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Technical Note

Updates in diagnostic and clinical laboratory immunology from the 30th annual meeting of the Association of Medical Laboratory Immunologists (AMLI)

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This Special Issue of the Journal of Immunological Methods features reviews and short communications from key presentations delivered at the 30th Annual Meeting of the Association of Medical Laboratory Immunologists (AMLI) that was held in Denver, Colorado, USA in August 13–16, 2017. The meeting was composed of five plenary sessions each addressing both the clinical and technical aspects of laboratory testing. The five plenary sessions were: 1. New Roles for an Old Entity: The Complement System, 2. Updates in Autoimmunity: Anti-neutrophil Cytoplasmic Antibody (ANCA), 3. Vaccination and Vaccine Response Assessment in Primary and Acquired Immune Deficiencies, 4. Aging and the Immune System, and 5. The Molecular Revolution in the Clinical Laboratory and Beyond.

The manuscripts contained in this Special Issue cover selected topics from these sessions. Each author attempted to describe the challenges and the relevant clinical features of each of these topics with emphasis on the role of the immunology laboratory in the diagnosis, prognosis and management of the underlying medical conditions.

1. Plenary-1: New roles for an old entity: the complement system

The article "The Impact of Eculizumab on Routine Complement Assays" by Willrich et al. (2018), describes the effects of increased eculizumab concentrations on various parameters of the complement assays.

The authors conducted CH50, AH50 and C5 assays using a set of serum samples that were spiked with known concentrations of eculizumab and a second set of the samples directly obtained from eculizumab treated patients. The authors observed the inhibition of complement function in these three assays when eculizumab concentration was greater than 100 μg/mL. The authors recommended using one of these routine complement function assay for monitoring of patients undergoing eculizumab therapy. They also observed that monitoring of eculizumab allow for the individualization of therapy which significantly reduces the financial burden of this expensive, but efficacious medication. This study also reported that the performance of the liposome methodology for CH50 and C5 function tests was more sensitive in the presence of eculizumab as compared to the Wieslab AH50 ELISA.

"The Effect on the Immunology Laboratory of the Expansion in Complement Therapeutics" by Dr. Ashley Frazer-Abel (2018) provides a review of the complement components including the regulatory components that are currently or have recently been targeted by drug development. This review focuses on how the current wave of drug development stands to impact complement testing in the clinical immunology laboratory. Two classes of approved drugs have already impacted the immunology laboratory, but there are many more with novel modalities and potential indications that are in various stages of development. Every pathway and almost every component of the complement is being targeted. Dr. Frazer Abel discusses how the emergence of these new drugs may impact complement testing in the diagnostic laboratory. In addition to understanding how new complement therapeutics may interfere with the measurement of routine complement component levels of C3/C4/C3a/Bb or Ba/sC5b-9 levels, and/or functional assays such as the CH50/AH50, it must be acknowledged that our current assays may not be sufficient to monitor the new therapeutics. In the very near future an individualized approach to complement testing based on the type of new therapeutic and the disorder being treated may be required.

Atypical Hemolytic Uremic Syndrome (aHUS) is a rare disease. Its diagnosis can be difficult as it is basically a diagnosis of exclusion in the absence of clear diagnostic criteria. This disease can present in both children and adults (mostly females) and is one of the thrombotic microangiopathy (TMA) disorders. aHUS is caused by hyper-activation of the alternative complement pathway due to over activation of C3 convertases and the loss of complement regulatory mechanisms. Half of the aHUS patients with renal injury progress to the end-stage renal

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disease. In this review Sridharan et al. (2018) present three cases (female adults) to highlight the clinical presentation, diagnosis and management of aHUS. The responses of these three aHUS patients treated with therapeutic plasma exchange (TPE) were variable. Two patients who were clinically unresponsive to TPE required eculizumab (targets C5 and inhibits terminal complex formation) treatment which dramatically improved their prognosis. The third patient did not initiate eculizumab based on the clinical recovery following the TPE treatment. The authors concluded that the first line therapy for children and symptomatic aHUS adult patients who do not respond to TPE is eculizumab. Furthermore, due to the complex nature of the disease, authors recommended that all clinical, genetic (mutations in complement proteins or their regulators), and complement serologic data (acquired autoantibodies) must be considered in the diagnosis of aHUS.

