy), HLA-B51 (Behcet's disease), and HLA-A29 (birdshot chorioretinopathy)
10. All the tests discussed so far represent the systemic immune response and do not depict the local immune response in the eye. Many uveitis entities, typically the infectious uveitis (e.g., recurrent toxoplasma retinochoroiditis, most viral uveitis) may have the immune response localized to the eye. Such infections are diagnosed by the Goldman–Witmer coefficient which demonstrates greater proportion of infection-specific antibodies in the eye compared to the serum.

To summarize, immunological tests can provide meaningful representation of systemic immune response in uveitis patients provided we understand the strengths and limitations of each test and use them judiciously.

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