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Review

Highlights of the 33rd annual scientific meeting of the Association of Medical Laboratory Immunologists (AMLI)

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ABSTRACT

The annual meeting of the Association of Medical Laboratory Immunologists (AMLI) was convened virtually over the month of August. Prior to the emergence of the COVID-19 pandemic, AMLI's scientific committee had chosen the following topics as the focus of its 2020 meeting: Histocompatibility Testing and Transplant Immunology; Secondary Immunodeficiency and Immunotherapy Monitoring; ANA Update; and Emerging Infectious Diseases and New Algorithms for Testing. Given the central role of the discipline in the evaluation of the host response to infection, it was apt to add a separate session on antibody testing for SARS-CoV-2 infections to the original program. The current report provides an overview of the subjects discussed in the course of this meeting.

Histocompatibility testing and transplant immunology

Hematopoietic cell transplantation (HCT) aims to replace the host's malignant, absent, or genetically defective cells through stable engraftment of donor stem cells after partial or complete ablation of the recipient's bone marrow by using a number of modalities, including radiation, chemotherapy and antibody therapy. The past few years have witnessed a rapid growth in the field of transplant immunology, in part due to innovations and refinements in histocompatibility testing, conditioning and post-transplant immune monitoring, and the emergence of novel modes of cellular therapy. Recent developments and best practices for histocompatibility testing, and pre- and post-transplant immune monitoring were highlighted in this session.

Medhat Z. Askar, MD, PhD, Baylor University Medical Center focused on current best practices in clinical assessment of histocompatibility between donors and recipients of HCT, allogeneic immune effector cell therapy, and post-transplant engraftment monitoring.

HCT remains the only curative treatment for several malignant and non-malignant diseases. Matching donor and recipient human leukocyte antigens (HLA) at HLA-A, B, C and DRB1 loci is the most critical factor affecting HCT clinical outcomes. The presence of a mismatch at any of these loci would increase the risk of acute graft versus host disease (GvHD), as well as overall transplant-related mortality. More recent studies have emphasized the role of donor age, and of donor specific antibodies (DSA) as additional factors in determining the success or failure of engraftment. Within the US, the chance of finding an HLA match for HCT differs by ethnic group. While individuals with a white European background have a 75% chance of finding an 8/8 match, others fare far worse in this respect, and therefore depend on alternative donor sources. This has led to an increasing use of haploidentical donors. Recently, the European Society for Blood and Marrow Transplantation has published consensus recommendations for donor selection in HLA haplo-identical HCT, plus detection and management of DSA in these transplants.

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Another emerging trend is the increasing use of allogeneic cellular therapy products. So far, immune effector therapy has been mainly based on ex-vivo engineering of the patient's own cells, precluding the need for histocompatibility matching. However, prior treatment of the patient with radiation therapy, chemotherapy and/or other modalities can impose limitations on autologous cell bioengineering. This has prompted the idea of bioengineering cells from healthy donors, which is subject to the same barriers as HCT, therefore raising the question of donor/recipient compatibility. Allogeneic immune effector cells collected from healthy donors offer advantages over autologous T cell therapies, including availability of healthy cells for production on demand which improves outcomes, reduction in cost due to the possibility of using a single donor for multiple recipients, and elimination of risks associated with inadvertent transfer of leukemic blasts.

Dr. Askar went on to define chimerism, the diagnostic significance of its subgroups, and the modes and indications of its laboratory assessment. Chimerism analysis is most frequently used to monitor engraftment, to detect relapse and to guide decisions on donor lymphocyte infusion. Most laboratories use the short tandem repeat (STR) method, some use quantitative polymerase chain reaction (qPCR) and a small number use next generation sequencing (NGS) for chimerism analysis. STR and qPCR have a sensitivity limit of 5% and 1% respectively; consequently, STR does not have adequate sensitivity for detecting relapse, or for decisions on donor cellular infusion.

Throughout the talk, Dr. Askar emphasized the precision and high resolution of NGS in assessing donors and recipients prior to transplant for either traditional HCT or allogeneic immune effector cell therapy, and for post-HCT chimerism analysis. NGS offers high throughput testing and full gene sequencing of many HLA loci, including non-coding sequences associated with differential levels of expression, facilitating identification of permissive mismatches. It also allows lineage-specific chimerism analysis to monitor immune reconstitution of specific hematopoietic lineages such as T cells, B cells, myeloid cells, NK cells and $CD34^+$ cells. Leveraging these testing technologies in diagnostic algorithms could improve clinical outcomes of various cellular therapy modalities.