2. Plenary 2: Updates in autoimmunity and anti-neutrophil cytoplasmic antibody (ANCA)

The review article by Naides (2018) "The Role of the Laboratory in the Expanding Field of Neuroimmunology: Autoantibodies to Neural Targets" focuses on the laboratory perspective of emerging and evolving field of autoimmune neurology. Targets for autoantibodies associated with autoimmune neurological disorders include paraneoplastic intracellular antigens, non-paraneoplastic neuronal antigens and glial cell surface antigens. The author focusses on the auto-antibodies involved in paraneoplastic neurologic syndromes, acquired neuromuscular junction disorders and autoimmune encephalitis. The review discusses the evolution of laboratory testing methodologies for antibodies (in serum and/or CSF) associated with autoimmune neurological disorders with coverage of "Euroimmune biochips", characteristics of specific autoantibodies and a brief summary of clinical presentations associated with each autoantibody. An evolving role for the proper measurement and classification of neural autoantibodies in clinical diagnoses is evidenced by the increasing number of novel neural autoantibodies and growing spectrum of neurological syndromes with overlapping clinical presentations. Due to the complex nature of these syndromes, the author recommends a testing algorithm which should start with broad autoantibody coverage (tissue indirect immunofluorescence or tissue-IFA) followed by technologies that allow for more specific autoantibody identification such as western blots, ELISA or newer methods such as line blots or single-analyte transfected cell substrate in IFA.

The article on "The clinical utility of anti-double-stranded DNA antibodies and the challenges of their determination" by Mummert et al. (2018), discussed potential causes for the lack of concordance in anti-double stranded DNA antibody levels measured between laboratories. Anti-dsDNA antibodies are polyclonal and contain a variety of different properties including mixed isotype, affinity, and avidity all directed against a biochemically complex antigen with multiple targets. Currently available diagnostic tests detect antibodies with quite different properties. This makes it more challenging to fully understand the clinical significance of these different antibodies in both the diagnosis of disease and in characterizing changes in disease activity. Studies show that high avidity anti-dsDNA antibodies are a relatively specific marker for SLE, constitute a risk factor for renal involvement and, moreover, antibody levels correlate with SLE disease activity. SLE symptoms are not very specific and therefore guidelines (ACR and SLICC) suggest that one test for anti-dsDNA antibodies may not be sufficient. The combination of a sensitive (in order not to miss any patients) and a highly specific assay should be considered. Available a-dsDNA tests range from very sensitive and less specific tests to very specific and less sensitive tests such as the Crithidia Luciliae Immunofluorescence Test (CLIFT). The authors recommend use of standardized automated methods, Fluoroenzyme immunoassay (FEIA) or preferably Chemiluminescence assay (CLIA), with good sensitivity and high specificity, for screening. This article also strongly recommends that questionable results (positive or negative) should be confirmed by a second method such as a highly specific CLIFT. It is a comprehensive review of the history of anti-dsDNA antibodies (a-dsDNA) from discovery to current use with a summary of the controversy and challenges involved in anti-dsDNA antibody testing.

3. Plenary 3: Vaccination and vaccine response assessment in primary and acquired immune deficiencies

The review by LaFon and Nahm (2018) covers guidelines for pneumococcal vaccines and the importance of currently available assays to measure the immune response to the latter (current gold standard test) in relation to vaccine efficacy (the amount and functional capacity of pneumococcal antibody present in serum to kill pneumococci) and in the clinical diagnosis of immune deficiency syndromes. This article addressed the details of different methodologies and their limitations with respect to clinical utility as a diagnostic test post vaccine in both primary and secondary immunodeficiency versus their application as a test for the evaluation of the efficacy of pneumococcal vaccines. This article also describes future directions for immunoassays used for pneumococcal vaccines and suggests laboratory professionals consider alternative polysaccharide vaccines for the evaluation of humoral immune deficiency.

As suggested in the above article, there is a growing concern regarding the different types of analytical methods used to measure the pneumococcal vaccine response (in the assessment of immune system functionality) due to poor concordance between different methodologies and their interpretation in different clinical scenarios. The review article by Antony Parker (2018) nicely delivers a message regarding the utilization of alternative polysaccharide vaccines for the evaluation of humoral immune deficiency. Salmonella typhi Vi polysaccharide (Typhim Vi® or ViCPS) is a potent immunogen to which few individuals have been exposed, making it an ideal tool for the assessment and evaluation of a "normal adaptive humoral immune response". This review delineates the advantages of evaluating a ViCPS vaccine response in the assessment of adaptive humoral immunity and suggests that the combined measurement of antibodies to Pneumovax®23 and Typhim Vi may have a wider scope for evaluation of immune function.

4. Plenary 4: Aging and the immune system

The review article by Amir A. Sadighi Akhaa (2018) provides a balanced and broad overview of effects of aging on the immune system focusing primarily on adaptive immunity. The article discusses in detail the effect of age on the immune cell repertoire, major cell intrinsic defects in lymphocytes as well as clinical and therapeutic implications of aging on the immune system. The concept of aging in the era of immune therapy for several cancers, and how age may affect this burgeoning therapeutic modality, as well as aging and outcomes as related to solid organ transplant are discussed. Akhaa provides a nice summary of "inflammaging" which is described as a persistent, low grade, sterile, systemic inflammation as a risk factor for morbidity and mortality in the elderly - including its implication in the pathogenesis of conditions such as osteoporosis, atherosclerosis, Alzheimer's disease and diabetes mellitus type II. "Inflammaging" is also discussed in the context of its relationship with frailty, "a state of cumulative decline in several physiological systems with a consequent decrease in homeostatic reserve and a disproportionate vulnerability to stressor events". The author addresses the lack of inclusion of elderly subjects in cancer immunotherapy trials and solid organ transplant trials. He proposes the inclusion of older individuals, both frail and in good general health as "an empirical imperative" necessary to understand how aging and senescence affects outcomes in this group of patients. The author quotes "data obtained from younger age groups cannot be automatically extrapolated to the elderly, nor will results acquired from older individuals with a good health status necessarily apply to those with

multiple co-morbidities or frailty".

5. Plenary 5: The molecular revolution in the clinical laboratory and beyond

The subtypes of influenza virus A (IVA) are important from a public health perspective. The major membrane glycoproteins, HA and NA of IVA, are critical to the virion life cycle and are the primary targets for the humoral immune response or vaccination. Guo et al. (2018), in their short analytical review highlight the use of bio-layer interferometry (BLI) biosensors to map and understand the immunogenicity of the surface protein epitopes of influenza virus A subtypes (H1, H3 and N9) in order to develop therapeutic monoclonal antibodies (mAbs), diagnostic tools, and vaccines. They have developed a site-directed mutagenesis epitope mapping system coupled with the use of BLI biosensor to determine epitopes of isolated mAbs against the HA or NA of influenza A viruses. In addition, mapping of the epitopes that bind to mAbs also offers a molecular understanding of the mAb function. Currently available methods for epitope mapping include structural or functional approaches. A site-directed mutagenesis epitope mapping system coupled with the use of BLI biosensor uses both structural and functional approaches and offers high resolution, ease of use, and quick results.

The article by Sherwood and Weimer (2018) titled "Characteristics, Properties, and Potential Applications of Circulating cell-free DNA in Clinical Diagnostics: A Focus on Transplantation" reviews the potential clinical utility of sequencing cell free DNA (cfDNA) in a variety of clinical circumstances with a focus in solid organ transplantation. The authors, based on published data, propose that the quantification of donor cfDNA can successfully serve as a marker to detect early rejection in solid organ transplants (especially heart transplants). Measurement of cfDNA can play a potential role in post-transplantation monitoring and therapeutic dosing as it is noninvasive, safe, cheap and reproducible. This review also provides a broad overview of the origins, properties and clinical utility of cfDNA measurements in other health conditions and potential sample sources. Finally the article addresses the challenges associated with the pre-analytic, analytic, and post-analytic variables that affecting cfDNA assessment.

6. Conclusion

Articles selected for publication in this Special Issue of the Journal of Immunological Methods represent areas of current and cutting edge immunological based treatments (complement therapeutics, therapeutic monoclonal antibodies, immune monitoring and disease management), immune physiological concepts (vaccine responses, immunity and aging) and potential cutting edge technologies that may influence the development of treatment, understanding and monitoring of disease (e.g. cell free DNA sequencing, new neural autoantibody testing). It is by understanding mechanisms of disease pathogenesis that novel new laboratory tests are developed, tested and implemented. The Diagnostic Immunology Laboratory represents a key component in the continuum of disease management, from diagnosis to disease management and the evaluation of novel therapeutics. The articles published in this special issue include a broad range of immune-mediated disorders and attempt to highlight the significance of the diagnostic immunology laboratory in disease management. The material presented in this special issue should be of interest to the practicing clinical immunologist, the laboratory director and the laboratory scientist involved in the growing field of applied and translational clinical immunology.

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