Susan E. Prockop, MD, Memorial Sloan-Kettering Cancer Center discussed predictable milestones of immune reconstitution that can be modified to improve transplant outcomes.

Within the past two decades, there has been a concerted effort to decrease short-term (viral reactivation, treatment related mortality and GvHD) and long-term toxicity in allo-HCT, and to achieve better disease control, all of which are contingent on effective immune reconstitution.

A period of profound immune compromise with defects in both innate and adaptive immunity is integral to allo-HCT. While the innate immune component is typically restored within the first month after transplant, restoration of adaptive immunity may require months to years. The pace and sequence of reconstitution in the adaptive arm varies by cell type, and can be affected by the source of the graft. In most transplant platforms, emergence of effective T cell immunity is the final phase of this process.

The gold standard for complete restoration of the adaptive immune system after HCT is demonstration of specific antibody production in response to vaccination. However, short of this, other milestones of immune reconstitution have been used to predict response to vaccination and to translate into improved overall survival. Earlier efforts took their cue from the HIV literature and validated a CD4+ T cell count of >200 cells/μl and in vitro proliferation of >70% to phytohemagglutinin (PHA) in comparison to normal as a predictor of response to vaccination. However, there was no reproducible system for predicting or augmenting this immune recovery.

More recently, a group at Utrecht (Princes Maxima/UMC, Netherlands) demonstrated that a CD4+ T cell count of 50 cells/μl within the first 100 days post-HCT can robustly predict improved overall survival, lower viral reactivation, and viral-related morbidity and mortality. This finding was subsequently validated in pediatric transplant recipients at Memorial Sloan Kettering, thereby underscoring its validity for both T cell replete and T cell depleted HCTs.

One strategy to improve the chance of having early and adequate CD4+ immune reconstitution is to individualize dosing in conditioning regimens. So far, tailored dosing of Busulfan, as well as optimizing the dose and timing of treatment with anti-thymoglobulin (ATG) and fludarabine have shown promise in this respect. This may lead to more consistent early CD4+ T cell immune reconstitution and to further improve allo-HCT outcomes in the future.

Timing of immune cell reconstitution depends on various factors, including source of the graft (mobilized peripheral blood, bone marrow, cord blood) and the patient conditioning regimen used. The immunology laboratory plays a critical role in monitoring immune cell reconstitution after HCT via different cellular assays. Flow cytometry is the prominent diagnostic tool used.

Evangelos Ntrivalas, MD, PhD, Memorial Sloan Kettering Cancer Center, discussed various immunophenotypic and functional flow cytometric assays used for this purpose. Immunophenotypic assays include identification and enumeration of immune cell subsets (T cells, B cells, NK cells) and their subpopulations. Cells of innate immunity (neutrophils, monocytes, NK cells) are the first to engraft followed by T cells. In most cases, the first T cells to be generated in a thymus-dependent pathway are $CD8^+$ T cells, which causes an initial inversion in the CD4:CD8 ratio. B cells emerge at different time points, and complete reconstitution of B cells can be delayed depending on various factors such as presence of chronic GvHD. Another important aspect of immune reconstitution after HCT is the cells' functional status, such as proliferation potential, cytotoxicity, and cytokine production. In ex vivo flow cytometry-based functional assays, mostly for T cells, cells are incubated with non-antigen-specific or antigen-specific stimuli to measure their biological function. These assays are used to evaluate the immune response and response to vaccination post-HCT. Flow cytometry is an evolving field and we should expect new cellular assays to emerge for monitoring immune cell function after HCT in the near future.

Swati Naik, MBBS, Baylor College of Medicine, discussed functional immune monitoring in allogeneic stem cell transplant recipients. Serious viral infections due to delayed immune reconstitution are a leading cause of morbidity and mortality after allo-HCT. Thus, many transplant centers prospectively track cellular immune recovery by evaluating absolute cell numbers and phenotypic profile of reconstituting T cell subsets, to identify individuals who are at highest risk of infection. Conventional assessments, however, fail to measure either antigen specificity or functional capacity of reconstituting cells - factors that correlate with endogenous antiviral protection. In a pilot study performed at Baylor College of Medicine, this limitation was addressed by prospectively investigating the tempo of endogenous immune reconstitution in a cohort of pediatric HCT patients using quantitative (flow cytometry) and qualitative (IFN-γ ELISpot) measures, which were correlated with presence or absence of infections associated with Cytomegalovirus (CMV), Adenovirus, Epstein-Barr virus (EBV), BK Virus, Human Herpes Virus-6 (HHV-6), Respiratory Syncytial Virus (RSV), Parainfluenza, Influenza and Human Metapneumovirus. Data demonstrating the influence of conditioning regimens on immune recovery and highlighting the differential impact of active viral replication on quantity and quality of reconstituting cells were discussed. A combination of quantitative and qualitative measures might enable distinction between patients who are likely to clear viral infections from those that are not. Further, these measures could help identify patients who might benefit from adoptive transfer of virus-specific T cells. Judicious use of phenotypic and functional monitoring strategies can help guide clinical care and personalized management of allo-HCT recipients with infections.

In conclusion, the session demonstrated the power to improve outcomes of HCT and adoptive cellular therapy by taking advantage of sophisticated algorithms for donor selection and consistent immune monitoring in the post-HCT period using both validated and exploratory

methods.

Secondary immunodeficiency and immunotherapy monitoring

The number of distinct monogenic primary immunodeficiencies (PIDs) or inborn errors of immunity (IEI) has grown exponentially over the past two decades. This has led to advances in diagnostic testing and improved recognition of clinical phenotypes in this group of diseases. By contrast, despite their higher overall prevalence, secondary immunodeficiencies caused by infection, malignancy, nutritional deficiencies or immunomodulatory therapies have remained a significant but often under-appreciated group of clinical conundrums. This session focused on a variety of underlying causes in this category.

Cullen M. Dutmer, MD, University of Colorado School of Medicine and Children's Hospital, Colorado, opened this session with a comparison of PID and secondary immunodeficiency. Due to considerable overlap between clinical presentations of PID and secondary immunodeficiency, an understanding of immune processes and the effect that infection, malignancy, disruption of physical barriers, or therapeutics may have on distortion of immune processes is critical to clinical and laboratory workup of these patients.

Recurrent, persistent or unusual infections may signal an underlying PID, an autoimmune process or malignancy. Alternatively, such infections might in fact precipitate a secondary immunodeficiency. While therapeutic agents, including signaling pathway inhibitors (e.g. Janus kinase [JAK] inhibitors), cytokine antagonists (e.g. secukinumab, dupilumab), B cell depleting therapies (e.g. rituximab), or checkpoint inhibitors or agonists (e.g. abatacept) play a critical role in treating an immediate pathological process, they may have unintended consequences and affect immune processes, thus resulting in secondary immunodeficiency or uncovering a pre-existing PID.

Dr. Dutmer illustrated the clinical conundrum of differentiating secondary immunodeficiencies from PIDs with two patients, both of whom presented with clinical features consistent with immunodeficiency, and whose laboratory findings included lymphopenia, perturbation of immunoglobulins, and compromised T cell function, but whose evaluation was confounded by previous treatment with a variety of immunosuppressive therapies. The importance of genetic testing in such patients was emphasized by the fact that one patient had a pathogenic variant in *RAG1*, and the other in *CARD11*. Thus, a detailed laboratory evaluation including genetic analysis is often necessary when evaluating secondary immunodeficiencies, as they may in fact be due to an underlying PID.

Sameer Parikh, MBBS, Mayo Clinic, focused on secondary immunodeficiency due to hematologic malignancy. He addressed the issue under three categories: tumor-induced defects; therapy-induced defects; and immunomodulatory effect(s) of cancer therapies.

Using chronic lymphocytic leukemia (CLL) as his main example, he explained the effect of tumor-induced immune defects on the course of the leukemia, risk of infection and emergence of secondary malignancies. A multi-variable analysis of risk factors in CLL identifies hypogammaglobulinemia as an independent predictor of time to first therapy, i.e. the lower the immunoglobulin levels, the sooner the patient should be treated. Similarly, immunoparesis observed in patients with dysproteinemias is a predictor of shorter overall survival in this group.

Patients with monoclonal B cell lymphocytosis (MBL), a precursor to CLL, show defective synapse formation between B cells and T cells which worsens with MBL's progression to CLL. Patients with MBL and CLL have a higher risk of infection than healthy individuals and are prone to secondary cancers including various common and rare solid tumors, therefore requiring routine screening for early detection of these conditions.

The use of immune profiling to stratify patients with acute myelogenous leukemia (AML) into immune-infiltrated and immune-depleted categories was discussed, noting that immune-infiltrated AML has a better response to therapy. Similarly, inflamed lymphomas show a better response to immune checkpoint blockade than their non-inflamed counterparts.

The highlighted therapy-induced defects were increased risk of pneumonia and herpes zoster infection due to impaired T cell function by proteasome inhibitors; neutropenia, NK cell depletion, and higher incidence of pneumonia and viral infections after treatment with the anti-CD38 antibody Daratumumab; and increased incidence of *pneumocytis jiroveci* pneumonia (PJP) despite normal CD4 T cell counts, and higher risk of aspergillosis through inhibition of NF-κB and NFAT pathways in macrophages in patients treated with the Bruton's tyrosine kinase (BTK) inhibitor Ibrutinib.

Finally, Dr. Parikh briefly discussed the potential effect of Linolidamide in improving vaccine responses in multiple myeloma and reversing T cell defects in CLL, and the differential immunodulatory effects of various Btk inhibitors based on their respective kinome profiles.

Thomas G. Boyce, MD, MPH, Marshfield Clinic, discussed immunodeficiency secondary to infectious diseases. He classified infections into those leading to long-term immune suppression, i.e. human immunodeficiency virus (HIV), and those with shorter-term effects, including measles, influenza, pertussis and bacterial sepsis. Bone marrow suppression due to certain viral infections, such as Parvovirus B19 and dengue virus was also noted.

The discussion on HIV included cellular tropism, time course of infection, virus reservoirs, immune evasion mechanisms, and hallmarks of chronic infection including hyperactivation, inflammation and immune exhaustion.

Measles-associated deaths are normally due to secondary infection. A decrease in delayed type hypersensitivity and T cell proliferation to mitogens, as well as Th2 cytokine polarization are documented in measles although their relative contribution to morbidity and mortality in comparison to risks associated with infancy and prior malnutrition is unclear.

Secondary bacterial pneumonia is a common feature of influenza infection. Numerous mechanisms have been proposed for this susceptibility, among which decreased neutrophil function and alteration of respiratory epithelium through sialidase activity are the main contending hypotheses.

Pertussis infection affects the immune system through several of its components: its filamentous hemagglutinin (FHA) mediates adherence to host cells and can induce regulatory T cells that secrete IL-10 and suppress Th1 responses. Its toxin (PT) delays neutrophil recruitment and antibody-induced bacterial clearance. The bacterium also causes TLR-4-mediated IL-10 production.

Bacterial sepsis was characterized as causing medium-term immunosuppression. Alterations in immune function after sepsis were discussed and lymphopenia and its severity on day 4 was highlighted as a predictor of mortality.

Dr. Boyce concluded by discussing the effect of SAR-CoV-2 on the immune system based on available literature. A list of tests for evaluating the immune system in patients suspected of secondary immunodeficiency following infection was provided.

Tiphanie P. Vogel, MD, PhD, Baylor College of Medicine and Texas Children's Hospital, Houston discussed recognition and subsequent monitoring of the consequences of therapeutic immune suppression in the context of autoimmunity. Treatment with corticosteroids and immunomodulators can result in severe infections (tuberculosis, bacterial, viral and invasive fungal infections). The effect of immunosuppressive agents on immune function was illustrated with cases of lupus, rheumatoid arthritis (RA), autoinflammatory disease, systemic-onset juvenile arthritis (JA) and vasculitis treated with a variety of immunosuppressive therapies. Pleotropic immunosuppressive effects of corticosteroids were illustrated in a lupus patient who presented with reduced NK cell cytotoxic function and hemophagocytic lymphohistiocytosis (HLH) secondary to EBV infection. This case emphasized that decreased NK cell cytotoxicity in HLH patients without a genetic cause should be reassessed at least 2 months after weaning off treatment to

exclude primary HLH. Dr. Vogel noted that administration of azathioprine during pregnancy for treatment of RA may result in low T cell receptor excision circles (TRECs) in the newborn. Examples of vasculitis and lupus patients who had received rituximab illustrated that B cell depletion could last up to six to twelve months following cessation of therapy. Addressing immune monitoring of cytokine levels for patients on anti-cytokine therapy, Dr. Vogel cautioned that assays that measure both free and antibody-bound IL-1β may lead to a falsely elevated IL-1β result in patients on Canakinumab (a monoclonal antibody that inhibits IL-1β). Dr. Vogel underscored the role of tailored immunotherapeutic regimens combined with regular clinical and laboratory immune evaluation before, during, and after treatment, and recommended vaccinating patients prior to initiating therapy.

This session emphasized the utility of genetic and immunological studies to differentiate PID from secondary immunodeficiency, described infectious, malignant and iatrogenic causes of secondary immunodeficiency, and underscored the need for careful evaluation and monitoring of patients on immunomodulatory therapies.

ANA update

Autoantibodies are valuable biomarkers for the diagnosis of systemic autoimmune rheumatic diseases. The antinuclear antibody (ANA)/anti-cell (AC) antibody testing by indirect immunofluorescence assay using HEp-2 cell substrates (HEp-2 IFA), commonly known as ANA test, is used worldwide in screening for autoantibodies. The ANA update session focused on initiatives to evaluate and harmonize the reporting of results for the ANA test. It included two presentations.

The first of these, a synopsis of the International Consensus on Antinuclear Antibody (ANA) Patterns (ICAP) initiatives, was presented by **Edward K. L. Chan**, PhD, University of Florida, Gainesville and co-coordinator of ICAP.

ICAP is an initiative of the Autoantibody Standardization Committee (ASC), itself a subcommittee of the International Union of Immunological Societies (IUIS) Quality Assessment and Standardization Committee. The goals of ICAP are to optimize usage of HEp-2 IFA patterns in patient care by promoting harmonization and better understanding of autoantibody test nomenclature as well as guidance on its interpretation and reporting.

In 2019, a survey was conducted to identify gaps in the assessment, interpretation and reporting of HEp-2 IFA in the clinical diagnostic laboratory. The results of this survey, presented at the 2019 AMLI meeting, revealed a lack of widespread familiarity with ICAP and other initiatives for increasing standardization in the interpretation and reporting of HEp-2 IFA results. It was concluded that to improve familiarity with ICAP and to further enhance HEp-2 IFA assessment, increased collaboration between ICAP and the clinical laboratory community is needed, particularly with respect to education and availability of reference materials.

Dr. Chan reviewed the ICAP goals and initiatives with an emphasis on the website and its role in bringing those goals to fruition. The ICAP website provides the consensus classification of 30 relevant HEp-2 IFA patterns with illustrative images and detailed information on the immunological and clinical relevance of each pattern. Currently, ICAP has over 3000 registered users from 183 countries and its content is available in 13 languages. So far, ICAP has developed one training module. Its website also has a frequently asked question section which includes both a list of previously asked questions and a link for users to submit questions. ICAP intends to develop additional training modules, translate its content into additional languages, and to continue discussion with stakeholders and world-wide users to achieve consensus for HEp-2 IFA interpretation and reporting.

In the second talk, **Mark H. Wener**, MD, University of Washington, Seattle discussed the results of a recent AMLI-sponsored performance survey to evaluate current practice and use of ICAP nomenclature for interpretation and reporting of HEp-2 IFA patterns on 12 well-characterized specimens. In all, 16 clinical laboratories [USA ($n = 13$) and Canada ($n = 3$)] and 8 in vitro diagnostic manufacturers participated. Dr. Wener's presentation focused on the results from the clinical laboratories. Based on the survey, most clinical laboratories can interpret the more commonly reported nuclear HEp-2 IFA patterns with distinct features, but there is room for improvement in the reporting of less common, complex and/or compound patterns. Factors hypothesized to contribute to the discordance in reporting such patterns include variability in staff experience/training, complexity of patterns, lack of experience in interpretation of patterns infrequently encountered, absence of some patterns in proficiency testing surveys, variability in microscope light sources, as well as variability in manufacturer reagents including slides. Endpoint titers were variable, spanning a median of 5 2-fold titers. The survey results underscore the need for collaboration between professional organizations such as ICAP and AMLI with clinical laboratories to provide additional training and optimize harmonization in the reporting of HEp-2 IFA patterns, as well as more collaborative efforts to improve consistency and quality of slides and reagents for HEp-2 IFA testing by all industry stakeholders.

Emerging infectious diseases and new algorithms for testing

The term "emerging and re-emerging disease" was coined by Joshua Lederberg as part of a corrective to the dominant "Eradicationist" vision of infectious diseases in mid- to late- twentieth century, which was espoused by Aidan Cockburn and Abdel Omran, both eminent epidemiologists, as well as MacFarlane Burnet, among many others. Laboratory testing plays a pivotal role in identifying emerging infectious diseases and by extension in their treatment. This session featured four speakers.

William T. Lee, PhD from the New York Department of Health discussed "Modified Two-Tiered Serological Testing for Lyme Disease". *Borrelia burgdorferi* sensu *lato* is carried by *Ixodes scapularis* and *Ixodes pacificus* in the US. Diagnosis is often challenging as 30–60% of infected patients do not show the classic erythema chronicum migrans, or bullseye rash. An estimated 10% of patients remain symptomatic despite treatment. Hence, laboratory diagnosis remains a key element in early diagnosis and treatment. Laboratory diagnosis may be made in one of two ways: (1) pathogen culture or molecular detection, or (2) a two-tier serology algorithm. While serum is typically more accessible, cerebrospinal fluid may be used in the setting of neuroborreliosis. In the typical patient, Borrelia IgM is detectable within days to weeks of infection and peaks within 3–6 weeks; IgG appears about 2 weeks post-infection and is long lived. The conventional serological two-tiered test consists of either an enzyme immunoassay (EIA) or indirect fluorescence assay (IIF); if either is positive or equivocal, the sample is reflexed to western blotting. If EIA is negative, a second sample obtained about 4 weeks after suspected infection will be tested by EIA. Early generation tests were sensitive, but not specific because the flagella antigen whole-cell lysates used differed in both the tick and the patient. The lack of specificity was resolved with the use of recombinant protein and peptide antigens. C6-based EIAs used a synthetic peptide of the invariant portion of VlsE. Recombinant PepC10-based EIAs used a conserved portion of outer surface protein C. The first generation of western blots similarly used whole cell lysates; second generation assays use recombinant proteins and peptides, decreasing background and improving densitometry. Dr. Lee ended his presentation by highlighting the recent update by the FDA to the testing algorithm, in which the reflex to western blot has been replaced with a second EIA.

Elitza S. Theel, PhD, Mayo Clinic then reported on "Tick-Borne Infections – Beyond Lyme Disease". Using a series of case studies, Dr. Theel covered 5 tick-borne illnesses, including parasites (Babesia), flavivirus (Powassan), and bacteria (Ehrlichia, Rickettsia, and Borrelia). With regard to diagnosis of these infections, several themes emerged from this presentation: First, knowledge of the geographical area in which an exposure could have occurred can be important in identifying the

potential tick species and infectious agent. Second, most general laboratory findings, such as thrombocytopenia and elevated liver enzymes are non-specific and are not very helpful for diagnosis. Third, blood smears can be useful in the diagnosis of some tick-borne infections and certain findings, such as the presence of morulae in monocytes with Ehrlichia, are very specific. However, blood smears generally suffer from low sensitivity. During the acute stage of infection, ≤7 days from exposure, reverse transcriptase PCR (RT-PCR) is the preferred diagnostic modality. RT-PCR has the advantage of high sensitivity while maintaining high specificity. However, a major disadvantage of molecular testing for tick-borne infections is limited availability. This testing may only be available through public health or reference laboratories, and long turn-around times may limit their clinical utility. Lastly, for individuals who are beyond the acute infection stage, serology testing becomes the method of choice. According to Dr. Theel, infection-specific antibody testing has the highest sensitivity in patients at least 7–10 days following the onset of symptoms. Serologic testing can be done by EIA, IIF, and, in the case of viruses, plaque reduction neutralization testing (PRNT). Serology testing is reported with a titer, and higher titer results are more clinically relevant for establishing the diagnosis. Also, results in which the titer of convalescent serum is at least 4-fold higher than the titer of serum obtained during the acute phase provides strong evidence for the causative infection.

Aileen Chang, MD, MSPH, George Washington University presented "Emerging Arbovirus Disease: Chikungunya, Mayaro Virus and Oropouche Virus, and Powassan Virus". Dr. Chang reviewed emerging viruses in the Americas spread by arthropods such as mosquitos and ticks. These viruses cause systemic acute illness. Chikungunya virus, spread by *Aedes aegypti* and *Albopictus* mosquitos, emerged in 2013 in the US, with cases of rash, arthralgia, arthritis, and occasionally complicated by uveitis, retinitis, myocarditis, hepatitis, nephritis, hemorrhage, meningoencephalitis, myelitis, Guillain-Barré syndrome, and cranial nerve palsies. The polyarthralgia, polyarthritis and tenosynovitis may become chronic. Mayaro virus, spread by *Haemagogus* mosquitoes of the Amazon jungle, emerged in the Americas and manifests similarly to Chikungunya. Dr. Chang highlighted that both viral infections may be difficult to distinguish from dengue. Oropuoche virus, transmitted by the *Culicoides paraensis* midge from sloth to man, circulates in Central and South America. Patients present with fever, headache, arthralgia, rash, and may progress to meningitis and/or encephalitis. Dr. Chang ended her presentation with a discussion of Powassan virus infections that have been reported in the US and Canada. Powassan virus is transmitted by *Ixodes* ticks from *Peromyscus leucopus* mice to humans. Infected individuals develop fever, headache, meningitis, encephalitis, and in 10% of infected patients death. Survivors may suffer chronically from recurrent headache, weakness, and cognitive impairment. Laboratory diagnosis for these viruses is typically molecular, but serology has also been employed. Treatment for these is supportive.

This session ended with a presentation by **Aaron C. Brault**, PhD titled "Epidemiology and Diagnostic Capacity for US Domestic Arboviruses: Role of Classical and Next Generation Technologies". Dr. Brault leads the Arbovirus Diagnostic and Reference Laboratory within the US Centers for Disease Control and Prevention. Most arboviruses of public health importance in the US, such as West Nile virus (WNV), St Louis encephalitis virus, and Eastern equine encephalitis virus, are transmitted by mosquitoes, although 2 other relevant viruses, Powassan and Colorado tick fever viruses, are transmitted through tick bites. Dr. Brault discussed the role of the Arboviral Diseases Branch in the epidemiology of these diseases, using WNV as an example. He showed data tracking the number of WNV cases by month and the annual incidence of WNV neuroinvasive disease by year, by age group, and by county across the US. Dr. Brault then presented the various methods used by the Arbovirus Diagnostic and Reference Laboratory, including molecular and serologic testing. The serologic testing methods include screening EIAs and microsphere immunoassays (MIAs) for IgG and IgM, with confirmatory testing by plaque reduction normalization test (PRNT). Importantly, Dr. Brault presented guidelines for interpretation of serologic results for acute and convalescent serum. Lastly, Dr. Brault described the role of the Arboviral Disease Branch in the discovery of new viruses, namely the Heartland and Bourbon arboviruses. Dr. Brault used these discoveries to highlight the importance of maintaining classical virology techniques while continuing to invest in new diagnostic technologies.

Antibody testing for COVID-19

On 30 December 2019, PCR assessment of samples from the lung of a patient with pneumonia in Wuhan confirmed the presence of a new type of coronavirus, since named SARS-CoV-2. Within a month, on 30 January 2020, WHO's International Health Regulations Emergency Committee declared a Public Health Emergency of International Concern (PHEIC) with respect to COVID-19. Despite guidelines issued by the National Academies in the aftermath of SARS, and simulations such as Exercise Cygnus in the United Kingdom and Crimson Contagion in the United States, governments and health systems have so far fallen short in tackling the COVID-19 pandemic.

The COVID-19 pandemic has highlighted for the public, as never before, the critical role of laboratory testing for infectious disease. For months the public has been inundated with information on diagnostic testing including terms such as "PCR", "antibodies" and "sensitivity and specificity". While the complexity of laboratory testing is possibly better appreciated, so are the limitations of testing and the regulatory environment with which laboratory tests are reviewed before they are put into use.

The diagnosis of COVID-19 disease relies upon detection of SARS-CoV-2 nucleic acid or antigen. Detection of SARS-CoV-2 components provides direct evidence of the virus in various sample types collected from patients. However, the performance of these tests depends on collection of appropriate samples at the appropriate time. Given these restrictions, no direct detection method routinely achieves 100% clinical sensitivity. Antibody testing provides an alternate approach to diagnose infection. However, the presence of antibody indicates exposure at some time in the recent or remote past and does not confirm current infection. This fact was appreciated from the start of COVID-19 testing and has limited the application of serologic testing for diagnosis to select clinical situations. Patients with later manifestations or prolonged infection, when viral nucleic acid or antigen may be below the limit of detection or are no longer present, might benefit from an antibody test. In addition, the administration of convalescent plasma as a therapeutic option for seriously ill COVID-19 patients led to the use of serologic testing to confirm the presence of SARS-CoV-2 antibody in convalescent plasma units. Finally, antibody testing is the method of choice for the conduct of studies to determine the prevalence of SARS-CoV-2 exposure in asymptomatic populations (seroprevalence).

While the FDA instituted a policy of review and granting of emergency use authorization (EUA) for molecular and antigen detection tests for diagnostic purposes, there was a sense of urgency to make testing broadly available. Since antibody testing was not considered a reliable diagnostic approach to the acute infection, FDA review of validation data was not initially required for the marketing of antibody tests. An initial, suboptimal approach to regulation of antibody tests for SARS-CoV-2 infection led to the proliferation of more than 100 serologic tests for patients suspected of COVID-19. Some of these tests ultimately proved poor in terms of their performance and, after later institution of FDA review of validation data, were ultimately removed from distribution. Many reliable tests remain and are available for use in patients with suspected infection or for sero-surveys to assess prevalence of infection.

Gabriel N. Maine, PhD, Beaumont Health, gave an overview of the technical and regulatory aspects of serologic testing for SARS-CoV-2 infection.

The SARS-CoV-2 virus encodes 2 proteins that have been used as antigens in serologic tests. The spike protein contains regions S1, that

includes the receptor binding domain of the virus, and the S2 region which participates in the fusion event of the virus and host cell. The receptor binding domain of the spike protein which is the likely antigen targeted by neutralizing antibodies, is also used. The nucleocapsid protein is immunogenic as well and used in several commercially available antibody tests. Three formats of assays are used including lateral flow (LFA), EIA and chemiluminescent immunoassay (CLIA). These tests have been developed to detect IgM, IgA and IgG antibodies, and also formatted as a polyvalent assay to detect SARS-CoV-2 antibody regardless of isotype.

The rapid development of a large number of tests with limited regulatory oversight, initially, led to significant concern over test performance as well as confusion about the role of antibody testing in general. At first, serologic test developers needed only to inform FDA they intended to market an antibody test and that it had been validated. That did not relieve laboratories of the responsibility to perform in house validations but did lead to the deluge of tests with varying performance characteristics. Dr. Maine described the process of validation and gave examples of validation results from 3 classes of tests. The FDA website lists available antibody tests that have EUA, of which many have quite good specificity. The sensitivity of antibody tests, as discussed by Dr. Maine, depends on the time of sample collection relative to the onset of symptoms. Prior to 21 days after onset, antibody tests have suboptimal sensitivity. Many tests approach 100% sensitivity beyond 21 days however.

This session ended with a useful discussion of the features of antibody testing that are yet to be resolved. Important questions included the clinical utility of antibody tests, the longer-term persistence of antibody, kinetics of antibodies in symptomatic versus asymptomatically infected individuals and whether prior infection with related viruses induces any immunologic protection from SARS-CoV-2 infection.

A frequent use of serologic testing for COVID-19 is for seroprevalence studies. While virus is only detectable during acute illness and for a relatively short time thereafter, antibodies are likely detectable for a much longer time and could be useful for tracking prevalence of SARS-CoV-2 infection. SARS-CoV-2 seroprevalence is variable. Factors contributing to this variance are likely epidemiologic in nature, but also entail specific performance characteristics of the test used. In particular, the positive predictive value of a test will vary highly depending on the test's specificity and the prevalence of the infection in the population tested.

In the next talk, **Matthew D. Sims**, MD, PhD, Beaumont Health, addressed the issue of seroprevalence. He described, in great detail, the development and implementation of a large seroprevalence study in the largest healthcare system in Michigan, Beaumont Health, and the challenges faced with the design and implementation of the study.

Their study had 3 aims: (1) determine the prevalence of antibody in health system employees; (2) determine the magnitude, durability and protection of the antibodies and (3) determine the suitability of asymptomatic individuals as convalescent plasma donors. In order to achieve these aims, an infrastructure able to test over 30,000 employees in a timely and accurate manner needed to be developed in a short amount of time.

At the heart of the process was development of a project team that provided oversight of the research details, clinical details, logistics, financial aspects and information technology. In total, a team of 400 individuals successfully launched the study in approximately 3 weeks. At the time of this meeting, they had enrolled over 22,000 participants with over 37,000 blood draws and 75,000 tests performed.

As one would expect there were many lessons learned. Communication was critical including daily huddles to foster interaction. A local institutional review board (IRB) that could provide rapid review was also critical. Finally, the laboratory had to adjust and accommodate to the demands and oversight associated with clinical trials as compared to routine clinical laboratory testing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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